CIRCUIT MECHANISMS OF NEURODEGENERATIVE DISEASES

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CIRCUIT MECHANISMS OF NEURODEGENERATIVE DISEASES

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EDITORIAL
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Editorial: Circuit Mechanisms of Neurodegenerative Diseases

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Editorial on the Research Topic

Circuit Mechanisms of Neurodegenerative Diseases

Neurodegenerative diseases (NDs), such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), or Amyotrophic lateral sclerosis (ALS) are histopathologically characterized by the formation of protein aggregates and the selective death of a defined population of neurons. These diseases have traditionally been viewed as being caused by the selective dysfunction of those vulnerable neurons via cell autonomous mechanisms. Current evidence, however, strongly implicates a causal involvement of altered neuronal circuit function in triggering and perpetuating the degenerative cascade. Recent methodological advances offered by newly developed genetic tools, *in vivo* electrophysiology and imaging techniques, enabled the selective assessment and manipulation of dedicated cell populations and neural circuits *in vivo*. Such approaches have provided evidence that circuit alterations result from complex changes at the level of synapses, intrinsic excitability of cells and disrupted connectivity within local microcircuits and between projection areas. This ultimately results in altered activity patterns of neurons, impaired information processing in a disease-stage dependent manner and eventual neuronal loss. Intriguingly, preclinical studies in rodent models suggest that many of those structural and functional alterations within a neuronal network are already found at early presymptomatic stages, long before typical markers of degeneration are detectable. These findings support the notion that circuit dysfunction is likely not only a consequence of degenerative processes in NDs, but can in fact represent a main driver of the pathology. This novel concept not only opens up new therapeutic avenues by shedding light into brain-region, and cell-type specific alterations occurring in a disease-stage specific manner; but moreover, it has the potential to identify novel diagnostic approaches and therapeutic windows.

In this Research Topic Werner et al. summarize the current status and opportunities of *in vivo* imaging using miniature fluorescence microscopes (miniscopes), which enables chronic monitoring of neuronal activity and the disentangling of circuit deficits in NDs. A number of review articles summarize the current knowledge about the impact of neurodegenerative processes on neural circuit function and development. As such, Blumenstock and Dudanova explore cell-type specific impairments in cortical and striatal circuits in HD, while Binda et al. summarize evidence arguing for developmental deficits in the maturation of cerebellar circuits in Spinocerebellar Ataxias. Gunes et al. recapitulate findings of altered excitability in various elements of motor circuits in ALS and Brunet et al. assemble evidence for cortical dysfunction as a main driver of ALS pathophysiology. Another set of papers addresses the circuit mechanism of memory loss in mouse models of dementia. To this end, Vyas et al. review the complex structural and functional changes of single neurons and neural circuits in hippocampus of mouse models of AD. Yeung et al. investigate the impact of amyloid-beta 42, one of the main molecular players in AD, on glutamatergic receptor and transporter expression

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in hippocampus. In a systematic review Rashidi-Ranjbar et al. revisit evidence for structural and functional impairments of corticolimbic circuits in late-life depression and dementia. Furthermore, in a hypothesis paper Janitzky argues for a compromised function of the locus coereleus (LC)—a brain region harboring noradrenergic projection neurons—in early stages of NDs. LC dysfunction results in persistent high tonic discharge, while the phasic discharge is impaired, which is hypothesized to diminish putative anti-inflammatory and neuroprotective effects conveyed by phasic LC firing.

With this Research Topic in Frontiers in Neuroscience, we aim to provide compelling recent evidence in support of the hypothesis of altered circuit function as a potent co-driver of various NDs. This volume contains original research articles as well as reviews providing an overview of the current knowledge and technological advances in the mentioned area of neurodegeneration.

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SS and SL were equally involved in the conceptualization and editing of the Research Topic.

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MINI REVIEW
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Circuit Mechanisms of Neurodegenerative Diseases: A New Frontier With Miniature Fluorescence Microscopy

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Neurodegenerative diseases (NDDs), such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD), are devastating age-associated brain disorders. Significant efforts have been made to uncover the molecular and cellular pathogenic mechanisms that underlie NDDs. However, our understanding of the neural circuit mechanisms that mediate NDDs and associated symptomatic features have been hindered by technological limitations. Our inability to identify and track individual neurons longitudinally in subcortical brain regions that are preferentially targeted in NDDs has left gaping holes in our knowledge of NDDs. Recent development and advancement of the miniature fluorescence microscope (miniscope) has opened up new avenues for examining spatially and temporally coordinated activity from hundreds of cells in deep brain structures in freely moving rodents. In the present mini-review, we examine the capabilities of current and future miniscope tools and discuss the innovative applications of miniscope imaging techniques that can push the boundaries of our understanding of neural circuit mechanisms of NDDs into new territories.

Keywords: neurodegenerative disorders, miniature fluorescence microscopy, miniscope, *in vivo* calcium imaging, deep brain imaging, longitudinal recording

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INTRODUCTION

Neurodegenerative diseases (NDDs), such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD), are prevalent and incredibly devastating diseases, with an estimated 6 million people affected by NDDs in the United States alone. A common risk factor for most NDDs is advancing age, and with a globally growing elderly population, the number of cases is expected to increase rapidly worldwide in the coming years. Substantial efforts have been undertaken to study NDDs, which have led to fundamental insights into these diseases. Still, the neurobiology that underlies NDDs is not well understood. With limited knowledge, therapeutic interventions have been developed to delay deterioration and provide temporary relief of symptoms, but there remains a tremendous need to improve treatments.

Although NDDs uniquely affect specific neuronal subpopulations, the accumulation of distinct protein-based macroscopic deposits that cause neuronal loss is a common hallmark of NDDs. In parallel with progressive neuronal loss, synaptic dysfunction, altered calcium homeostasis, aberrant neural activity, abnormal neuronal plasticity, and impairment in large-scale neural circuits are well-documented features of many NDDs (Palop and Mucke, 2016; Frere and Slutsky, 2018; Pchitskaya et al., 2018; Jackson et al., 2019). The interdependence between abnormal protein depositions, neuronal loss, and dysfunctional neural networks remains to be determined. Nevertheless, growing evidence suggests that many neurological deficits in NDDs may reflect functional impairment in neural circuits rather than neuronal loss (Palop et al., 2006; Seeley et al., 2009), suggesting that targeting reversible neural circuits holds therapeutic potential and deserves substantial consideration (Laxton et al., 2010; Bakker et al., 2012; Canter et al., 2016).

Due to technical challenges in performing longitudinal studies on individual neurons in large-scale populations from subcortical brain regions in freely moving animals, our current understanding of the neural circuit mechanisms that underlie NDDs and associated symptoms remains limited. In the present mini-review, we focus on the potential of the miniature fluorescence microscope (miniscope), a recently developed *in vivo* calcium imaging technology (**Figure 1**; Aharoni and Hoogland, 2019), to provide new insights into dysfunctional neural circuits that contribute to the pathogeneses of NDDs. We first discuss a brief history of the miniscope, and then describe specific capabilities of the miniscope with potential applications to study microcircuit dysfunction in models of NDDs.

A BRIEF HISTORY OF THE MINISCOPE

The observation of *in vivo* calcium transients using microscopy would not be possible without calcium indicators (Grienberger and Konnerth, 2012). Genetically encoded calcium indicators (GECIs; Miyawaki et al., 1997), and GCaMP-family GECIs in particular (Nakai et al., 2001; Tian et al., 2009; Chen et al., 2013), have proved to be incredibly important tools, and have played important roles in defining what can be achieved with calcium imaging. Ongoing advancements in calcium sensors continue to push beyond current limitations, and with activity sensors also moving beyond calcium, voltage, neurotransmitter, and ion sensors will help to shape a very different future for fluorescence microscopy (**Table 1**; Lin and Schnitzer, 2016; Deo and Lavis, 2018).

TABLE 1 | Selected genetically encoded indicators.

Type	Sensor examples	Reference
Calcium	GCaMP6	Chen et al., 2013
	GCaMP7	Dana et al., 2019
	jRGECO1	Dana et al., 2016
	XCaMPs	Inoue et al., 2019
Voltage	QuasAr2	Hochbaum et al., 2014
	ArcLight	Kwon et al., 2017
Dopamine	$GRAB_{DA}$	Sun et al., 2018
	dLight1	Patriarchi et al., 2018
Glutamate	SF-iGluSnFR	Marvin et al., 2018
Acetylcholine	GACh	Jing et al., 2018
Potassium	KIRIN1	Shen et al., 2019

Coinciding with advancements in fluorescent activity reporters has been progress in imaging instrumentation. Since the development of two-photon microscopy (Denk et al., 1990), studies examining calcium activity have increased dramatically. Imaging cortical activity through cranial windows in head-fixed animals using a variety of behaviors has provided many new insights into the neuronal encoding of behavior (Yang et al., 2009; Komiyama et al., 2010), and the emergence of Gradient Index (GRIN) lenses, which relay images from one end to the other to provide an optical interface to the brain, has allowed for endoscopic imaging of deep brain regions (Barretto et al., 2009; Yang et al., 2019; Zhang et al., 2019). Examination of neuronal activity during behavior in freely moving animals was then made possible by the development of fiber-optic based microscopes (Helmchen et al., 2001, 2013; Flusberg et al., 2008; Sawinski et al., 2009) and miniature fluorescence microscopes with two-photon (Helmchen et al., 2001; Sawinski et al., 2009; Liang et al., 2017; Zong et al., 2017) or epifluorescent light sources (Ghosh et al., 2011).

The first miniscope was developed by Ghosh et al. (2011), which included a number of unique features. The miniscope was head-mounted with fluorescence excitation and detection onboard rather than away from the microscope, and also bypassed cost limitations of table-top setups by using commercially available and inexpensive technologies. The miniscope offers many unique advantages, including the ability to perform recordings that are cell-type specific, large-scale (hundreds of cells can be recorded), and longitudinal in nature [cells can be recorded for months (Zhang et al., 2019)]. These features provide a unique tool to study NDDs, as the miniscope can image cells in microcircuits during the development of NDD-like pathophysiology and behavior in animal models.

IN VIVO DEEP BRAIN IMAGING IN FREELY MOVING ANIMALS

Many behavioral manifestations of NDDs have been modeled in animals (Gitler et al., 2017), and understanding the microcircuitry that encodes disease-related aberrant behaviors could provide essential insights into the neurobiology underlying NDDs. However, observing neuronal activity during behaviors in freely moving animals has been technically challenging. Two-photon imaging using table-top setup approaches requires head fixation, which limits the usability to certain behavioral assays. Moreover, differences in neural activity have been reported in head-fixed animals compared to freely moving animals, suggesting that head-restrained and natural conditions have different neural correlates (Ravassard et al., 2013; Aghajan et al., 2015). Various instruments have been developed to

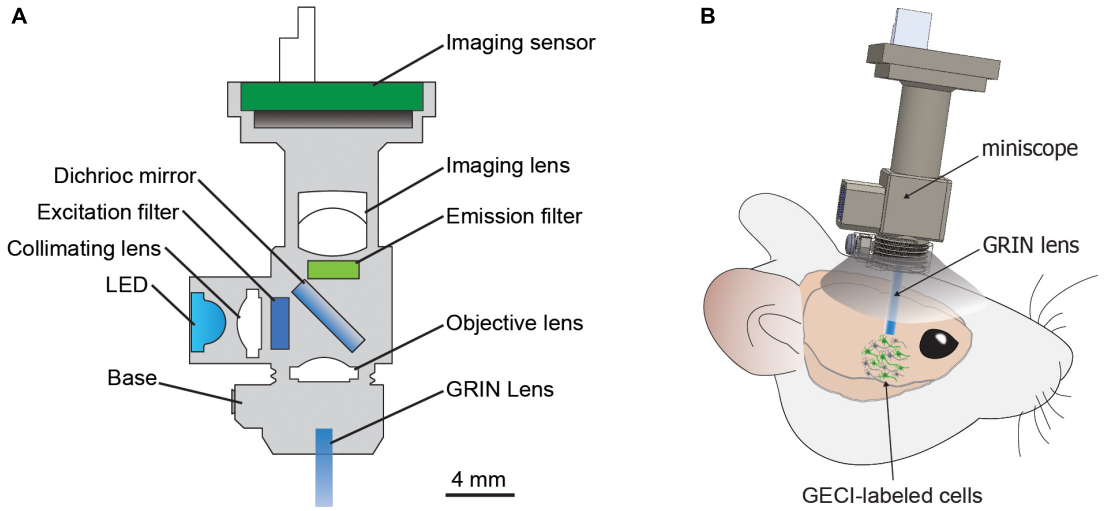

FIGURE 1 | The miniscope. **(A)** Basic components of miniScope and GRIN lens system. **(B)** Schematic for a mounted miniScope with the GRIN lens aimed at GECI-labeled cells in a deep brain structure. GRIN, gradient index lens; GECI, genetically encoded calcium indicator.

examine neuronal activity during behavior in freely moving animals (Helmchen et al., 2001, 2013; Flusberg et al., 2008; Sawinski et al., 2009; Liang et al., 2017; Zong et al., 2017), but the miniscope offers a unique option that is accessible, compact, and affordable (**Figure 1** and **Table 2**; Aharoni and Hoogland, 2019).

An additional technical challenge is accessing deep brain structures that are preferentially affected in many NDDs. Until recently, the ability of fluorescence microscopy to monitor neuron activity at subcellular resolution has been limited to superficial depths of ∼1 mm (Mittmann et al., 2011). This is due to light scattering that occurs in tissue (Ji, 2014), as well as tissue damage and inflammatory responses that occurs when removing overlaying tissue to provide optical access to a structure of interest. Imaging neurons in deeper brain structures is more practical with endoscopic techniques, which include insertion of lenses into the brain. The use of smaller-diameter GRIN lenses can reduce tissue damage (Bocarsly et al., 2015; Yang et al., 2019) and minimize the effect on behavior (Lee et al., 2016), which can be further improved with the use of robotic surgical instruments (Liang et al., 2019).

TABLE 2 | Open-source miniscopes.

Miniscope	Developer	Platform	Reference
UCLA miniscope	UCLA	http://miniscope.org/index.php/Main_Page	Cai et al., 2016
FinchScope	Boston University	https://github.com/gardner-lab/FinchScope	Liberti et al., 2016
miniScope	NIDA	https://github.com/giovannibarbera/miniscope_v1.0	Barbera et al., 2016
CHEndoscope	University of Toronto	https://github.com/jf-lab/chendoscope	Jacob et al., 2018
cScope	Princeton	https://github.com/PrincetonUniversity/cScope	Scott et al., 2018

LONGITUDINAL TRACKING OF INDIVIDUAL CELLS TO STUDY NDDs

Aging is a leading risk factor for many NDDs. While each NDD demonstrates unique spatiotemporal patterns of degeneration, the often-slow advancement of these diseases provides a similarly distinct challenge for researchers to study. A major cause of age-related degeneration is the accumulation of disease-specific misfolded proteins that lead to progressive loss of neuronal function (Hung et al., 2010; Chiti and Dobson, 2017). However, changes in patterned neural and circuit activity have also been observed in most NDDs and are believed to contribute to the onset and progression of disease states (Palop et al., 2006; Seeley et al., 2009).

Hyperexcitability in Early Stage of NDDs

Hyperexcitability has been observed in a number of NDDs. Cortical and peripheral hyperexcitability has been well-documented in clinical studies of ALS patients (Mills and Nithi, 1997; Vucic and Kiernan, 2006; Vucic et al., 2008; Williams et al., 2013; Menon et al., 2015), and is among the earliest pathologies identified in ALS patients (Bae et al., 2013) and during early stages of ALS-like pathology in rodent models (van Zundert et al., 2008; Zhang et al., 2016; Marcuzzo et al., 2019). Therefore, it is presumed that neuronal hyperexcitability is an important factor that leads to neuronal loss in ALS. In AD, although hypoactivity is a key feature of late stages of the disease (Braak and Braak, 1991; Nestor et al., 2003), paradoxical hyperactivity has been observed in multiple brain regions of AD patients during pre-symptomatic stages

(Sperling, 2007; Dickerson and Sperling, 2008; Miller et al., 2008; Pihlajamaki et al., 2009; Yassa et al., 2010). Furthermore, chronic aberrant increases in excitatory neuronal activity have been detected in the hippocampal and cortical regions in AD mouse models (Palop et al., 2007; Busche et al., 2008), and increasing neural activity has been demonstrated to directly promote the production and secretion of amyloid-β (Aβ) peptide (Cirrito et al., 2005, 2008; Bero et al., 2011), whose excessive accumulation appears to play a causal role in AD (Stargardt et al., 2015). However, how hyperexcitable neurons encode behavior and contribute to disease progression remains unknown.

Therapeutic Potential of Targeting Hyperactivity

Targeting hyperactivity in mouse models of AD and ALS has been demonstrated to improve signatures of neural injury (Sanchez et al., 2012; Yuan and Grutzendler, 2016; Zhang et al., 2016; Haberman et al., 2017), and, importantly, clinical trials aiming to reduce hippocampal hyperactivity showed positive effects in patients with amnestic cognitive impairment (Bakker et al., 2012). The most widely prescribed drug for ALS, riluzole, reduces excitatory synaptic activity in the brain (Doble, 1996), and memantine, a therapeutic drug approved for use in late-stage AD, functions as a NMDA receptor antagonist to counteract hyperactive glutamatergic circuits (Zhu et al., 2013). But still, there remains a need to determine how these drugs affect neuronal activity to provide therapeutic relief to disease-related behavioral symptoms. Examining the activity of individual neurons across time could provide insights into how microcircuits encode pathophysiological behaviors as diseases advance. The miniscope is able to track individual neurons for several months during behaviors in freely moving animals (Liang et al., 2018; Zhang et al., 2019), providing an opportunity to examine how changes in activity in individual neurons within a microcircuit across long periods of time affect behavior.

IMAGING SPECIFIC CELLULAR SUBTYPES TO STUDY NDDs

Each NDD has a signature pattern of degeneration progression. Particular circuits are vulnerable, and specific subtypes of cells within circuit-associated brain regions are targeted. In combination with transgenic rodent lines and/or cell-type specific and inducible promotors for vectors in gene therapy, specific neuron subtypes, and other cell-types in the brain can be imaged with the miniscope. This capability provides exciting opportunities for new insights into how neuronal subtypes encode pathology-related behaviors in models of NDDs.

Imaging Dopaminergic, Cholinergic, and Medium Spiny Neurons

The manifestation of PD symptoms is believed to be the cause of dopaminergic neuron loss in the substantia nigra pars compacta (SNc) that leads to an imbalance in basal ganglia direct and indirect pathways (McGregor and Nelson, 2019). Studies with parkinsonian rodents have linked activity changes to discrete neuronal populations with potentially distinct contributions to and/or alleviation of parkinsonian motor symptoms (Mallet et al., 2016; Parker et al., 2018; Ryan et al., 2018; Sagot et al., 2018). Using transgenic Cre-driver mouse lines and a commercial miniscope, Parker et al. (2018) reported that medium spiny neurons (MSNs) in the direct and indirect pathways displayed bidirectional changes in firing rate in parkinsonian mice. Firing rates in MSNs, along with behavioral abnormalities, were reversed by administration of L-DOPA or a dopamine receptor D_2 receptor agonist (Parker et al., 2018). While this study demonstrated some of the capabilities of miniscope imaging, numerous questions still remain related to firing rate, pattern, and synchronization of neurons in the SNc and other brain regions that are believed to contribute to PD pathology.

Selective vulnerability of MSNs in the basal ganglia is a characteristic of HD (Ross and Tabrizi, 2011). MSNs are unique in that they not only receive dopaminergic input from the midbrain but also glutamatergic input from the cortex and other brain regions. Direct imaging of MSN activity in the basal ganglia would provide useful clues to determine intrinsic properties of MSNs that may lead to vulnerability in HD.

Neuromodulatory system dysfunction has been linked to a number of NDDs, but how specific neuromodulatory systems encode behavior in models of NDD has yet to be examined. Selective loss of cholinergic neurons have been observed in postmortem brains of AD patients (Whitehouse et al., 1981; Mesulam et al., 2004), and reductions in cholinergic markers are strongly correlated with cognitive decline in PD patients (Perry et al., 1985). Importantly, clinical studies support beneficial effects of cholinesterase inhibitor treatment in both PD and AD patients (Emre et al., 2004; Zhu et al., 2013), suggesting a need to better understand how cholinergic neuron dysfunction contributes to symptoms in PD, AD, and other NDDs.

Imaging Specific Inhibitory Interneurons

γ-aminobutyric acid (GABA) plays a central role in regulating neuronal excitability and maintaining balanced network activity, and inhibitory interneuron abnormalities also contribute to a number of NDDs. Inhibitory interneuron dysfunction has been suggested to contribute to network hypersynchrony (Palop and Mucke, 2016) that underlies frequent epileptic activity that occurs in AD patients (Vossel et al., 2016, 2017), as deficits in inhibitory interneurons result in impaired oscillatory rhythm and network hyperactivity in AD mouse models (Baglietto-Vargas et al., 2010; Villette et al., 2010; Verret et al., 2012; Hamm et al., 2017). In addition, reductions of subpopulations of inhibitory interneurons have been described in AD mouse models and postmortem brains of AD patients (Ramos et al., 2006; Takahashi et al., 2010; Albuquerque et al., 2015; Mahar et al., 2016). Dysfunctional interneurons have also been linked to upper motor neuron hyperexcitability in an ALS/FTD model

(Zhang et al., 2016). Imbalance of excitation-to-inhibition (E/I) due to interneuron impairment has been suggested to be a potential common driver for AD and ALS, as well as other NDDs (Do-Ha et al., 2018; Ambrad Giovannetti and Fuhrmann, 2019). Studies on inhibitory interneurons using miniscope imaging in NDD models could shed light on this theory and provide information on inhibitory interneurons in NDD pathogenesis.

Imaging Specific Glial Cells

A variety of glial cells have complex reciprocal interactions with neurons to support their functions and are active participants in neural circuit refinement (Paolicelli et al., 2011; Schafer et al., 2012; Risher et al., 2014; Tani et al., 2014). For instance, subsets of astrocytes in the basal ganglia release glutamate to selectively activate homotypic synapses to regulate neural networks (Martin et al., 2015). Dysfunctional glial cells contribute to the disease progression in both HD and ALS through non-cell-autonomous toxicity (Yamanaka et al., 2008; Haidet-Phillips et al., 2011; Creus-Muncunill and Ehrlich, 2019). Neuroinflammation that results from chronic activation of immune responses are often mediated by microglia and occur in susceptible regions, and are believed to contribute to age-related degeneration (McGeer and McGeer, 2004; Cappellano et al., 2013). Microglial activation and reactive astrogliosis are observed in many NDDs, and reactive astrocytes lose many normal functions and gain new abnormal functions. Synapse elimination by microglial and astrocytes may contribute to the loss of presynaptic terminals and dendritic spines (Chung et al., 2015; Henstridge et al., 2019), one of the earliest features associated with cognitive impairment in many NDDs (Terry et al., 1991; Scheff et al., 2006). Glial cells may selectively remove excitatory or inhibitory synapses to compensate for disease-associated changes at specific brain regions (Hong et al., 2016; Aono et al., 2017; Paolicelli et al., 2017), or may act as a primary driver for the E/I imbalance by directly inducing excessive synaptic pruning (Serrano-Pozo et al., 2013; Rodriguez et al., 2014). Studying glial activity though miniscope calcium imaging might provide useful information on the mechanism of how glial dysfunctions contribute to NDDs.

NEW FRONTIERS IN MINISCOPE IMAGING

The current generation of miniscopes offers many exciting possibilities for the study of NDDs. Innovation of the miniscope has been dramatically accelerated by open-source sharing of miniscope projects (**Table 2**; Aharoni and Hoogland, 2019), with functional improvements that hold the promise of new frontiers. The next generation of miniscopes and fluorescence sensors boast capabilities to distinguish two neuron subtypes simultaneously, manipulate cellular activity while imaging, image cellular events with non-calcium indicators, and record electrical properties (Deo and Lavis, 2018; Aharoni and Hoogland, 2019).

GECIs With Spectrally Separable Wavelengths

In the case of NDDs, it would be advantageous to simultaneously image a preferentially affected cell-type population, as those described above, while also imaging surrounding cell-types. This would provide information to understand how dysfunction of a vulnerable cellular population not only affects behavior, but also the other cells in the local microcircuit. Development of GECIs (**Table 1**) with spectrally separable wavelengths (Chen et al., 2013; Dana et al., 2016; Inoue et al., 2019) provide potential to examine the concomitance of two distinct neuronal populations correlated to specific behaviors, which can be accomplished a number of ways, including using a Cre-On/Cre-off approach (Saunders et al., 2012).

GECIs with distinct wavelengths also provide an opportunity to concurrently image and manipulate neural activity. Miniscope imaging offers correlational insight into neural activity that encodes behavior, but manipulating neural activity concurrently with imaging can offer insight into causal roles of cell populations within microcircuits (Stamatakis et al., 2018). This task can be achieved by using light-driven channels that have minimal overlapping spectra with a fluorescent activity sensor. One could also model dysregulated neuronal firing observed in NDDs with optogenetics while recording surrounding neuron activity during behavior.

Non-calcium Fluorescence Activity Sensors

Fluorescence imaging has focused primarily on measuring intracellular calcium transients. However, florescent sensors for dopamine transients (Patriarchi et al., 2018; Sun et al., 2018), glutamate transients (Marvin et al., 2013, 2018), intracellular potassium ion concentration (Shen et al., 2019) and voltage signals (Lin and Schnitzer, 2016; Deo and Lavis, 2018), among others, have been developed for imaging (**Table 1**), with more to come. Many NDDs feature a variety of aberrant signaling, including dysfunction of dopamine and glutamate (Heath and Shaw, 2002; Hynd et al., 2004; Lewerenz and Maher, 2015; Schwab et al., 2015; Poewe et al., 2017), which provides an abundance of opportunities to study NDDs with these cutting-edge sensors. For example, dopamine sensors could be used to image dopamine transients in the SNc during progressive loss of dopaminergic neurons.

Extensive evidence suggests that many NDDs may be characterized by alterations in single-cell firing patterns and dyssynchronization between circuit nodes. Electrophysiological techniques currently offer unparalleled temporal resolution, but are unable to record spatiotemporal dynamics of action potential activity in ensembles with single-neuron resolution (Henze et al., 2000; Obien et al., 2014). Fluorescence imaging infers neural activity with high sensitivity but is traditionally unable to accurately resolve individual action potentials during high frequency firing due to slow decays. Engineering of voltage-responsive fluorophores is advancing

rapidly with hopes of providing fluorescence responses that can greatly improve temporal resolution (Deo and Lavis, 2018; Quicke et al., 2019).

CONCLUSION

Capable of performing cell-type specific recordings on large-scale populations longitudinally, the miniscope offers opportunities to ask new and exciting questions in NDDs. Studies utilizing miniscope imaging are increasing rapidly across disciplines of neuroscience but are not commonly being used in NDD models. It is our hope that this mini-review, while by no means exhaustive, brings attention to the powerful capabilities of miniscope imaging, and offers a glimpse into potential questions that could be answered with miniscope experiments in studies of NDDs.

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The Acute Effects of Amyloid-Beta$_{1-42}$ on Glutamatergic Receptor and Transporter Expression in the Mouse Hippocampus

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Alzheimer's disease (AD) is the leading type of dementia worldwide. Despite an increasing burden of disease due to a rapidly aging population, there is still a lack of complete understanding of the precise pathological mechanisms which drive its progression. Glutamate is the main excitatory neurotransmitter in the brain and plays an essential role in the normal function and excitability of neuronal networks. While previous studies have shown alterations in the function of the glutamatergic system in AD, the underlying etiology of beta amyloid (Aβ_{1-42}) induced changes has not been explored. Here we have investigated the acute effects of stereotaxic hippocampal Aβ_{1-42} injection on specific glutamatergic receptors and transporters in the mouse hippocampus, using immunohistochemistry and confocal microscopy 3 days after Aβ_{1-42} injection in aged male C57BL/6 mice, before the onset of neuronal cell death. We show that acute injection of Aβ_{1-42} is sufficient to induce cognitive deficits 3 days post-injection. We also report no significant changes in glutamate receptor subunits GluA1, GluA2, VGluT1, and VGluT2 in response to acute injection of Aβ_{1-42} when compared with the ACSF-vehicle injected mice. However, we observed increased expression in the DG hilus and ventral stratum (str.) granulosum, CA3 str. radiatum and str. oriens, and CA1 str. radiatum of the GluN1 subunit, and increased expression within the CA3 str. radiatum and decreased expression within the DG str. granulosum of the GluN2A subunit in Aβ_{1-42} injected mice compared to NC, and a similar trend observed when compared to ACSF-injected mice. We also observed alterations in expression patterns of glutamatergic receptor subunits and transporters within specific layers of hippocampal subregions in response to a microinjection stimulus. These findings indicate that the pathological alterations in the glutamatergic system observed in AD are likely to be partially a result of both acute and chronic exposure to Aβ_{1-42} and implies a much more complex circuit mechanism associated with glutamatergic dysfunction than simply glutamate-mediated excitotoxic neuronal death.

Keywords: amyloid beta, glutamate receptor, glutamate transporter, hippocampus, Alzheimer's disease

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INTRODUCTION

Beta amyloid (Aβ) is a ∼4 kDa peptide product derived from the cleavage of amyloid precursor protein (APP). In normal physiology, the APP molecule can be cleaved by two different secretases; cleavage by alpha and gamma secretase yields non-neurotoxic fragments (Sadigh-Eteghad et al., 2015), while Aβ is generated from the cleavage of APP through the beta and gamma secretase pathway (Selkoe, 1998). This Aβ can further aggregate into larger polymeric structures, including oligomers, protofibrils, and amyloid fibrils, each of which exhibit different functional properties (Finder and Glockshuber, 2007). Amyloid plaques are formed from the assembly of insoluble amyloid fibrils, whereas amyloid oligomers are soluble and appear to exhibit much higher cytotoxicity, perhaps due to their soluble nature (Dahlgren et al., 2002). Both amyloid plaques and soluble amyloid oligomers have been implicated in the pathogenesis of Alzheimer's disease (AD).

AD is a major neurodegenerative disorder characterized by the presence and accumulation of two pathological hallmarks: (Aβ) aggregates and neurofibrillary tau (Glenner and Wong, 1984; Grundke-Iqbal et al., 1986). The amyloid cascade hypothesis is one of the earliest and leading hypotheses in relation to both the initiation and progression of AD. There is contention as to which form of Aβ is responsible for the pathophysiological responses seen in AD, although current data point toward smaller soluble Aβ oligomers as playing the most critical role, with amyloid plaques contributing to but not essential in the pathogenesis of AD (Sakono and Zako, 2010).

Glutamate comprises a major excitatory system within the CNS, and has a critical role in a variety of homeostatic and neurological processes. It acts on a variety of receptors, broadly categorized as ionotropic and metabotropic. Ionotropic receptors include the *N*-methyl-D-aspartate receptor (NMDAR), alpha-amino-3-hydroxy-5-methyl-isoxazolepropinoic acid receptor (AMPAR), and kainate receptor classes. The metabotropic class of receptors are subdivided into three functionally distinct groups; group I are coupled with phospholipase C, while group II and III are coupled with adenylyl cyclase (Kew and Kemp, 2005). While ionotropic receptors are present predominantly on the post-synaptic membrane, metabotropic receptors have been found to be expressed on both neuronal and glial cells (Niswender and Conn, 2010). The differential spatial localization of these two receptor subtypes appear to facilitate differential activation of receptors in proportion to the amount of glutamate released from the presynaptic space (Nusser et al., 1994). Vesicular glutamate receptors (VGluTs), categorized into VGluT1 and VGluT2, are present at presynaptic neurons and are vital in maintaining vesicular glutamate concentrations (Fremeau et al., 2001).

The glutamatergic system has also been heavily implicated in the pathogenesis of AD, however the relationship between Aβ and glutamatergic dysfunction is still not well understood. Studies have shown associations between glutamatergic dysfunction and Aβ exposure, with Aβ exposure associated with the endocytosis of NMDARs and AMPARs (Snyder et al., 2005; Hsieh et al., 2006). Although changes in expression of components of the glutamatergic system in AD have been noted in previous studies, there has been little examination on whether this is due to direct Aβ interaction, secondary to downstream effects, or associated with other pathways altogether.

The importance of investigating acute changes lies in the possibility of early phenomena not captured in later stages of Aβ interaction. Such physiological changes have been observed in human patients, with an increase in glutamatergic synapses observed in mildly cognitively impaired patients and subsequent reduction in AD patients, potentially reflecting a compensatory mechanism (Bell et al., 2007). Intracerebroventricular injection of Aβ oligomers in rats has been associated with memory deficits and cholinergic neuron loss in the acute setting. Acute exposure of rats to Aβ oligomers for 1, 3, 7, 21 days has shown pathophysiological alterations, including delayed increase in activated microglia and a decreased cholinergic neuronal number observed at day 21 (Wong et al., 2016). There are a limited number of studies that have examined acute $A\beta_{1-42}$ -induced behavioral deficits (Kim et al., 2016; Kasza et al., 2017). Therefore, we have performed a thorough behavioral examination 3 days post-$A\beta_{1-42}$ injection. Furthermore, there have not yet been studies examining acute effects of Aβ exposure on glutamatergic function. This is the first comprehensive anatomical study to characterize the subregion- and cell layer-specific effect of acute $A\beta_{1-42}$ administration on the expression of specific glutamate receptors and transporters in the mouse hippocampus.

MATERIALS AND METHODS

$A\beta_{1-42}$ Preparation

Method for preparation of $A\beta_{1-42}$ is as described in Wilson C. M.Sc. Thesis, University of Otago (2007) and Kwakowsky et al. (2016). In short, $A\beta_{1-42}$ is produced as a recombinant protein fused with maltose binding protein (MBP) in *Escherichia coli*. This strategy utilizes the solubilizing character of the MBP (product of the MalE gene) to ensure expression of soluble protein at high concentration (Kapust and Waugh, 1999). After expression in bacteria, the product was purified on an amylose column to which the MBP segment of the protein binds. Following binding to amylose resin, the pure fusion protein was eluted from the resin with maltose and concentrated by ammonium sulfate precipitation. Carrier MBP was cleaved off the fusion protein by Factor X protease, and the released $A\beta_{1-42}$ isolated and further purified by hydrophobic chromatography with 0–50% v/v acetonitrile/0.1% v/v TFA, using FPLC. Fractions containing pure $A\beta_{1-42}$ were detected immunologically with an antibody against residues 17–24 of $A\beta_{1-42}$ and lyophilized to remove solvent. Mass spectrometry confirmed the expected molecular ion for the desired product. Prior to stereotaxic intrahippocampal injection, $A\beta_{1-42}$ was dissolved in Artificial cerebrospinal fluid (ACSF) and 'aged' at 37°C for 48 h to facilitate the formation of toxic soluble aggregates. The optimal incubation time for preparations of $A\beta_{1-42}$ to produce the highly toxic oligomers varies from preparation to preparation but is generally is 48–120 h. Western blots of an aging profile of $A\beta_{1-42}$ are shown in **Figure 1**, analyzed both on non-dissociating gels (A),

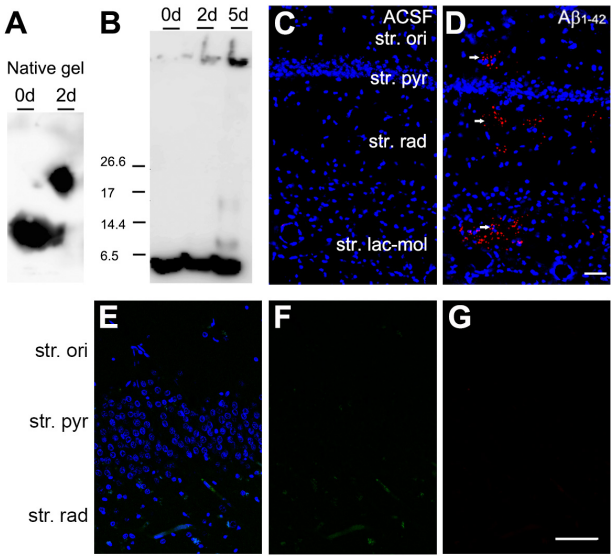

FIGURE 1 | Aging of $A\beta_{1-42}$, and detection at the injection site with Aβ immunolabeling. **(A)** Western Blot of a non-dissociating gel after aging $A\beta_{1-42}$ for 48 h. The $A\beta_{1-42}$ monomer, and possible aggregates were detected with antibody 4G8. **(B)** Western blot of an SDS dissociating gel after aging $A\beta_{1-42}$ for up to 120 h. Bands detected with antibody 4G8 can be seen at the sizes of monomer, dimer and tetramer and a higher order aggregate. **(C,D)** $A\beta_{1-42}$ immunolabeling (red on **D**) in the CA1 region of the mouse hippocampus after $A\beta_{1-42}$ injection [**(C,D)** Hoechst (blue); **(D)** $A\beta_{1-42}$ (red)]. Scale bar, 50 = μm. **(E,F)** The omission of the primary antibodies resulted in complete absence of the immunoreactivity [**(E)** Hoechst (blue), goat anti-rabbit Alexa Fluor 488 (green), goat anti-guinea pig Alexa Fluor 647 (red); **(F)** goat anti-rabbit Alexa Fluor 488 (green); **(G)** goat anti-guinea pig Alexa Fluor 647 (red)]. Scale bar: 70 = μm.

where the monomer decreases and an oligomer appears by 48 h, and SDS gels (B) where the dimer and trimer of $A\beta_{1-42}$ are seen at 5 days as well as a higher molecular weight oligomer. Only aggregates from misfolded $A\beta_{1-42}$ are deduced to be SDS insoluble (Hillen, 2019), explaining why SDS gels show lower amounts with the less stably-aggregated species dissociated by the SDS. Following gel electrophoresis of $A\beta_{1-42}$ samples on a 12.5% acrylamide gel run under non-dissociating conditions, or a 16% acrylamide 'peptide' (Kolby) gel under dissociating conditions with SDS, samples were transferred to a PVDF membrane at 100 V for 1 h. After brief staining with Ponceau red to mark peptide markers, the PVDF was immersed with rocking for 2 h at room temperature (RT) in 1% milk powder in Tris-buffered saline, 0.1% Tween (TBS-T) (blocking solution), and then with primary antibody, 4G8 diluted in blocking solution, overnight at 4°C. Following washing 3 min × 10 min with TBS-T the blot was incubated with secondary antibody (1:5000 anti mouse HRP in TBS-T) at RT 1 h with rocking. After wash 3 min × 10 min with TBS-T signals were developed with the ECI reagent.

Animals and Tissue Preparation

All experiments were approved and performed in accordance with the regulations of the University of Otago and the University of Auckland Animal Ethics Committees. Mice were housed under standard laboratory conditions and maintained in a 12 h light–dark cycle at the Hercus Taieri Resource Unit, University of Otago and Vernon Jensen Unit, the University of Auckland with food and water ad lib. Prior to surgery, 18 months old C57BL/6 male mice were anesthetized by subcutaneous injection of 75 mg/kg ketamine and 1 mg/kg domitor. Bilateral hippocampal stereotaxic surgery was performed, with coordinates for injection determined relative to the bregma (anterior–posterior, −2.0 mm; medial-lateral, ±1.3 mm; dorsal-ventral, −2.2 mm) with 1 μL 20 μM aggregated $A\beta_{1-42}$ or ACSF injected at a speed of 0.1 μl/min. Following surgery, 1 mg/kg antisedan was subcutaneously administered to reverse anesthesia. Naïve control (NC) animals did not undergo any surgical procedures. Mice used in this study were categorized into three groups: NC (n = 6), ACSF-injected (n = 12), and $A\beta_{1-42}$-injected (n = 12).

After 3 days post-$A\beta_{1-42}$ injection, animals were deeply anesthetized via overdose of ketamine and domitor and perfused transcardially with 20 mL of ice-cold 4% paraformaldehyde in phosphate buffer (pH 7.6). For the $A\beta_{1-42}$ immunohistochemistry experiment animals were perfused transcardially after 90 min of $A\beta_{1-42}$ injection. Brains were removed and post-fixed in paraformaldehyde solution for 2 h at RT and then incubated in 30% sucrose in Tris-buffered saline (TBS) solution overnight at 4°C. Four sets of 30-μm thick coronal brain sections were cut using a freezing microtome. The sections were then stored in antifreeze solution at −20°C until use.

Fluorescent Immunohistochemistry

Free-floating fluorescent immunohistochemistry was used to examine the expression of glutamate receptors AMPAR, NMDAR, and glutamate transporters VGluT1, VGluT2. Immunohistochemistry was performed as described by Kwakowsky et al. (2016). Tissue sections were blocked using 1% (v/v) goat serum in 0.05M tris buffered saline (TBS)/0.3% v/v Triton/0.25% w/v BSA (TTB) for 1 h at RT. Sections were then washed in TBS for 3 min × 10 min and incubated for 72 h in primary antibody specific for glutamate receptors and transporters at 4°C (**Table 1**). Specificity of the primary antibodies has been tested using western blotting and reported previously for each of the antibodies GluA1 (Zhu et al., 2017; Song et al., 2019), GluA2 (Banerjee et al., 2013; Hussain and Davanger, 2015), GluN1 (Morimura et al., 2017; Seigneur and Südhof, 2018), GluN2A (Atkin et al., 2015; Konstantoudaki et al., 2016), VGluT1 (Venniro et al., 2017; Nakano et al., 2018), VGluT2 (Hernández et al., 2015; Nakano et al., 2018), and $A\beta_{1-42}$ (Kwakowsky et al., 2016) (**Figures 1A–D**). Following 3 min × 10 min washes in TBS, the sections were incubated at RT for 1 h in secondary antibodies goat anti-mouse Alexa Fluor 647 (1:500, A21236, Thermo Fisher, Waltham, MA, United States), goat anti-rabbit Alexa Fluor 488 (1:500, A11034, Thermo Fisher), and goat anti-guinea pig Alexa Fluor 594 (1:500, A11076, Thermo Fisher) diluted in TTB. Sections were then washed in 3 min × 10 min TBS prior to 15 min RT incubation of Hoechst nuclei counterstain (1:10000, H3570 Thermo Fisher) diluted in TTB followed by 3 min × 10 min TB wash. Sections with the primary antibody omitted were run in tandem with each

TABLE 1 | Primary antibodies used in this study.

Immunogen	Source, host, species, catalog number	Dilutions
KLH-conjugated linear peptide corresponding to human glutamate receptor 1 at the cytoplasmic domain	Millipore, Rabbit, AB-1504	1:200
Peptide fragment corresponding to amino acid residues of rat AMPA receptor 2	Alamone, Rabbit, AGC-005	1:500
Recombinant protein corresponding to AA 660 to 811 from rat GluN1	Synaptic Systems, Mouse, 114-011	1:200
Peptide GHSHDVTERELRN(C), corresponding to amino acid residues 41–53 of rat NMDA Receptor 2A	Alamone, Rabbit, AGC-002	1:500
Amino acid segment from C-terminal of mouse VGluT1 protein	Frontier Institute, Guinea Pig, VGluT-GP-Af570	1:200
559–582 amino acid segment from C-terminal of mouse VGluT2	Frontier Institute, Guinea Pig, VGluT-GP-Af810	1:1000
Whole $A\beta_{1-42}$ peptide	Thermo Fisher Scientific, Rabbit, PA3-16761	1:500
Peptide corresponding to amino acid residues 17–24 of $A\beta_{1-42}$ (4G8).	Sigma, Mouse, A1349	1:300

experiment. The omission of the primary antibodies resulted in complete absence of the immunoreactivity (**Figures 1E,F**). Sections were mounted in gelatin, air dried overnight at RT, rehydrated, cover slipped with Mowiol mounting medium, and sealed with nail varnish.

Behavioral Testing

Behavioral testing was performed to elucidate the effects of $A\beta_{1-42}$ on the cognitive performance of the mice using behavioral tests that target different types of hippocampal-dependent memories, including long-term spatial memory [novel object alteration (NOALT) and novel object recognition test (NORT)], as well as non-spatial memory (passive avoidance test). The NOALT and NORT behavioral tests were started at 9 AM and the passive avoidance test 11 AM, and behavioral analysis was performed using the tracking image analyzer system EthoVision XT 9 (Noldus).

Novel Object Alteration Test (NOALT)

The NOALT test was performed in a square arena that was surrounded by non-transparent plexiglass walls (25 cm × 29 cm × 25 cm). Each mouse was placed in the arena individually and given 10 min to habituate to the environment. Next, two identical objects were introduced in the arena at designated locations, and the mice were given 5 min to interact with and explore the objects. The following day (24 h later), one of the identical objects was placed in a new location, and the behavior of the mice was recorded over a 5 min testing period. The testing apparatus was cleaned between animals with 5% acetic acid to minimize olfactory cues. The discrimination ratio (DR) for a novel over a familiar object was calculated as follows: time spent near the object at the new position minus the time spent near the object at the old position, divided by time spent near the object at the new position plus the time spent near the object at the old position.

Novel Object Recognition Test (NORT)

Novel object recognition test was performed in the same arena as the NOALT. Animals were allowed to explore a set of two identical objects for a 10 min period, afterward the mice were returned to their cages. The next day (24 h later) the animals were presented with a similar set of objects but one object was novel to them; they were allowed to freely explore the objects again for a 5 min period. The amount of time spent to explore the new object is considered as an index of recognition memory. The DR for a novel over a familiar object was calculated as follows: time near a new object minus the time near the old object, divided by time near the new object plus the time near the old object (Kwakowsky et al., 2016).

Passive Avoidance Test

The passive avoidance test was performed following the NOALT or NORT. This associative learning task was conducted in a two-compartment box made of one bright compartment and one dark compartment (16 cm × 18 cm). During habituation, the mouse was placed in the bright compartment, and the mouse gained access to the dark compartment. When the mouse entered the dark compartment the door was closed, and the mouse was briefly administered a 0.3-mA electric shock on the foot for 2 s as an aversive stimulus. After 30 s the animal was returned to its home cage. Three hours later, the animal was returned to the bright compartment with the sliding door open. The animal now had the option to avoid or enter the dark compartment. The latency period before the mouse entered the dark compartment was measured.

Imaging and Analysis

Imaging was conducted using a Zeiss 710 confocal laser-scanning microscope (Carl Zeiss, Jena, Germany). Regions and layers were differentiated based on cell type and relative location, utilizing Hoechst staining. Integrated density measurements were undertaken using ImageJ. The size of the measured areas as follows: 21,352 μm^2 for the CA1 region, 4,761 μm^2 for the CA3 region, and 12,295 μm^2 for the DG. Specifically, intensity measurements were taken in the regions of the stratum (str.) pyramidale, str. radiatum and str. moleculare of the CA1 and CA3 regions, and the hilus, str. moleculare, and str. granulosum of the DG. The experimenter was blinded to avoid any potential bias during image acquisition and analysis.

Statistical Analysis

Data in all experiments are expressed as mean ± SEM. To examine the differences between groups, a Kruskal–Wallis test was conducted for the data obtained, using Graph-Pad Prism software (GraphPad Software, San Diego, CA, United States; RRID:SCR_002798) with a p-value of $p < 0.05$ considered significant, as the data did not meet the assumptions of parametric tests assessed by the D'Agostino-Pearson omnibus

and Brown-Forsythe tests. Adobe Photoshop CC 2017 (Adobe Systems Software, San Jose, CA, United States) was used to prepare the figures.

RESULTS

Expression of AMPA Receptor Subunits in the Hippocampal CA1, CA3, and Dentate Gyrus Regions

The GluA1 receptor subunit displayed diffuse staining within the str. radiatum and str. oriens, with marked immunoreactivity localized to cellular processes within the str. pyramidale of the CA3 (**Figure 2**). Isolated localization to pyramidal cell bodies can be seen through all three layers of the CA3, although mainly concentrated within the str. pyramidale. The CA1 showed strong dense immunoreactivity within the str. oriens and str. radiatum, with relatively decreased staining within the str. pyramidale cells. Within the DG, immunoreactivity was diffuse within the str. moleculare, with staining localized to cellular bodies within the str. granulosum. In particular, the hilus displayed neuronal body staining, with otherwise weak diffuse immunoreactivity. There were no significant expression changes in GluA1 receptor subunit in any of the treatment groups compared to control in all three hippocampal regions analyzed (**Figures 3A–K**).

GluA2 showed diffuse uniform staining within the str. radiatum and str. oriens of the CA3, with greater localization to neuronal bodies within the str. pyramidale (**Figures 4Ba–f**). The CA1 region exhibited similar staining patterns, localized to the cell bodies within the str. pyramidale, with diffuse staining throughout the str. oriens and str. radiatum (**Figure 4A**). In addition, immunoreactivity was localized to dendritic processes within the str. radiatum. Immunoreactivity within the DG was more diffuse within the str. moleculare, in contrast to the str. granulosum, which displayed more localized labeling surrounding cell bodies (**Figure 4C**). Labeling was also strong surrounding some neuronal cell bodies within the hilar region. There was a significant ($p = 0.0400$) increase in immunoreactivity of the GluA2 subunit within the injection plane of the DG hilus in ACSF-injected mice compared to control (**Figure 5I**). Increases in GuA2 subunit expression were also seen in the CA3 str. oriens ($p = 0.0276$) and DG ventral str. moleculare ($p = 0.0236$) in Aβ-injected mice when compared to naïve controls (**Figures 5D,J**). The ACSF-injected group showed the same trend of expression changes as the Aβ-injected group and there are no significantly different changes between these groups in any of the regions examined indicating an injection effect (**Figures 5D,I,J**). No other regions elicited any significant changes in GluA2 subunit expression between NC, ACSF-injected, and $A\beta_{1-42}$-injected mice (**Figures 5A–C,E–H,K**).

Expression of NMDA Receptor Subunits in the Hippocampal CA1, CA3, and Dentate Gyrus Regions

GluN1 immunoreactivity appears localized to the membrane of str. pyramidale cells in the CA1, with reduced staining within the str. radiatum and str. oriens (**Figure 6A**). When compared to the NC group, immunoreactivity within Aβ-injected mice demonstrated much stronger immunoreactivity both at a diffuse level within the str. radiatum and str. oriens, as well as a stronger labeling surrounding cellular bodies which extends to some cells within the str. oriens (**Figures 6Ac–f**). Comparison between Aβ-injected and NC mice showed a statistically significant increase in expression within the caudal plane of the CA1 str. oriens ($p = 0.0414$) and str. radiatum ($p = 0.0262$) (**Figures 7A,C**), as well as the injection plane of the CA1 str. pyramidale ($p = 0.0286$) and str. radiatum ($p = 0.0091$) (**Figures 7B,C**). Increases were also seen within the ACSF-injected group compared to NC group within the injection plane of the str. oriens ($p = 0.0216$) and str. pyramidale ($p = 0.0286$) (**Figures 7A,B**). Overall, this indicates an increase in immunoreactivity of GluN1 subunits within all three layers of the CA1, particularly within the str. radiatum and the str. oriens. However, no significant differences in immunoreactivity were seen within any of the three layers of the CA1 between ACSF-injected and Aβ-injected mice. Expression changes within the str. oriens and str. pyramidale were responses to a microinjection stimulus, while in the str. radiatum GluN1 expression showed a trend toward increased expression in Aβ-injected mice compared to the ACSF-injected mice (**Figure 7C**).

GluN1 immunoreactivity within the CA3 followed a similar distribution, with staining strongest within the str. pyramidale, and limited punctate staining within the str. oriens and radiatum (**Figure 6B**). Results show a significant increase in expression of GluN1 receptor subunits within the injection plane of the str. oriens ($p = 0.0037$) and both injection ($p = 0.0033$) and caudal planes ($p = 0.0148$) of the str. radiatum in the CA3 in Aβ-injected mice compared to NC mice (**Figures 7D,F**). A similar trend in GluN1 expression was observed between Aβ-injected and ACSF-injected mice, although this did not reach statistical significance. Immunolabeling within the str. pyramidale appeared similar between NC, ACSF-injected and Aβ-injected mice (**Figure 7E**).

In the DG region, GluN1 immunoreactivity in NC mice followed a similar pattern seen within CA1 and CA3, with specific cellular staining within the dorsal and ventral str. granulosum (**Figure 6C**). ACSF-injected and Aβ-injected mice, however, showed a much stronger immunostaining that had a more diffuse picture within the hilus and str. moleculare, whilst retaining the specific cellular staining within the str. granulosum layers seen in NC mice. In addition, Aβ-injected and ACSF-injected mice displayed increased neuronal staining within the hilar area. Quantification revealed an increase in immunoreactivity within both the injection and caudal plane of the hilus (injection $p = 0.0090$; caudal plane $p = 0.0353$) and ventral str. granulosum (injection plane $p = 0.0154$; caudal plane $p = 0.0372$) in Aβ-injected mice compared to NC mice (**Figures 7I,K**). Similarly, increases in immunoreactivity were observed within the caudal plane of the ventral str. moleculare ($p = 0.0315$) (**Figure 7J**), although this was not statistically significant within its dorsal counterpart (**Figure 7G**). These changes were induced by the microinjection stimulus but Aβ further increased the expression of GluN1 in the hilus and ventral str. granulosum in Aβ-injected mice compared to ACSF-injected mice, although this

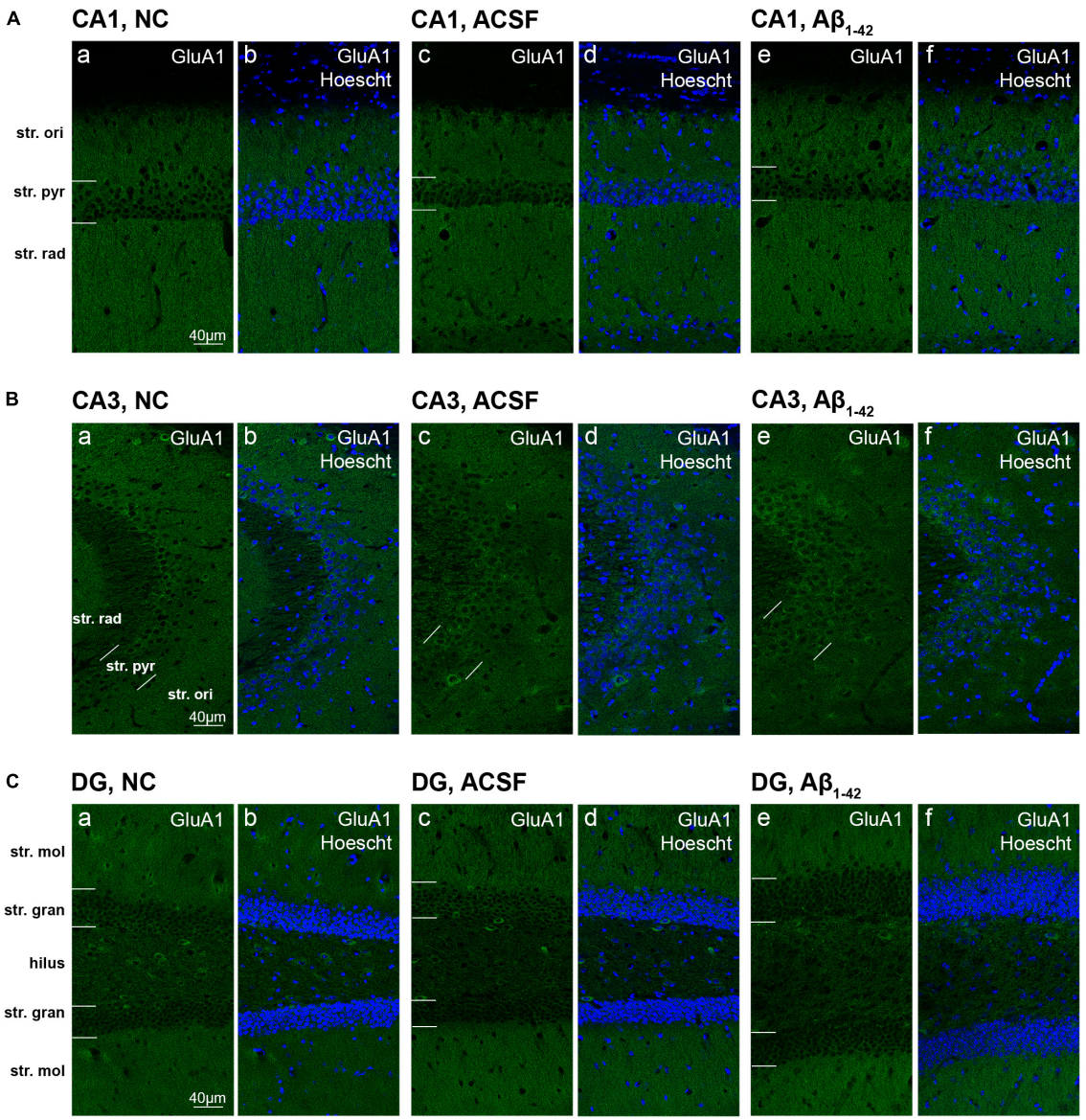

FIGURE 2 | GluA1 expression in the mouse hippocampus 3 days post-injection. **(A–C)** Representative confocal images show GluA1 (green) and Hoescht (blue) immunofluorescence for NC **(a,b)**, ACSF-injected **(c,d)**, and $A\beta_{1-42}$-injected mice **(e,f)** in the CA1 **(A)**, CA3 **(B)**, and DG **(C)** regions of the hippocampus. Scale bars = 40 μm.

increase did not reach significance (**Figures 7I,K**). No significant changes in immunoreactivity levels were seen within the dorsal str. granulosum (**Figure 7H**).

Similar to GluN1 immunoreactivity, the GluN2A receptor subunit was localized to the str. pyramidale of the CA1, however appeared to display increased labelling of the cell bodies in comparison to a more membrane-associated pattern seen in GluN1 immunostaining (**Figure 8A**). In addition, sparse dendritic-like staining could be observed within the str. pyramidale and str. radiatum. This was also observed within the CA3 region (**Figure 8B**), and within the str. granulosum layers of the DG (**Figure 8C**). The hilar region of the DG also exhibited strong cellular immunoreactivity. Some Aβ-injected specimens revealed specific localization of immunoreactivity to cellular bodies and their associated processes within the DG hilus and str. moleculare, with an increase in diffuse immunolabeling within the str. moleculare layers (**Figure 8C**). The densitometry analysis however showed that GluN2A staining remained largely robust within all layers of the CA1, CA3, and DG in NC, ACSF-injected, and Aβ-injected mice (**Figures 9A–K**). In contrast, a decrease in immunolabeling was detected (p = 0.0083) in the caudal plane of the DG dorsal str. granulosum in Aβ-injected mice compared to NC mice (**Figure 9H**). An increase (p = 0.0195) in immunoreactivity was also seen in the caudal plane of the CA3

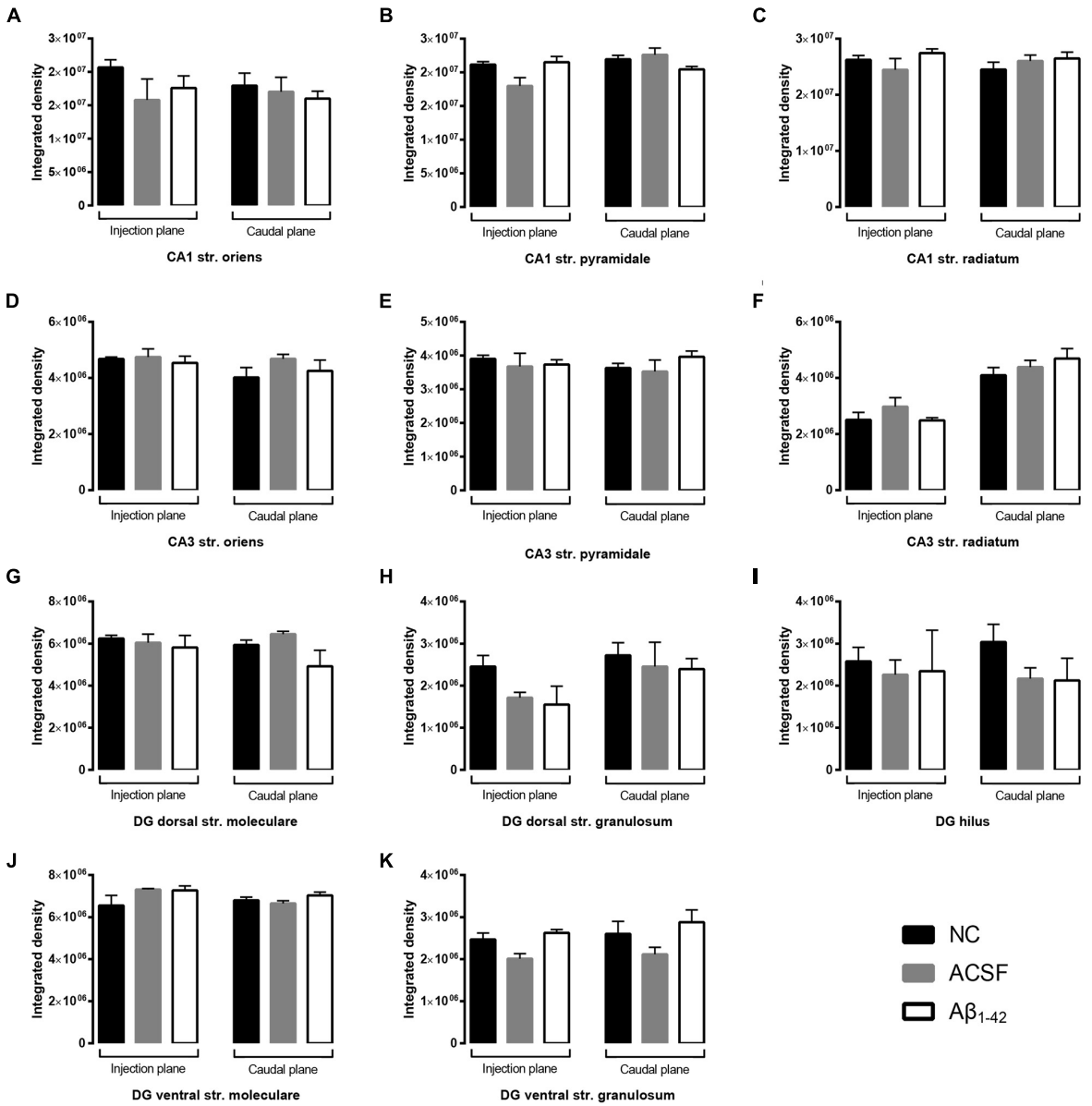

FIGURE 3 | $A\beta_{1-42}$ injection does not alter levels of GluA1 expression in the mouse hippocampus 3 days post-injection. **(A–K)** Graphs show quantification of GluA1 optical density in the str. oriens (str. ori), str. pyramidale (str. pyr), and str. radiatum (str. rad) of the CA1 and CA3 regions, and the hilus, str. moleculare (str. mol), and str. granulosum (str. gran) of the DG region. Data are expressed as mean ± SEM (Unpaired Mann–Whitney test; n = 6 NC, 6 ACSF injected mice and 6 $A\beta_{1-42}$-injected mice). NC, naïve control; ACSF, ACSF-injected; $A\beta_{1-42}$, $A\beta_{1-42}$-injected mice.

str. radiatum in Aβ-injected mice compared to NC (**Figure 9F**). A similar trend in GluN2A expression was observed between Aβ-injected and ACSF-injected mice, although this did not reach statistical significance (**Figures 9F,H**).

Expression of Vesicular Glutamate Transporters in the Hippocampal CA1, CA3, and Dentate Gyrus Regions

VGluT1 transporter staining in the CA1 is largely diffuse, with some faint localization to neuronal bodies particularly in the str. pyramidale (**Figure 10A**). VGluT1 immunoreactivity within the CA3 was mainly punctate within the str. radiatum and the str. pyramidale, with localization to cellular membranes within both the str. radiatum and str. oriens (**Figure 10B**). Expression of VGluT1 vesicular transporters appeared to be well-preserved in ACSF- and Aβ-injected mice 3 days post-injection within all three layers of the CA1 and CA3 regions (**Figures 11A–F**).

Immunoreactivity within the DG demonstrated a strong punctate staining within the hilus and a weaker staining was observed within the str. granulosum (**Figure 10C**). The str. moleculare exhibited a stronger diffuse staining than the str.

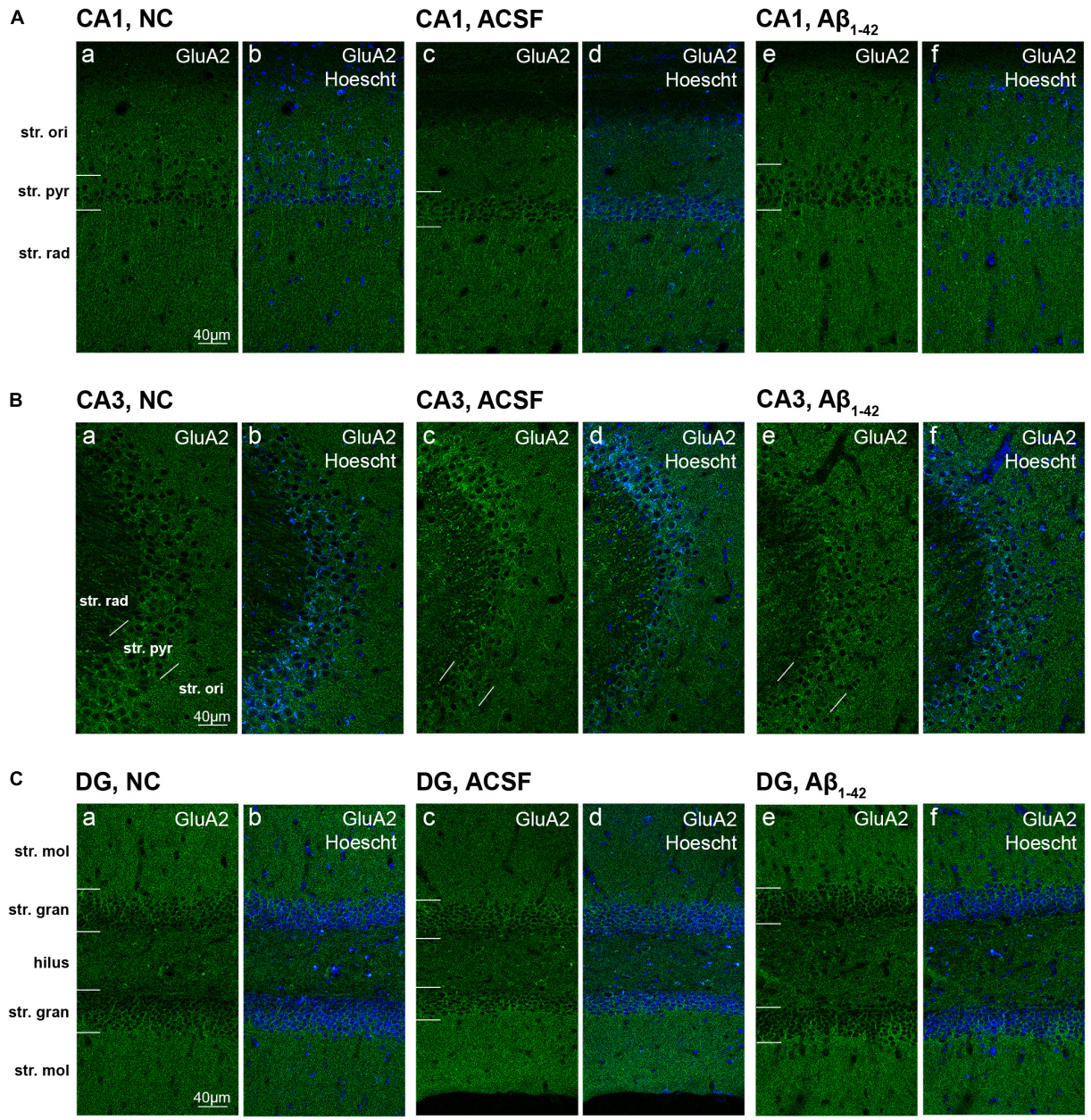

FIGURE 4 | GluA2 expression in the mouse hippocampus 3 days post-injection. **(A–C)** Representative confocal images show GluA2 (green) and Hoescht (blue) immunofluorescence for NC **(a,b)** ACSF-injected **(c,d)** and $A\beta_{1-42}$-injected mice **(e,f)** in the CA1 **(A)**, CA3 **(B)**, and DG **(C)** regions of the hippocampus. Scale bars = 40 μm.

granulosum, where staining was sparser and more localized to cellular membranes. Within the caudal plane of the DG, we found a significant increase (p = 0.0203) in VGluT1 expression within the ventral str. moleculare (**Figure 11J**), with a similar trend observed between Aβ-injected and ACSF-injected mice. We found a significant decrease (p = 0.0325) in GluN2A expression within the hilar region in Aβ-injected mice compared to NC (**Figure 11I**) and also observed a significant decrease (p = 0.0262) within the injection plane of the DG dorsal str. moleculare (**Figure 11G**) in ACSF-injected mice compared to NC mice; a similar trend was observed for the Aβ-injected group compared to NC mice, indicating an injection induced decrease. No significant changes in VGluT1 transporter expression was quantified in the dorsal and ventral str. granulosum layer (**Figures 11H,K**).

The VGluT2 transporter displayed similar punctate staining within the CA1 and CA3, however, staining was localized to the str. pyramidale, with reduced reactivity within the str. oriens and str. radiatum which do not appear to be associated with cellular bodies (**Figures 12A,B**). We

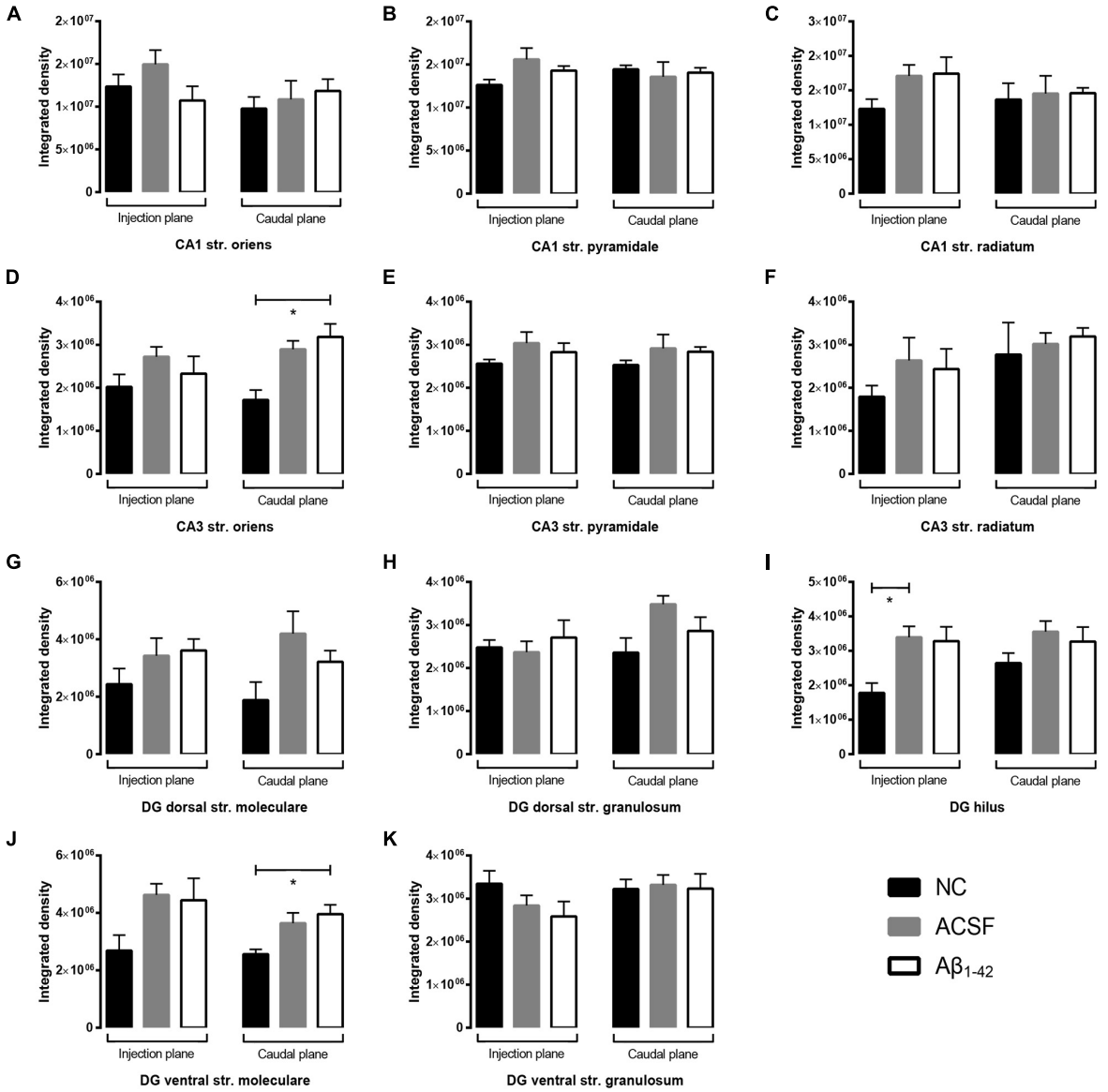

FIGURE 5 | $A\beta_{1-42}$ injected mice show altered hippocampal GluA2 expression within the CA1 when compared to NC mice. **(A–K)** Graphs show quantification of GluA2 optical density in the str. oriens (str. ori), str. pyramidale (str. pyr), and str. radiatum (str. rad) of the CA1 and CA3 regions, and the hilus, str. moleculare (str. mol), and str. granulosum (str. gran) of the DG region. Data are expressed as mean ± SEM (*$p < 0.05$, Unpaired Mann–Whitney test; $n = 6$ NC, 6 ACSF injected mice and 6 $A\beta_{1-42}$-injected mice). NC, naïve control; ACSF, ACSF-injected; $A\beta_{1-42}$, $A\beta_{1-42}$-injected mice.

report no VGluT2 transporter protein expression changes within the CA1 and CA3 regions between NC, ACSF-injected, and Aβ-injected mice (**Figures 13A–F**). In contrast to VGluT1 expression patterns, there was an absence of VGluT2 immunolabeling within the hilar region of the DG (**Figure 12C**). VGluT2 immunoreactivity was punctate within the str. granulosum, localized to cellular membranes, whilst the str. moleculare demonstrated much more diffuse staining with a lack of punctate reactivity. We found a significant decrease ($p = 0.0298$) in VGluT2 expression within the ventral str. granulosum of the DG in Aβ-injected mice compared to NC (**Figure 13K**) and a similar trend was observed between Aβ-injected and ACSF-injected mice. In all other layers of the DG, we did not detect any significant changes in VGluT2 expression between NC, ACSF-injected, and Aβ-injected mice (**Figures 13G–J**).

$A\beta_{1-42}$-Induced Cognitive Changes at Day 3 Post-injection

To elucidate the effect of $A\beta_{1-42}$ treatment on cognitive function the NOALT and NORT tests for long-term spatial-memory, and passive avoidance test for non-spatial memory were performed (**Figure 14**).

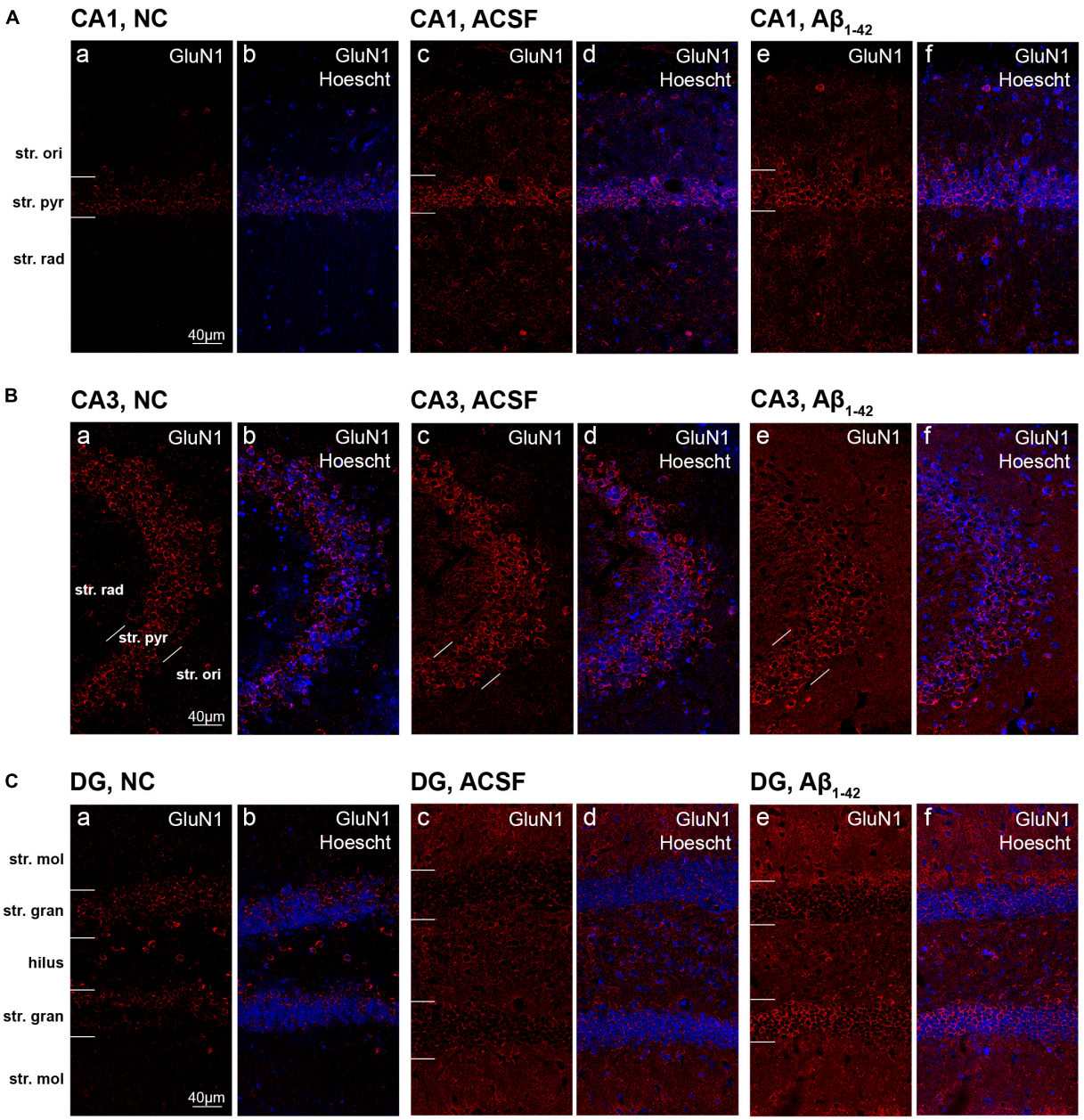

FIGURE 6 | GluN1 expression in the mouse hippocampus 3 days post-injection. **(A–C)** Representative confocal images show GluN1 (red) and Hoescht (blue) immunofluorescence for NC **(a,b)**, ACSF-injected **(c,d)**, and $A\beta_{1-42}$-injected mice **(e,f)** in the CA1 **(A)**, CA3 **(B)**, and DG **(C)** regions of the hippocampus. Scale bars = 40 μm.

At day 3 post-injection, $A\beta_{1-42}$-injected mice demonstrated significant spatial memory impairment compared with ACSF-injected (p = 0.0098) and NC mice (p = 0.0350) in the NOALT (**Figure 14A**). The significantly lower DR found in $A\beta_{1-42}$-injected mice compared with ACSF-injected and NC mice indicated that $A\beta_{1-42}$-injected mice could not detect changes in the location of object that had been moved.

At day 3 post-injection, $A\beta_{1-42}$-injected mice showed significant spatial memory impairment compared with ACSF-injected (p = 0.0082) and NC (p = 0.0399) mice in the NORT (**Figure 14B**). The significantly lower DR found in $A\beta_{1-42}$-injected mice compared with ACSF-injected and NC mice, indicates that $A\beta_{1-42}$-injected mice could not discriminate between familiar and novel objects.

$A\beta_{1-42}$-injected mice showed no significant difference in non-spatial memory performance when compared with the ACSF-injected and NC mice (**Figure 14C**). In phase 3 of the passive avoidance test (post-shock 3 h), similar latency (time taken to enter the dark chamber) was found in the control and $A\beta_{1-42}$-injected mice (**Figure 14C**).

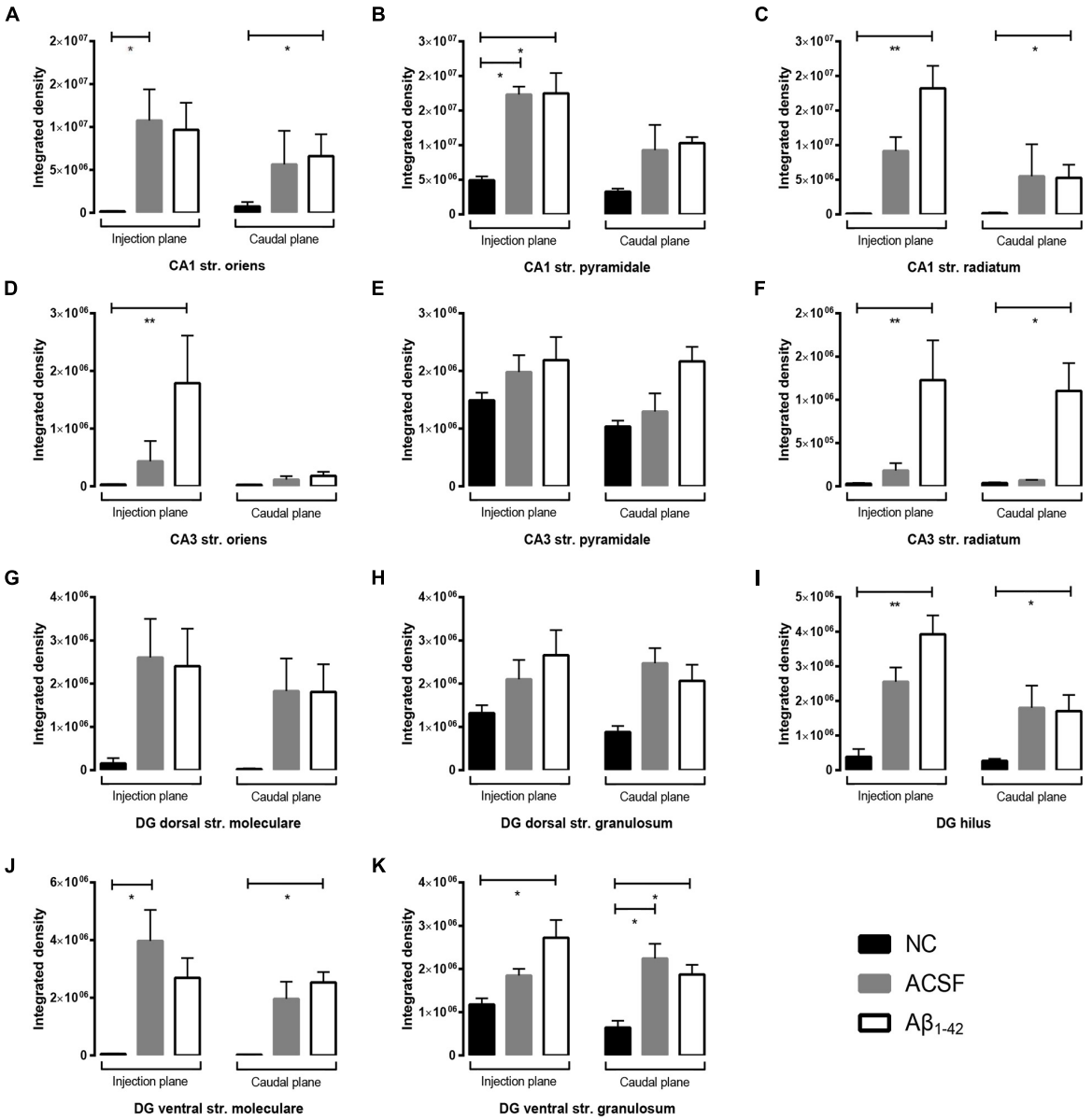

FIGURE 7 | Aβ $_{1-42}$ injection causes altered GluN1 expression within the mouse hippocampus 3 days post-injection. **(A–K)** Graphs show quantification of GluN1 optical density in the str. oriens (str. ori), str. pyramidale (str. pyr), and str. radiatum (str. rad) of the CA1 and CA3 regions, and the hilus, str. moleculare (str. mol), and str. granulosum (str. gran) of the DG region. Data are expressed as mean ± SEM (*$p < 0.05$, **$p < 0.01$, Unpaired Mann–Whitney test; n = 6 NC, 6 ACSF-injected mice and 6 $A\beta_{1-42}$-injected mice). NC, naïve control; ACSF, ACSF-injected.

DISCUSSION

Although glutamatergic dysfunction has been reported in chronic neurodegenerative conditions such as AD, the precise effect Aβ has within the hippocampal environment is not well-understood. Previous studies have investigated effects of Aβ on neuronal conduction and excitability, but these mainly involve *in vitro* cell cultures. In addition, previous studies have not examined the localization and neuroanatomical expression of glutamatergic components in relation to specific regional and cell layers within the hippocampus. The present study demonstrates the effect of Aβ-injection on components of the glutamatergic system within specific regions and cell layers of the mouse hippocampus 3 days post-injection. Importantly, it also serves to quantify these effects on the glutamatergic system, and animal's behavior in response to acute exposure to Aβ in an *in vivo* setting.

AMPA Receptor Expression Alterations 3 Days Post-Aβ Injection

The GluA1 receptor subunit demonstrated robust expression patterns in the acute setting post Aβ-injection. Previous

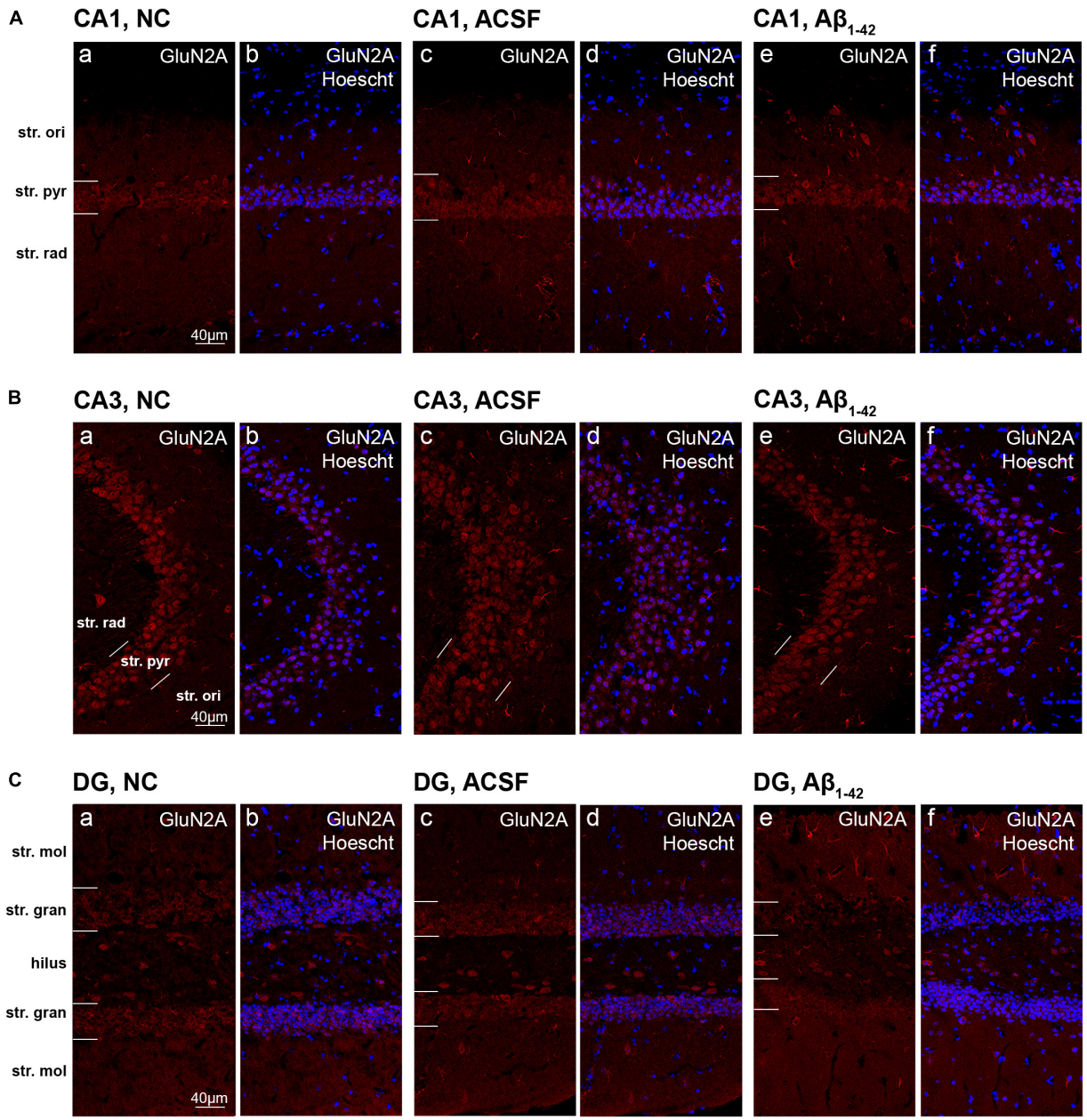

FIGURE 8 | GluN2A expression in the mouse hippocampus 3 days post-injection. **(A–C)** Representative confocal images show GluN2A (red) and Hoescht (blue) immunofluorescence for NC **(a,b)**, ACSF-injected **(c,d)**, and $A\beta_{1-42}$-injected mice **(e,f)** in the CA1 **(A)**, CA3 **(B)**, and DG **(C)** regions of the hippocampus. Scale bars = 40 μm.

studies have indicated a loss in GluA1 expression post-Aβ exposure, secondary to a loss of scaffolding proteins at the post-synaptic membrane due to a variety of Aβ-mediated processes. Application of $A\beta_{1-40}$ to cortical primary neurons and neuronal cultures resulted in a decrease in PSD-95, GluA1 and GluA2 (Almeida et al., 2005; Roselli et al., 2005). From this, it is postulated that degradation of PSD-95 as a result of Aβ application results in a concomitant decline in GluA1 expression. We were, however, unable to demonstrate loss of either GluA1 or GluA2, implying that the processes involved are a result of either chronic changes, or changes that only occur acutely in the artificial culture environment. A study by Zhang et al. (2018) demonstrated AMPAR internalization in human cultured primary neurons after application of Aβ, which was associated with an increase in AMPAR ubiquination (Zhang et al., 2018). This study involved Aβ treatment for 4 h, and whilst this represents an acute neuronal response, the *in vitro* nature of the experiment makes it difficult to

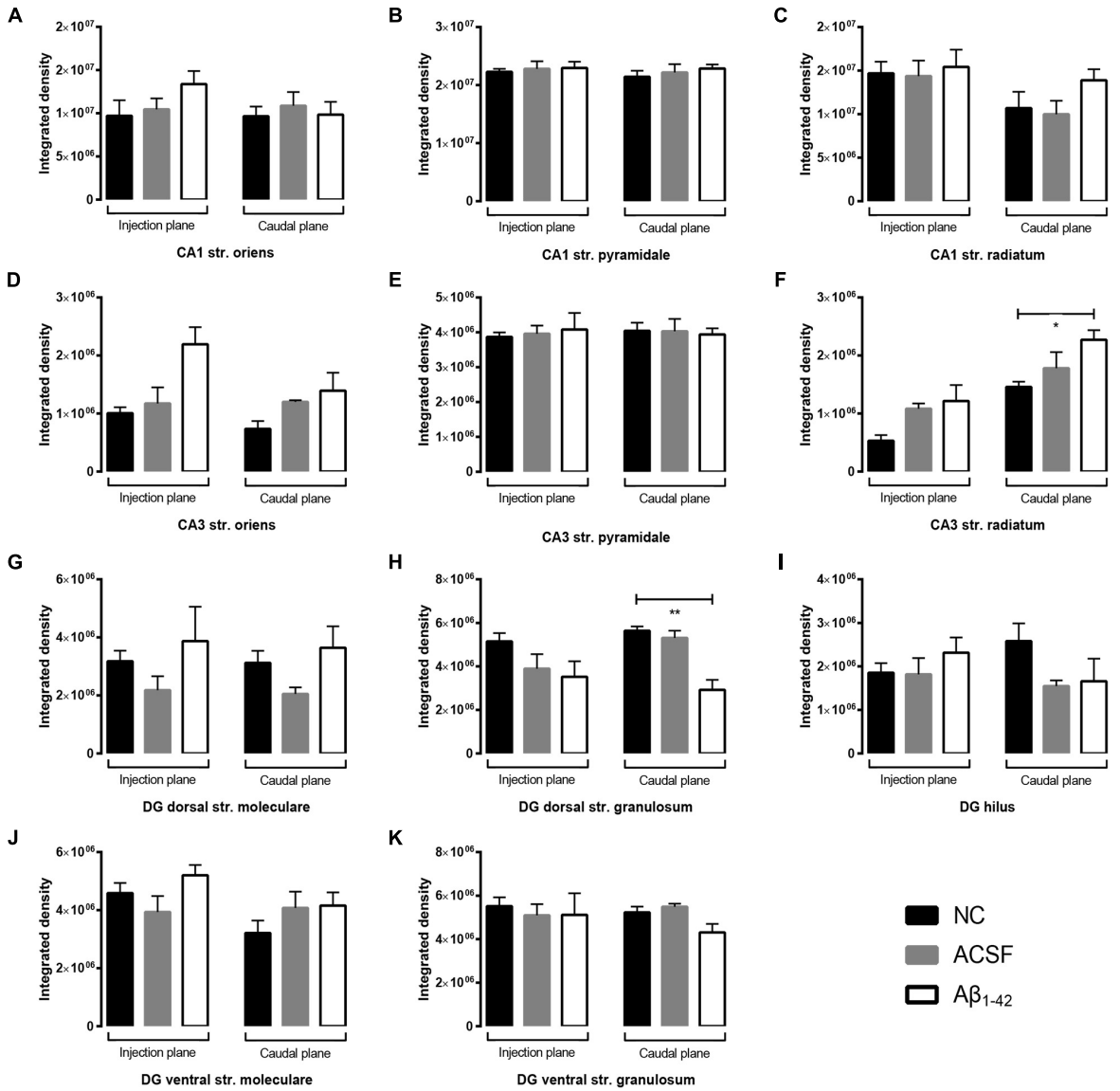

FIGURE 9 | $A\beta_{1-42}$ injected mice show altered hippocampal GluN2A expression within the CA3 and DG regions when compared to NC mice. **(A–K)** Graphs show quantification of GluN1 optical density in the str. oriens (str. ori), str. pyramidale (str. pyr), and str. radiatum (str. rad) of the CA1 and CA3 regions, and the hilus, str. moleculare (str. mol), and str. granulosum (str. gran) of the DG region. Data are expressed as mean ± SEM (*$p < 0.05$, **$p < 0.01$, Unpaired Mann–Whitney test; $n = 6$ NC, 6 ACSF-injected mice and 6 $A\beta_{1-42}$-injected mice). NC, naïve control; ACSF, ACSF-injected.

extrapolate this to the physiological microenvironment of the brain. Similarly, Hsieh and colleagues demonstrated a loss of surface GluA1 and GluA2 after expression of β-CTF, the penultimate precursor of Aβ, at 22 h in CA1 hippocampal slices (Hsieh et al., 2006). This experimental design is still very limited in its capacity to represent acute neurotoxic effects seen in the cerebral setting.

Studies involving transgenic mouse models have also yielded similar results, reporting decreased GluA1 expression or a reduction in AMPA currents in mice overexpressing APP (Hsia et al., 1999; Almeida et al., 2005; d'Amelio et al., 2011), but transgenic models seek to replicate the chronic changes seen with clinical disease, thus does not offer any indication of potential acute changes. However in another study, Whitcomb et al. (2015) demonstrated application of oligomerized Aβ induced a rapid increase in AMPAR-mediated synaptic transmission 30 min after Aβ exposure, with an associated increase in surface expression of GluA1 in biotinylated hippocampal slices as rapidly, with no change in GluA2/3 expression.

While current literature indicates Aβ is involved in the downregulation of AMPARs and NMDARs, our results and results from Whitcomb et al. (2015) suggest another role of Aβ in the acute setting. Whitcomb et al. (2015) demonstrated

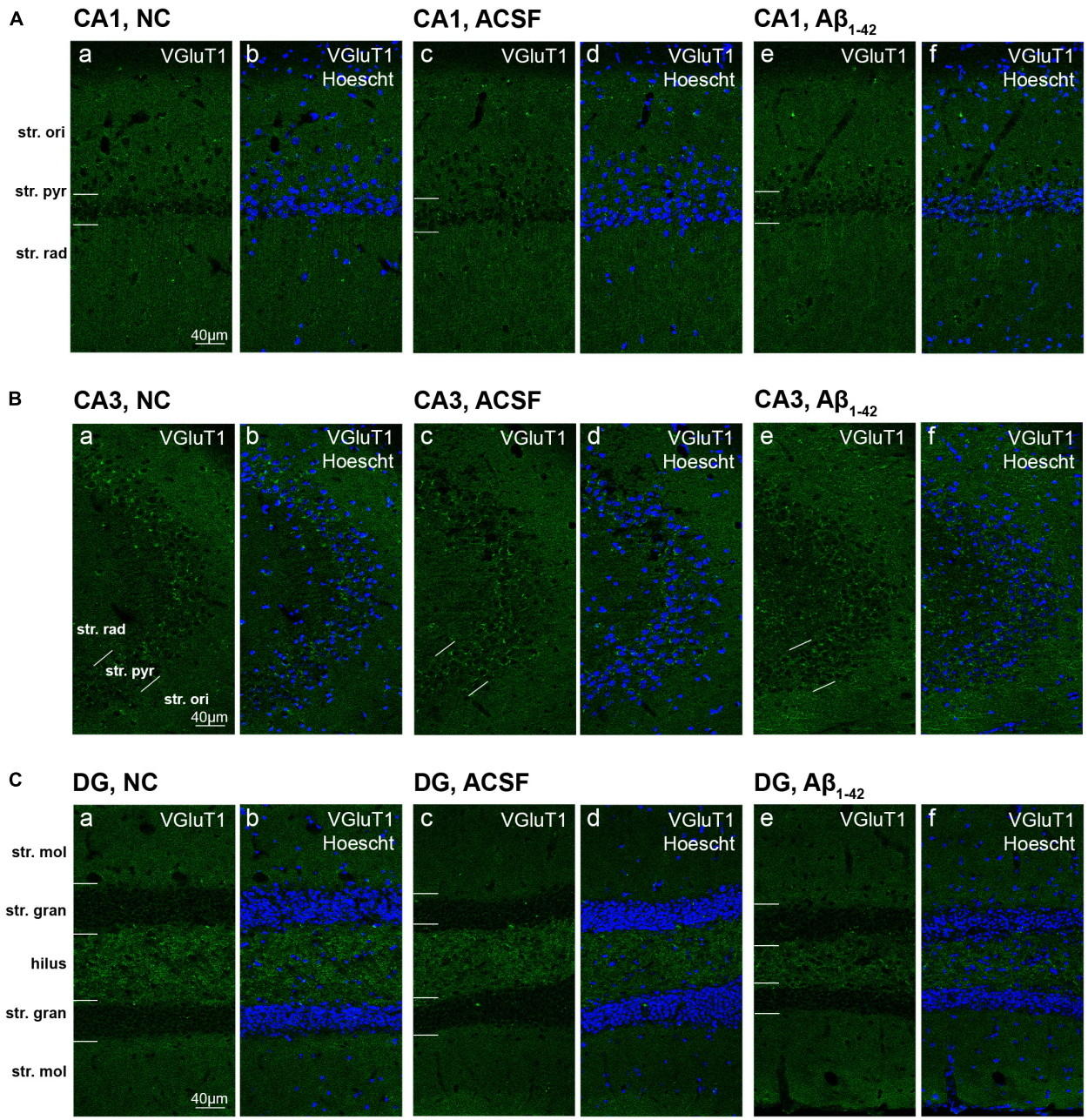

FIGURE 10 | VGluT1 expression in the mouse hippocampus 3 days post-injection. **(A–C)** Representative confocal images show VGluT1 (gree) and Hoescht (blue) immunofluorescence for NC **(a,b)**, ACSF-injected **(c,d)**, and $A\beta_{1-42}$-injected mice **(e,f)** in the CA1 **(A)**, CA3 **(B)**, and DG **(C)** regions of the hippocampus. Scale bars = 40 μm.

amelioration of Aβ effects through inhibition of CaMKII, postulating a novel interaction between Aβ and CaMKII and PKA. CaMKII and PKA mediate phosphorylation, insertion, and synaptic stabilization of AMPARs (Opazo et al., 2012). As such, it is possible that in early disease, Aβ acts at normal physiological levels to stabilize and increase GluA1 receptor subunit expression at synaptic sites through potentiation and interaction with intrinsic molecules such as CaMKII and PKA. Our study demonstrates a timepoint later than studies reporting increased GluA1 expression and prior to studies showing decreased GluA1 expression, potentially indicating a chronological biphasic response to Aβ. Possibly, a rapid increase in AMPAR expression could present as an instantaneous acute response to neurotoxic exposure, which is followed by a secondary chronic phase resulting in reduction of AMPAR surface expression through a series of Aβ-driven mechanisms, including and not limited to ubiquination, dephosphorylation, and endocytosis. As such, our finding of no expression changes

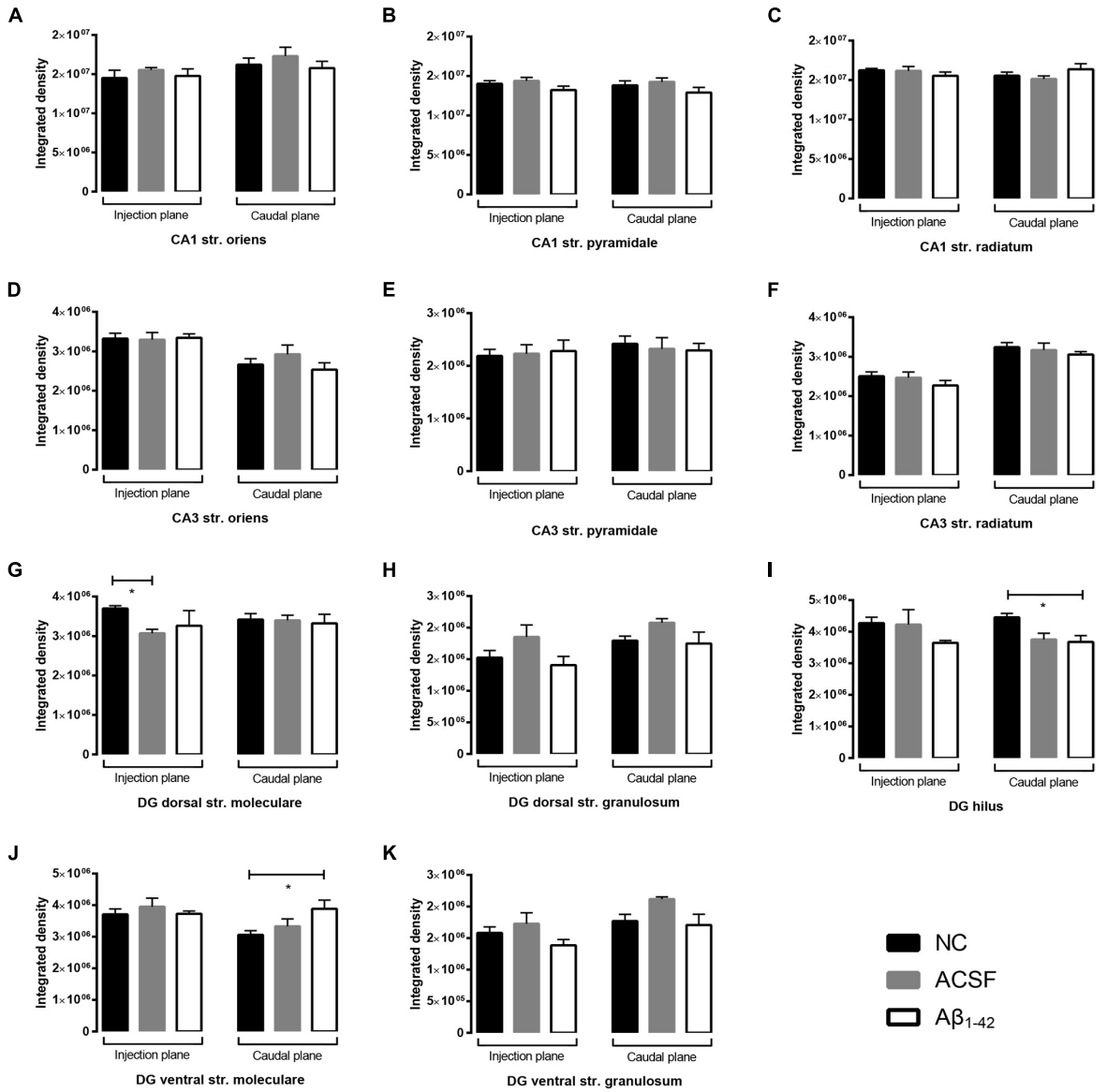

FIGURE 11 | $A\beta_{1-42}$ injected mice show altered hippocampal VGluT1 expression within the DG when compared to NC mice. **(A–K)** Graphs show quantification of VGluT1 optical density in the str. oriens (str. ori), str. pyramidale (str. pyr), and str. radiatum (str. rad) of the CA1 and CA3 regions, and the hilus, str. moleculare (str. mol), and str. granulosum (str. gran) of the DG region. Data are expressed as mean ± SEM (*$p < 0.05$, Unpaired Mann–Whitney test; $n = 6$ NC, 6 ACSF injected mice and 6 $A\beta_{1-42}$-injected mice). NC, naïve control; ACSF, ACSF-injected.

could be due to either no alterations in the early acute stages of Aβ administration, or a timepoint where dynamic expression changes have equilibrated.

NMDA Receptor Expression Alterations 3 Days Post-Aβ Injection

There have been many studies characterizing the effect of acute Aβ administration on NMDAR-mediated currents (Domingues et al., 2007; Alberdi et al., 2010; Mezler et al., 2012). Our findings indicate varying degrees of increased GluN1 receptor subunit expression particularly within different layers of the CA3 region of the mouse hippocampus 3 days post-Aβ injection. In addition, alterations in expression were seen between ACSF-injected and NC mice. Similar to results seen in the AMPAR subunits, this was largely unexpected, as most current literature indicate Aβ's primary inhibitory effect is on synaptic activity, in addition to its role in increasing ubiquination and internalization of NMDARs (Snyder et al., 2005). The NMDAR GluN1 subunit is an essential component of all functional NMDARs, therefore its homogenous expression can be used as a proxy for the number of NMDARs expressed at synaptic sites.

Currently, literature is still conflicting on Aβ's effect on NMDAR activity, with some studies indicating Aβ-mediated

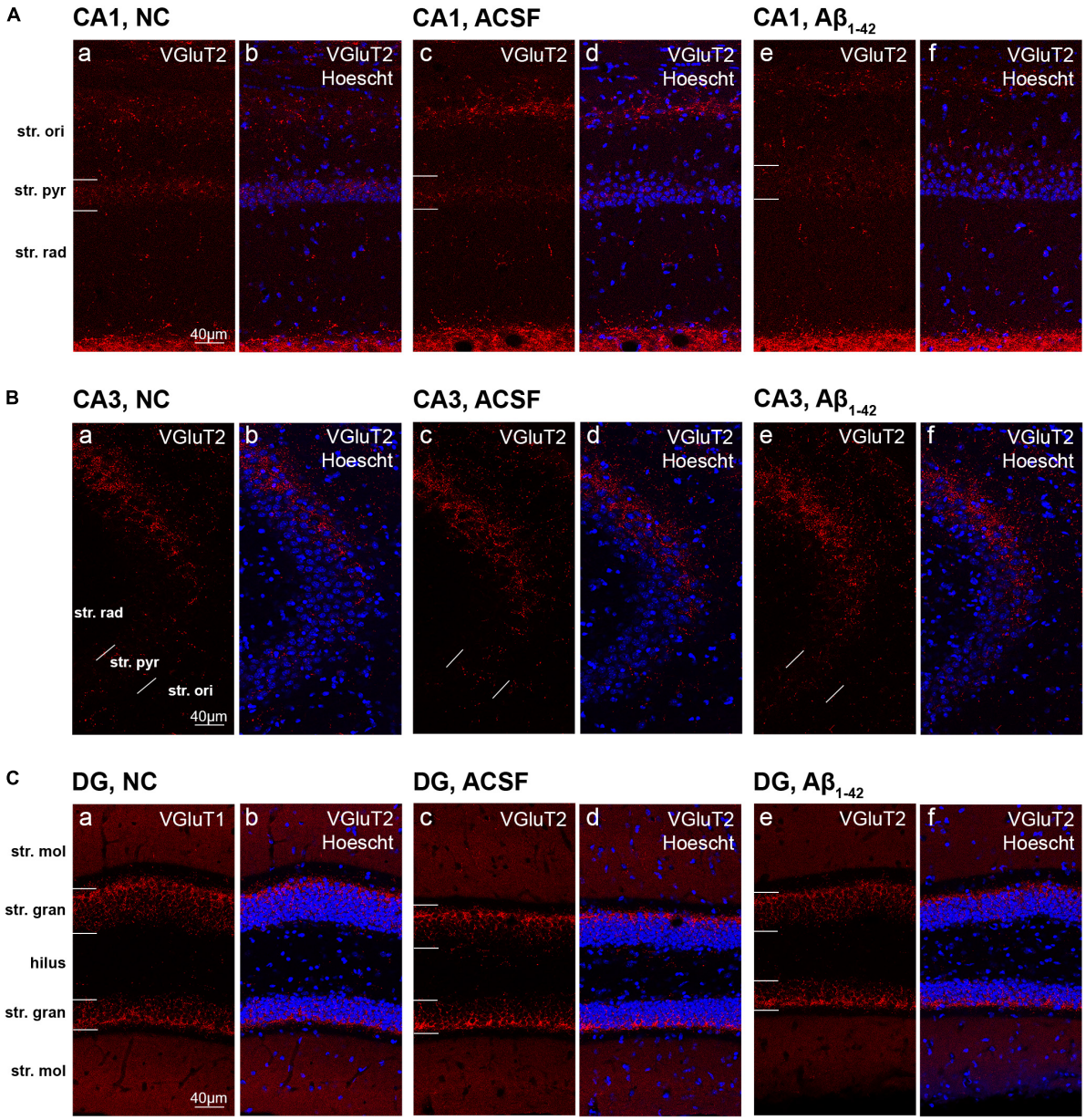

FIGURE 12 | VGluT2 expression in the mouse hippocampus 3 days post-injection. **(A–C)** Representative confocal images show VGluT2 (red) and Hoescht (blue) immunofluorescence for NC **(a,b)**, ACSF-injected **(c,d)**, and $A\beta_{1-42}$-injected mice **(e,f)** in the CA1 **(A)**, CA3 **(B)**, and DG **(C)** regions of the hippocampus. Scale bars = 40 μm.

aberrant activation of NMDARs resulting in increasing concentrations of cytosolic Ca^{2+} (Texidó et al., 2011), whilst others demonstrate Aβ-mediated selective inhibition of NMDAR activity (Zhang et al., 2009). This may be due to the different Aβ fragments used, or other experimental parameters present, in these studies (Zhang et al., 2009; Texidó et al., 2011).

Cullen et al. (1996) demonstrated reduced NMDAR synaptic transmission in the rat hippocampus more than 24 and 48 h after being intracerebroventricularly injected with Aβ, postulating that the delayed reduction in glutamatergic function may be due to an initial over-activation of NMDAR mediated synaptic transmission, reflecting a potentially biphasic response. As functional changes are only seen 24 h after Aβ exposure, expression changes, which involve more complex cellular pathways, may take a longer period to occur. In keeping with this hypothesis, $A\beta_{1-42}$ intrahippocampal injection has been associated with a relative increase in GluN1 mRNA and protein expression 10 days post-injection compared to control mice, the extent of expression increase correlated in a dose-dependent manner (Peng et al., 2017). Our results indicate that such changes

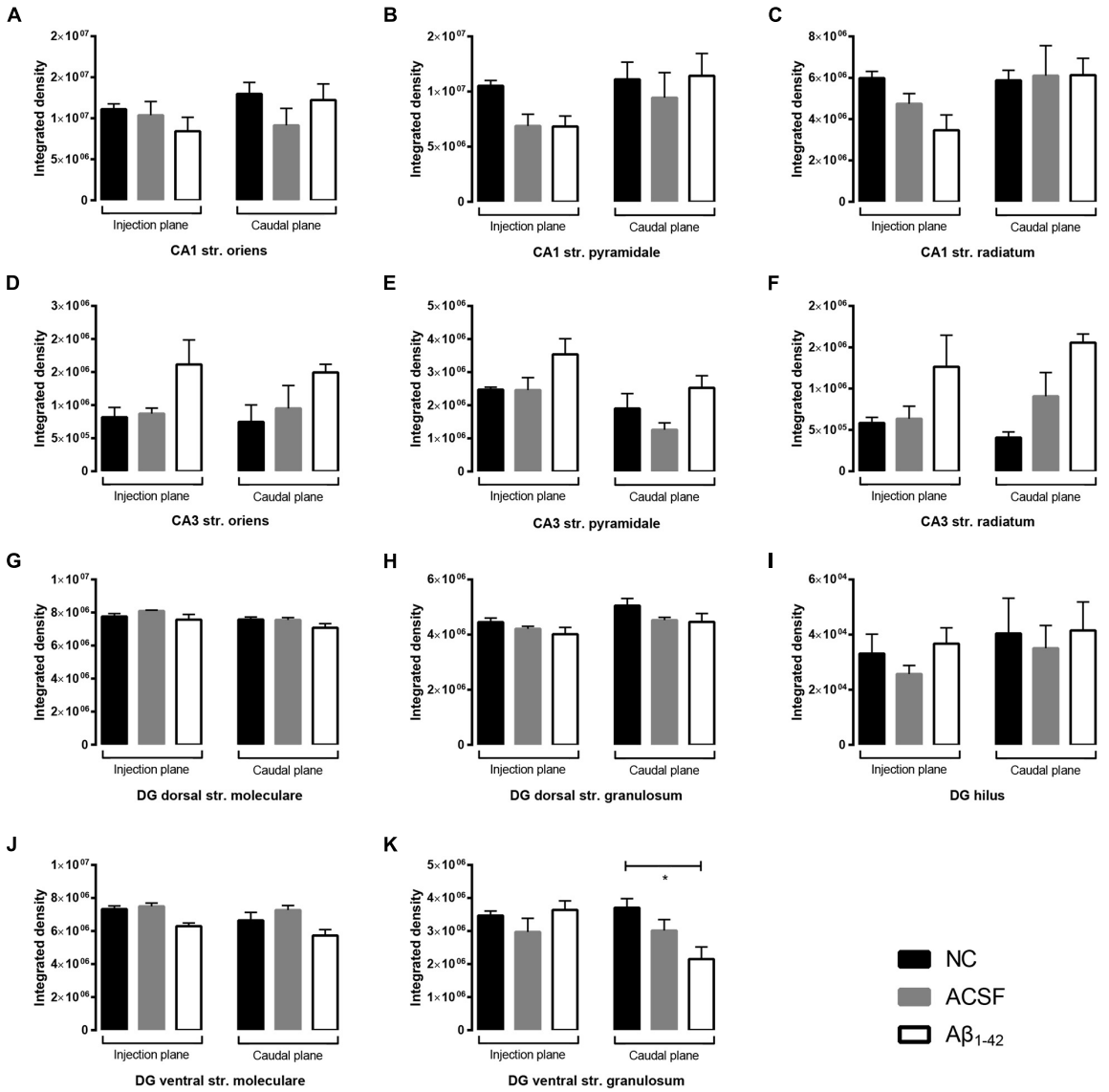

FIGURE 13 | $A\beta_{1-42}$ injected mice show altered hippocampal VGluT2 expression within the DG when compared to NC mice. **(A–K)** Graphs show quantification of VGluT2 optical density in the str. oriens (str. ori), str. pyramidale (str. pyr), and str. radiatum (str. rad) of the CA1 and CA3 regions, and the hilus, str. moleculare (str. mol), and str. granulosum (str. gran) of the DG region. Data are expressed as mean ± SEM (*$p < 0.05$, Unpaired Mann–Whitney test; $n = 6$ NC, 6 ACSF injected mice and 6 $A\beta_{1-42}$-injected mice). NC, naïve control; ACSF, ACSF-injected.

occur much earlier and can be evident 3 days post-injection. As a result, our anatomical findings of increased GluN1 subunit expression may be what follows immediately from the acute functional excitatory response, and may occur prior to the delayed reduction in AMPAR surface expression noted in other studies (Dewar et al., 1991; Chang et al., 2006; Hardt et al., 2014; Guntupalli et al., 2016). Furthermore, as illustrated prior, many studies involve *in vitro* experimentation, which does not take into account possible *in vivo* physiological mechanisms which may be neuroprotective and prevent NMDAR expression reduction in the acute setting. Studies demonstrating reduced NMDAR surface levels with acute (up to 3 days) exposure to Aβ have all been performed *in vitro* in hippocampal slice neurons and primary cortical neurons (Snyder et al., 2005; Hsieh et al., 2006).

The two most rigorously studied NMDAR subunits include the GluN2A and GluN2B, which have been implicated in disease processes (Ferreira et al., 2012; Tackenberg et al., 2013). The expression of these subunits dictate receptor function, and also the receptor's response to physiological insults, such as exposure to toxic Aβ. For example, Aβ initiated GluN2B-containing NMDAR activation is able to suppress GluN2A-containing NMDAR activity (Liu et al., 2010). Despite literature suggesting significant disruptions to NMDAR composition and

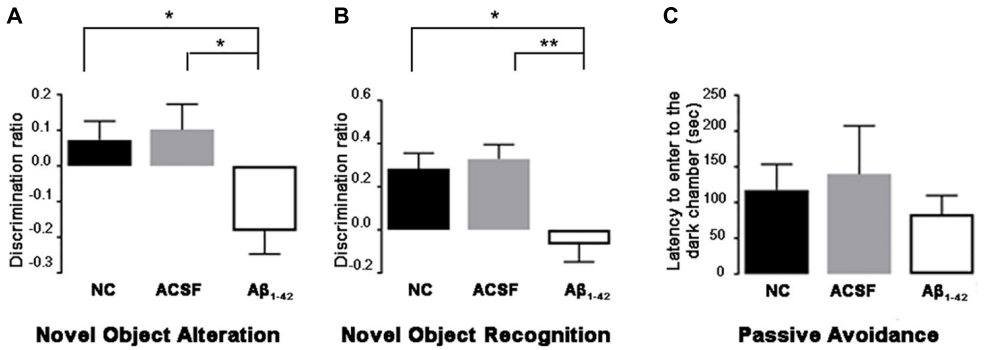

FIGURE 14 | $A\beta_{1-42}$-injected mice showed long-term spatial memory impairment revealed by the novel object alteration **(A)**, novel object recognition **(B)** tests. Non-spatial memory remained unchanged in $A\beta_{1-42}$-injected mice compared with controls **(C)**. Data are expressed as mean ± SEM (one-way ANOVA, Bonferroni's *post hoc* test) (*$p < 0.05$; **$p < 0.01$, $n = 12$).

activity with Aβ interaction, our findings have demonstrated insubstantial changes in GluN2A expression in response to acute injection of Aβ.

VGluT Expression Alterations 3 Days Post-Aβ Injection

Our findings indicate only minor changes in VGluT1 and VGluT2 transporter expression within the mouse hippocampus, although overall the transporters appear relatively robust after acute exposure to Aβ. While studies have identified the VGluTs as being preferentially affected in amyloidopathies such as AD, there is a lack of research examining acute Aβ effects on this transporter system. Studies have demonstrated a preferential accumulation of Aβ in glutamatergic neurons, with increased Aβ within synaptosomes co-labeled with both VGluT1 and Aβ (Sokolow et al., 2010). This study, however, does not examine the cause for this accumulation, and does not offer any insight into the potential mechanisms involved in this change. This accumulation of Aβ in AD has been shown to result in selective decline in VGluT1 expression (Rodriguez-Perdigon et al., 2016). Rodríguez-Moreno and Lerma (1998) noted a reduction in both glutamatergic terminals and VGluT1 levels in hippocampal cell cultures exposed to Aβ, with intracerebroventricular administration of $A\beta_{1-42}$ resulting in altered synaptic plasticity and neuroinflammation.

On examining the Aβ-injection effect, Canas et al. (2014) demonstrated a preferential decrease in density of both VGluT1 and VGluT2 transporters in mice 15 days post-Aβ administration. In support of this, mice expressing the apoE4 gene demonstrated a reduction in VGluT1 levels in hippocampal neurons in conjunction with accumulation of Aβ and hyperphosphorylated tau (Liraz et al., 2013). This, however, represented a chronic accumulation of Aβ, which, while able to mimic possible chronic mechanisms and the pathological sequelae of apoE4 expression, is not able to be extrapolated to show the effect of acute Aβ insult on VGluT1 expression and function.

In our study there was only a minor increase in VGluT1 expression in the DG ventral str. moleculare and a decrease in VGluT2 expression in the str. granulosum in the Aβ-injected mice. This study demonstrates the robustness of the vesicular glutamate transport system, indicating changes noted in other studies are a result of longer more chronic exposure to Aβ.

The observed significant long-term spatial memory impairment is in line with studies conducted in the past examining the acute effect of Aβ injection on cognitive memory and function (Kim et al., 2016; Kasza et al., 2017). Mice treated with acute intracerebroventricular Aβ displayed statistically significant spatial memory impairment in Y maze test 3 days post-injection (Kim et al., 2016). Rats displayed impaired spatial memory on Morris water maze test and impaired synaptic plasticity 7 days post-intracerebroventricular $A\beta_{1-42}$ injection (Kasza et al., 2017) but this timepoint might reflect more long-term consequences of the neurotoxic insult. We show no acute $A\beta_{1-42}$-induced deficits in non-spatial memory performance and this is in line with findings in transgenic AD mouse models displaying these type of impairments only after extended periods of Aβ exposure. While the glutamatergic system is most likely involved in acute $A\beta_{1-42}$-induced memory deficits, the robustness of the expression of receptor subunits and transporters indicate that other mechanisms might be involved which have to be further elucidated. Evaluating gene expression or other markers of glutamatergic signaling, e.g., proteins of the post-synaptic density, may be the focus of future research to deepen the knowledge into glutamatergic alterations by the Aβ protein and provide more information into disease mechanisms causing cognitive deficits.

CONCLUSION

The results detailed in this study provide evidence on acute and focal effects of $A\beta_{1-42}$ on memory function and the expression of components of the glutamatergic system in the mouse hippocampus. Importantly, although the glutamatergic system in early exposure is relatively robust against $A\beta_{1-42}$-induced neurotoxic changes, even minor alterations in specific receptor subunit and transporter expression

could lead to significant pathophysiological outcomes which is why glutamatergic changes in response to Aβ warrants further investigation.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article.

ETHICS STATEMENT

The animal study was reviewed and approved by the University of Otago and the University of Auckland Animal Ethics Committees.

AUTHOR CONTRIBUTIONS

JY, TP, WT, KP, and AK performed the research. AK, WT, RF, and HW designed the research. JY, TP, WT, HW, RF, and AK wrote the manuscript.

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REVIEW
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Cortical and Striatal Circuits in Huntington's Disease

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Huntington's disease (HD) is a hereditary neurodegenerative disorder that typically manifests in midlife with motor, cognitive, and/or psychiatric symptoms. The disease is caused by a CAG triplet expansion in exon 1 of the huntingtin gene and leads to a severe neurodegeneration in the striatum and cortex. Classical electrophysiological studies in genetic HD mouse models provided important insights into the disbalance of excitatory, inhibitory and neuromodulatory inputs, as well as progressive disconnection between the cortex and striatum. However, the involvement of local cortical and striatal microcircuits still remains largely unexplored. Here we review the progress in understanding HD-related impairments in the cortical and basal ganglia circuits, and outline new opportunities that have opened with the development of modern circuit analysis methods. In particular, *in vivo* imaging studies in mouse HD models have demonstrated early structural and functional disturbances within the cortical network, and optogenetic manipulations of striatal cell types have started uncovering the causal roles of certain neuronal populations in disease pathogenesis. In addition, the important contribution of astrocytes to HD-related circuit defects has recently been recognized. In parallel, unbiased systems biology studies are providing insights into the possible molecular underpinnings of these functional defects at the level of synaptic signaling and neurotransmitter metabolism. With these approaches, we can now reach a deeper understanding of circuit-based HD mechanisms, which will be crucial for the development of effective and targeted therapeutic strategies.

Keywords: Huntington's disease, cortex, basal ganglia, neural circuits, genetic mouse models, *in vivo* calcium imaging, optogenetics

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INTRODUCTION

Huntington's disease (HD) is a devastating movement disorder that affects about 1 in 10,000 people. Among the heterogeneous group of neurodegenerative diseases, it takes a special role based on its strictly genetic cause, i.e., an autosomal dominant mutation of the huntingtin (*HTT*) gene on chromosome 4 (The Huntington's Disease collaborative research group, 1993). The repetition of a CAG codon above a number of 35 translates into an expanded polyglutamine (polyQ) tract in the HTT protein, and causes a cascade of pathological events manifesting in psychiatric, cognitive and motor symptoms. The disease usually starts in midlife, with age of onset inversely correlating to CAG repeat number (Ross et al., 2014), and follows the course of three consecutive stages. The initial stage is typically characterized by mood disorder, cognitive deficits, and subtle motor impairments. In the second stage, excessive, abrupt, and involuntary movements (chorea) become the dominant symptom, while motor skills such as gait, swallowing, and

speech rapidly deteriorate. Cognitive capacities also continue to decline, culminating in dementia. In the third stage, severe weight loss and overall deterioration of health occurs and choreic movements are replaced by bradykinesia and rigidity. Finally, death becomes imminent 15 to 20 years after disease onset.

Pathologically, HD is characterized by neurodegeneration of the basal ganglia, which is particularly severe in the striatum. Prominent atrophy also occurs in the neocortex, the main input region of the striatum, and in advanced disease stages other brain regions become affected as well (Waldvogel et al., 2015). Striatal atrophy mainly results from the loss of GABAergic spiny projection neurons (SPNs), also known as medium spiny neurons, while cortical neurodegeneration is most pronounced in the motor and premotor areas and primarily affects cortical pyramidal neurons (CPNs), also referred to as principal cells. Importantly, changes in neuronal function occur long before overt cell death is observed, suggesting that circuit alterations underlie the early symptoms of the disease.

In this review we will outline the current understanding of circuit mechanisms of HD based on investigations in available genetic mouse models. As classical electrophysiological studies in HD models have been extensively reviewed elsewhere (Raymond et al., 2011; Galvan et al., 2012; Bunner and Rebec, 2016; Plotkin and Goldberg, 2018), our main emphasis will be on the most recent developments in the field enabled by technological advances in circuit analysis, such as long-term *in vivo* imaging, *in vivo* multi-channel electrophysiology, optogenetics, and systems approaches for unbiased characterization of transcriptomic and proteomic changes. With these tools at hand, in the next few years it should be possible to not only accurately describe the HD-related defects in cortical and basal ganglia circuits, but also attempt to ameliorate them through cell type-specific activity manipulations.

GENETIC MOUSE MODELS OF HD

A number of HD mouse models have been created over the years since the discovery of the causal mutation in the *HTT* gene. These models have been reviewed in detail elsewhere (Brooks and Dunnett, 2013; Pouladi et al., 2013), and here we will only highlight the ones that are most frequently used for the study of HD-related circuit defects (**Table 1**). They can be divided into truncated and full-length models, the latter including transgenic and knock-in lines. Truncated models are all transgenic and express an N-terminal fragment of HTT with a pathological polyQ stretch. The R6 lines were the first HD mouse lines to be generated and are among the best studied. The R6/2 mouse line contains ∼150 CAG repeats and shows an aggressive phenotype with very early neurophysiological, histological, and behavioral alterations and a lifespan of only ∼3–5 months (Mangiarini et al., 1996).

Transgenic full-length models of HD express full-length human mutant HTT (mHTT) and generally exhibit a slower disease progression than truncated models. The yeast artificial chromosome (YAC) transgenic strategy was used to generate several mouse lines, named corresponding to their number of CAG repeats: YAC18 (control), YAC46, YAC72, and YAC128 (Hodgson et al., 1999; Slow et al., 2003). The YAC128 mouse exhibits striatal followed by cortical atrophy and mimics human disease progression by displaying first a hyperkinetic and later a hypokinetic phenotype (Slow et al., 2003). The bacterial artificial chromosome (BAC) HD model carries 97 mixed CAA-CAG repeats, shows reduced cortical and striatal volume and progressive motor impairments (Gray et al., 2008).

Knock-in HD mouse models provide stronger construct validity than transgenic models, as the CAG expansion is inserted into the native murine *Htt* locus, thereby more closely resembling the genetic context of HD patients. In these mice, brain atrophy and motor defects slowly emerge in a protracted manner. Among the knock-in models are the HD allelic series mice with various CAG tract lengths, including CAG140 and the widely used zQ175 line (Menalled et al., 2002, 2003, 2012; Heikkinen et al., 2012). Although no model perfectly reproduces all the aspects of the human disease, the major findings on circuit phenotypes have been quite consistent between various transgenic and knock-in lines, strengthening the confidence that mouse models can deliver important insights into pathogenic mechanisms of this disorder.

CIRCUITS AFFECTED IN HD

Two brain regions most vulnerable to HD are the basal ganglia and the neocortex, which are extensively connected to each other (**Figure 1**). The neocortex contains two major neuron types: CPNs, which constitute ∼80% of all cortical neurons, and interneurons, which account for the remaining 20% (Defelipe et al., 2013; Huang, 2014). CPNs are excitatory glutamatergic neurons with long-range projections connecting cortical areas to each other or to subcortical structures. Interneurons are inhibitory GABAergic cells with mostly local connections. Based on the almost non-overlapping expression of molecular markers, cortical interneurons are subdivided into three main populations with distinct morphology, electrophysiological properties, layer distribution and function: parvalbumin (PV)-positive, somatostatin (SST)-positive and 5HT3a-receptor-positive cells (Tremblay et al., 2016). PV cells are known to synapse onto or close to the soma of CPNs and exert very fast and strong inhibition onto their target cells (Pfeffer et al., 2013; Hu et al., 2014), whereas SST cells form synapses on more distal dendrites (Wang et al., 2004). 5HT3aR cells are very heterogeneous, with a major subclass of this population expressing the marker vasointestinal peptide (VIP). VIP cells preferentially synapse onto SST interneurons (Pfeffer et al., 2013) (**Figure 1**).

The basal ganglia are a group of subcortical structures including the striatum, globus pallidus (GP), ventral pallidum, substantia nigra (SN), and subthalamic nucleus (STN). Within the basal ganglia, striatum is the region receiving most of the long-range input, including extensive glutamatergic innervation from CPNs (**Figure 1**). Although these afferents come from virtually all cortical areas, and are involved in many types of sensory, cognitive and motor functions, here we will focus on the motor circuitry relevant for HD, including motor cortical areas and the dorsolateral striatum. Another source of

TABLE 1 | Selected genetic HD mouse models*.

Model	Type of genetic manipulation	References
R6/2	Transgenic, human exon 1 fragment with ~150 CAG repeats under human HTT promoter	Mangiarini et al., 1996
YAC128	Transgenic, human full-length HTT with 128 CAG repeats	Slow et al., 2003
BACHD	Transgenic, human full-length HTT with 97 mixed CAA-CAG repeats	Gray et al., 2008
CAG140	Knock-in, chimeric mouse/human exon 1 with 140 CAG repeats inserted into the murine Htt locus	Menalled et al., 2003
zQ175	Knock-in derived from the CAG140 line, 188 CAG repeats	Menalled et al., 2012

This table is not intended to give a complete overview of existing mouse models of HD, but only contains the models widely used for circuits studies.

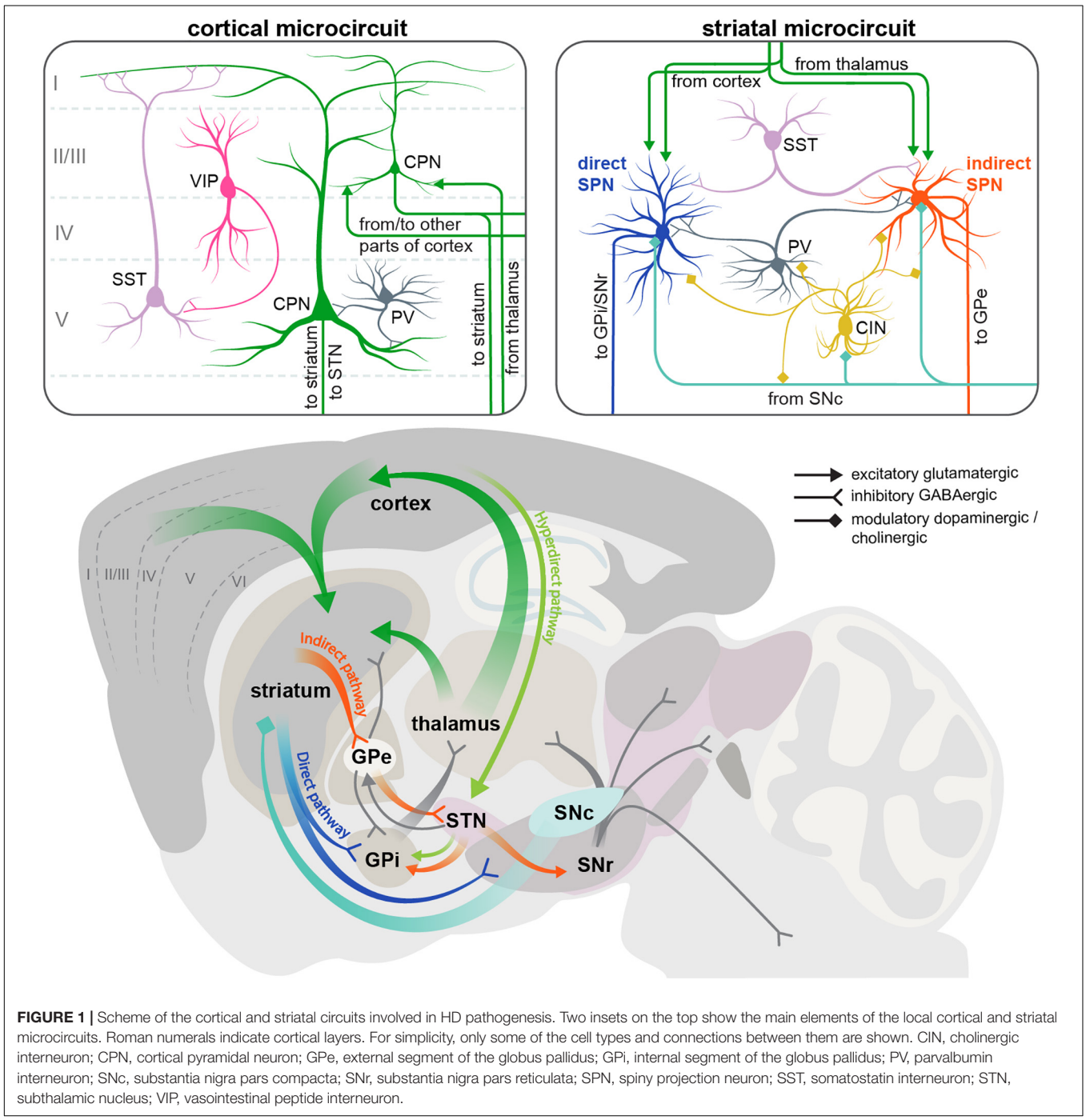

FIGURE 1 | Scheme of the cortical and striatal circuits involved in HD pathogenesis. Two insets on the top show the main elements of the local cortical and striatal microcircuits. Roman numerals indicate cortical layers. For simplicity, only some of the cell types and connections between them are shown. CIN, cholinergic interneuron; CPN, cortical pyramidal neuron; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; PV, parvalbumin interneuron; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SPN, spiny projection neuron; SST, somatostatin interneuron; STN, subthalamic nucleus; VIP, vasointestinal peptide interneuron.

glutamatergic afferents to the striatum is the thalamus. Apart from these excitatory inputs, striatum also receives abundant modulatory dopaminergic afferents from the substantia nigra pars compacta (SNc).

GABAergic SPNs account for > 90% of striatal neurons and are subdivided into two populations of approximately equal size, giving rise to the two main striatal projections. The D1 dopamine receptor-expressing SPNs form the direct pathway, and are therefore referred to as dSPNs. This pathway projects directly to the output nuclei of the basal ganglia: the internal part of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). The D2 dopamine receptor-positive SPNs form the indirect pathway and are referred to as iSPNs. This polysynaptic pathway connects to the output nuclei indirectly via the external segment of the globus pallidus (GPe) and STN (**Figure 1**) (Alexander and Crutcher, 1990). GPi and SNr consist of GABAergic neurons that have pacemaker properties and maintain tonic activity, continuously inhibiting their target cells in the ventral anterior and ventral lateral nuclei of the thalamus (Gerfen and Surmeier, 2011; Plotkin and Goldberg, 2018). Thalamic nuclei in turn send glutamatergic projections to the frontal cortex, forming the cortico-basal ganglia-thalamo-cortical loop. The direct and indirect striatal projections have opposing effects on the activity of the GABAergic cells in the GPi and SNr, and thereby on the overall output of the basal ganglia. The direct pathway inhibits the GPi/SNr activity and therefore has a net excitatory effect on the thalamus and cortex, facilitating execution of motor programs. Conversely, the indirect pathway disinhibits the GABAergic neurons in the GPi/SNr, leading to reduced activity of the thalamic and cortical neurons, and suppression of undesired movements (Alexander and Crutcher, 1990; Gerfen and Surmeier, 2011). It should be noted that this simple model of two antagonistic striatal pathways has been refined in the recent years by *in vivo* studies demonstrating simultaneous activation of dSPN and iSPN cell clusters during motion initiation, as well as similar correlation of their activity with locomotor behavior, suggesting a more sophisticated functional arrangement of basal ganglia circuits than previously thought (Cui et al., 2013; Barbera et al., 2016; Klaus et al., 2017; Parker et al., 2018).

In addition to the direct and indirect pathways, a third, hyperdirect pathway exists that bypasses the striatum and connects the frontal cortex to the output nuclei via glutamatergic neurons of the subthalamic nucleus (STN) (**Figure 1**). Like the indirect projection, this pathway also has a net inhibitory action on the thalamus and cortex. However, it conveys signals faster than the indirect pathway, and is believed to be important for precise timing of motor program initiation (Nambu et al., 2002).

Apart from SPNs, striatal microcircuits include multiple groups of local interneurons: cholinergic interneurons (CINs) and several types of GABAergic cells that can be distinguished by the expression of molecular markers such as parvalbimun (PV), neuropeptide Y (NPY), neuropeptide Y/somatostatin/nitric oxide synthase (NPY/SST/NOS), calretinin (CR), and tyrosine hydroxylase (TH). Overall, striatal interneurons receive similar types of afferents as striatal SPNs (glutamatergic from cortex and thalamus, and dopaminergic from SNc), and provide feedforward inhibition onto SPNs, modulating their activity on different time scales; however, each interneuron subtype has distinct connectivity and physiology. Striatal PV interneurons are fast-spiking cells (hence also referred to as FS interneurons) that preferentially target the soma and proximal dendrites of SPNs, providing fast and strong inhibitory inputs. All other GABAergic interneurons fire at lower rates and form synapses on distal SPN dendrites (Straub et al., 2016; Plotkin and Goldberg, 2018). CINs in turn modulate the activity of GABAergic cells as well as SPNs (**Figure 1**). There are also multiple connections between different striatal interneuron subtypes that are just beginning to be uncovered (Lee et al., 2017; Plotkin and Goldberg, 2018).

Although striatal interneurons provide the major source of GABAergic inhibition to SPNs, other inhibitory inputs also exist. Both dSPNs and iSPNs send collateral projections to other SPNs belonging to both pathways (Gittis and Kreitzer, 2012). Another layer of inhibitory connectivity is added by the reciprocal feedback projections between different nuclei of the basal ganglia that complement the unidirectional cortico-basal ganglia-thalamo-cortical loop described above (Plotkin and Goldberg, 2018). The following sections explain the specific defects that have been described in all these circuits during disease progression in various HD mouse models. A brief summary of these defects is given in **Figures 2**, **3**.

Cortical Circuits

Cortical degeneration and dysfunction significantly contribute to impairments in motor and executive functions and cognitive abilities observed in HD. This is underlined by detailed neuropathological studies in human post-mortem brains which revealed a reduction in overall cortical area and cortical white matter, associated with a marked cell loss. It is well-established that CPNs are particularly vulnerable to HD, however region-specific degeneration of interneurons also occurs (Kim et al., 2014; Mehrabi et al., 2016). Interestingly, neuronal cell loss in the cortex correlates with CAG repeat numbers (Halliday et al., 1998), as well as with the clinical symptomatology, such that patients with primarily motor symptoms show a prominent reduction in cell numbers in the primary motor cortex (Thu et al., 2010).

A growing body of evidence also highlights the fact that structural and functional alterations in the cortex precede neuronal loss by several years. The cortex of HD mutation carriers shows progressive regional thinning in a topographically predictable manner already up to 15 years before the onset of motor symptoms (Rosas et al., 2005, 2008; Nopoulos et al., 2010). On a functional level, one of the earliest events in HD is increased cortical excitability and impaired GABA-mediated cortical inhibition, as shown by transcranial magnetic stimulation studies already in the presymptomatic phase of the disease (Nardone et al., 2007; Schippling et al., 2009; Philpott et al., 2016; Agarwal et al., 2019).

Similar findings were also reported and further extended in HD mouse models, which exhibit multiple morphological and electrophysiological abnormalities of cortical neurons. Morphologically, dysmorphic dendrites and loss of dendritic spines on CPNs was observed in R6/2 and knock-in mice (Klapstein et al., 2001; Laforet et al., 2001). Chronic *in vivo*

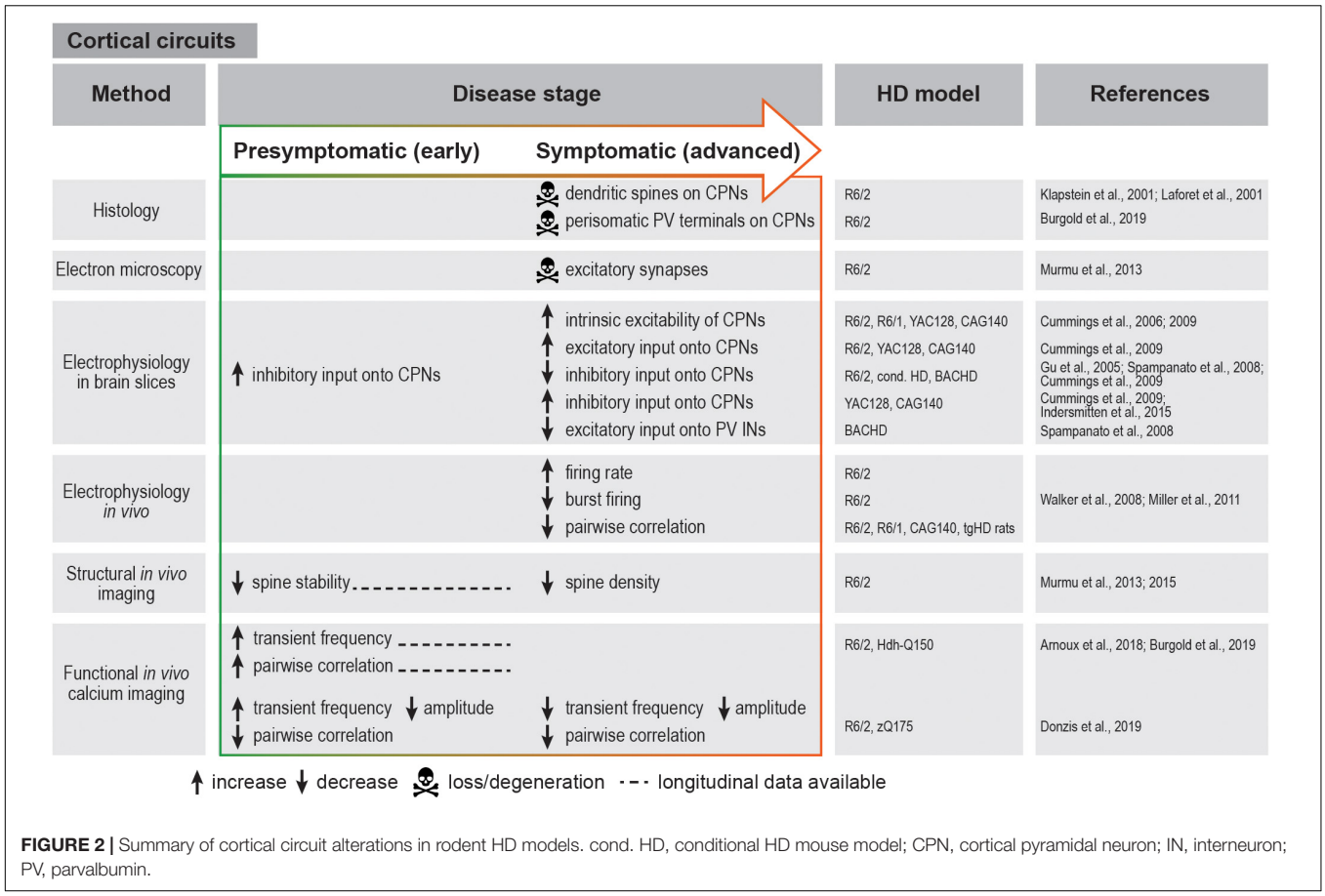

FIGURE 2 | Summary of cortical circuit alterations in rodent HD models. cond. HD, conditional HD mouse model; CPN, cortical pyramidal neuron; IN, interneuron; PV, parvalbumin.

structural imaging furthermore demonstrated impaired spine turnover and a progressive loss of persistent spines in the somatosensory cortex (Murmu et al., 2013). Sensory deprivation exacerbated the loss of persistent spines and impaired stabilization of newly gained spines, suggesting that mHTT promotes maladaptive synaptic plasticity (Murmu et al., 2015).

Electrophysiological studies in HD mouse models revealed several changes in the basic membrane properties of CPNs, such as an increase in input resistance, decrease in cell membrane capacitance and depolarized resting membrane potential (Cummings et al., 2006, 2009; Stern, 2011). These alterations are known to affect synaptic plasticity and to make cells more excitable and therefore prone to excitotoxic damage. In addition to the elevated intrinsic excitability, there is an increase in excitatory inputs to CPNs. Thus, behaviorally phenotypic R6/2 mice present higher frequency of spontaneous excitatory post-synaptic potentials (EPSCs) and larger amplitudes of evoked EPSCs in layer II/III CPNs. Likewise, increased excitatory drive was also observed in the YAC128 and CAG140 models. Changes in inhibitory post-synaptic currents (IPSCs) seem to be more complex and variable between models: in R6/2 mice, IPSC frequency is initially increased in presymptomatic animals, but markedly declines at an advanced stage, while it is increased at an advanced stage in two different full-length mouse models (Cummings et al., 2009) (**Figure 2**).

In vivo electrophysiological recordings in R6/2 mice also showed faster and less variable firing rates in the medial prefrontal cortex. In addition, the firing pattern was temporally altered, with mostly individual spikes instead of coordinated bursts that typically occur in the WT cortex (Walker et al., 2008; Miller et al., 2011) (**Figure 2**). Although it should be noted that these parameters were not affected in knock-in HD mice, the observations in R6/2 animals are consistent with the possibility that the cortical activity is overall increased, but becomes "noisy" and less structured, possibly leading to impaired information processing in the cortex.

Recent advances in imaging techniques have allowed a more detailed investigation of cortical network dysfunction in large cell populations at single-cell resolution in a living animal. Using *in vivo* calcium imaging in transgenic and knock-in HD mice, we and others have demonstrated increased frequency of calcium transients at the premanifest disease stage and at disease onset, indicative of higher firing rates in CPNs as also suggested by electrophysiological recordings (Arnoux et al., 2018; Burgold et al., 2019; Donzis et al., 2019). In contrast, a decrease in calcium transient frequency and amplitude was observed in advanced-stage R6/2 and zQ175 knock-in mice (Donzis et al., 2019) (**Figure 2**). Although further studies spanning presymptomatic and symptomatic stages in the same mouse will be required to reconcile these observations, taken together, the presently

available findings suggest dynamic changes in the cortical network during disease progression. An important advantage of chronic imaging is the possibility to longitudinally follow activity dynamics of single identified cells during disease course. This enabled us to show that a large fraction of neurons in the primary motor cortex of R6/2 mice become more active before the onset of motor symptoms, and maintain higher activity as disease progresses (Burgold et al., 2019).

In addition to the well-established perturbations of CPN activity, there is significant evidence supporting the involvement of cortical GABAergic interneurons in HD progression. Studies in conditional HD mouse models pointed to the importance of mHTT expression in cortical interneurons in addition to CPNs and the role of cell-cell interactions between CPNs and interneurons in the development of full-fledged cortical HD pathology and behavioral defects (Gu et al., 2005). Moreover, certain behavioral phenotypes were attributed specifically to cortical interneuron dysfunction (Dougherty et al., 2014). Electrophysiological recordings in HD mouse brain slices demonstrated reduced inhibitory inputs onto CPNs, along with reduced excitatory inputs onto PV interneurons and an altered probability of GABA release (Gu et al., 2005; Spampanato et al., 2008; Cummings et al., 2009). In agreement with these findings, a reduction in perisomatic PV terminals around CPNs was observed in both R6/2 mice and *post-mortem* HD patient tissue (Burgold et al., 2019). Altogether, these studies suggest that weakened inhibition might play a role in HD-related cortical network dysfunction. It should be noted that apart from the few studies on PV interneurons (Spampanato et al., 2008; Dougherty et al., 2014), the contribution of other major cortical interneuron types to HD is still largely unexplored.

The relevance of understanding interneuron function in HD becomes even more apparent when we appreciate their role in fine-tuning the activity of neuronal networks. GABAergic neurons promote fast spike synchrony between excitatory neurons and serve as pacemakers for generating neuronal oscillations, temporally defined cortical rhythms produced by coordinated activity of the network and important for cognitive functions. Thus, autaptic self-connectivity among PV neurons drives cortical oscillations in the gamma frequency range (Connelly, 2014; Deleuze et al., 2019). SST interneuron in turn are important for beta oscillations, and drive long-distance coherence across cortical areas (Chen et al., 2017; Veit et al., 2017). Interneurons are also believed to sculpt the functional flexibility of cortical circuits, which is a key factor for shaping behavior (Berke et al., 2004; Buzsáki et al., 2012; Cardin, 2019).

Studies in HD mouse models reach to some degree contradictory conclusions concerning cortical network synchrony assessed by correlations of simultaneously recorded neuron pairs. *In vivo* electrophysiology demonstrated reductions in spike synchrony in both transgenic and knock-in HD models regardless of disease stage (Walker et al., 2008; Miller et al., 2011; Stern, 2011), and implicated these deficits in behavioral alterations (Walker et al., 2011). While reduced pairwise synchrony was also observed in one *in vivo* calcium imaging study (Donzis et al., 2019), other studies described an increase in pairwise neuronal correlation as one of the earliest alterations in both transgenic and knock-in HD mice (Arnoux et al., 2018; Burgold et al., 2019). These initial insights call for further investigation to clarify the spatial and temporal manner in which network synchrony is affected in HD.

Corticostriatal Projection

Changes in the corticostriatal connections are among the earliest events in disease progression, which occur before any signs of cell death can be detected, and presumably underlie the subtle motor deficits in premanifest HD (Reading et al., 2004; Unschuld et al., 2012; Hintiryan et al., 2016; Reiner and Deng, 2018). Disconnection from cortical afferents likely plays a major role in the subsequent dysfunction of the downstream striatal circuits. The breakdown of corticostriatal communication in HD has been extensively studied, and for detailed information the reader is referred to several excellent reviews available on this topic (Miller and Bezprozvanny, 2010; Raymond et al., 2011; Estrada-Sánchez and Rebec, 2013; Plotkin and Surmeier, 2015; Bunner and Rebec, 2016; Rebec, 2018). The conclusion that emerged from electrophysiological studies in slices as well as *in vivo* in multiple HD mouse models is that alterations in corticostriatal connections occur in two phases, with increased glutamate release and SPN hyperexcitation at the presymptomatic stage, followed by SPN silencing at the symptomatic stage (**Figure 3**) (Klapstein et al., 2001; Cepeda et al., 2003; Rebec et al., 2006; Joshi et al., 2009; André et al., 2011b; Miller et al., 2011; Raymond et al., 2011; Indersmitten et al., 2015; Rothe et al., 2015). The elevated cortical activity observed early in disease causes an increased excitatory drive onto striatal SPNs. Excess glutamate release from the cortical synaptic terminals leads to sustained activation of extrasynaptic NMDA receptors, triggering apoptotic mechanisms in the SPNs (DiFiglia, 1990; Okamoto et al., 2009; Milnerwood et al., 2010). Conversely, the decrease in SPN activity at a later stage is due to progressive loss of cortical inputs. Morphological investigations show a decrease in corticostriatal synaptic terminals in symptomatic animals (Deng et al., 2013), as well as progressive decline in spine densities on SPN dendrites (Indersmitten et al., 2015).

In vivo electrophysiological studies in freely behaving mice also analyzed local field potentials (LFPs), transient extracellular signals generated by large populations of neurons. These recordings uncovered altered synchrony between the cortical and striatal networks in HD (Hong et al., 2012; Naze et al., 2018). However, it is still unknown how cortical activity shapes firing patterns in the striatum. A study combining wide-field calcium imaging in the cortex with simultaneous multielectrode recordings in the dorsal striatum elucidated some of the principles of corticostriatal activity coupling during a behavioral task (Peters et al., 2019). Such cellular resolution studies would be invaluable to obtain a more comprehensive picture of corticostriatal miscommunication in HD.

An important function of the corticostriatal afferents is providing trophic support for SPNs, which are dependent on brain-derived neurotrophic factor (BDNF) for survival. BDNF is mainly produced in the cortex and delivered to the striatum anterogradely via CPN axons (Altar et al., 1997). mHTT

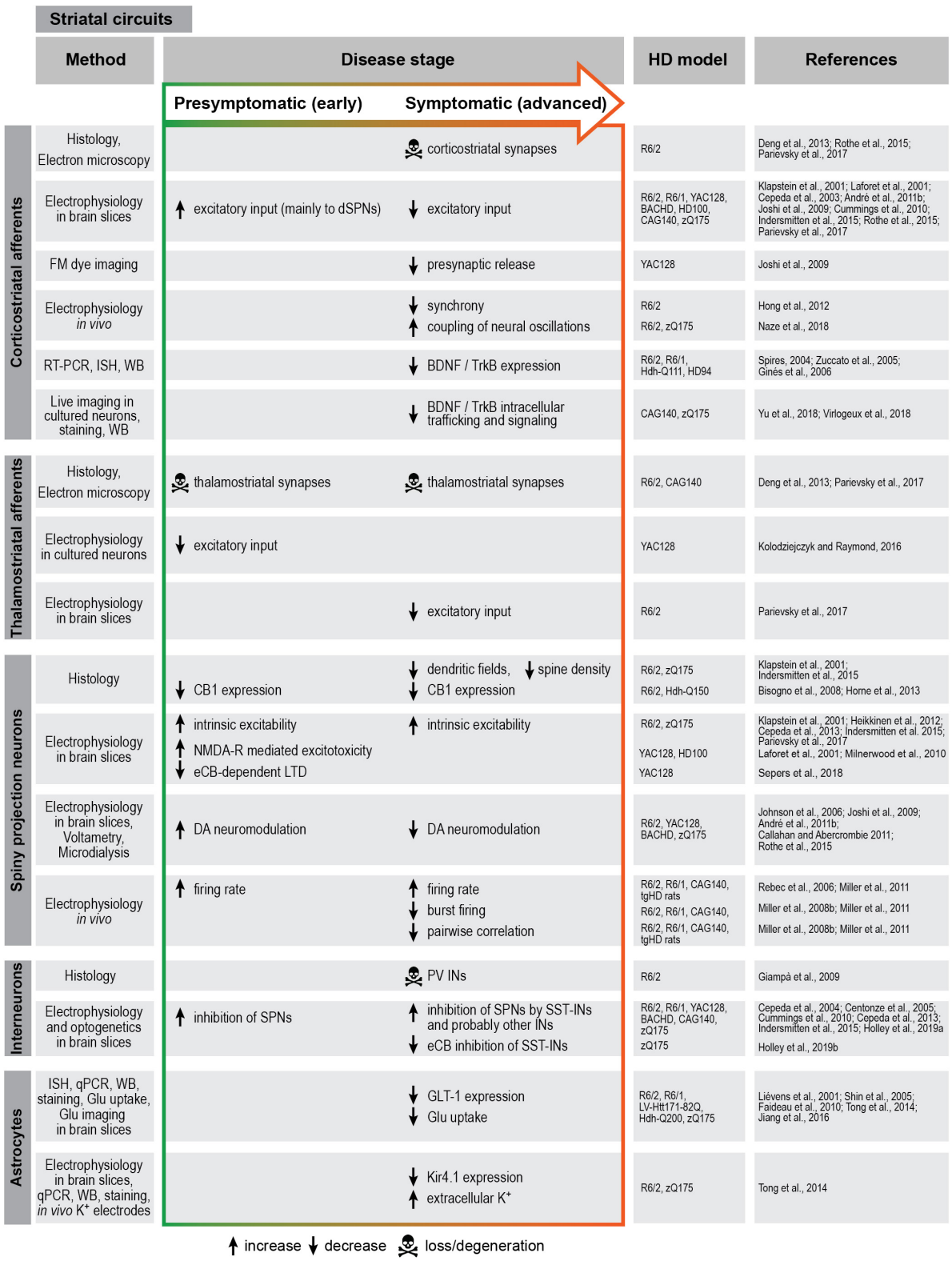

FIGURE 3 | Summary of striatal circuit alterations in rodent HD models. BDNF, brain-derived neurotrophic factor; CB1, cannabinoid receptor 1; DA, dopamine; eCB, endocannabinoid; Glu, glutamate; HPLC, high-pressure liquid chromatography; IN, interneuron; ISH, *in situ* hybridization; LTD, long-term depression; NMDA-R, *N*-methyl-D-aspartate receptor; PV, parvalbumin; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; SPN, spiny projection neuron; SST, somatostatin; TrkB, tropomyosin receptor kinase B; WB, Western blot.

decreases the levels of BDNF and its receptor tropomyosin-related kinase B (TrkB) in human (Ferrer and Blanco, 2000; Zuccato, 2001) and mouse brain (Spires, 2004; Zuccato et al., 2005; Ginés et al., 2006). Moreover, impaired transport and reduced release of BDNF has been observed in cortical neurons of zQ175 mice (Yu et al., 2018). Genetic reduction of BDNF in mice leads to striatal degeneration and expression profile similar to human HD, arguing for a major contribution of insufficient trophic support to striatal degeneration in HD (Strand et al., 2007).

Abundant evidence of corticostriatal miscommunication and impaired BDNF trophic support in HD obtained from studies in brain slices and *in vivo* was also confirmed in a recent *in vitro* approach using compartmentalized microfluidic chambers to reconstitute corticostriatal connectivity in a dish (Virlogeux et al., 2018). Consistent with previous findings, this study highlighted the important contribution of cortical afferents to the functional alterations observed in the post-synaptic striatal neurons.

It should be noted that in addition to the corticostriatal projection, the glutamatergic thalamostriatal afferents are also affected in various HD mouse models (**Figure 3**) (Kolodziejczyk and Raymond, 2016; Parievsky et al., 2017) and might be impaired even earlier than the cortical inputs (Deng et al., 2013). However, our knowledge about their significance in disease is still scarce.

Basal Ganglia Circuits

Among the striatal cells, iSPNs are the most vulnerable to mHTT and are the first ones to degenerate (Reiner et al., 1988; Deng et al., 2004). This results in disinhibition of the thalamic target neurons, which is believed to underlie the hyperkinetic symptoms in HD. At a later stage, when dSPNs also succumb to disease, dyskinesia is replaced by akinesia and muscle stiffness (Albin et al., 1989; Plotkin and Goldberg, 2018; Reiner and Deng, 2018). Long before overt cell death occurs, SPNs start showing functional abnormalities. Some of the well-established early alterations in SPNs across various HD models are a depolarized membrane potential, an increase in input resistance, and hyperexcitability (Klapstein et al., 2001; Cepeda et al., 2003, 2013; Raymond et al., 2011; Heikkinen et al., 2012; Indersmitten et al., 2015; Dvorzhak et al., 2016; Parievsky et al., 2017). In addition, the two types of SPNs exhibit differential changes in glutamatergic, GABAergic inputs, and dopaminergic modulation, leading to an overall increased activation of dSPNs in particular (André et al., 2011a,b; Galvan et al., 2012; Deng et al., 2014). These functional changes are believed to be crucial to the imbalance of the direct and indirect pathway early in disease. It should be noted that recent demonstration of overlapping dSPNs and iSPNs activity patterns *in vivo* during motion (Cui et al., 2013; Barbera et al., 2016; Klaus et al., 2017; Parker et al., 2018) raised the possibility that disturbances occurring in HD likely go beyond a mere increase or decrease in the firing rates of one or both SPN types.

In vivo recordings of spontaneous activity in the striatum of freely behaving HD mice and rats revealed population-level impairments in striatal activity with a reduction in pairwise correlations and coincident bursts (Miller et al., 2008b, 2010). To obtain a more precise picture of these disturbances, it will be necessary to monitor the activity of different neuronal populations at a cellular resolution. However, while striatal circuits in HD models have been extensively explored in electrophysiological studies, their subcortical location has presented a certain difficulty for imaging. The development of head-mounted miniature microscopes that give optical access to deep brain structures should accelerate progress in this area (Werner et al., 2019). For example, calcium imaging in the striatum of a pharmacological mouse model of Parkinson's disease revealed an imbalance of dSPNs and iSPN activity rates along with more complex changes in the spatiotemporal activity patterns and motion encoding that were specific to the iSPN population (Parker et al., 2018). It will be exciting to see what kind of changes can be uncovered by similar studies in freely behaving HD mice.

While the main focus in HD research has been on interplay between the direct and indirect pathways, more recent studies have also revealed abnormalities in the STN function that might contribute to motor impairments. *In vivo* extracellular recordings combined with electrocorticography demonstrated increased excitability of STN neurons in presymptomatic YAC128 animals, and reduced cortico-subthalamic coherence in symptomatic YAC128 and R6/2 mice. In addition, there was an overall decline in spontaneous activity and altered firing pattern of STN neurons (Callahan and Abercrombie, 2015a,b). These findings suggest that early hyperexcitability and later disconnection from the cortex is a general feature of cortico-basal ganglia projections in HD, affecting corticostriatal as well as corticosubthalamic pathways. Moreover, an *ex vivo* study in brain slices from two further mouse models, zQ175 and BACHD, confirmed reduced activity of STN neurons and attributed this reduction to an increased activation of NMDA receptors, resulting oxidant stress, and activation of K_{ATP} channels (Atherton et al., 2016). In addition to these functional changes, age-dependent loss of STN neurons was shown in human patients as well as HD mice (Lange et al., 1976; Guo et al., 2012; Atherton et al., 2016).

Striatal inhibitory circuits also show multiple defects in HD animals. Electrophysiological recordings demonstrated a consistent increase in GABAergic transmission onto SPNs of different mouse models, which in some cases occurred already at the presymptomatic stage (Cepeda et al., 2004, 2010, 2013; Centonze et al., 2005; Cummings et al., 2010; Indersmitten et al., 2015; Hsu et al., 2018). This increase in striatal inhibition, together with the loss of excitatory inputs, contributes to silencing of SPNs as disease progresses. Interestingly, the alterations in GABAergic inputs were distinct for the two types of SPNs, with a stronger effect in iSPNs found in three different mouse models at a symptomatic stage (André et al., 2011b; Galvan et al., 2012; Cepeda et al., 2013). These differences likely exacerbate the imbalance between the direct and indirect pathways.

Although the increase in inhibition onto SPNs is well-established, the underlying cell types and circuit mechanisms still remain to be deciphered. In contrast to SPNs, most local interneurons are relatively spared in HD, with the exception of PV interneurons, which are reduced in numbers in human patients and R6/2 mice (Giampà et al., 2009; Reiner et al., 2013; Reiner and Deng, 2018). A detailed analysis of PV cells

in the zQ175 mouse model furthermore uncovered changes in morphology, physiological properties, and connectivity (Holley et al., 2019a). A recent study combining activity manipulation of PV interneurons with simultaneous calcium imaging of PV cells and SPNs in freely moving mice pointed to the function of PV cells in facilitating execution of movement (Gritton et al., 2019); it is therefore tempting to speculate that dysfunction and loss of striatal PV interneurons may play a causal role in HD-related akinesia, a hypothesis still to be tested in HD model animals.

Even though unchanged in numbers, other types of interneurons are also likely to be functionally affected in HD (Reiner and Deng, 2018). Recent studies started tackling the contributions of different interneuron types using genetic targeting and circuit manipulation tools (Cepeda et al., 2013; Holley et al., 2015, 2019b; Tanimura et al., 2016). Thus, optogenetic silencing of SST-positive interneurons [also referred to as low-threshold spiking (LTS) interneurons] in brain slices suggested a major contribution of this cell type to the increased GABAergic inhibition in symptomatic zQ175 and R6/2 mice (Holley et al., 2019b). In addition, the feedback inhibitory connections between SPNs are also partially severed, and their pattern is altered in HD mice, with a substantial number of abnormal bidirectional connections between SPN pairs (Cepeda et al., 2013). How this rewiring of SPNs contributes to their dysfunction remains a subject for future research.

Taken together, accumulated evidence points to biphasic alterations in SPNs as a result of multiple impairments in local and extrastriatal inputs: after initial hyperexcitation at the early stage, with disease progression the excitation/inhibition balance tips toward increased inhibition (**Figure 3**) (Galvan et al., 2012; Indersmitten et al., 2015).

NEUROMODULATION BY DOPAMINE AND ENDOCANNABINOIDS

Dopaminergic Modulation

Alterations in glutamate-driven flow of information from the cortex to the striatum play a key role in the onset and progression of HD. Yet, the corticostriatal system is tightly regulated by dopamine (DA), a monoamine neuromodulator, which, together with GABA signaling, provides crucial counterbalance and adds flexibility to glutamatergic excitation of SPNs. In addition to the disturbances of glutamate and GABA signaling described above, many HD symptoms are therefore associated with altered dopaminergic modulation.

The dopaminergic circuit anatomy as well as dopaminergic signaling alterations in HD are described in detail in a number of excellent articles (André et al., 2010; Gerfen and Surmeier, 2011; Tritsch and Sabatini, 2012; Chen et al., 2013; Gardoni and Bellone, 2015; Rangel-Barajas and Rebec, 2016; Koch and Raymond, 2019). Two major dopaminergic pathways that innervate cortical and striatal areas and both show alterations in HD are the nigrostriatal and mesocorticolimbic pathways. The nigrostriatal pathway projects from the SNc to the dorsal striatum and is implicated in cognitive function and flexibility as well as in the control of movement (Bäckman and Farde, 2001; Groenewegen, 2003; Sleezer and Hayden, 2016; Andres and Darbin, 2018). In contrast, the mesocorticolimbic pathway originates from dopaminergic neurons in the ventral tegmental area (VTA) and ascends to the ventral striatum (or nucleus accumbens) and large areas of the frontal cortex. The mesocorticolimbic pathway plays a prominent role in motivation and reward-driven behavior.

DA can modulate the function of both excitatory CPNs and inhibitory SPNs on several levels, such as the probability of neurotransmitter release, the post-synaptic receptor sensitivity to the neurotransmitter (e.g., NMDA and AMPA receptors), and post-synaptic integration and ensuing excitability (Tritsch and Sabatini, 2012).

Intracellular DA signaling is mediated by a family of G-protein coupled receptors (D1–D5 DA receptors), which are grouped into two classes referred to as D1-like and D2-like. Upon receptor activation, one of the main targets of the recruited heterotrimeric G proteins is protein kinase A (PKA), which is positively or negatively coupled to D1- and D2-like receptors, respectively. As a consequence, the two classes of DA receptors drive complimentary cellular effects. The two types of SPNs show differential expression of DA receptors, with D1 receptors strongly enriched in dSPNs and D2 receptors in iSPNs (Tritsch and Sabatini, 2012). D1- and D2-like receptors exert opposite actions on presynaptic glutamate release and post-synaptic glutamate receptor currents: D1-like receptor signaling enhances glutamate release, depolarizes dSPNs, and increases their activity, while D2-like receptor signaling inhibits glutamate release, hyperpolarizes iSPNs, and leads to a decrease in their activity (Gerfen and Surmeier, 2011; Tritsch and Sabatini, 2012). An optimal level of dopaminergic modulation and balanced DA transmission between D1- and D2-like receptors is required for efficient motor function and behavioral flexibility (Chen et al., 2013).

In *post-mortem* HD brains, ∼40% dopaminergic neuron loss can be observed in the SNc along with a significant decrease in dopaminergic terminals in the striatum and loss of DA transporter (DAT) (Oyanagi et al., 1989; Suzuki et al., 2001). Transcriptional dysregulation of DA receptors in the striatum has also been reported in HD patients and HD models, with both D1- and D2-like receptors being reduced (Richfield et al., 1991; Weeks et al., 1996; Bibb et al., 2000; Ariano et al., 2002; Petersén et al., 2002; Pouladi et al., 2012).

During early disease progression, SPN loss is largely limited to striosomes (Hedreen and Folstein, 1995). As these cells project to the SNc, the death of these inhibitory neurons is thought to initially hyperactivate the nigrostriatal pathway, contributing to chorea and other clinical HD symptoms. First evidence for altered DA transmission came from the observation that pharmacologically increasing DA signaling in HD patients worsens chorea, whereas reducing DA leads to akinesia (Bird, 1980; Spokes, 1980). A wealth of following studies showed that progression of HD is accompanied by biphasic changes in DA inputs that are intertwined with changes in glutamate neurotransmission (**Figure 3**). The early symptomatic stage characterized by chorea is reflected by excessive glutamate and DA release, leading to a selective activation of the direct pathway

and disinhibition of the thalamus. At a later stage, when chorea is replaced by hypoactivity, a lack of sufficient glutamate and DA signaling leads to the silencing of the direct pathway and inhibition of the thalamus (Johnson et al., 2006; Joshi et al., 2009; André et al., 2010, 2011b; Callahan and Abercrombie, 2011; Galvan et al., 2012; Rothe et al., 2015; Covey et al., 2016; Koch and Raymond, 2019).

Additionally, D2-mediated DA signaling onto striatal CINs generally reduces acetylcholine release and thereby dampens the inhibition of dSPNs. In HD models, despite the survival of CINs in the striatum, a dysregulation of acetylcholine release has been reported (Reiner and Deng, 2018). By this mechanism, increased levels of DA would exacerbate the imbalance toward activation of the direct pathway, and promote the development of HD symptoms (Smith-Dijak et al., 2019).

In cortical neurons, which receive both dopaminergic and glutamatergic input, a disturbed signal-to-noise ratio and reduced range over which DA and glutamate can be modulated impairs both cognitive and motor functions (Kiyatkin and Rebec, 1999; Dallérac et al., 2011). An interesting theoretical framework suggested that increased neural noise would therefore lead to inflexibility of brain activity and as a consequence, behavioral adaptations to environmental challenges would be impaired (Hong and Rebec, 2012).

Importantly, HD is often accompanied by a range of psychiatric symptoms, such as mood disorders, aggression, compulsive behavior, psychotic episodes, apathy, and sexual disorders (Roos, 2010; Tabrizi et al., 2013; Martinez-Horta et al., 2016). A lack of interest in life activities and depression are the most common mood symptoms of HD, appear early and continue during HD progression, thereby becoming one of the most disabling symptoms. This group of psychiatric symptoms has been linked to dysfunctional activity of PFC (Epping and Paulsen, 2011; Grace, 2016), which receives prominent dopaminergic input via the mesocorticolimbic pathway and plays a key role in reward. Impaired D1 receptor-mediated DA transmission was suggested to be involved in depression-like behaviors in HD (Renoir et al., 2012), and loss of function of D2-SPNs in the ventrolateral striatum causes motivational deficits without affecting spontaneous behavior or reward preference (Tsutsui-Kimura et al., 2017). Presymptomatic zQ175 mice further exhibit suppressed motivation to work for reward and compromised dopaminergic encoding of reward delivery in the nucleus accumbens (Covey et al., 2016).

Modulation by Endocannabinoids

Endocannabinoids (eCBs), such as arachidonoylethanolamide (AEA) and 2-arachidonylglycerol (2-AG), are small lipophilic neuromodulators that are released from the post-synapse to diffuse locally and act retrogradely on presynaptic CB1 receptors where they inhibit neurotransmitter release. In the context of HD, decreased CB1 expression can be detected early in disease in both humans and animal models (**Figure 3**) (Denovan-Wright and Robertson, 2000; Glass et al., 2000; Dowie et al., 2009; Van Laere et al., 2010; Horne et al., 2013). Furthermore, changes in the levels of endogenous endocannabinoids have been reported (Bisogno et al., 2008), and CB1 knockout worsens motor performance in HD mice (Blázquez et al., 2011; Mievis et al., 2011). mHTT-dependent loss of CB1 furthermore disinhibits GABA neurotransmission in SPNs, and is associated with progressive decline of motor and cognitive function in HD models (Blázquez et al., 2011; Chiarlone et al., 2014).

eCB signaling plays important roles in synaptic plasticity at corticostriatal synapses. It modulates DA signaling to control flexible goal-oriented and reward-driven behavior (Hilário et al., 2007; Cui et al., 2015; Gremel et al., 2016; Augustin and Lovinger, 2018), two processes that are compromised in HD (Lawrence et al., 1996; Curtin et al., 2015). In the striatum, eCBs drive long-term depression (LTD) at both excitatory and inhibitory synapses (Huang et al., 2001). Striatal DA signaling, in contrast, is not modulated directly by eCBs, but is disinhibited indirectly via decreased GABAergic release at CB1-expressing inhibitory afferents. A recent study showed that accumbal eCB signaling inhibits CIN-driven DA release, whereas CIN activation recruits production of 2-AG, thereby providing negative feedback. Critically, 2-AG mobilization modifies DA-dependent reward-driven behavior (Mateo et al., 2017), and it has been shown that motivational deficits in HD mouse models can be normalized by pharmacologic elevation of 2-AG signaling at CB1 receptors (Covey et al., 2018). Additionally, attenuated LTD at corticostriatal synapses could be restored by inhibiting the degradation of 2-AG (Sepers et al., 2018), and viral delivery of CB1 ameliorated some of the cellular dysfunction observed in R6/2 mice (Chiarlone et al., 2014; Naydenov et al., 2014).

Activation of CB1 receptors seems to be neuroprotective, possibly via inducing expression of BDNF (Blázquez et al., 2015). It is furthermore suggested to play a role in the differential vulnerability of iSPNs vs. dSPNs by protecting dSPNs in particular (Ruiz-Calvo et al., 2018), although the mechanism of this selectivity is not yet clear. Therefore, stabilization of dopaminergic and endocannabinoid neuromodulatory systems are attractive targets for novel drugs treating HD.

ROLE OF ASTROCYTES IN HD-RELATED CIRCUIT DYSFUNCTION

In addition to the changes in various neuronal cell types described above, astroglia has recently emerged as an important contributor to neuronal dysfunction in HD (Khakh et al., 2017). Indeed, mice selectively expressing mHTT in astrocytes exhibit age-dependent motor deficits and shortened life span (Bradford et al., 2009; Meunier et al., 2016). Astrocytic alterations have been so far mostly studied in the striatum, where astrocytes seem to play a crucial role in HD-related excitotoxicity via at least two interrelated mechanisms.

First, astrocytes are equipped with glutamate transporters and are responsible for the rapid clearance of extracellular glutamate released at synapses (Ransom and Ransom, 2012). A well-established HD phenotype in humans and mice is the loss of the astrocytic glutamate transporter GLT1 (also called EAAT2) (Arzberger et al., 1997; Liévens et al., 2001; Behrens, 2002; Shin et al., 2005; Faideau et al., 2010; Cao et al., 2019), which is probably due to transcriptional inhibition in the presence of

mHTT (Bradford et al., 2009). Reduced expression of GLT-1 results in impaired glutamate uptake and increased extracellular glutamate levels (**Figure 3**) (Liévens et al., 2001; Behrens, 2002; Shin et al., 2005; Faideau et al., 2010; Estrada-Sánchez and Rebec, 2012). Accordingly, increasing GLT-1 expression rescues glutamate uptake and improves behavioral phenotypes in HD mice (Miller et al., 2008a). It should be noted that this well-established view was recently challenged by an *in vivo* imaging study of glutamate dynamics, which showed normal glutamate uptake in the striatum of HD mice (Parsons et al., 2016), a controversy that remains to be resolved.

Second, astrocytes play an important role in extracellular K^+ buffering (Ransom and Ransom, 2012). This function is also disturbed in HD, as expression of mHTT leads to a downregulation of the Kir4.1 inwardly rectifying potassium channel on the cell membrane of astrocytes, causing a shift in the distribution of K^+ ions across the membrane (**Figure 3**). The resulting increase in K^+ concentration in the extracellular space is likely to be at least one of the underlying causes of depolarized membrane potential and elevated excitability of SPNs (Tong et al., 2014). In addition, it also depolarizes the membrane potential of astrocytes and reduces the electrogenic uptake of glutamate through the GLT-1 transporter, further impairing extracellular glutamate clearance (Dvorzhak et al., 2016). A recent imaging study with genetically encoded calcium and glutamate sensors in brain slices of HD mice not only confirmed the prolonged presence of extracellular glutamate after cortical stimulation, but also demonstrated profound alterations of Ca^{2+} signaling in astrocytes (Jiang et al., 2016). Interestingly, those defects could be partially rescued by restoring Kir4.1 expression in astrocytes, probably via improved GLT-1 expression and/or function (Dvorzhak et al., 2016; Jiang et al., 2016).

INSIGHTS FROM SYSTEMS BIOLOGY STUDIES

While electrophysiology and imaging have been useful to describe the nature and time course of neural circuit dysfunction in HD models, the molecular links between the HTT mutation and neuronal miscommunication for a long time remained elusive. Recently, powerful systems biology approaches have provided important insights into these matters through large-scale and unbiased screens at the transcriptomic and proteomic levels. Next generation RNA-sequencing studies performed in HD mouse models and human HD patient induced pluripotent stem cell (iPSC)-derived or directly converted neural cultures have all highlighted dysregulation of synaptic genes. In particular, there was a downregulation of transcripts involved in the post-synaptic scaffold, neurotransmitter signaling, Ca^{2+} signaling, long-term synaptic plasticity, as well as reduced transcription of neuronal activity-regulated genes (Langfelder et al., 2016; HD iPSC Consortium, 2017; Veldman and Yang, 2018; Victor et al., 2018). Importantly, these changes also hold true at the proteomic level (Langfelder et al., 2016; Hosp et al., 2017; Skotte et al., 2018). Quantitative mass spectrometry analysis in R6/2 mice demonstrated a progressive decline of both excitatory and inhibitory synaptic proteins (Burgold et al., 2019), in line with morphological and functional defects described for both types of synapses. Interestingly, synapse-related proteins are also abundantly present in the mHTT interactome (Shirasaki et al., 2012) and within insoluble mHTT inclusion bodies (Hosp et al., 2017), suggesting that at least some of the synaptic defects are directly caused by the mutant protein, rather than being a secondary consequence of deteriorating neuronal health.

Systems biology studies furthermore deepened our understanding of astrocytic dysfunction in HD mice, not only by validating the reduction in glutamate transporters and K^+ channels, but also by unveiling mHTT-induced metabolic disturbances in astrocytes (Langfelder et al., 2016; Skotte et al., 2018; Diaz-Castro et al., 2019). Among the altered astrocytic proteins were those involved in the glutamate-glutamine-GABA neurotransmitter metabolic cycle. Isotope labeling experiments in brain slices confirmed impaired astrocytic synthesis and/or release of glutamine, which serves as a precursor for GABA production in neurons and could play a role in the weakened striatal inhibition (Skotte et al., 2018).

OPEN QUESTIONS AND FUTURE DIRECTIONS

The impairments that occur in the principal neuron types in the cortex (CPNs) and striatum (SPNs) have been thoroughly characterized in the last two decades. Recent investigations have also highlighted the contribution of astrocytes to circuit dysfunction in HD. Despite these significant advances in the knowledge about circuit mechanisms of HD, major questions remain that will be particularly critical to address. The microcircuit mechanisms involving cortical and striatal interneurons are still largely unexplored. Moreover, it is not clear how low-level changes in synaptic connectivity and neuronal activity relate to higher-level changes such as network synchronization and LFP oscillations across brain areas. It is further not known whether and how such changes give rise to behavioral symptoms. Progress in this area will be facilitated by new techniques like multi-channel electrophysiology and deep brain imaging that permit simultaneous recording of large neuronal ensembles over prolonged periods of time. Manipulation of neuronal activity *in vivo* using optogenetic or chemogenetic approaches will be crucial to decipher the causal role of distinct circuit elements in pathomechanisms of HD.

Moreover, viewing the described cell types as homogenous populations is clearly an oversimplification. Single-cell RNA-sequencing (scRNA-seq) studies are revealing an ever-greater diversity of cortical neurons (Zeisel et al., 2015; Tasic et al., 2016, 2018) and challenging the classical subdivision of MSNs into two discrete D1 and D2 classes (Gokce et al., 2016). How this complex picture of cellular diversity is altered in diseases including HD is completely unknown. Extension of the scRNA-seq approach to HD models and human HD tissue will therefore be helpful to shed more light on the involvement of different neuronal and non-neuronal cell types and to tackle the enigmatic issue of differential vulnerability to mHTT.

In vitro co-culture systems have been useful in delineating the contribution of various afferents to SPN dysfunction

(Kolodziejczyk and Raymond, 2016; Virlogeux et al., 2018). The availability of HD neural cultures either derived from iPSCs or directly converted from patients' fibroblasts (HD iPSC Consortium, 2017; Victor et al., 2018) now opens the possibility of extending such *in vitro* approaches to human neurons, thus providing a convenient and physiologically relevant platform for drug testing. In addition, brain organoids (Lancaster et al., 2013) and further modifications of this technique that promote establishment of functional neuronal connectivity (Giandomenico et al., 2019) offer another promising way to bring *in vitro* modeling of disease-related circuit impairments closer to the *in vivo* setting.

At the molecular level, unbiased systems approaches including transcriptomics and proteomics have delivered important insights into the molecular underpinnings of neuronal dysfunction in HD. However, extensive further work will be required to link these molecular alterations to discrete functional deficits.

In conclusion, the development of novel methodologies allowing to monitor and manipulate neuronal activity in large populations of neurons during natural behavior, and to detect gene expression changes at a single-cell level, will enable a more complete understanding of circuit mechanisms of HD, and might open the doors for designing more refined therapies for this devastating disorder.

AUTHOR CONTRIBUTIONS

Both authors wrote and edited the manuscript.

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Cerebellar Development and Circuit Maturation: A Common Framework for Spinocerebellar Ataxias

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Spinocerebellar ataxias (SCAs) affect the cerebellum and its afferent and efferent systems that degenerate during disease progression. In the cerebellum, Purkinje cells (PCs) are the most vulnerable and their prominent loss in the late phase of the pathology is the main characteristic of these neurodegenerative diseases. Despite the constant advancement in the discovery of affected molecules and cellular pathways, a comprehensive description of the events leading to the development of motor impairment and degeneration is still lacking. However, in the last years the possible causal role for altered cerebellar development and neuronal circuit wiring in SCAs has been emerging. Not only wiring and synaptic transmission deficits are a common trait of SCAs, but also preventing the expression of the mutant protein during cerebellar development seems to exert a protective role. By discussing this tight relationship between cerebellar development and SCAs, in this review, we aim to highlight the importance of cerebellar circuitry for the investigation of SCAs.

Keywords: cerebellum, spinocerebellar ataxia, Purkinje cell degeneration, cerebellar circuits, circuit maturation

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INTRODUCTION

Spinocerebellar ataxias (SCAs) are a large family of movement disorders characterized by the progressive loss of motor coordination, muscle tone and control, with a broad spectrum of severity ranging from mild gait and posture problems to death. Moreover, several comorbidities including pyramidal features, peripheral neuropathy, tremor, dystonia, Parkinsonian features, myoclonus, epilepsy, and dementia occur frequently in SCAs. The 47 as of now identified SCAs are dominantly inherited diseases, and a growing number of genes are implicated, revealing two major pathological alterations; (1) Microsatellite repeat expansions coding for CAG generating polyglutamine (PolyQ) repeats: SCA1, SCA2, SCA3, SCA6, SCA7, SCA17 and Dentatorubral-pallidoluysian atrophy DRPLA, and non-coding repeats: SCA8, SCA10, SCA12, SCA31, SCA36, SCA37). (2) Single-gene point mutations as observed in SCA5, SCA11, SCA13-16, SCA18, SCA19/22, SCA20-23, SCA26, SCA27-29, SCA34-35, SCA38, SCA40-46 (Ashizawa et al., 2018; Pilotto and Saxena, 2018).

Genes causing SCAs code for a large variety of proteins including ion channels (Riess et al., 1997; Zhuchenko et al., 1997; Waters et al., 2006; Lee et al., 2012; Coutelier et al., 2015; Fogel et al., 2015; Morino et al., 2015), transcription factors and repressors (Orr et al., 1993; Koide et al., 1999; Lin et al., 2018), scaffolding proteins (Ikeda et al., 2006; Tsoi et al., 2014), or signaling kinases (Chen et al., 2003; Houlden et al., 2007), phosphatases (Holmes et al., 1999), and receptors (Storey et al., 2001; Hara et al., 2004; Hara et al., 2008; Huang et al., 2012; Watson et al., 2017). Despite this heterogeneity, SCA patients share a slow dramatic degeneration of the cerebellum and its afferent and efferent systems, suggesting a common point of interception, wherein SCA causing mutations converge and promotes the onset of these neurodegenerative diseases. Interestingly, wiring defects of the cerebellar circuitry are a common finding in SCAs and they appear at the early asymptomatic phase (Ebner et al., 2013; Hansen et al., 2013; Dulneva et al., 2015; Jayabal et al., 2017). In preclinical rodent models of SCA1, inhibiting the expression of the mutant ATXN1 protein during cerebellar postnatal development exerted a protective effect on the ataxic phenotype (Serra et al., 2006) and ameliorated synaptic transmission in the cerebellar cortex (Zu et al., 2004; Barnes et al., 2011). Therefore, cerebellar development seems to be highly vulnerable to the expression of mutant SCA-causing molecules, and the resulting compromised cerebellar compartmentation, patterning and circuit wiring could have a direct impact on the severity of the disease. In this review, we focus on the possible role of SCA genes in cerebellar development in an attempt to define a new framework for SCAs.

THE CEREBELLUM

The cerebellum is traditionally known for its role in motor control and more recently its involvement in higher cognitive functions has been recognized (Koziol et al., 2014; Adamaszek et al., 2017).

The cerebellum receives two major excitatory inputs: mossy fibers (MFs) and climbing fibers (CFs). MFs originate from several pre-cerebellar nuclei in the brainstem and spinal cord, and they carry vestibular, motor, sensory and proprioceptive information relayed by the granule cells (GCs). GC axons ascend into the molecular layer (ML), where they bifurcate into parallel fibers (PFs) that run longitudinally to the cerebellar lobule, contacting several Purkinje cells (PCs) on their way. Each PC receives up to 10^5–10^6 excitatory PF synaptic contacts, however, one PF makes only one to two synapses onto a single PC (Napper and Harvey, 1988). PF activity induces simple spike discharge in PCs; moreover, PCs spontaneously fire action potentials, and this pacemaker activity has been recorded in cerebellar slices during the pharmacological blockade of excitatory inputs (Hausser and Clark, 1997) and in cultured PCs (Nam and Hockberger, 1997). Notably, CFs only originate from the inferior olive nuclei (IO) in the brainstem and establish monoinnervation on mature PCs. CF activation signature in PCs is a high frequency burst of spikes known as complex spike (Eccles et al., 1966). CFs provide teaching signals to the cerebellum that drive cerebellar-mediated motor learning. The simultaneous activation of CF and PFs leads to long-term depression at the PF to PC synapses (Ito et al., 1982), while the sole activation of GCs promotes long-term potentiation at these synapses (Lev-Ram et al., 2002; Coesmans et al., 2004). Long-term plasticity at these excitatory synapses provides a major molecular platform for supporting cerebellar-mediated motor learning (De Zeeuw et al., 1998; Boyden et al., 2006; Hansel et al., 2006; Schonewille et al., 2010; Ly et al., 2013). Traditionally, CFs are known to carry sensory/motor errors but more recently, they have also been shown to mediate reward and predictive signals (Ohmae and Medina, 2015; Heffley et al., 2018; Kostadinov et al., 2019; Larry et al., 2019).

Anatomically, three parts are recognized in the cerebellum: the cerebellar cortex, the white matter and the deep cerebellar nuclei (DCN). The cerebellar cortex is the most superficial structure and organized into three layers (from the most superficial): the ML, the Purkinje cell layer (PCL), and the granule cell layer (GCL) (**Figure 1**).

The ML accommodates the complex and ramified dendritic tree of PCs together with two types of GABAergic interneurons (molecular layer interneurons, MLIs): Basket cells (BCs) and Stellate cells (SCs). BCs synapse onto PC soma and its axon initial segment in a characteristic structure known as *pinceau* (Ango et al., 2004; Blot and Barbour, 2014), while SCs innervate PC dendrites. MLIs receive excitatory inputs from PFs and they provide feed-forward inhibition, which modulates PC spike output (Mittmann et al., 2005; Brown et al., 2019), calcium influx and long-term plasticity (Binda et al., 2016). Moreover, CF activation also recruits MLIs via a spillover mechanism (Szapiro and Barbour, 2007). PC somas define the PCL: PCs are the sole output of the cerebellar cortex and they send GABAergic projections to the DCN and vestibular nuclei. The PCL also hosts the small vertically oriented pear-shaped soma of Candelabrum cells (CaCs). CaCs have one or two long dendrites that enter the ML, and several short dendrites localized below the GCL. The axon of these neurons runs horizontally and is characterized by multiple vertical branches ascending to the ML (Laine and Axelrad, 1994). In the *Macaca* monkey, the immunohistochemical analysis of the cerebellum provided evidence for the release of glycine and GABA neurotransmitters by these neurons (Crook et al., 2006), however, CaCs targets and inputs still remain elusive.

Together with GCs, the GCL also contains glutamatergic interneurons, the Unipolar Brush cells (UBCs) that establish excitatory inputs onto GCs and on other UBCs (Nunzi et al., 2001). UBCs mainly receive excitatory inputs from a single MF carrying vestibular information and they are particularly enriched in the posterior cerebellum and flocculus (Rossi et al., 1995). UBCs also receive inhibitory synaptic inputs from Golgi Cells (GoCs) (Dugue et al., 2005). Glycinergic/GABAergic interneurons also populate the GCL and these include GoCs and Lugaro Cells (LCs). GoCs provide the sole inhibitory input to GCs, via feed-forward and a feedback inhibitory loops (Kanichay and Silver, 2008). LCs have spindle-shaped cell bodies (Laine and Axelrad, 1996) and they innervate the MLIs (Laine and Axelrad, 1998), GoCs (Dieudonne and Dumoulin, 2000; Dumoulin et al., 2001), and PCs (Dean et al., 2003). A prominent feature of these

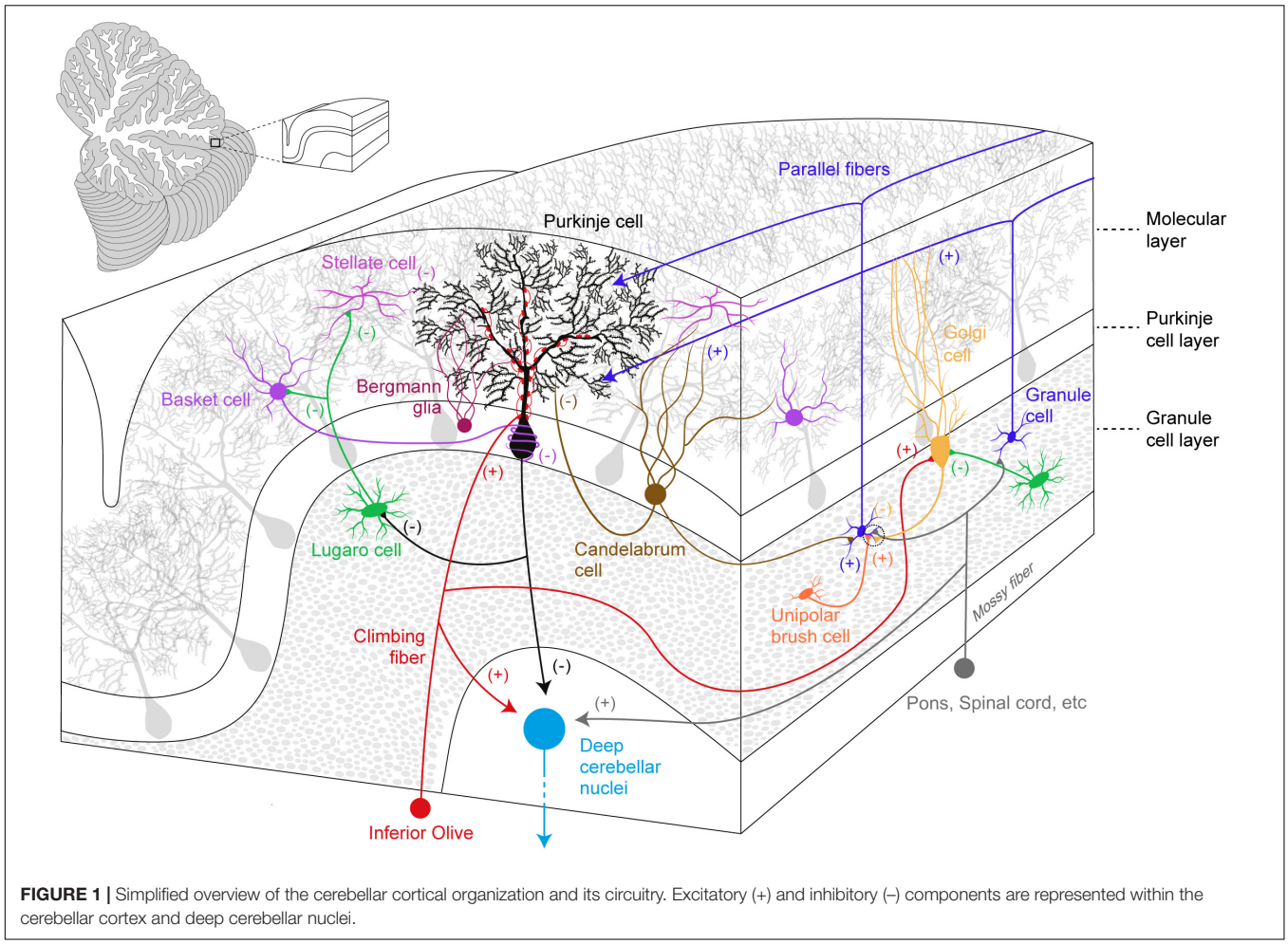

FIGURE 1 | Simplified overview of the cerebellar cortical organization and its circuitry. Excitatory (+) and inhibitory (–) components are represented within the cerebellar cortex and deep cerebellar nuclei.

interneurons is their activation by serotonin, which induces firing in an otherwise silent cell (Dieudonne and Dumoulin, 2000; Dumoulin et al., 2001).

PURKINJE CELLS DEVELOPMENT, CONNECTIVITY AND FUNCTIONAL DEFICITS IN SCAs

PCs originate in the ventricular zone around embryonic day E10.5-E13.5 in the mouse; by E14, they form a multicellular layer called the PC plate, a temporary structure which is replaced by a monolayer organization at early postnatal stage. PCs are characterized by an extensive dendritic tree, which develops during the first three postnatal weeks in the mouse (Sotelo and Dusart, 2009). The expression or the absence of specific molecular markers in PCs (Ex. ZebrinII (ZII)/Aldolase C) defines the compartmentation of the cerebellar cortex, organized in an alternate series of positive/negative parasagittal PC stripes (Brochu et al., 1990). The timing of PC birth determines their molecular fate, with early born (E10-E11.5) PCs becoming ZII+ cells and late-born (E11.5-E13.5) PCs destined to have a ZII− phenotype (Miale and Sidman, 1961). The spatial arrangement of CFs and MFs terminals follow PC patterning in the cerebellar cortex. Notably, immunolabelling of IO subnuclei in the rat revealed the preferential targeting of strongly ZII+ parasagittal bands by CFs originating from the subnucleus a of the caudal medial accessory olive (c-MAO), the rostral part of the MAO (r-MAO) and the dorsal and ventral lamellas of the principal olive (d-PO and v-PO, respectively). In contrast, ZII− bands of PCs are targeted by the subnucleus b of the c-MAO (Sugihara and Shinoda, 2004). Also, spinocerebellar and cuneocerebellar MFs terminate in the anterior cerebellum in complementary parasagittal bands with a defined distribution relative to the ZII± bands in this region (Ji and Hawkes, 1994; Valera et al., 2016). PC patterning plays a role in the definition of the final topographic map of cerebellar excitatory afferents and its modifications are mirrored by the altered spatial arrangement of CF and MF terminals (Blatt and Eisenman, 1988; Sillitoe et al., 2010; Reeber et al., 2013).

Activity-dependent mechanisms influence cerebellar compartmentation, the fine delineation of afferents topographic map and circuitry wiring. Of note, the targeted silencing of PC-specific neurotransmission affected the organization of ZII stripes, and altered spinocerebellar MF patterning (White et al., 2014). Furthermore, monoinnervation of PCs via CF relies on

activity-dependent calcium influx as revealed by the impairment in the biased strengthening of a single CF in $Ca_v2.1$ (P/Q type) PC-targeted knockout (KO) mice (Hashimoto et al., 2011; Kawamura et al., 2013).

Purkinje cells are also involved in the final maturation of the cerebellum: during the early postnatal period, PC-mediated Sonic Hedgehog (SHH) signaling drives the proliferation of GC progenitors within the External Granule cell Layer (EGL) (Wechsler-Reya and Scott, 1999) and the level of SHH signaling is an important determinant for cerebellar foliation (Corrales et al., 2006).

Several SCA-linked mutations directly or indirectly affect PC activity, calcium dynamics and dendrite development. They are therefore potentially harmful to cerebellar compartmentation, wiring and afferent organization.

PC firing defects have been described in several SCA models. The expression of the mutant ATXN1 in SCA1 PCs or ATXN2 in SCA2 PCs reduced their firing frequency (Hansen et al., 2011; Dell'Orco et al., 2015) and this decrease was also associated with an increase in PC firing variability in the SCA2 mouse model (Kasumu et al., 2012). Furthermore, *in vivo* analysis of complex spike activity in SCA2 mice revealed a reduction in the complex spike frequency in response to pharmacological IO stimulation by systemic harmaline injection (Egorova et al., 2018).

Deletion or mutation in SPTBN2 gene (β-III spectrin) (Ikeda et al., 2006) causes SCA5, and β-III knockout in mice resulted in transient and resurgent sodium current reduction in PCs, leading to a decrease in PC spontaneous firing. Furthermore, PC single spike frequency was altered while no difference in complex spikes was detected (Perkins et al., 2010). Moreover, PCs presented reduced dendritic surface area, impaired mono-planar dendritic arborization and reduced spine density (Gao et al., 2011). An impaired dendritic development and spine density reduction is also a feature of SCA14 as demonstrated in cultured PCs expressing PKCγ harboring the SCA14-causing S119P mutation (Chen et al., 2003; Seki et al., 2009).

SCA6 is caused by a PolyQ tract expansion in the alternatively spliced exon 47 of the *CACNA1A* gene encoding for the α1A subunit of $Ca_v2.1$ voltage gated calcium channels (Zhuchenko et al., 1997). The PolyQ expansion alters $Ca_v2.1$ physiology via decreased channel expression in PCs, concomitantly reducing P/Q type calcium channel-mediated currents in SCA6 knock-in mice model. These modifications, however, were independent from the length of the PolyQ expansion (Watase et al., 2008). The presence of an internal ribosome entry site (IRES) within the $Ca_v2.1$ mRNA permits the expression of a C-terminus fragment (CT) containing the PolyQ tract. While the CT carrying the normal PolyQ tract serves as a transcription factor in PCs and promotes neurite outgrowth, the expression of the CT with the extended pathogenic PolyQ causes gait abnormalities and significant ML thinning (Du et al., 2013). Furthermore, *in vivo* recordings support a higher incidence of irregular PC firing in mice specifically expressing the pathogenic CT in PCs (Mark et al., 2015).

Three KCNC3 single mutations (R420H, R423H, and F448L) have been identified in families affected by SCA13 (Waters et al., 2006; Figueroa et al., 2011). KCNC3 encodes for $K_v3.3$ potassium channel, expressed in PCs where it participates in CF-mediated complex spike formation (Zagha et al., 2008). Additionally, based on computer modeling, $K_v3.3$-mediated conductance is proposed to interact with resurgent sodium currents in order to drive PC spontaneous firing (Akemann and Knopfel, 2006). R420H substitution in the S4 helix of $K_v3.3$ leads to the loss of the channel-mediated current via a dominant negative effect (Waters et al., 2006), while R423H and F448L mutations affect the gating properties of the channel. In the $K_v3.3$ KO SCA13 mouse model, PCs exhibited reduced spontaneous firing rate (Akemann and Knopfel, 2006) and lentiviral-mediated expression of $K_v3.3$ R424H (murine homolog of human $K_v3.3$ R423H) in cultured PCs led to impaired dendritic development (Irie et al., 2014). The missense mutation F145S in the Fibroblast Growth Factor 14 (FGF14) causes SCA27 in humans through a dominant negative effect (van Swieten et al., 2003; Brusse et al., 2006), and FGF14 ablation induces ataxia in mice (Wang et al., 2002). FGF14 modulates resurgent sodium currents in PCs (Yan et al., 2014) and FGF14 KO mice present a higher number of PCs lacking spontaneous firing when compared to WT (Shakkottai et al., 2009).

ALTERATIONS IN CIRCUIT WIRING IN THE CEREBELLAR CORTEX OF SCAs

The cerebellum undergoes a profound change in terms of maturation and synaptic refinement during the first postnatal weeks, a process mediated by the activity of several molecules. During this period, CFs and PFs establish their final innervation territory onto PCs. Electrophysiological studies in mice revealed that at postnatal day 3 (P3), PC soma display multiple/polyinnervation by CFs. At this stage of development, these multiple CF synapses display similar synaptic strength, but thereafter they undergo functional differentiation. This diversification plateaus at P7 with PCs receiving both a strong CF input and several weaker ones. The weaker CFs are characterized by a lower multivesicular release probability. P/Q type voltage-gated calcium channel activity is required for CF diversification; $Ca_v2.1$ deletion in PCs prevents the selective biased strengthening of a single CF, and multiple CF innervation onto PCs persists throughout the cerebellar development (Hashimoto et al., 2011; Kawamura et al., 2013). All CFs at this stage synapse onto PC soma and only by P9-10, the strongest CF, starts to translocate from the soma to PC dendrites (Hashimoto et al., 2009). Following the functional differentiation and as cerebellar development proceeds, the strong/winner CF is further strengthened via an anterograde signaling involving the C1ql1–Bai3 (brain-specific angiogenesis Inhibitor 3) pathway (Kakegawa et al., 2015). Moreover, retrograde signaling mediated by the secreted semaphorin 3A (Sema3A)–Plexin A4 and Progranulin–Sortilin 1 pathways participate in the strengthening and/or the maintenance of CFs inputs (Uesaka et al., 2014; Uesaka et al., 2018). By the end of the second postnatal week, the majority of PCs (60%) show monoinnervation by a strong CF while the remaining PCs display polyinnervation by a strong CF and one or more weaker CFs (Hashimoto and Kano, 2003). At

this time point, while the winner CF proceeds further onto the PC dendritic tree, somatic synapses of weak CFs are progressively eliminated (Hashimoto et al., 2009).

The establishment of CF monoinnervation on PCs requires extensive pruning of synapses. An early study in the x-irradiated agranular cerebellum in the rat supports two consecutive phases of elimination: an early GC independent phase (from P7 to around P11 in the mouse) and a late phase (from P12 to around P17 in the mouse) that relies on the correct establishment of PF synapses onto PCs (Crepel et al., 1981). Indeed, impairing PF to PC synapse formation by deleting the glutamate receptor δ2 (GluRδ2) caused a marked reduction in PF synaptic contacts on PC dendrites by the second postnatal week (Kurihara et al., 1997). Additionally, persistent CF-mediated PC polyinnervation (Hashimoto et al., 2001) and its invasion into PF territory i.e., the distal portion of PC dendritic tree was observed (Hashimoto et al., 2001; Ichikawa et al., 2002). Several molecules and signaling pathways participate in CF synapse elimination process. These involve Serotonin 3A receptors (5-HT_{3A}) (Oostland et al., 2013), C1Ql1-Bai3 signaling (Kakegawa et al., 2015), IGF-I-mediated signaling (Kakizawa et al., 2003), mGluR1 (Kano et al., 1997; Levenes et al., 1997), PKCγ (Kano et al., 1995), phospholipase Cβ4 (Hashimoto et al., 2000), semaphorin 7A (Sema7A)-Plexin C1 signaling (Uesaka et al., 2014), and Sema7A-Integrin B1 (Uesaka et al., 2014). Ligand-receptor signaling such as BDNF-TrkB signaling (Bosman et al., 2006; Johnson et al., 2007; Sherrard et al., 2009; Choo et al., 2017), NMDA receptors (Rabacchi et al., 1992; Kakizawa et al., 2000), GluRδ2 receptors (Kurihara et al., 1997), and P/Q type voltage gated calcium channels (Miyazaki et al., 2004; Hashimoto et al., 2011) are also implicated. GABAergic transmission (Nakayama et al., 2012), the immediate early gene Arc (Mikuni et al., 2013), Ca/Calmodulin Kinase IV (Ribar et al., 2000), αCa/Calmodulin Kinase II (Hansel et al., 2006) plays an important role in this process. Lastly, glutamate transporter GLAST (Watase et al., 1998; Miyazaki et al., 2017), the brain-specific receptor-like proteins BSRPs (Miyazaki et al., 2006) and myosin Va (Takagishi et al., 2007) are also involved (see **Figure 2** for complete list of molecules involved in this process). Molecular pathways and players determining CF strengthening and synaptic refinement are susceptible to SCA-causing molecules/mutations and several studies have identified problems in CF to PC synaptic transmission (Smeets and Verbeek, 2016). Physiological, morphological and developmental abnormalities of these synaptic inputs can precede PC degeneration (Duvick et al., 2010; Barnes et al., 2011; Ebner et al., 2013; Ruegsegger et al., 2016).

The pharmacological manipulation of the Insulin-like growth factor I (IGF-I) pathway *in vivo* revealed the involvement of this hormone in the early phase of CF synapse elimination and strengthening. Application of exogenous IGF-I to the cerebellum at P8 caused an increase in the percentage of PCs displaying CF-mediated polyinnervation in young adult mice; and no significant effect when the IGF-I signaling was augmented during the late phase of synaptic pruning (P12). Moreover, together with the strengthening of the winner CF, the enhancement of weaker CF-mediated excitatory responses was also observed. In agreement with these results, inhibiting IGF-I signaling by local application of antisera against IGF-I and its receptor decreased EPSCs elicited by activation of the winner CF (Kakizawa et al., 2003). The analysis of transcriptional changes in murine models of SCA1 and SCA7 revealed the conserved downregulation of mRNA coding for the insulin-like growth factor binding protein 5 (IGFBP5) (Beattie et al., 2006), leading to enhanced IGF-I receptor activation probably via increased IGF-I availability (Gatchel et al., 2008). The modulation of the IGF-I signaling pathway by the mutant-ATXN1 therefore could interfere with CF strengthening and synapse elimination. Indeed, CF polyinnervation of young adult PCs and the impaired pruning of somatic CF synapse have been identified in SCA1 (Ebner et al., 2013). Nevertheless, a detailed analysis of the process of CF synapse elimination during cerebellar development is required to underpin SCA1- associated defects in CF wiring and function.

The retrograde BDNF to TrkB signaling in the developing cerebellum contributes to the late phase of CF synapse elimination. The PC-targeted knockout of BDNF resulted in a significant increase in the percentage of PCs showing polyinnervation by CFs at P16-P19, while no difference in innervation was detected prior to this time point. Similar results were obtained when the expression of TrkB was specifically downregulated in CFs (Choo et al., 2017). Accordingly, qRT-PCR analysis of post-mortem cerebellar samples from SCA6 patients revealed a significant decrease in the expression of BDNF transcripts (Takahashi et al., 2012), and an impairment in synaptic elimination of weaker CFs in the $Ca_V2.1$[84Q] SCA6 mouse model (Jayabal et al., 2017).

The mGluR1 cascade participates in postnatal maturation of CFs and mice lacking mGlur1 (Kano et al., 1997) or its downstream effectors PLCβ4 (Hashimoto et al., 2000) and PKCγ (Kano et al., 1995) displayed impairments in the late phase of synapse elimination. Importantly, the PC-specific expression of a splice variant of mGluR1 in mGluR1 knockout mice was effective in rescuing the late phase of CF synapse elimination (Ichise et al., 2000). Several lines of evidence indicate that the activation of mGluR1 receptors at PF to PC synapse is a major player at this stage of CF development (Hashimoto and Kano, 2013). mGluR1 signaling defects at PF to PC synapses have been identified in rodent models of SCA1 (Shuvaev et al., 2017), SCA3 (Konno et al., 2014), and SCA5 (Armbrust et al., 2014). The reduced amplitude of the mGluR1-dependent slow EPSCs component (Hartmann et al., 2008), the reduced dendritic calcium transients mediated by the mGluR1-PLCβ-IP3 receptor pathway activation and the impairment of short (Brown et al., 2004) and long-term plasticity (Ichise et al., 2000) have been demonstrated in these models. Furthermore, mGluR1 targeting to the PC dendritic spines was affected and a more diffuse expression of this metabotropic receptor was observed in SCA3 models (Konno et al., 2014) and SCA5 (Armbrust et al., 2014). On the contrary, the increased basal calcium concentration in PCs is likely to be responsible for the enhancement of mGluR1 signaling via a positive feedback loop in SCA2 (Meera et al., 2017).

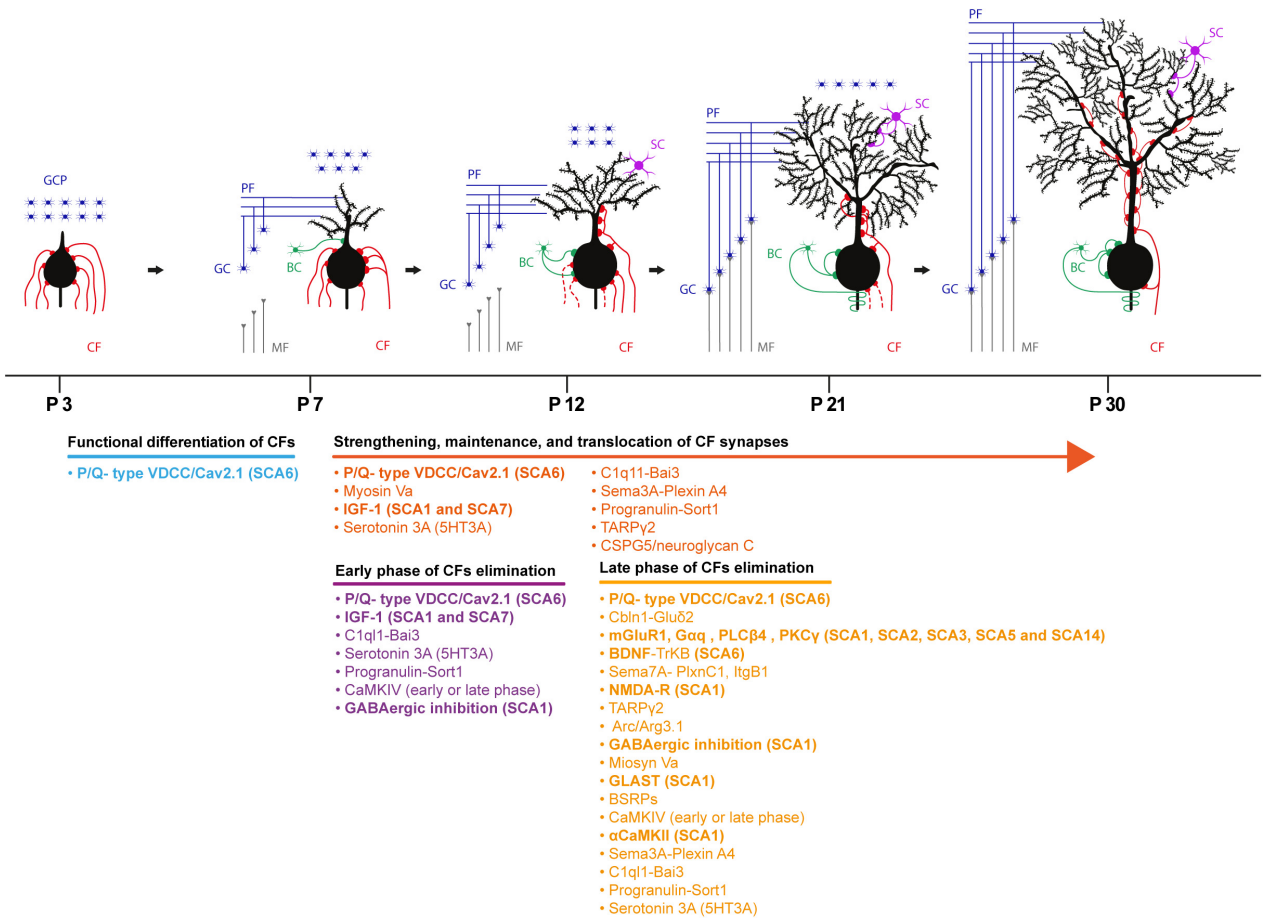

FIGURE 2 | Schematic depicting the developmental synaptogenesis and refinement of CF to PC synapses. Four representative phases of the cerebellar circuit maturation are described with the molecular underpinnings involved in the process. Bold molecules indicate molecules implicated and identified in different SCAs in both human and rodent models. GCP, granule cell progenitor; CF, climbing fiber; MF, mossy fiber; GC, granule cell; PF, parallel fiber; BC, basket cell; SC, stellate cell.

mGluR1 signaling is a potential target for pharmacological interventions. Acute treatment of SCA1 mice via a single injection of the mGluR1 positive allosteric modulator Ro0711401 resulted in a significant long-lasting improvement of motor performance (Notartomaso et al., 2013). Exploiting mGluR1-$GABA_B$ functional coupling to enhance mGluR1 signaling (Tabata and Kano, 2006) was also proven effective in rescuing motor impairment in SCA1 mice (Shuvaev et al., 2017). Moreover, in the doxycycline-regulated conditional transgenic mouse model of SCA1, the improved motor performance of symptomatic mice due to the inhibition of mutant-ATXN1 expression was associated with restored mGluR1 expression levels in dendritic spines (Zu et al., 2004).

Missense mutations in PKCγ, the downstream mGluR1 effector, causes SCA14 (Chen et al., 2003). The expression of the SCA14-associated PKCγ-S119P mutant via *in vivo* lentiviral-mediated transduction of cerebellar neurons impaired CF synapse elimination, resulting in an increased number of PCs exhibiting polyinnervation. Importantly, this deficit in CF maturation appeared only by the expression of the PKCγ mutant during the time window of cerebellar development (Shuvaev et al., 2011). Among the proteins that contribute to the late phase of elimination, the GLAST glutamate transporter, NMDA receptors and αCamKII have been shown to be affected in SCA1. The decreased expression of the GLAST glutamate transporter in Bergmann glia (Cvetanovic, 2015), and the aberrant activation of extra synaptic NMDA receptors (Iizuka et al., 2015) in the cerebellum are involved in disease progression. Moreover, the decreased activation of αCamKII has been observed in SCA1 mouse model (Ruegsegger et al., 2016).

PF to PC synapse formation and transmission are also affected in SCAs. Abnormal PF invasion of CF innervation territory onto PCs is associated with the PC-specific expression of the pathogenic ATXN1-[30Q]-D766 mutant (Ebner et al., 2013); and the reduced strength of PF to PC synapse has also been reported in the SCA1[82Q] mouse model (Hourez et al., 2011). In mixed rat primary cerebellar cultures containing GCs and PCs, transfection of GCs with the SCA27 causing splice variant FGF14b-F150S mutant negatively modulated calcium currents in GCs and impaired PF to PC synaptic transmission, as demonstrated by the reduced EPSCs amplitude evoked in PCs by GCs (Yan et al., 2013). In agreement

with these *in vitro* results, a reduced PC responsiveness to PF stimulation was also observed in acute cerebellar slices from the FGF14 knockout SCA27 mouse model, likely caused by a presynaptic deficit in glutamate release (Tempia et al., 2015).

The involvement of altered glutamatergic transmission in SCAs is well established, but the role of GABAergic transmission in these diseases remains poorly investigated. Interestingly, the GABA transporter 1 (GAT1) KO mouse (Chiu et al., 2005) and the vesicular GABA transporter (VGAT) KO mouse (Kayakabe et al., 2013) display poor motor coordination and an ataxic gait. Moreover, the Ax^J cerebellar ataxia mouse model is characterized by PCs expressing high levels of the ionotropic $GABA_A$ receptor ($GABA_AR$) and enlarged IPSCs (Lappe-Siefke et al., 2009). These findings therefore support a possible role of GABAergic transmission in ataxia onset and in disease progression. Indeed, postmortem analysis of brain tissue from SCA1 patients revealed a prominent increase in the BC to PC synapses (Edamakanti et al., 2018).

DISCUSSION

Key molecules and molecular pathways involved in PC development, activity and cerebellar afferent synaptic organization are affected in SCAs highlighting the possible role of cerebellar development and circuit wiring in the pathobiology of these neurodegenerative diseases. As the earliest manifestation of several SCAs, developmental defects characterize the conserved long silent phase of the pathology. However, their causative vs. protective role in the onset of ataxia and degeneration requires further investigation. Results from the conditional SCA1 mouse model supports their detrimental role, since motor impairment is partially rescued by delaying the expression of mutant ATXN1 until cerebellar development is complete (Serra et al., 2006). Nevertheless, if and how developmental deficits exacerbate the severity of the disease in other SCAs has still to be elucidated and further experiments are required.

A so far poorly explored area in the SCA research field is the investigation of the downstream effects of cortical wiring deficits on the final output stage of the cerebellum that generates the abnormal commands underlying motor impairments. The DCN is the main output of the cerebellum, and it receives GABAergic projections from PCs and excitatory inputs from CFs (Sugihara et al., 1999) and from MF collaterals (Shinoda et al., 1992; Matsushita and Gao, 1997; Matsushita and Xiong, 1997; Wu et al., 1999). Stimulation of the IO mediates the rebound increase in the firing of excitatory DCN neurons following an initial PC-mediated pause in their activity (Hoebeek et al., 2010). DCN activity is also effectively modulated by PC synchronicity that mediates time-locked spiking and rising of the firing frequency as well (Person and Raman, 2012a). Mechanisms of PC synchronizations are not fully elucidated, but experimental evidence supports the role of CF, GC to PC transmission and/or PC collaterals (Person and Raman, 2012b). By affecting the cortical synaptic organization of the cerebellum and PC activity, SCAs molecules could therefore strongly impact DCN activity and cerebellar neuronal coding; the so far identified defects in SCAs predict negative effects on the rebound firing properties of the DCN and PC synchronicity that require further and much needed investigation.

AUTHOR CONTRIBUTIONS

FB CP, and SS wrote the review. SS supervised the overall project. All authors read and commented on the final version of the review.

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REVIEW
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Hippocampal Deficits in Amyloid-β-Related Rodent Models of Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that is the most common cause of dementia. Symptoms of AD include memory loss, disorientation, mood and behavior changes, confusion, unfounded suspicions, and eventually, difficulty speaking, swallowing, and walking. These symptoms are caused by neuronal degeneration and cell loss that begins in the hippocampus, and later in disease progression spreading to the rest of the brain. While there are some medications that alleviate initial symptoms, there are currently no treatments that stop disease progression. Hippocampal deficits in amyloid-β-related rodent models of AD have revealed synaptic, behavioral and circuit-level defects. These changes in synaptic function, plasticity, neuronal excitability, brain connectivity, and excitation/inhibition imbalance all have profound effects on circuit function, which in turn could exacerbate disease progression. Despite, the wealth of studies on AD pathology we don't yet have a complete understanding of hippocampal deficits in AD. With the increasing development of *in vivo* recording techniques in awake and freely moving animals, future studies will extend our current knowledge of the mechanisms underpinning how hippocampal function is altered in AD, and aid in progression of treatment strategies that prevent and/or delay AD symptoms.

Keywords: hippocampus, Alzheheimer's disease, mouse models, synaptic plasticity, circuit changes

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INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease affecting more than 40 million people worldwide (Alzheimer's Disease International, 2018). AD is clinically characterized as a progressive impairment of memory and other cognitive functions, eventually leading to dementia and death (Förstl and Kurz, 1999; Holtzman et al., 2011). There are three stages of AD: (1) "preclinical" asymptomatic phase, (2) mild cognitive impairment where the first symptoms including changes in mood and behavior, confusion, and some memory loss become evident, and (3) dementia in which patients demonstrate deficits in multiple cognitive domains that are severe enough to produce loss of function (Förstl and Kurz, 1999; Jack et al., 2010; Sperling et al., 2011). Post-mortem AD brain tissue is characterized by pathological markers including amyloid plaques, tau neurofibrillary tangles, vascular damage from the plaque deposition, and profound neuronal cell loss (Blessed et al., 1968; Katzman and Saitoh, 1991; Selkoe, 1991; for a review see Uylings and de Brabander, 2002). There are currently no cures for AD or dementia, and any treatments available

are only palliative, therefore, many groups internationally are working to further understand the pathophysiology of AD in order to develop potential treatment strategies.

The hippocampus is widely studied in AD as this brain region is essential for forming new memories, and the progressive degeneration of neurons in the hippocampus responsible for short-term memory loss is a hallmark effect of AD (West et al., 1994, 2004; Fox et al., 1996). Microscopic changes in the hippocampus also precede behavioral symptomology in AD patients (for a review see Mufson et al., 2015) and mouse models; therefore, this review will focus on hippocampal deficits observed in AD.

There are two categories of AD, the early-onset familial AD generally occurring before 65 years of age, and the late-onset sporadic AD occurring after the age of 65. Data from extensive human genetic, histopathological, biomarker and animal model studies indicates that the 39–42 amino-acid (aa) peptide amyloid-β plays a prime role in the pathogenesis of familial and sporadic AD (Oddo et al., 2003; Haass and Selkoe, 2007; Bertram et al., 2010; Jack et al., 2010, 2013; Bateman et al., 2012). Here we will focus on the pathological effects of amyloid-β in AD. The 42aa amyloid-β protein is a hydrophobic peptide with an ominous tendency to assemble into long-lived polymers, and this excessive accumulation and deposition of amyloid-β is hypothesized to underlie the cascade of events that ultimately lead to cell death (Selkoe, 1991; Hardy and Higgins, 1992; Hardy and Selkoe, 2002). Early-onset AD is associated with mutations in the amyloid precursor protein (APP) gene, the presenilin 1 (PS1), and the presenilin 2 gene, which increases the production of the 42aa isoform of amyloid-β, an isoform more closely associated with the development of amyloid plaques than the shorter isoforms (Goate et al., 1991; Borchelt et al., 1996; Duff et al., 1996; Citron et al., 1997; Steiner et al., 1999). The strongest risk factor of late-onset sporadic AD is the ε4 allele of apolipoprotein E (a protein involved in the fat metabolism), which also significantly increases the burden of amyloid plaques in the brain (Verghese et al., 2011). Although familial AD cases represent only approximately 5% of AD cases, they have been critical for understanding the molecular mechanisms of AD, and importantly, similar mechanisms occur in sporadic AD.

Rodent models of AD have been extensively studied to examine neurological changes and to test therapeutic strategies that cannot directly be tested in humans. A frequently used single mutation AD model is the PDAPP model, expressing the $A\beta PP_{V717F}$ mutation more commonly known as the $A\beta PP_{Ind}$ mutation (Games et al., 1995). To more accurately replicate the human AD pathology, many mouse AD models have multiple mutations. Double mutant models include the Tg2567 and APP23 mouse models which both express the $A\beta PP_{Swe}$ ($A\beta PP_{K670N/M671L}$) mutation but with different promoters (Hsiao et al., 1996; Sturchler-Pierrat et al., 1997). Other multi-mutation models are the TgCRND8 and J20 mouse models expressing the $A\beta PP_{Swe,Ind}$ mutations, the APP/PS1 mice with the $A\beta PP_{Swe}/PS1_{M146L}$, $A\beta PP_{Swe}/PS1_{P264L}$, $A\beta PP_{Swe}/PS1_{L166P}$or the $APP_{Swe}/PS1\Delta E9$ mutation, the 5xFAD model with $A\beta PP_{Swe,Lnd,Flo}/PS1_{M146L,L286V}$ mutation, and the 3xTg-AD triple transgenic mouse model with the $A\beta PP_{Swe}/Tau_{P301L}/PS1_{M146V}$ mutations (Holcomb et al., 1998; Mucke et al., 2000; Chishti et al., 2001; Flood et al., 2002; Oddo et al., 2003; Oakley et al., 2006; Radde et al., 2006; Lindström, 2007; Hall and Roberson, 2012; Graybeal et al., 2015). Additional models include transgenic mice expressing the human APP (hAPP), as well as the SAMP8 mouse model that spontaneously develops AD (Mucke et al., 1996; Morley et al., 2000). Although each of these models displays some deficits associated with AD, the difficulty has been generating models where disease progression reaches stage 3 within the shorter lifespan of rodents. For this reason, double and triple knockout models that show faster rates of disease progression are often favored in the field.

While late-stage AD is characterized by profound neuronal loss, more subtle neuronal changes occur early in AD progression including synapse loss and circuit changes, which have been well correlated with cognitive impairments (Davies et al., 1987; Masliah et al., 1991; Terry et al., 1991; for a reviews see Selkoe, 2002; Palop et al., 2006; Palop and Mucke, 2010). Changes in synaptic function, plasticity, neuronal excitability, brain connectivity, and excitation/inhibition imbalance all have profound effects on circuit function, and are thought to exacerbate disease progression in AD. These circuit changes may be crucial targets for slowing or even preventing disease progression before widespread cellular loss has occurred. Changes in circuit function in AD lead to a high co-morbidity with epilepsy, even in the early stages of disease (Vossel et al., 2013; Subota et al., 2017; for reviews see Noebels, 2011; Vossel et al., 2017). Moreover, seizures worsen disease progression (Volicer et al., 1995) whereas anti-epileptic drugs improve memory impairments in individuals with mild cognitive impairment (Bakker et al., 2015).

HIPPOCAMPAL DEFICITS IN AMYLOID-β-RELATED ALZHEIMER'S DISEASE

Behavioral Deficits

Memory impairments are a major feature of AD that are crucial to replicate in rodents to accurately model the disease. Spatial memory encodes information about ones environment and orientation, and is commonly impaired in AD patients (Henderson et al., 1989; Cherrier et al., 2001). A common behavioral paradigm examining spatial memory in mice is the Morris water maze (MWM). Using the MWM it has been demonstrated that mouse models of AD show impaired spatial memory performance (Chen et al., 2000; Cayzac et al., 2015; Rorabaugh et al., 2017; Bergin et al., 2018). In addition, APP mice require considerably more training to reach the same level of competency as the control mice (Zhao et al., 2014). Furthermore AD-associated behavioral deficits have been observed in other hippocampal-dependent tests such as the radial maze, T-maze, olfactory tubing maze, and in the novel object recognition test (Klevanski et al., 2015; Baranger et al., 2017; Biasibetti et al., 2017). Female 3xTg-AD mice perform worse in working memory tests than males which is correlated with a higher amyloid-β

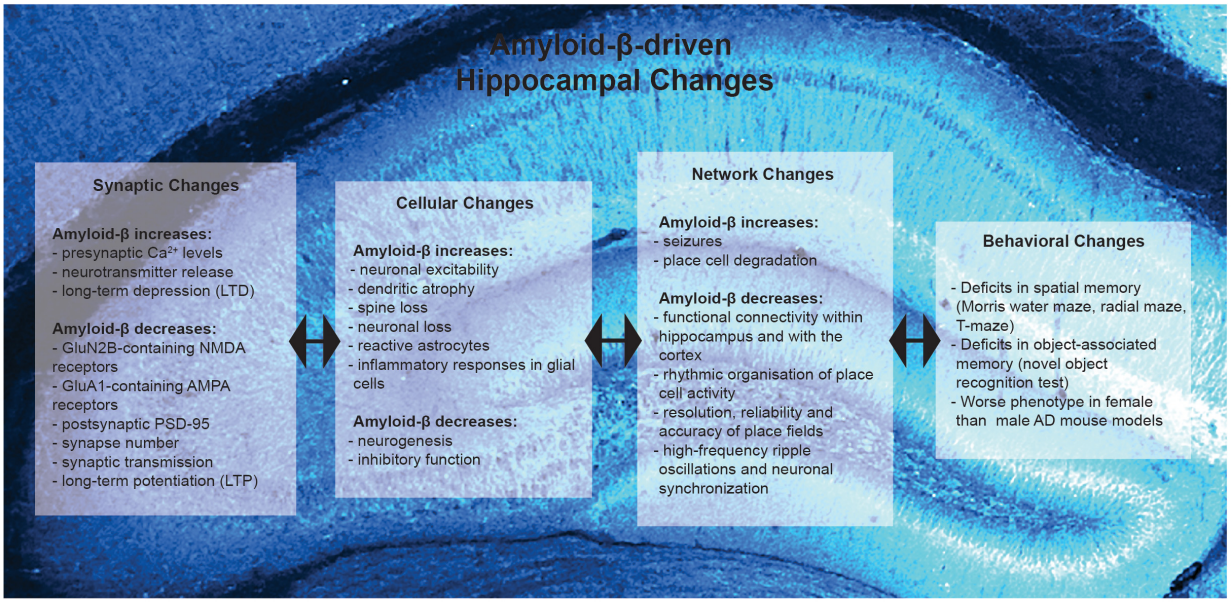

FIGURE 1 | Summary of Amyloid-β-driven hippocampal changes in rodent models of Alzheimer's disease. Amyloid-β drives changes at the synaptic, cellular, network and behavioral level.

load, and is reflective of the higher prevalence of AD in women (Carroll et al., 2007, 2010). Similarly female APP/PS1/tau triple-transgenic mice perform worse than males in the MWM, which is correlated with increased amyloid-β and tau load (Yang et al., 2018). These studies and others demonstrate that mouse models of AD ubiquitously present hippocampal-dependent behavioral deficits (**Figure 1**), and also reflect the gender differences in AD prevalence observed in human patients.

Changes in Glutamatergic Synapse Function

Changes in synapse function are a vital aspect during the early stages of AD progression with amyloid-β playing a complex role as, in addition to its effects on synapses, amyloid-β levels are regulated by synaptic activity. Synapse loss is highly correlated with cognitive impairments in AD (Terry et al., 1991). Synapse loss is also correlated with amyloid-β burden (Terry et al., 1991), and many studies have focused on how amyloid-β influences presynaptic function, postsynaptic receptors and proteins, and consequently synapse function. However, neuronal and synaptic activity also influence the metabolism of amyloid-β (Kamenetz et al., 2003). Furthermore, the extracellular concentration of amyloid-β is critical in determining whether it will aggregate into toxic species (Kamenetz et al., 2003). Consequently, the areas of the brain with the highest basal rates of metabolic and neuronal activity develop the most amyloid-β plaques (Buckner et al., 2005). Synaptic activity rapidly regulates interstitial fluid amyloid-β levels *in vivo* and correlates with local amyloid-β burden (Cirrito et al., 2005). Extracellular amyloid-β levels have been linked to synaptic vesicle release, suggesting that the synaptic amyloid-β levels are regulated presynaptically (Cirrito et al., 2005). Amyloid-β evokes sustained increases in presynaptic Ca^{2+}, and acts as a positive endogenous regulator of neurotransmitter vesicle release probability at hippocampal synapses (Abramov et al., 2009). These studies indicate that amyloid-β increases neurotransmitter release and the consequent hyperactivity further leads to more amyloid-β and its subsequent aggregation, resulting in a positive feedback loop (which has been proposed to be a major feature of AD; for a review see Doig, 2018). However, β-amyloid can also lead to depletion of presynaptic proteins involved in neurotransmitter release such as dynamin (Kelly et al., 2005; for a review see Honer, 2003).

Amyloid-β effects multiple postsynaptic proteins and there is evidence that correcting postsynaptic changes can improve impairments in mouse models of AD. More than 90% of synaptic oligomeric amyloid-β is colocalized in the postsynaptic density (Lacor et al., 2004). Amyloid pathology appears to progress in a neurotransmitter-specific manner with the glutamatergic and cholinergic terminals being the most vulnerable, whereas GABAergic terminals appear to be more resilient (for a review see Bell and Claudio Cuello, 2006). In early stages of AD, amyloid-β disrupts neuronal signaling via glutamatergic and acetylcholine receptors (Dougherty et al., 2003; Abramov et al., 2009). Amyloid-β regulates N-methyl-D-aspartate receptor (NMDAR) trafficking (Snyder et al., 2005) and oligomeric amyloid-β leads to a selective loss of GluN2B-containing NMDAR function (Kessels et al., 2013). Increases in the intracellular domain of APP (AICD), which occur in AD, affect NMDAR composition by increasing the prevalence of GluN2B containing receptors (Pousinha et al., 2017). Furthermore, increased ACID reduces excitability of CA1 neurons and impairs spatial memory (Pousinha et al., 2019). Amyloid-β induces NMDAR-dependent degradation of postsynaptic density 95 (PSD-95) at glutamatergic

synapses (Roselli et al., 2005). In addition, accumulation of amyloid-β in APP mutant neurons reduces synaptic PSD-95 and GluA1 (Almeida et al., 2005). Interestingly, restoration of PSD-95 levels can rescue memory deficits in $AbPP_{Swe}$/PS1 mice (Bustos et al., 2017). Therefore, amyloid-β also acts postsynaptically to reduce the expression of glutamatergic receptors and proteins, which is directly linked to cognitive impairments in AD.

These amyloid-β-induced pre and post-synaptic alterations consequently impair glutamatergic synaptic transmission in several mouse models of AD. Amyloid-β depresses synaptic transmission, and this was initially noted in APP_{Ind} mice which displayed severe impairments in synaptic transmission between hippocampal CA3 and CA1 cells (Hsia et al., 1999; Kamenetz et al., 2003). Additionally, the APP/PS1 model of AD, which overexpresses mutant human genes for APP and PS1, display deficits in synaptic transmission at a younger age than Tg4510 mice, which overexpress the mutant human Tau gene (Gelman et al., 2018). Therefore, amyloid-β plays a dominant role in causing synaptic deficits in the hippocampus, from the structural to the functional level (**Figure 1**).

In summary, amyloid-β increases presynaptic transmitter release but its postsynaptic negative effects override these leading to impaired synaptic function in AD. However, many of these studies examined the influence of amyloid-β using via external application *in vitro*. Thus, more *in vivo* studies are required to decipher the influence of intrinsically-released amyloid-β on synapse function, and to understand the temporal relation between AD-associated presynaptic and postsynaptic changes in the hippocampus. Exactly how these complex synaptic changes affect circuit function also remains somewhat a mystery. Nevertheless, disrupted synapse function could directly impact the ability of synapses to undergo synaptic plasticity, which in turn could underlie the memory deficits characteristic of AD.

Changes in Synaptic Plasticity

Synaptic plasticity has been well studied in AD as deficits in the ability of synapses to undergo changes in strength could be responsible for memory deficits. There is good consensus in the field that impaired synaptic strengthening is a key feature of AD as deficits in long-term potentiation (LTP) occur in many mouse models of AD (Nalbantoglu et al., 1997; Chapman et al., 1999; Gruart et al., 2008; Gengler et al., 2010; Klevanski et al., 2015; Gelman et al., 2018; Viana da Silva et al., 2019). Even transgenic mice expressing only the carboxy-terminal 104 amino acids of APP display deficits in the maintenance of LTP (Nalbantoglu et al., 1997). On the contrary, amyloid-β facilitates synapse weakening in the form of long-term depression (LTD) and depotentiation *in vivo* (Kim et al., 2001). Amyloid-β drives the loss of surface α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid receptors (AMPAR) by employing signaling pathways of LTD and can also lead to reduced synaptic NMDAR currents (Hsieh et al., 2006; Kessels et al., 2013). The synaptic depression and memory deficits induced by amyloid-β require the AMPAR subunit GluA3, as they are absent in GluA3 knockout mice (Reinders et al., 2016). This suggests that amyloid-β initiates removal of GluA3-containing AMPARs from synapses leading to synaptic and memory deficits (Reinders et al., 2016). Taken together, synaptic strengthening is impaired and synaptic depression is enhanced in AD (**Figure 1**) (for a review see Mucke and Selkoe, 2012).

Upon investigation of the mechanisms affecting synaptic plasticity in AD, several genes and pathways have been implicated. APP/PS1 mice showed reduced expression of synaptic plasticity genes such as *Arc, Zif268, NR2B, GluR1,* and *Homer-1a* (Dickey et al., 2003). In transgenic mice producing hAPP, dentate granule cells in particular were vulnerable to disruption of Arc expression as well as reductions in actin-binding protein α-actinin-2, which was tightly correlated with reductions in Fos and calbindin, shown previously to reflect learning deficits in these hAPP mice (Palop et al., 2005). Amyloid-β precursor protein (β-APP) fragments and amyloid-β oligomers impair LTP *in vivo*, and in hippocampal slices, this process is mediated via activation of several different kinases, such as c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as metabotropic glutamate receptor type 5 (Cullen et al., 1997; Walsh et al., 2002; Wang et al., 2004; Klyubin et al., 2012). LTP deficits in APP/PS1 mice are also linked to disruption of the hippocampal pro-opiomelanocortin (POMC)/melanocortin 4 receptor (MC4R) circuit as the suppression of hippocampal MC4R activity exacerbated LTP impairments in these mice, and is alleviated by activation of the hippocampal MC4R-coupled Gs signaling and POMC/MC4R activity (Shen et al., 2016). MC4R activation also rescues amyloid-β-induced synaptic dysfunction thereby implicating the POMC/MC4R as a potential therapeutic target to rescue synaptic dysfunction in AD. Contextual fear conditioning deficits in aged 5XFAD mice is associated with different expression of hippocampal proteins than normal aging (Neuner et al., 2017). Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to AD-related cognitive deficits (Palop et al., 2003). Overall, these reflect amyloid-β's multi-faceted disruption of processes involved in synaptic plasticity in AD.

Several studies have examined whether rescuing synaptic plasticity deficits in mouse models of AD can improve behavioral symptoms. Neuron-specific postnatal deficiency of PS1 prevented amyloid pathology and rescued LTP in $AβPP_{Ind}$ mice but failed to prevent cognitive deficits observed in the object recognition test in these mice (Dewachter et al., 2002), suggesting that LTP deficits do not underlie all behavioral deficits in this model. Another approach of preventing LTP deficits in AD mice included activation of *Wnt* signaling as several studies have shown *Wnt* signaling activation to facilitate LTP in wildtype mice (Chen et al., 2006; Beaumont et al., 2007; Cerpa et al., 2011). Vargas et al. (2014) found that chronic activation of *Wnt* signaling enhanced basal excitatory synaptic transmission, facilitated LTP and improved episodic memory in APP/PS1 mice (Vargas et al., 2014). In attempts to rescue synaptic plasticity deficits in AD mice, Cissé et al. (2011) regulated NMDAR function using receptor tyrosine kinase EphB2 which phosphorylates NMDARs via Src-mediated tyrosine phosphorylation (Dalva et al., 2000; Henderson et al., 2001; Takasu et al., 2002; Chen et al., 2008;

Cissé et al., 2011). The phosphorylation status of NMDAR subunits is correlated with cognitive performance, and levels of EphB2 and tyrosine-phosphorylated NMDARs are depleted in hAPP mice (Sze et al., 2001; Palop et al., 2005, 2007; Simón et al., 2009). Reversing EphB2 depletion in the dentate gyrus reversed LTP and memory impairments in hAPP mice (Cissé et al., 2011). Furthermore, neutralization of adenosine A_{2A} receptors could restore associative CA3 LTP and revert memory deficits in APP/PS1 mice (Viana da Silva et al., 2016). Chronic intranasal administration of Colivelin (a novel and strong humanin derivative) reduced amyloid-β deposition in the hippocampus, rescued suppression of hippocampal LTP, and prevented AD-associated behavioral impairments in APP/PS1 mice (Wu et al., 2017). In addition, increasing levels of the secreted APPα (sAPPα, an alternative cleavage product of APP that has neuroprotective and neurotrophic properties) completely reversed deficits in LTP and spatial memory tasks in APP_{Swe}/PS1ΔE9 mice (Tan et al., 2018). Therefore, it is important to keep in mind the protective roles of some biproducts of APP (for a review see Montagna et al., 2017), although the pathological biproducts of APP are often the focus in AD literature. Together these studies demonstrate potential for therapeutics that target LTP and its downstream pathways using a range of different methods to provide behavioral improvements. It is currently unknown whether these treatment strategies are applicable to idiopathic AD.

Neurogenesis

Neurogenesis in the adult hippocampus is a dynamic process that continuously changes the dentate gyrus, and is important for hippocampal plasticity, learning, and memory (Altman and Das, 1965; Eriksson et al., 1998; Aimone et al., 2011; Gu et al., 2012). Adult hippocampal neurogenesis consists of three main stages: proliferation, differentiation, and survival (Dard et al., 2019). Controversy exists in the literature as to whether hippocampal neurogenesis is increased or decreased in mouse models of AD (Rodríguez et al., 2008; Demars et al., 2010; Hamilton et al., 2010; Krezymon et al., 2013; for reviews see Wirths, 2017; Dard et al., 2019). However, altered neurogenesis must still provide some cognitive benefit as conditional ablation of adult neurogenesis in APP_{Swe}/PS1ΔE9 mice worsened behavioral performance in contextual conditioning and pattern separation tasks (Hollands et al., 2017). Together these studies suggest that deficits in adult neurogenesis may contribute to the pathology of AD, and points toward the possibility of increasing neurogenesis or using neural stem cells transplantation as an approach for preventing AD-associated changes in neuronal circuitry. In support of this idea Richetin et al. (2017) demonstrated that amplification of mitochondrial function rescued adult neurogenesis in APP/PS1 mice, and overexpression of the pro-neuronal marker Neurod1 increased dendritic growth and spine formation, and consequently rescued spatial memory in these mice (Richetin et al., 2017). Furthermore, neural stem cell engrafts into APP/PS1 mice were able to restore memory and promote endogenous neurogenesis and synaptic remodeling in these mice (Zhang et al., 2017).

Changes in Neuronal Excitability, and Excitation/Inhibition Imbalance

AD-associated alterations have also been observed beyond the synapse, with hyperexcitability of hippocampal neurons observed both *in vitro* and *in vivo*. Hippocampal neurons show increased excitability in the 3xTg-AD model due to altered Kv2.1 potassium channel function (Frazzini et al., 2016). Similarly, neurons of aged SAMP8 mice are hyperexcitable and show altered voltage-dependent Ca^{2+} currents (Wang et al., 2017). In APP/PS1 mice hyperexcitability has been linked to structural degeneration of dendrites (Šišková et al., 2014). The dendritic structure is known to determine the electrical properties of neurons as it defines the input-to-output conversion, therefore, when dendritic integrity is impaired neuronal function is aberrant (Šišková et al., 2014). These *in vitro* demonstrations of hyperexcitability in AD were later confirmed *in vivo* in APP/PS1 mice using two-photon imaging in the hippocampus (Busche et al., 2012). Neuronal hyperactivity in the hippocampus *in vivo* was correlated with soluble amyloid-β levels (Busche et al., 2012). Together, these studies demonstrate that hippocampal hyperexcitability is a common feature of different mouse models of AD (for a review see Busche and Konnerth, 2015). In addition to alterations in neuronal excitability, mouse models of AD demonstrate deficits in γ-aminobutyric acid (GABA) pathways and altered excitation/inhibition balance leading to seizures. In APP/PS1 mice deficits in the GABAergic pathway and feed forward inhibition are age-dependent (Oyelami et al., 2016; Viana da Silva et al., 2019). In hAPP mice parvalbumin interneuron dysfunction and reduced levels of voltage-gated sodium channel subunit Nav1.1 have also been linked to abnormal oscillatory rhythms, network synchrony and cognitive function (Verret et al., 2012). Furthermore, APP/PS1 mice show somatostatin-positive interneuron axon loss, enhanced spine turnover, and impaired learning–dependent spine gain in association with memory deficits in these mice (Schmid et al., 2016). Similarly, soluble amyloid-β oligomers increase neuronal excitability by disrupting glutamatergic/GABAergic balance in the hippocampus, and this could be prevented by increasing GABA tone or partially blocking NMDAR activity (Lei et al., 2016). Moreover, APP/PS1 mice are also susceptible to seizures, the frequency of which is correlated with the load of amyloid-β plaques (Minkeviciene et al., 2009; Busche et al., 2012; Reyes-Marin and Nuñez, 2017). Seizure activity appears to trigger compensatory mechanisms in the dentate gyrus of hAPP mice as enhanced synaptic inhibition and GABAergic sprouting have been observed (Palop et al., 2007). Furthermore, synaptic and cognitive deficits in hAPP and APP23 mice are reversed by antiepileptic drugs which suppress neuronal network dysfunction (Bromley-Brits et al., 2011; Sanchez et al., 2012). Together these data show that deficits in inhibition leading to overexcitation and seizures is commonly seen in mouse models of AD and contributes to our understanding of epilepsy co-morbidity in AD patients.

Overall these studies show that inhibition is reduced in AD, which combined with hyperactive excitatory neurons massively shifts the ratio toward excess excitation leading to seizures (**Figure 1**), which negatively impact cognition.

Increased excitability may eventually promote the excitotoxic damage observed in the AD brain. Therefore, restoration of excitation/inhibition balance may hold therapeutic potential in AD. The relation between the synaptic and plasticity changes to hyperexcitable networks seems counterintuitive as weaker synapses, impaired strengthening, and enhanced depression should lead to reduced network activity. However, changes in dendritic structure and activity levels can increase excitability of neurons leading to action potentials being triggered by fewer inputs. Other homeostatic changes, such as inhibitory alterations, that aim to restore activity levels may overcompensate and fail to restore balance.

Astrocytic Changes

Alterations in glial function have also been observed in AD, and growing evidence shows that glial changes may precede neuronal changes and behavioral impairment noted in the progression of AD (Heneka et al., 2010; for a review see De Strooper and Karran, 2016). Astrogliosis is a universal feature of AD brains (Nagele et al., 2004; Rodríguez et al., 2009; Heneka et al., 2010, 2013). Inflammatory responses in glial cells contribute to the pathogenesis of AD, and several studies have highlighted specific therapeutic targets for the treatment of AD, such as targeting the inflammasome NLRP3 or RIPK1, an enzyme abundantly expressed in microglia (Heneka et al., 2013; Ofengeim et al., 2017). Pathological astroglial changes have been shown to be prevented by environmental enrichment in PDAPP-J20 transgenic mice (Beauquis et al., 2013). Additionally, it was identified that gamma frequency entrainment could recruit both glial and neuronal responses to attenuate AD-associated pathology (Iaccarino et al., 2016). Reactive astrocytes likely play a role in clearing damaged synapses and dendrites, however, they are limited in their ability to fully clear away debris (Gomez-Arboledas et al., 2018). The role of astrocytes in synaptic plasticity is also affected in AD (for a review see Singh and Abraham, 2017). Therefore, preventing glial pathology may represent a new therapeutic intervention for AD, and preventing abhorrent glial changes can be achieved by altering network activity, either naturally by changing the environment or artificially by stimulation.

Changes in Brain Connectivity and Circuit Function

Brain connectivity and circuit function are disrupted in AD, in part due to synaptic and neuronal loss (**Figure 1**). At the synaptic level, amyloid-β induced LTD results in loss of dendritic spines (Hsieh et al., 2006; Wei et al., 2010). Amyloid-β-induced synapse loss and dendritic spine abnormalities have been noted by other studies in several mouse models of AD, such as the APP mice, APP/PS1 mice, PDAPP, and Tg2576 mice (Lanz et al., 2003; Spires et al., 2005; Shankar et al., 2007; Knafo et al., 2009). In hippocampal slice cultures from APP_{SDL} mice, spine loss was accompanied by changes in spine shape from mushroom to stubby spines (Tackenberg and Brandt, 2009; for a review see Tackenberg et al., 2009). Use of adeno-associated virus to express oligomeric amyloid-β in the hippocampus also resulted in spine loss (Forner et al., 2019). Interestingly, extracellular amyloid-β lead to a greater reduction in stubby spines than intracellular overexpression, while other spine types were equally affected (Forner et al., 2019). Amyloid-β pathology also results in dendritic abnormalities and atrophy. High-resolution confocal microscopy has revealed that, in the PSAPP mouse model of AD, dendrites passing within 40 μm of amyloid deposits displayed loss of dendritic spines, shaft atrophy, varicosity formation, and sprouting (Tsai et al., 2004; Grutzendler et al., 2007). Similarly, post-mortem human brains from AD patients also display similar dendritic alterations (Merino-Serrais et al., 2013), further emphasizing that amyloid deposits and their surroundings microenvironments are toxic to dendrites.

The hippocampal CA1 subregion is particularly more susceptible to AD-associated atrophy in comparison to CA2 or CA3 subregions (West et al., 2000; Frisoni et al., 2008; Apostolova et al., 2010). Selective neuronal death in brain regions most affected by AD has also been demonstrated in APP mice, and this was directly correlated with amyloid plaque formation (Calhoun et al., 1998). As a result, mouse models of AD demonstrate decreased functional connectivity within the hippocampus as well as the cortex, as examined by resting state fMRI and optical intrinsic signal imaging technique, respectively (Bero et al., 2012; Shah et al., 2013). Furthermore, functional coupling between the hippocampus CA1 region and medial frontal cortex is also impaired in mouse models of AD (Zhurakovskaya et al., 2019).

Spatial memory deficits in AD mice are attributed to changes in circuit function due to altered cellular responses in the hippocampus. At a cellular level, place cells play a critical role in spatial memory and these have been shown to be affected in AD. Place fields from control mice become spatially restricted and stable after repeated exposures of a new environment; however, APP mice produce a spatial code of lower resolution, reliability and accuracy (Zhao et al., 2014). Furthermore, hippocampal place cell degradation and MWM training deficits correlate with amyloid-β plaque burden, respectively in Tg2576 and PDAPP mouse models of AD (Chen et al., 2000; Cacucci et al., 2008). A lack of learning dependent changes in place cells in APP-PS1 mice has been correlated with impaired action-reward association tasks in a spatially defined environment (Cayzac et al., 2015). Impairments in rhythmic organization of place cell activity have also been observed in the 3xTg mouse model of AD, and may contribute to the unstable spatial representation and spatial memory deficits (Mably et al., 2017). Furthermore, in young rTg4510 mice high-frequency ripple oscillations and neuronal synchronization are reduced even though place fields of hippocampal CA1 cells are largely normal (Ciupek et al., 2015). Impaired cellular and network activity in the hippocampus therefore appear to contribute to spatial memory deficits in mouse models of AD.

Alterations in networks in other brain regions which are connected to the hippocampus are also observed in AD. For example cortical principle cells become hyperexcitable at the early stages of amyloid pathology, and via the thalamo-cortical pathway, drive thalamic cells too (Gurevicius et al., 2013; Busche et al., 2015). This precedes hippocampal electrophysiological abnormalities, and is hypothesized to underlie the network

reorganization which leads to epileptic seizures (Palop et al., 2007; Minkeviciene et al., 2009).

Treatment Strategies in Amyloid-β-Related AD

The prevention of behavioral deficits in AD mice has been studied extensively with a variety of different approaches: For example: Learning and age-related memory deficits can be prevented in APP/PS1, TgCRND8, Tg2576, and PDAPP mice with immunization against the amyloid-β peptide (Janus et al., 2000; Morgan et al., 2000; Dodart et al., 2002; Kotilinek et al., 2002). Such immunizations reduce pathological changes including plaque formation in PDAPP mice (Schenk et al., 1999). In 3xTg-AD mice, immunizations against amyloid-β have also been shown to act at the synaptic level by reducing synaptic impairments (Baglietto-Vargas et al., 2018). In this same AD mouse model, accumulation of intraneuronal amyloid-β precedes plaque and tangle pathology. Using immunotherapy to clear intraneuronal amyloid-β pathology rescued the early cognitive deficits seen in the MWM (Billings et al., 2005). Re-emergence of the amyloid-β pathology could again lead to cognitive deficits, implicating intraneuronal amyloid-β in the onset of cognitive dysfunction (Billings et al., 2005).

Despite billions of dollars being invested into drug development for AD, over 100 compounds have failed in clinical trials (Mehta et al., 2017). These potential disease-modifying drugs fall into four categories: monoclonal antibodies, gamma secretase inhibitors, tau aggregation inhibitors, and symptomatic treatments. Some examples of previously failed clinical trials include (i) bapineuzumab, one of the first monoclonal amyloid-β antibodies to reach phase 3 clinical trials, but unfortunately was found to have no significant clinical benefit (Salloway et al., 2014), (ii) solanezumab, which despite demonstrating an excellent safety profile and low incidence of vasogenic edema, failed to meet primary and secondary endpoints in the phase2B-3A study (Doody et al., 2014; Siemers et al., 2016), (iii) crenezumab did not show a significant benefit in treatment in comparison to placebo in a phase 2 trial (Miller, 2012), and (iv) gantenerumab did not meet a significant clinical efficacy endpoint in phase 3 trials at its administered dosage (Ostrowitzki et al., 2017). However, more recently, Aducanumab, a human monoclonal antibody that is selective for aggregated forms of amyloid-β has been examined as a potential treatment for amyloid-β-associated pathologies. *In vivo* multiphoton imaging of calcium homeostasis in aged Tg2576 mice demonstrated that acute topical application of aducanumab to the brain resulted in clearance of amyloid plaques, and chronic systemic administration ameliorated calcium overload and restoring intracellular calcium to control levels (Kastanenka et al., 2016). Aducanumab also restored NMDAR GluN1 and GluN2A subunit-expressing cell numbers to wildtype levels, thus indicating a potential restoration of neuronal network function and cognitive function in these mice. Phase I clinical trials using Aducanumab demonstrated an acceptable safety and tolerability profile of the drug, and it was shown to reduce amyloid deposition in the brain in a dose- and time-dependent manner (Ferrero et al., 2016; Sevigny et al., 2016). Phase III clinical trials were performed in 3200 individuals across 20 countries, but early analyses showed no promising effects of Aducanumab in decreasing amyloid burden or improving symptomology in patients and thus the study has been halted (Selkoe, 2019). However, longitudinal studies are required to investigate any potential long-term benefits of antibodies against amyloid-β.

Genetic, social, environmental, and pharmacological approaches have also been used to prevent behavioral deficits in AD models. For example, development of memory deficits was prevented in APP_{Swe}/PS1ΔE9 mice by constitutive deletion of the amyloid-β-binding cellular prion protein (Gimbel et al., 2010). Conditional deletion of PrP^{c} at 12 or 16 months of age completely rescued MWM deficits, novel object recognition, and passive avoidance test in APP_{Swe}/PS1ΔE9 mice, together with reversal of hippocampal synapse loss (Salazar et al., 2017). Memory deficits in APP/PS1 mice could be rescued by social interaction, and this effect was linked to increased levels of BDNF in the hippocampus (Hsiao et al., 2014). In addition, environmental enrichment led to reduced amyloid-β levels and amyloid deposition in APP_{Swe}/PS1ΔE9 (Lazarov et al., 2005). Environmental enrichment also changes the function of microglia in a way that prevents their inflammatory response to human soluble amyloid-β oligomers (Xu et al., 2016). Recently it was demonstrated that environmental enrichment and voluntary exercise revives adult neurogenesis, reverses AD-associated memory deficits, and prevents amyloid-β seeding (representing early stages of plaque formation) via activated phagocytic microglia cells (Ziegler-Waldkirch et al., 2018). Therefore, prolonged environmental enrichment could protect against AD by regulating the brain's innate immune system. 5xFAD mice displayed improved cognitive abilities, decreased amyloid plaque and neuroinflammation in the entorhinal cortex after treatment with RS67333, a partial 5-HT_4R agonist, for 4 months (Baranger et al., 2017). Genetic reduction of tau expression has also been shown to prevent behavioral impairments and neuronal deficits (Roberson et al., 2007; Vossel et al., 2010). Similarly, expression of truncated versions of tau that lack dendritic localization has beneficial effects in APPswe transgenic mice (Ittner et al., 2010), fitting with evidence that shows the amyloid-β-induced mis-localization of endogeneous tau into dendrites is detrimental (Zempel et al., 2010). These are just a few of the treatment strategies that have shown promise in animal trials, however, there are not yet many that have translated well in human trials. However manipulating circuit function still holds promise for future treatments (for a review see Canter et al., 2016).

FUTURE DIRECTIONS IN UNDERSTANDING CIRCUIT FUNCTION IN AMYLOID-β-RELATED ALZHEIMER'S DISEASE

Given the complex multifaceted nature of the identified issues in AD it is becoming increasingly important to understand changes

in brain networks *in vivo*. Examining circuit dynamics during behavior will give the next breakthroughs in our understanding. To date there have been several studies that have taken advantage of *in vivo* two-photon imaging to better understand circuit changes in the hippocampus and other brain regions. For example *in vivo* studies that have examined hyperactivity near plaques in both hippocampus and cortex (Busche et al., 2008, 2012) have identified significant heterogeneity in cell responses within the same brain region. In the visual cortex neuronal hyperactivity has been shown to affect function (Grienberger et al., 2012). Hyperactive neurons exhibited poor orientation tuning, which was correlated with impaired performance in visual-pattern discrimination (Grienberger et al., 2012). Furthermore, visual experience driven-expression of Arc is impaired in AD mice, providing further *in vivo* evidence of altered memory processes (Rudinskiy et al., 2012). Astrocytes in the cortex have also shown synchronous hyperactivity and intercellular calcium waves in APP/PS1 mice (Kuchibhotla et al., 2009). In future studies it will be crucial to understand the link between aberrant neuronal and glial activity *in vivo* in AD mice.

Examining *in vivo* dynamics of axons and dendrites longitudinally with disease progression and/or treatment also offer promise for understanding complex changes in AD models. For example, long-term imaging revealed how axon pathology proceeds around amyloid-β plaques in APP-PS1 mice (Blazquez-Llorca et al., 2017). Axons near plaques appeared swollen before becoming disconnected, over a time course of several months (Blazquez-Llorca et al., 2017). In addition, instability of dendritic spines and axonal boutons near plaques was revealed in this way and could be prevented by γ-secretase inhibitor treatment (Liebscher et al., 2014). Further studies are needed to reveal not just structural but also functional changes in dendrites and axons using calcium and voltage imaging *in vivo*. Furthermore *in vivo* imaging in freely moving animals using miniaturized microscopes is another exciting possibility for future studies (for a review see Werner et al., 2019).

Activation of specific subsets of neurons using channel rhodopsin is also an important approach to understand circuit changes in AD. By utilizing learning-dependent expression of channel rhodopsin it is possible to label memory engram cells (Ryan et al., 2015). It is then possible to re-activate these cells ontogenetically and trigger memory retrieval (Ryan et al., 2015). This approach has been used to restore fear memory in young AD mice (Roy et al., 2016). More studies are required to increase our understanding of the specific pathways involved in memory deficits in AD so that treatments can be targeted to the right networks at the right time in disease progression.

In conclusion, hippocampal deficits in synapse and neuronal function manifest into behavioral abnormalities in mouse models of AD. However, more research and consensus in the field are required to completely understand hippocampal deficits in AD. With the increasing development of *in vivo* recording techniques in awake and freely moving animals, future studies will extend our current knowledge about how hippocampal function is altered in AD by combining network imaging with behavior. It will be crucial to identify network changes early and treat them before pathology becomes widespread. However, because network changes likely contribute to disease progression this could lead to future treatments that prevent AD symptoms from worsening.

AUTHOR CONTRIBUTIONS

YV, JM, and JC wrote the manuscript.

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SYSTEMATIC REVIEW
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Evidence for Structural and Functional Alterations of Frontal-Executive and Corticolimbic Circuits in Late-Life Depression and Relationship to Mild Cognitive Impairment and Dementia: A Systematic Review

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Depression is a risk factor for developing Alzheimer's disease and Related Dementia (ADRD). We conducted a systematic review between 2008 and October 2018, to evaluate the evidence for a conceptual mechanistic model linking depression and ADRD, focusing on frontal-executive and corticolimbic circuits. We focused on two neuroimaging modalities: diffusion-weighted imaging measuring white matter tract disruptions and resting-state functional MRI measuring alterations in network dynamics in late-life depression (LLD), mild cognitive impairment (MCI), and LLD+MCI vs. healthy control (HC) individuals. Our data synthesis revealed that in some but not all studies, impairment of both frontal-executive and corticolimbic circuits, as well as impairment of global brain topology was present in LLD, MCI, and LLD+MCI vs. HC groups. Further, posterior midline regions (posterior cingulate cortex and precuneus) appeared to have the most structural and functional alterations in all patient groups. Future cohort and longitudinal studies are required to address the heterogeneity of findings, and to clarify which subgroups of people with LLD are at highest risk for developing MCI and ADRD.

Keywords: late-life depression, mild cognitive impairment, Alzheimer's disease, frontal-executive, corticolimbic, diffusion-tensor imaging, resting-state functional MRI, brain network

INTRODUCTION

Alzheimer's Disease and Related Dementias

The number of individuals with Alzheimer's disease and related dementia (ADRD) is expected to rise to 82 million in 2030 and 152 million in 2050 (WHO Fact Sheet, 2019). One in every 2–3 people over the age of 85 will develop ADRD (Hebert et al., 2013). In the 2011 guideline, "Alzheimer's dementia" is referred to what was "Alzheimer's disease" under the 1984 guidelines. Alzheimer's dementia is referring to the dementia stage of the Alzheimer's disease continuum that starts with

initial brain changes leading to cognitive and physical symptoms over years (Association Alzheimer's, 2018). Delaying disease onset or progression by even 1 year can lead to a significant reduction of the global burden of disease (Brookmeyer et al., 2007). Absent a breakthrough drug, the "lowest hanging fruit" for delay or prevention of ADRD consists of several modifiable risk factors. These include depression, diet, type 2 diabetes mellitus, midlife hypertension, current smoking, cognitive inactivity, physical inactivity, and traumatic brain injury (Daviglus et al., 2010; Baumgart et al., 2015; Xu et al., 2015; Clare et al., 2017).

Depression and ADRD: The Epidemiological Connection

Cohort studies and meta-analyses have shown that late life depression (LLD) increases the risk for dementia in general, and AD in particular (Jorm, 2001; Ownby et al., 2006; Diniz et al., 2013). More specifically, depression is a risk factor for the progression from normal cognition to MCI, and from MCI to dementia (Diniz et al., 2013; Brailean et al., 2017).

Depression is currently the leading cause of disability and disease worldwide (WHO Fact Sheet, 2019). With the increased rates of depression and the aging of the world's population (Compton et al., 2006) the incidence of depression in late life will rise (Chapman and Perry, 2008; Zivin et al., 2013). Older adults with depression develop mild cognitive impairment (MCI)—an intermediate stage between normal cognition and AD with a ratio of 6–15% per year (Farias et al., 2009; Petersen et al., 2009). More strikingly, one-third to one-half of older individuals with depression have a concomitant diagnosis of MCI, far higher than the general population (Butters et al., 2004; Bhalla et al., 2006; Barnes and Yaffe, 2011). However, the relative risk among studies varies. Discrepancies may be due to study population differences or methodologic differences between the various studies (Byers and Yaffe, 2011; Köhler et al., 2011). Alternatively, whether different age of onset of depression [i.e., early-onset depression (EOD)] with an age of onset below 60, as opposed to late-onset depression (LOD; Brodaty et al., 2001; Hashem et al., 2017) would influence the progression to AD remains to be elucidated (Edwards et al., 2019). For instance, a large-scale retrospective cohort study reported a 20 and 70% adjusted hazard of dementia in midlife and late-life depressive symptoms respectively (Barnes et al., 2012). Furthermore, the risk may vary due to depression severity, or its successful treatment (Almeida et al., 2017).

Cognitive Impairment in LLD, MCI, and AD Is Shared Across Memory and Executive Function Domains

Cognitive impairment, specifically in executive function and information processing speed corresponding to the frontal-executive circuit, and in episodic memory corresponding to the corticolimbic circuit, are common findings among LLD, MCI, and AD (Butters et al., 2008; Koenig et al., 2015; Liao et al., 2017). Episodic memory, executive function, and processing speed, are the main impaired cognitive domains in individuals with aMCI (Alexopoulos et al., 2012; Bai et al., 2012), LLD+MCI, and AD (Geerlings et al., 2008; Li H. J. et al., 2015). This impairment usually occurs to a greater extent in individuals with aMCI, LLD+MCI, and AD, relative to individuals with LLD (Bai et al., 2012; Wang L. et al., 2012; Li et al., 2014; Shimoda et al., 2015). In studies using a comprehensive neuropsychological battery, cognitive impairment in LLD, compared to HC, was observed especially in episodic memory, executive function, and processing speed (Bai et al., 2012; Koenig et al., 2015; Chen et al., 2016; Liao et al., 2017; Li W. et al., 2017). However, other cognitive findings in LLD are variable. For instance, a longitudinal study has reported poor cognitive performance in individuals with depression at baseline, compared to a HC group, and greater decline in episodic memory, attention-working memory, and executive function after both 3 and 12 months. Non-remitters had greater decline in executive function after 12 months (Riddle et al., 2017). Furthermore, LLD compared to HC groups have shown to have impairment in executive function, but no difference in verbal and visuospatial memory (Dybedal et al., 2013), or impairment in attention, and memory functions, but no difference in total cognitive function, processing speed, and executive function (Yue et al., 2015). Other studies have found no differences in the cognitive profile of LLD and HC groups (Alexopoulos et al., 2012), at baseline or over 1 year follow up (Li X. et al., 2017). The discrepancies may be due to differences in the cognitive tests that were used in these studies. A recent meta-analysis synthesized the evidence on cognitive impairment associated with depression in older adults and found that LLD was significantly associated with global cognitive impairment (John et al., 2018). However, the majority of studies in this meta-analysis had used the Mini-Mental State Examination (MMSE), a cognitive screening test that has ceiling effects in LLD and lacks sensitivity to subtle changes in specific cognitive domains (Tombaugh and McIntyre, 1992; Rajji et al., 2009). Thus, this meta-analysis could not report how LLD influences impairment in different cognitive domains, such as memory, executive function, or information processing speed (John et al., 2018).

The Potential Mechanisms Linking LLD and ADRD

In 2008, we proposed a conceptual model of the potential etiopathological mechanisms linking late-life depression (LLD) and ADRD (Butters et al., 2008). We hypothesized that three main pathways might explain the neurobiology linking depression with ADRD: a vascular pathway; aligning by and large with executive dysfunction, an inflammation pathway; aligning largely with episodic memory impairment, and an amyloid pathway, serving to reduce time to clinical dementia symptoms. The "vascular hypothesis" suggests that cerebrovascular disease (CVD) may lead to structural damage, particularly to frontal-striatal or frontal-executive circuits (Alexopoulos, 2003; Diniz et al., 2013) that in turn leads to executive dysfunction in both depression and ADRD (Alexopoulos et al., 1997; Krishnan et al., 1997). The "inflammation hypothesis" posits that the higher levels of cortisol (due to hyperactivity of the hypothalamic-pituitary-adrenal axis) (Paul, 2001) and proinflammatory cytokines (Diniz et al., 2010; Hermida et al.,

2012) associated with depression lead especially to hippocampal volume loss (i.e., damage to the corticolimbic circuit) and cognitive deficits (e.g., episodic memory) in older adults (Lee et al., 2007; Peavy et al., 2007). Finally, we proposed that increased amyloid production seen in preclinical studies and in pre- and post-mortem human studies of depression (Osorio et al., 2014; Mahgoub and Alexopoulos, 2017) may lead to increases in ADRD neuropathology. While 50% of individuals with LLD present with beta amyloid within the AD range (Butters et al., 2008), recent data do not support a role for amyloid in the depression-dementia link (Diniz et al., 2015; Nascimento et al., 2015; Morin et al., 2019). Each of these processes can independently or collectively contribute to brain injury burden, lower cognitive reserve, and lead to neurodegeneration and cognitive loss. This conceptualized "multiple pathways model" and associated mechanisms (some fixed, some progressive) may lead to heterogeneous cognitive outcomes—i.e., "normal" cognition, MCI, Alzheimer's dementia (AD), vascular Dementia (VaD), or mixed AD with cerebrovascular disease.

Progress in the Assessment of Brain Circuits and Networks

We can now interrogate brain circuits through the use of newer structural MRI techniques and identify networks with functional MRI. Unlike the original MRI studies of LLD or ADRD, these newer methods can use broadly adopted acquisition sequences, large sample sizes, and longitudinal follow-up. In contrast to the earlier focus on specific brain regions, recent studies interrogating brain circuits or networks can test whether there is overlap in the neural circuits implicated in LLD and in MCI or dementia. Thus, we conducted a systematic review of brain circuits reported to be impaired in LLD to complement and extend the mechanistic model proposed by Butters et al. (2008) more than 10 years ago.

Objectives and Hypotheses

We conducted a review to evaluate the evidence in overlap in alteration of brain circuits and networks and the associated cognitive impairment between depression and ADRD. We focused on two neuroimaging modalities: diffusion-weighted imaging (DWI) measuring white matter tract disruptions and resting-state functional MRI (rs-fMRI) measuring alterations in network dynamics. Based on the model proposed by Butters et al. (2008) and subsequent studies (Bhalla et al., 2009; Wu et al., 2011; Andreescu et al., 2013; Diniz et al., 2013, 2016; Boccia et al., 2015; Smagula et al., 2015), we hypothesized that evidence would support similar structural and functional disruptions in two circuits: (i) the frontal-executive circuit subserving executive function and the executive control network (ECN) and (ii) the corticolimbic circuit subserving episodic memory and the default mode network (DMN) in LLD and MCI or ADRD. We also explored whether the evidence would suggest similar or different structural or functional changes mechanisms in the two groups of individuals with LLD: those with early-onset depression (EOD; age of onset before 60 recurring in late life and those with late-onset depression (LOD; age of onset after 60) (Brodaty et al., 2001; Hashem et al., 2017).

Overview of Identifying Structural Brain Circuit Using Diffusion-Weighted Imaging (DWI)

DWI estimates water molecule diffusion and their directionality. The most common DWI measures are fractional anisotropy (FA), a potential marker of axonal structural integrity; and mean diffusivity (MD) a reflection of rotationally invariant variations within the intracellular and extracellular space (Winston, 2012). Pathological damage can be detected by decreased FA and increased MD. FA and MD can be measured locally using a predefined region of interest (ROI) analysis or globally with voxel-wise tract-based spatial statistics (TBSS) (Smith et al., 2006). Tractography is a DWI-based computational reconstruction method for the mapping of discrete fiber tracks, using defined originating regions ("seeds") and statistical procedures; it can follow the trajectories of white-matter tracts *in vivo* and infer the underlying structural connectome of the human brain (Jbabdi et al., 2015). DWI can provide other measures to indirectly index white matter health or pathology; however, this review focused on the most commonly used FA and MD metrics.

Overview of Identifying Functional Brain Network Using Resting-State fMRI (rs-fMRI)

Brain activity and network connectivity at rest can be measured using rs-fMRI. While a variety of analytical strategies are available to study resting-state network connectivity, this review focuses on three common approaches: (1) seed-based analysis: a hypothesis-driven approach where an a priori seed region of interest is initially selected and a brain connectivity map is calculated by detecting temporal correlation, i.e., synchronous co-activation, between that seed and all other regions in the brain (Lee M. H. et al., 2013); (2) an independent component analysis (ICA): a non-hypothesis, data-driven and more complex approach that evaluates the whole brain and decomposes it into a set of independent components, each depicted as a functional map (Van Den Heuvel et al., 2009; Wang N. et al., 2012); and (3) regional homogeneity (ReHo): an approach that evaluates the similarity or synchronization between the time series of a given voxel and its nearest neighbors (Zang et al., 2004).

Additionally, some DWI and rs-fMRI studies use more advanced data analysis techniques, including network and graph theory analyses, which quantify whole brain topology and its organization features (Bullmore and Sporns, 2009).

METHODS

Search Strategy and Data Sources

A systematic literature search was conducted for relevant articles published between 2008 and October 2018 in accordance with the PRISMA statement (Moher et al., 2009). Our search terms comprised three blocks; the first search block included keywords relating to depression in older populations. The second search block contained keywords for neuroimaging methods, MRI in particular. To reduce the number of irrelevant hits, a third search

block was added, which contained keywords related to specific brain regions and two main networks: the executive control network (ECN) and default mode network (DMN), subserved by the frontal-executive and corticolimbic circuits respectively. In addition, the reference lists of previous systematic reviews and meta-analyses were scanned for articles of interest. The search criteria were conducted in three electronic databases: the Medline/PubMed (**Table 1**), PsycInfo, and EMBASE. The search strategy was reviewed and approved by a librarian at the Center for Addiction and Mental Health, after modifying the search terms for each database (**Figure 1**).

Screening Procedure

After excluding duplicates, all articles identified by the search were screened based on their title or abstract when needed (D.M.) to identify studies using MRI in individuals with LLD. Twenty percent of all articles were additionally screened by a second rater (N.R.R.) to assess the consistency of screening. Then, all retained articles were screened again (N.R.R.), based on their abstracts or full text when needed, to identify studies meeting the eligibility criteria.

Inclusion and Exclusion Criteria

A study was included if: (1) the study was published in an English language peer-reviewed journal; (2) the study individuals were 55 years or older; (3) the study sample included older individuals diagnosed with a major depressive disorder by a psychiatrist according to DSM or ICD criteria (the depressive episode could be current or in remission); (4) the study included a group of "healthy controls" (HC) with no psychiatric diagnosis; (5) DWI or rs-fMRI was collected and analyzed for both LLD and HC individuals; (6) the results were presented specifically in terms of involved or uninvolved brain circuits. We did not include studies focused exclusively on MCI or dementia but we also included studies that, in addition to individuals with LLD and HC, included a group with MCI or LLD+ MCI, since these studies provided a rare opportunity for "head-to-head" comparison of brain structure and function and further exploration of our hypotheses. We excluded studies that were focused on (1) depressive symptoms exclusively; (2) apathy; and (3) clinical trials (if the baseline/prior to treatment data was available, data were included).

Data Extraction/Abstraction

Data from manuscripts meeting the inclusion criteria were extracted and entered into a database that included authors, year of publication, age group, study type, gender composition, sample size, depression status (active or remitted), diagnostic criteria or instrument used to assess depression, presence of other groups (i.e., MCI or LDD+MCI), neuroimaging modality, image analysis methods, and a brief description of findings. One study was excluded at this stage, as we detected potential plagiarism, which was reported to the editor-in-chief of both the original journal (Yin et al., 2016b) and the second journal in which the same data were published by different authors (Zhu et al., 2018).

Quality Assessment

A modified version of the Newcastle-Ottawa Scale (NOS) (**Table 2**) was used to assess the quality of all studies included in the data-synthesis (Wells et al., 2016). The question on representativeness of cases was removed as all cases were consecutive. The scale was modified to check the adequacy of sample size per group, and $n = 30$ was set as the minimum required number per group (Pajula and Tohka, 2016). Since cognitive deficits are important in the link between depression and ADRD, one question was added to assess the quality of cognitive assessment in the studies. In addition, an extra point was given to studies that had used a comprehensive battery to evaluate cognitive performance in both cases and controls. Points were summed and ranged from 0 to 8; and the overall quality of the studies was categorized into weak ($\leq$3), fair (4–5), or good (6$\leq$). Among 37 studies included in the systematic review, 20 were categorized as good quality, 15 were fair quality, and 2 were low quality.

Our quality assessment revealed that 32.4% of included studies had only used MMSE for cognitive screening that lacks sensitivity to subtle changes in cognition and has been shown to be influenced by ceiling and floor effects (Tombaugh and McIntyre, 1992). As a result, the confounding effect of cognitive impairment and LLD was indistinguishable in these studies. The Newcastle-Ottawa Scale for each study is presented in **Supplementary Tables 1**, **2**.

RESULTS

Selected Studies

Our search identified a total of 4,104 references in the three databases (**Figure 1**). After removal of duplicates, 3,962 unique citations remained. After screening by title 3,854 were excluded because they focused on psychiatric disorders other than LLD. All remaining citations were screened by their abstract and 33 studies met our eligibility criteria (i.e., the excluded studies did not use DWI or rs-fMRI, reported solely treatment response, did not include a healthy control group, or were conference presentations). As per above, one study was excluded at this stage, as we detected potential plagiarism. Finally, five studies were obtained by cross-reference of previous systematic reviews and meta-analyses and were incorporated into the final pool. Finally, 37 studies met all of our eligibility criteria:15 used DWI; 18 used rs-fMRI; and 4 used both DWI and rs-fMRI (Sexton et al., 2012a; Tadayonnejad et al., 2014; Yin et al., 2016a; Harada et al., 2018). Seven studies included groups other than LLD and controls: three included individuals with MCI (Bai et al., 2012; Xie et al., 2013; Chen et al., 2016); one included individuals with LLD plus amnestic-MCI (LLD+aMCI) (Li et al., 2015b); one included individuals with LLD plus memory deficit (LLD+MD) (Mai et al., 2017); and two included both individuals with aMCI, and LLD plus aMCI (Li et al., 2014, 2015a). Overall, the 37 studies included 1,140 individuals with LLD; 1,211 healthy controls; 173 aMCI; 53 LLD+aMCI; and 15 LLD+ MD. The results are presented below based on the imaging modality (DWI or rs-fMRI).

TABLE 1 | Search blocks: MeSH terms adapted for Medline.

Geriatric depression concept	Neuroimaging concept	Neural/Circuitry concept
1. exp Depressive Disorder 2. exp Depression/ 3. [(late-life or late-onset or "late in life" or geriatric) adj3 depress*].mp. 4. or/1–3	5. Neuroimaging/ or diffusion tensor imaging/ or exp functional neuroimaging/ 6. Magnetic resonance imaging/ or diffusion magnetic resonance imaging/ or echo-planar imaging/ 7. or/5–6	8. Cognitive reserve/ or executive function/ 9. Frontal lobe/ or prefrontal cortex/ or exp hippocampus/ 10. Neural pathways/ or internal capsule/ or perforant pathway/ 11. Default mode network*.mp. 12. (Executive control or executive function* or cognitive control or Corticolimbic or frontal-executive or neural pathway* or default mode network*).mp. 13. or/8–12

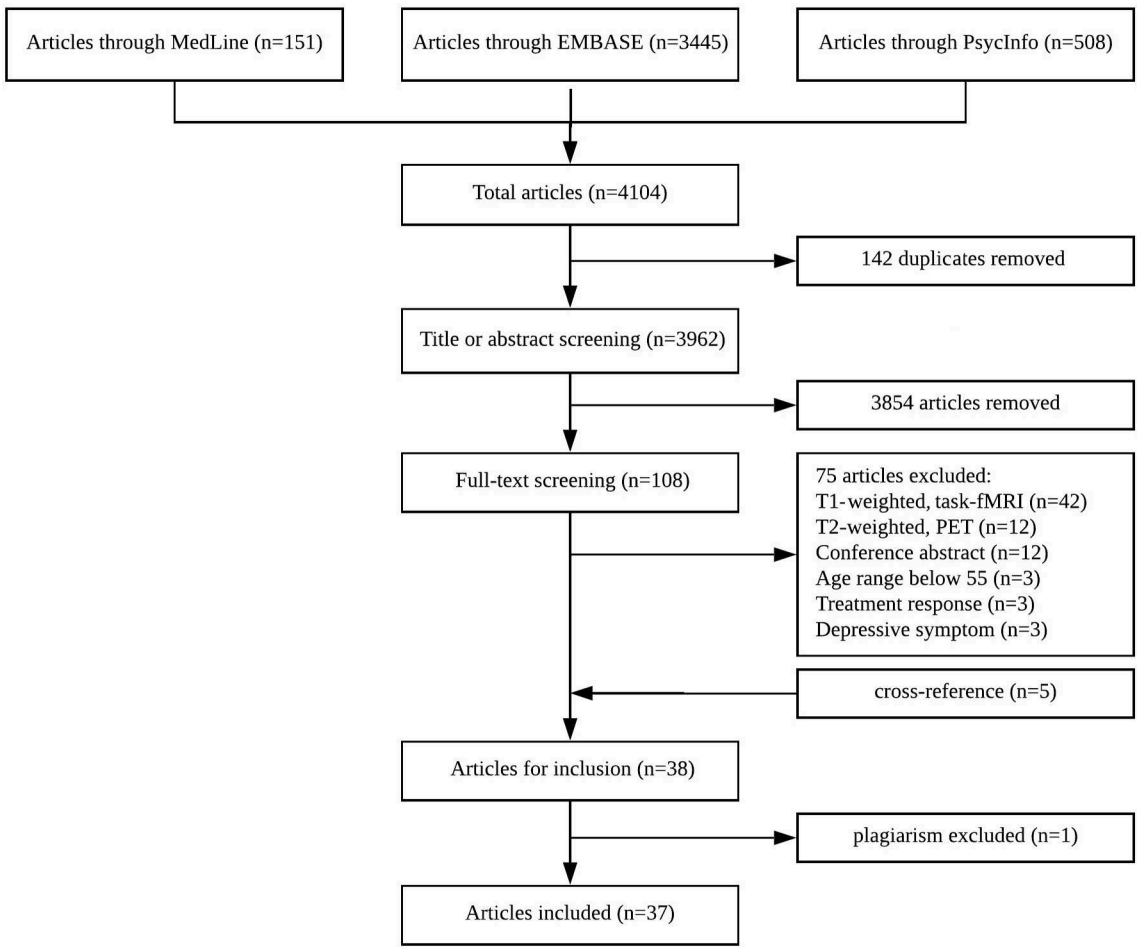

FIGURE 1 | PRISMA flowchart.

DWI Studies

Of 17 DWI studies, five used the ROI analysis method, three used tractography, nine used Tract-Based Spatial Statistics (TBSS), four used network and graph theory analyses, and one study compared apparent fiber density (AFD) and thickness of a single ROI (corpus callosum) in the LLD and HC groups (**Table 3**). Three DWI studies included additional comparison groups (MCI or LLD+MCI) (**Table 4**).

DWI Studies Using ROI Analysis

Seven studies compared white matter integrity using ROI analysis of which six compared differences between individuals with LLD and an HC group, and one across individuals with LLD, aMCI,

and LLD+aMCI, and an HC group (Li et al., 2014). Additionally, three studies conducted TBSS (Mettenburg et al., 2012; Sexton et al., 2012a; Harada et al., 2016), and tract-specific voxelwise analysis (Li et al., 2014) presented in the section DWI Studies Using TBSS.

Individuals with LLD vs. HC groups, showed disruption in white matter integrity in frontal-executive (Shimony et al., 2010; Yuan et al., 2010), and corticolimbic tracts (Shimony et al., 2010; Yuan et al., 2010; Harada et al., 2016). However, these studies have also found no significant difference between individuals with LLD and an HC group in other frontal-executive (Shimony et al., 2010; Yuan et al., 2010; Sexton et al., 2012a) and corticolimbic tracts (Yuan et al., 2010; Sexton et al., 2012a). One study reported no difference in the FA values or thickness, but a lower AFD in the corpus callosum [frontal-executive] (Emsell et al., 2017). See **Table 3** for further details.

One other study compared individuals with non-remitting and remitting LLD and an HC group and found lower FA in the splenium of the corpus callosum (frontal-executive) and the cingulate bundles and higher RD in the right uncinate fasciculus (corticolimbic) in individuals with non-remitting depression (but not in individuals with remitting depression) compared to the HC group (Mettenburg et al., 2012) (**Table 3**).

In the study comparing white matter integrity across LLD, aMCI, LLD+aMCI, and HC groups, individuals with LLD demonstrated higher MD and/or AD in the fornix, the corpus callosum (frontal-executive), the cingulum-hippocampus part, the uncinate fasciculus (corticolimbic) vs. the HC group. However, there was no significant difference between individuals with LLD and aMCI or LLD+aMCI (Li et al., 2014). See **Table 4** for further details.

TABLE 2 | Modified Newcastle-Ottawa scale.

Modified Newcastle-Ottawa scale	
	Ascertainment of diagnosis (LLD, MCI, LLD+MCI)
1	Independent validation (1+ person or process) to ensure diagnostic accuracy using DSM-IV or 5
0	No independent validation process
0	No description
	Control definition
1	Explicitly states that controls have no history of mental illness
0	Not specified
	Selection of controls
1	Community controls
0	Selected from a specific population (e.g. hospitals)
0	No description
	Sample size is adequate
1	Minimum of 30 per group
0	Less than 30 per group
	Comparability of groups on the basis of the design or analysis (LLD, MCI, MCI+LLD, HC)
1	Groups are matched for age or gender and/or analyses are adjusted for age or gender
0	No description of comparability based on factors of interest
	Assessment of cognitive state in patients and controls
1	General cognitive tests were used (MMSE, CDR)
1	Comprehensive cognitive tests were used (additional point)
0	No assessment reported
	Correction for multiple comparisons
1	Clear description of process to correct for multiple comparisons in analyses
0	No description of process to correct for multiple comparisons in analyses
8	6+/8 good, 4+/8 moderate, 3-/8 poor

DWI Studies Using TBSS

Nine studies used TBSS analyses of white matter, of which eight compared individuals with LLD to an HC group and one across four groups (LLD, HC, aMCI, LLD+aMCI). Two studies found no significant difference in FA or MD value (Bezerra et al., 2012; Harada et al., 2016); and three reported lower FA only at uncorrected threshold (Colloby et al., 2011; Sexton et al., 2012a; Tadayonnejad et al., 2014). Four studies reported loss of white matter integrity at corrected threshold in individuals with LLD vs. the HC group. In these studies, the loss of white matter integrity was found in both the frontal executive tracts (i.e., fornix, corpus callosum) (Mettenburg et al., 2012; Li et al., 2014), and the corticolimbic tracts (i.e., cingulate bundles, uncinate fasciculus, posterior cingulate gyrus, parahippocampal gyrus, uncinate fasciculus) (Alves et al., 2012; Mettenburg et al., 2012; Guo et al., 2014; Li et al., 2014). In the tract-specific voxelwise analysis of the selected ROIs, individuals with aMCI and LLD+aMCI demonstrated similar findings compared to the HC group (Li et al., 2014). See **Tables 3**, **4** for further details.

DWI Studies Using Tractography

Three DWI studies used tractography to compare white matter integrity in individuals with LLD to a HC group. Two studies connected one or two corticolimbic [posterior cingulate cortex/precuneus (PCC/Pcun), left anterior cingulate cortex (l-ACC)] regions to one or two frontal-executive [dorsal anterior cingulate cortex (dACC), thalamus, posterior superior temporal gyrus (pSTG)] regions. In the first study, individuals with LLD showed a lower FA and higher radial diffusivity (RD) in the tracts connecting the PCC/Pcun with the dACC, but no differences in the tracts connecting the PCC/PCun with the thalamus (Yin et al., 2016a). In the second study, both individuals with current and remitted LLD had a higher MD, RD, AD but no difference in FA compared to the HC group in the tract connecting l-ACC to pSTG (Harada et al., 2018). Finally, a corticolimbic tract study connecting bilateral uncinate fasciculus tracts and bilateral cingulum tracts, revealed a loss of white matter integrity in the right uncinate fasciculus and bilateral cingulum tracts in individuals with LLD (Charlton et al., 2014). See **Table 3** for further details.

DWI Studies Using Network and Graph Theory Analysis

Four DWI studies used network analysis (Li X. et al., 2017; Mai et al., 2017) and graph theory analysis methods (Bai

TABLE 3 | DWI studies with LLD and HC only.

References	N (LLD/HC)	Study type	Depression status	Analysis method	Frontal-executive findings LLD vs. HC	Corticolimbic findings LLD vs. HC	Other findings LLD vs. HC
Emsell et al. (2017)	107 (55/52)	Cross-sectional	Current	ROI (FA, AFD, thickness) CC[1]	Lower AFD, No difference FA or thickness in CC[1]	–	–
Yuan et al. (2010)	70 (37/33)	Cross-sectional	Remitted LOD>60	ROI (FA) gCC[2], sCC[3], SLF[4], CB[5], IFOF[6]	Lower FA in gCC No difference FA in sCC, SLF	Lower FA in posterior cingulum No difference FA in middle cingulate bundles	Lower FA in IFOF
Shimony et al. (2010)	96 (73/23)	Cross-sectional	Current	ROI (MD, RA)	Higher MD and lower RA in superior, middle, inferior frontal, medial and lateral orbital frontal ROIs Lower RA in temporal, parietal ROIs	Higher MD in dorsal, anterior, ventral cingulate	Lower RA in occipital, and motor ROIs
Harada et al. (2016)	106 (45/61)	Cross-sectional	Current	TBSS (FA, MD)	–	–	NS
				ROI (FA) UNC[7]	–	Lower FA in left UNC	No WMH difference
Mettenburg et al. (2012)	67 (51/16)	Cross-sectional	22 Remitted, 29 non-remitted	TBSS (FA, MD, AD, RD)	Corrected Voxel-wise comparison Sig clusters of lower FA and Greater extent of higher MD within CC[1] Higher RD in CC[1], and bilateral subcortical parietal/temporal white matter	Sig clusters of lower FA and greater extent of higher MD in confluent sections of the CB[5], Higher RD in CB[5], UNC[7] and right Pcun[9] regions	**Remitted vs. non-remitted** NS
				ROI (FA, MD, AD, RD) CB[5], gCC[2], bCC[11], sCC[3], and UNC[7]	**Non-remitted vs. HC** Higher RD in gCC[2], sCC[3], bCC[10], Lower FA only in sCC[3] **Remitted vs. HC** A similar trend, but did not reach significance	**Non-remitted vs. HC** Lower FA, higher RD in right uncinate region **Remitted vs. HC** Higher RD in right UNC	**Remitted and non-remitted vs. HC** No difference FA in gCC, bCC

(Continued)

TABLE 3 | Continued

References	N (LLD/HC)	Study type	Depression status	Analysis method	Frontal-executive findings LLD vs. HC	Corticolimbic findings LLD vs. HC	Other findings LLD vs. HC
Sexton et al. (2012a,b)[a,b]	61 (36/25)	Cross-sectional	Current or remitted	TBSS (FA, RD, AD)	–	–	Widespread lower FA in 36% of skeleton voxels at $P < 0.05$ and 16% at $P < 0.01$ 38% percent of voxels with lower in FA had higher DR, but no difference in DA
				ROI (FA, RD, AD) gCC[2], bCC[10], sCC[3], SLF[4], CB[5], UNC[7], ATR[11], ILF[12], CST[13], fornix	Lower FA at *uncorrected* threshold in ATR[11], sCC[3], SLF[4] (more than 50% of voxels at $p < 0.05$)	Lower FA at *uncorrected* threshold in UNC[7] (more than 50% of voxels at $p < 0.05$)	Lower FA at *uncorrected* threshold in CST[13] (more than 50% of voxels at $p < 0.05$) No WMH[8] differences
Alves et al. (2012)	35 (17/18)	Cross-sectional	Current	TBSS (FA, MD)	–	Lower FA at *corrected* threshold In right PCC[14]	
Guo et al. (2014)	30 (15/15)	Cross-sectional	Current	TBSS (FA)		Lower FA at *corrected* threshold in PGH[15]	
Bezerra et al. (2012)	83 (47/36)	Cross-sectional	Current	TBSS (FA)			No significant difference in FA or MD
Tadayonnejad et al. (2014)[a]	25 (10/15)	Cross-sectional	Current	TBSS (FA, MD)	20% reduction in FA only at *uncorrected* threshold In right forceps minor of the CC[1]		
Colloby et al. (2011)	68 (38/30)	Cross-sectional	Current or remitted	TBSS (FA, MD)	Lower FA only at *uncorrected* threshold in middle temporal	lower FA only at *uncorrected* threshold in PGH[9]	lower FA only at *uncorrected* threshold in fusiform gyri
Harada et al. (2018)[a]	46 (16/30)	Cross-sectional	Current and remitted (same individuals)	Probabilistic tractography between the ACC[16] and pSTG[17] sphere	**Current and remitted LLD vs. HC** higher MD, RD, AD, but no difference in FA in the left ACC–pSTG **Current LLD vs. remitted LLD** No difference		

(Continued)

TABLE 3 | Continued

References	N (LLD/HC)	Study type	Depression status	Analysis method	Frontal-executive findings LLD vs. HC	Corticolimbic findings LLD vs. HC	Other findings LLD vs. HC
Yin et al. (2016a)[a]	71 (32[c]/39)	Cross-sectional	Current (no antidepressant therapy 6 months prior to the study)	Tractography-deterministic for DMN[19] [+PCC[14]/Pcu[9] seed FC]	lower FA and higher RD in tracts connecting PCC[14]/Pcu[9] with dACC[18] No difference FA in tracts connecting PCC[14]/Pcu[9] with the thalamus		-
Charlton et al. (2014)	46 (23/23)	Cross-sectional	Current	Tractography - deterministic (FA, MD, AD, RD) Bilateral UNC[5] and cingulum tracts	-	Loss of white matter integrity in the right UNC[5] (FA, MD, AD, RD), left cingulum (MD, RD) and right cingulum (MD, AD, RD)	-
Li X. et al. (2017)	48 (24/24) **1 year follow-up** 29 (10/ 19)	Cohort 1-year follow-up	Remitted	Between-hemisphere connectivity and graph theory analysis (Probabilistic tractography- AAL ROIs)	**LLD hub regions (but not in HC)** L-ITG[20], and L- MTG[21]	–	**LLD hub regions** left calcarine fissure and surrounding cortex **HC hub regions** R-SMA[22] **LLD vs. HC** Significantly lower between-hemisphere connectivity, global efficiency, global circuitry strength Sig higher shortest path length **1-year follow up LLD** Lower global efficiency and strength and increased shortest path length
Charlton et al. (2015)	76 (28/48)	Cross-sectional	Current (2 weeks medication free)	Graph theory	**LLD hub regions** Stronger prefrontal region **HC hub regions** stronger right temporal regions	–	No significant differences on global metrics

[a] *studies that had reported findings in both rs-fMRI and DWI.*
[b] *DWI findings were reported in two separate publications.*
[c] *participants were over 55 y/o*
[1] *corpus callosum (CC);* [2] *genu of corpus callosum (gCC);* [3] *splenium of corpus callosum (sCC);* [4] *superior longitudinal fasciculus (SLF);* [5] *cingulate bundle (CB);* [6] *inferior fronto-occipital fascicle (IFOF);* [7] *uncinate fasciculus (UNC);* [8] *White-Matter Hyperintensity (WMH);* [9] *precuneus (Pcun);* [10] *body of corpus callosum (bCC);* [11] *anterior thalamic radiation (ATR);* [12] *inferior longitudinal fasciculus (ILF);* [13] *corticospinal tract (CST);* [14] *posterior cingulate cortex(PCC);* [15] *parahippocampal gyrus (PGH);* [16] *anterior cingulate cortex (ACC);* [17] *posterior superior temporal gyrus (pSTG);* [18] *dorsal anterior cingulate cortex (dACC);* [19] *default mode network(DMN);* [20] *inferior temporal gyrus(ITG);* [21] *middle temporal gyrus(MTG);* [22] *supplementary motor area (SMA).*

TABLE 4 | DWI studies with LLD, HC, and additional comparison groups.

References	N (LLD/HC/MCI/ LLD+aMCI)	Study type	Depression status	Analysis method	Frontal-executive findings	Corticolimbic findings	Other findings
Li et al. (2014)	84 (20/33/18/13)	Cross-sectional	Current	ROI (FA, MD, AD, RD) CCG[1], CGH[2], CC[3], fornix, UNC[4]	Corrected $p < 0.05$ **LLD vs. HC** Higher MD, AD, RD in fornix higher AD in CC[3] **LLD+aMCI vs. HC** Higher MD, AD and RD in fornix	**LLD vs. HC** higher MD, AD, RD in CGH[2], higher AD in UNC[4] tracts **aMCI vs. HC** higher MD, RD in CGH[2] **LLD+aMCI vs. HC** higher MD, AD, RD in CGH[2]	
				Tract-specific voxelwise in the five ROIs	**LLD vs. HC** Higher FA in CC tracts Higher MD in fornix tracts **LLD vs. aMCI** Higher MD in CC[3] tracts **LLD vs. LLD+aMCI** Lower FA in CC[3] tracts **aMCI vs. HC** Lower FA, higher MD in fornix tracts **LLD+aMCI vs. HC** Lower FA, higher MD in fornix tracts **LLD+aMCI vs. aMCI** Lower FA, higher MD in CC[3] tracts Higher MD in fornix tracts	**LLD vs. HC** Lower FA in UNC[4] tracts Increased MD CGH[2] **LLD vs. aMCI** Lower MD in CGH and UNC[4] **LLD vs. LLD+aMCI** Higher MD in UNC, CCG[1] **aMCI vs. HC** Lower FA in UNC[4] Lower FA, higher MD in CGH[2] **LLD+aMCI vs. HC** Lower FA, higher MD in UNC[4] Higher MD CGH[2] **LLD vs. aMCI** Lower MD in CGH and UNC **LLD vs. LLD+aMCI** Higher MD in UNC[4], and CCG[1] **LLD+aMCI vs. aMCI** Lower FA in UNC tracts **LLD+aMCI vs. all other** Lower FA in bilat-CGH[2]	
Mai et al. (2017)	69 (24/30/-/15[a])	Cross-sectional	Current	Network analysis probabilistic tractography (AAL atlas regions)	**LLD+MD vs. LLD** Decreased connections nodes: Frontal (R-IFG-operc[5], R-L-IFG- triang[6], L-inf-ORB[7], R-mid-TPO[8]), subcortical (R-L-Put[9], R-L-Thal[10]), and temporal (L-MTG[11])	**LLD+MD vs. LLD** Decreased connections nodes: paralimbic (R-med-SFG[12], R-sup.med-ORB[13], R-ACC[14], L-MCC[15], R-Pcun[16]), subcortical (R-L-Hippo[17])	**LLD vs. HC** Stronger local connection, lower circuitry density **LLD vs. LLD+MD** Stronger local connection **LLD and LLD+MD vs. HC** Weaker rich-club connection **LLD vs. LLD+MD** Stronger local connection **LLD+MD vs. LLD and HC** Weaker connective strength, lower shortest path length, global efficiency, fault tolerant efficiency
Bai et al. (2012)	103 (35/30/38/-)	Cross-sectional	Remitted	Graph theory	**LLD and aMCI vs. HC** Lower nodal efficiency in frontal and parietal cortices	**LLD hub region** Left Pcun[16] **LLD vs. aMCI** Higher nodal efficiency in PCC[18]	**LLD vs. HC** Lower circuitry strength global efficiency, higher absolute path length LLD vs. aMCI: no difference

[a]*memory deficit LLD (LLD+MD).*

[1]Cingulum-cingulate gyrus tract (CCG); [2]Cingulum-hippocampus tract (CGH); [3]corpus callosum (CC); [4]uncinate fasciculus (UNC); [5]opercular part of the inferior frontal gyrus (IFG-operc); [6]triangle part of the inferior frontal gyrus (IFG-triang); [7]orbital part of the inferior frontal gyrus (inf-ORB); [8]temporal pole of middle temporal gyrus (mid-TPO); [9]putamen (Put); [10]thalamus (Thal); [11]middle temporal gyrus (MTG); [12]medial part of the superior frontal (med-SFG); [13]medial orbital part of the superior frontal gyrus (med-sup-ORB); [14]anterior cingulate cortex (ACC); [15]middle cingulate cortex (MCC); [16]precuneus (Pcun); [17]hippocampus (Hippo); [18]posterior cingulate cortex (PCC).

et al., 2012; Charlton et al., 2015). In these four studies, T1-weighted images were used either as seed regions for probabilistic tractography or to generate label maps after tracking the whole brain at each voxel. Global efficiency is simply the average of the efficiencies over all pairs of vertices (node connections). Clustering coefficient is the overall level of clustering in a network, i.e., the average degree to which nodes in a graph tend to cluster together. Network strength is a measure of the connectivity of a graph, where a greater value means more connection strength from one node to its neighbor nodes. The average path length is the average number of steps along the shortest paths for all possible pairs of network nodes (**Tables 3**, **4**).

Global metrics

Two studies compared LLD individuals with current depression to a HC group, and reported either no difference in the global metrics (Charlton et al., 2015), or weaker rich-club connection, decreased shortest path length, stronger local connection and lower network density in individuals with LLD (Mai et al., 2017). Two other studies compared individuals with remitted LLD to a HC group and found reduced global efficiency and global strength in individuals with LLD (Bai et al., 2012; Li W. et al., 2017). In addition, similar disruption of the global metrics (i.e., lower global efficiency, weaker connection strength, lower shortest path length) was found in the LLD+memory deficit (LLD+MD) (Mai et al., 2017), and individuals with aMCI compared to the HC group (Bai et al., 2012). However, individuals with LLD had a stronger local connection compared to individuals with LLD+ memory deficit (Mai et al., 2017), and showed no difference compared to individuals with aMCI (Bai et al., 2012).

Hub regions and nodal efficiency

In addition to global metrics differences, the studies reviewed here reported alteration in the hub distribution and nodal efficiency in individuals with LLD compared to the HC group. For instance, a reduction of nodal efficiency in frontal and parietal cortices was found in the remitted LLD group (Bai et al., 2012), however LLD individuals with current depression showed stronger prefrontal region hubs (Charlton et al., 2015). Furthermore, regions from both frontal-executive (i.e., Inferior and middle temporal gyri) and corticolimbic circuits (i.e., precuneus) were identified as hub regions in the individuals with LLD but not in the HC group (Bai et al., 2012; Li W. et al., 2017).

In the comparison between individuals with LLD and aMCI, those with LLD had a higher nodal efficiency in the posterior cingulate (PCC) (Bai et al., 2012). In the study by Mai et al. (2017) individuals with LLD compared to LLD+MD showed increased nodal connection in the frontal, paralimbic, subcortical, and some parietal and temporal regions, which belong to the frontal-executive and corticolimbic circuits.

rs-fMRI Studies

We included 22 rs-fMRI studies; 10 used seed-based functional connectivity (FC); two used ReHo (regional homogeneity); one used both seed-based FC and ReHo, two used ICA; two used graph theory analysis; and five used other methods (**Table 5**). Four studies had additional comparison groups included in their analysis (**Table 6**). In this review, we focused on two main functional networks associated with frontal-executive and corticolimbic circuit, the executive control network (ECN) and the default mode network (DMN).

rs-fMRI Studies Using Seed-Based Connectivity

Ten rs-fMRI studies conducted seed-based connectivity analysis, using a variety of ROIs as seed regions. From the frontal-executive circuit, seed regions were the anterior cingulate cortex, dorsolateral prefrontal cortex (Alexopoulos et al., 2012), and cerebellum (Alalade et al., 2011; Yin et al., 2015). From the corticolimbic circuit, seed regions were the posterior cingulate cortex [PCC]/precuneus [Pcun] (Wu et al., 2011; Alexopoulos et al., 2012; Yin et al., 2016a), hippocampus (Xie et al., 2013; Shu et al., 2014; Wang et al., 2015), and amygdala (Yue et al., 2013; Li et al., 2015b). All studies reported projections of the seed region to ECN, DMN, or both networks. One study used dorsal raphe nucleus (DRN) as the seed region and found significantly lower functional connectivity (FC) in the bilateral PCC in the LLD individuals with current depression vs. the HC group (Ikuta et al., 2017) (**Table 5**).

In the study using anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (dlPFC) as the seed region, the LLD individuals with current depression showed decreased FC in the frontoparietal and temporal areas. In addition, remitters vs. non-remitters demonstrated greater FC in the dorsal ACC, dlPFC, and parietal cortices (frontal-executive) (Alexopoulos et al., 2012) (**Table 5**).

In two studies using cerebellum as the seed region, the LLD individuals with current depression vs. the HC group, showed decreased FC in the frontal-executive regions including the dlPFC (Alalade et al., 2011; Yin et al., 2015), caudate, putamen, and parietal cortex, as well as corticolimbic regions including the PCC (Alalade et al., 2011; Yin et al., 2015), dmPFC (Alalade et al., 2011), and increased FC in the ventromedial prefrontal cortex (vmPFC) and ACC (Yin et al., 2015) (**Table 5**).

In three studies where PCC/Pcun was used as the seed region, the LLD individuals with current depression compared to the HC group showed decreased FC (Yin et al., 2016a) or increased FC (Wu et al., 2011; Alexopoulos et al., 2012) in the frontal-executive regions [i.e., thalamus, dorsomedial prefrontal (dlPFC), dorsal anterior cingulate cortex (dACC)]. In addition, decreased (Wu et al., 2011) and increased FC (Alexopoulos et al., 2012) was found in the corticolimbic regions (i.e., subgenual ACC, ventromedial PFC).

Three studies used hippocampus or subregions of hippocampus as the seed region and compared the functional connectivity (FC) between individuals with LLD and HC group. One study compared FC in the LLD individuals with current depression to non-depressed individuals and found increased FC and decreased anti-correlation FC in the frontal-executive regions [i.e., dorsolateral prefrontal cortex (dlPFC)] and increased FC in the corticolimbic regions [i.e., posterior cingulate cortex, dorsomedial prefrontal cortex (dmPFC)] (Xie et al., 2013). This study included individuals with MCI that showed decreased FC in both frontal-executive [i.e., dlPFC,

TABLE 5 | rs-fMRI studies with LLD and HC only.

References	N (LLD/HC)	Study type	Depression status	Analysis method	Frontal-executive findings LLD vs. HC	Corticolimbic findings LLD vs. HC	Other findings LLD vs. HC
Ikuta et al. (2017)	95 (33/62)	Cross-sectional	Current	Seed dorsal raphe nucleus (DRN)	–	Lower FC between DRN and bilat. PCC[1]	–
Yin et al. (2015)	71 (32 LOD/39)	cross-sectional	Current (no antidepressant therapy 6 months prior to study	Seed (Cerebellum)	Decreased FC in left dlPFC[2], and bilateral cerebellum	Increased FC in vmPFC[3] and ACC[4] Decreased FC in PCC[1]	Increased FC in SMA[5] and bilat. SMG[6]
Alalade et al. (2011)	29 (11/18)	Cross-sectional	Current	Seed (Cerebellum)	Decreased FC 1. R-Crus II_{Exec} with R-dlPFC[2] 3. R-Crus I_{DMN} with R-head of the caudate, R-insula/putamen, 4. VI_{Limbic} with R-IPC[7] 5. L-$Vermis_{Limbic}$ with L-vlPFC[8]	Decreased FC 1. R-Crus II_{Exec} seed R- dmPFC[9] 2. bilal-Crus I_{Exec1} R-Crus II_{Exec2}, and left $Vermis_{Limbic}$ with vmPFC[3] 3. VI_{Limbic} with PCC[1]	Decreased FC between left 1. Lobule V_{Motor} with L-dlPFC[2] and vlPFC[8] 2. R-Crus I_{DMN} and L-FFG[10]
Alexopoulos et al. (2012)	26 (16/10)	12-week clinical-trial. (Only baseline findings were extracted).	Current	Seed (ACC[4], dlPFC[2]) seed L-dACC[11] with L-dlPFC seed L-dlPFC[2] with bilat. IPC[7]	Decreased FC	–	–
				Seed (PCC[1]/Pcun[8])	Increased FC in lateral parietal regions and L-Pcun[12]	Increased FC in DMN and sACC[13], and vmPFC[3]	–
Yin et al. (2016a)[a]	71 (32/39)	Cross-sectional	Current (no antidepressant therapy 6 months prior to the study)	Seed (PCC[1]/Pcun[8]) for DMN[14]	Decreased FC with and dACC[11] and thalamus	Both LLD and HC showed typical distribution of the DMN	–
Wu et al. (2011)	24 (12/12)	12-week clinical-trial. Only baseline findings were extracted.	Current	Seed (PCC[1]) (low cognitive load, event-related task)	Increased FC in the dmPFC[9] and OFC[15]	Decreased FC in sACC[9]	Higher WMH
Shu et al. (2014)	60 (31/29)	Cross-sectional	Remitted	Seed (Hippo[16])	**R-hippo positive** Circuit: increased FC to R-pMTG[17] **R-hippo negative** Circuit: decreased FC to R-SPL[18]	**R-hippo positive** Circuit: decreased FC to L-MFG[18] **L- hippo positive** Circuit: decreased to L-mPFC[19] and MFG[18] **L-hippo negative** Circuit: increased FC to R-MFG/SFG[20]	**R-hippo positive** Circuit: increased FC to LG[21] and cuneus and decreased FC to SMA[5] **L-hippo negative** Circuit: decreased FC to R-IOG[22]/LG[21]
					APOE ε4 carrier vs. non-carrier **right hippo**: Decreased positive FC to ITG[23] and R-IFG[24]; and decreased negative FC to L-IPL[25].	**APOE ε4 carrier vs. non-carrier** **left hippo:** Decreased positive FC to bilat. mPFC[19]/ACC[4] regions **right hippo**: Decreased positive FC to bilat. mPFC	**APOE ε4 carrier vs. non-carrier** **left hippo**: Decreased negative FC to R-SMG[26]; and increased positive FC to bilat. insula. **right hippo**: Increased positive FC to L-Insula

(Continued)

TABLE 5 | Continued

References	N (LLD/HC)	Study type	Depression status	Analysis method	Frontal-executive findings LLD vs. HC	Corticolimbic findings LLD vs. HC	Other findings LLD vs. HC
Wang et al. (2015)	30 (14/16)	Cohort - 21 months follow up	Remitted	Seed (6 Hippo subregions)	**Both baseline and follow-up vs. HC** All Hippo sub-regional seeds showed lower and less diffuse FC with parietal cortex **From baseline to follow-up** Nearly all Hippo subregions showed increased FC mainly with mainly frontal cortex	**From baseline to follow-up** Greater decrease in the left CA[27] FC with the bilat PCC[1]/Pcun[12]	–
Yue et al. (2013)	44 (22/22)	Cross-sectional	Current (first onset medication-naïve)	Seed amygdala (Amy)	L- Amy positive circuit: decreased FC with R-MFG[18]	L- Amy positive circuit: reduction FC with L-SFG[20]	L- Amy negative circuit: increased FC in R-postCG[28] R-Amy negative circuit: reduction FC in R-MOG[29]
				ReHo maps	Decreased ReHo in R-MFG[18]	decreased ReHo in L-SFG[20]	–
Chen et al. (2012)	30 (15[b]/15)	Cross-sectional	Current, treatment-naïve	ReHo maps	Increased ReHo in L-STG[30], L-Crus I cerebellum	Deceased ReHo in R-Pcun[12]	–
Yuan et al. (2008)	32 (18/14)	Cross-sectional	Remitted[c]	ReHo maps	Deceased ReHo in L-MFG[18], R-STG[30] and MTG[31] Increased ReHo in bilat-putamen	Deceased ReHo in bilat SFG[20] and bilat Pcun Increased ReHo in R-SFG[20]	Deceased ReHo in R- FFG[10] and R-postCG[28] Increased ReHo in L- postCG[28]
Li W. et al. (2017)	68 (39/29)[d]	Cross-sectional	Current	Voxelwise ICA (ECN[32], DMN, SN[33])	**Intra-circuit intrinsic FC comparisons** **ECN**: - Increased positive FC in bilat. IPL[25] - Decreased negative FC in bilat insula, sACC[13], and MCC[33] - Decreased negative FC and reversal FC pattern to positive in bilat-PCC[1], L-MTL[34], PHG[35], Hippo, Amy **DMN**: - Decreased positive FC in R-SFG[24] - Decreased negative FC in L-Insula and R-SPL[18] - Increased negative FC in L-PHG[35] **SN**: - Decreased positive FC in R-dlPFC[2] **Intrinsic circuit connectivity matrix**: 1. Decreased positive inter-circuit FC between bilat-ECN and subcortical-DMN 2. Decreased negative FC between L-ECN and SN (insula). 3. Increased inter-circuit FC between L-ECN and post-DMN + reversal from negative to positive FC 4. Increased positive FC between L-ECN and dACC[11]		
Sexton et al. (2012a)	61 (36/25)	Cross-sectional	Current or remitted	dual regression ICA	NS in ECN	NS in DMN	–

(Continued)

TABLE 5 | Continued

References	N (LLD/HC)	Study type	Depression status	Analysis method	Frontal-executive findings LLD vs. HC	Corticolimbic findings LLD vs. HC	Other findings LLD vs. HC
Harada et al. (2018)[a]	46 (16/30)	Cross-sectional	Current and remitted (same individuals)	FC ACC ∼ pSTG[36]	**current LLD vs. HC** NS **remitted LDD vs. HC** decreased FC in L-ACC–pSTG **current LLD vs. remitted LLD** NS		–
Yue et al. (2015)	32 (16/16)[e]	Cross-sectional	Current	ALFF	**Effect of disease:** ALF widely distributed over CePL[37], CeAL[38], and R-SPL[18]	**Effect of disease:** ALFF widely distributed over L-middle-OFC **Effect of disease*frequency:** distributed over R-SFG[20]	**Effect of disease:** ALFF widely distributed over L-SOG[39]
Hou et al. (2016)	68 (31/37)[f]	Cross-sectional	Current	Voxel-mirrored homotopic connectivity (VMHC)	Lower VMHC in STG[30], CePL[37]	Lower VMHC in SFG[20]	Lower VMHC in postCG[28] and preCG[40]
Tadayonnejad et al. (2014)[a]	25 (10/15)	Cross-sectional	Current	Pairwise BOLD signal averages correlations after Fisher's r-to-z transformations	Lower FC between R-Accumb[41] and R-mOFC[42])	Lower FC between R-rACC[43] and bilat-SFG[20]	–
Yin et al. (2016b)	64 (33/31)[g]	Cross-sectional	Remitted	Graph theory of DMN	–	Decreased FC in DMN	Abnormal global topology increased characteristic path length and reduced global efficiency of DMN

[a] Studies that had reported findings in both rs-fMRI and DWI.

[b] Illness duration was less than 1 year, and individuals were treatment-naive. In the current review we excluded results of 15 young EOD and 15 young HCs (mean age 24).

[c] Individuals were in the first depressive episode and the age of onset was over 60 years, and remitted for more than 6 months before the enrollment.

[d] Did not exclude significant anxiety or mild cognitive impairment as long as the primary diagnosis was LLD.

[e] Individuals with first onset after 60 years and medication-naïve.

[f] First depressive episode and the age of onset was over 55 years, Pearson's correlation analysis was conducted.

Between each pair of time series within symmetrical interhemispheric voxels. The computed correlation coefficients were Fisher z-transformed to obtain a VMHC z-map for statistical analyses.

[g] Duration of illness was less than 5 years and a medication-free period for all individuals was longer than 3 months prior to the assessment, GT threshold: 0.10 to 0.40 using an increment of 0.01.

[1]Posterior cingulate cortex (PCC); [2]dorsolateral prefrontal cortex (dlPFC); [3]ventromedial prefrontal cortex (vmPFC); [4]anterior cingulate cortex (ACC); [5]supplementary motor area (SMA); [6]supramarginal gyrus (SMG); [7]Inferior parietal cortex (IPC); [8]ventrolateral prefrontal cortex (vlPFC); [9]dorsomedial prefrontal cortex (dmPFC); [10]fusiform gyrus (FFG); [11]dorsal anterior cingulate cortex (dACC); [12]precuneus (Pcun); [13]subgenual anterior cingulate cortex (sACC); [14]default mode network (DMN);[15]orbitofrontal cortex (OFC); [16]hippocampus (Hippo); [17]posterior middle temporal gyrus (pMTG); [18]superior parietal lobule (SPL); [19]medial prefrontal cortex (mPFC); [20]superior frontal gyrus (SFG); [21]lingual gyrus (LG); [22]inferior occipital gyrus (IOG); [23]inferior temporal gyrus (ITG); [24]inferior frontal gyrus (IFG); [25]inferior parietal lobule (IPL); [26]middle frontal gyrus (MFG); [27]cornu ammonis (CA); [28]post central gyrus (postCG); [29]middle occipital gyrus (MOG); [30]superior temporal gyrus (STG); [31]executive control network (ECN); [32]salient network (SN); [33]middle cingulate cortex (MCC); [34]medial temporal lobe (MTL); [35]parahippocampal gyrus (PHG); [36]posterior superior temporal gyrus (pSTG); [37]Posterior lobe of cerebellum (CePL); [38]anterior lobe of cerebellum (CeAL); [39]superior occipital gyrus (SOG); [40]precentral gyrus (preCG); [41]nucleus accumbens (Accumb); [42]medial orbitofrontal cortex (mOFC); [43]rostral anterior cingulate cortex (rACC).

TABLE 6 | rs-fMRI studies with LLD, HC, and additional comparison groups.

References	N (LLD/HC/MCI/ LLD+MCI)	Study type	Depression status	Analysis method	Frontal-executive findings	Corticolimbic findings	Other findings
Xie et al. (2013)	72 (18/25/17/12	Cross-sectional	Current	Seed (Hippo)	**LLD vs. non-depressed** **Left hippo**: Decreased anti-correlation R-dlPFC[1] **Right hippo**: Increased FC in bilat-Thal[2], and R-Lent[3], decreased anti-correlated FC in l-dlPFC and d-striatum[4] **MCI vs. non-CI** **Left hippo**: Decreased + and – FC in bilat-dlPFC, IPC[5], L- pMTG[6]; and R-dACC[7] and SPC[8]. **Right hippo**: Decreased + and - FC in bilat-aTP[9] and IPC[5]; L-vlPFC[10], pMTG; R-ITC[11]. **Interactive effect of LLD*MCI** **Right hippo**: Found in bilat-MOG[12], L-dACC, R-dlPFC/dACC cluster	**LLD vs. non-depressed** **Left hippo**: Increased FC bilat-PCC[13], and R-dmPFC[14] **Right hippo**: Increased FC in L-Hippo **MCI vs. non-CI** **Left hippo**: Decreased + and – FC in bilat-dmPFC **Right hippo**: Decreased + and – FC in bilat-aTP[9], PHG[15] **Interactive effect LLD*MCI** **Right hippo**: Found in vmPFC[16]	**LLD vs. non-depressed** **Right hippo**: Decreased anti- correlated FC in bilat-MOG[13] **MCI vs. non-CI:** **Left hippo**: Decreased + and – FC in bilat-RSC[17]; L-postCG[18]
Li et al. (2015b)	63 (25/26/-/15)	Cross-sectional	Current	Seed (Amygdala)	**LLD vs. HC** Decreased FC in R-SPL[19], MFG[20], IFG[21], ITG[22] Increased in cerebellar vermis **LLD+aMCI vs. HC** Decreased FC in bilat-SPL, R-IFG, MFG; and MTG[23] Increased FC in IPL[24] **LLD+aMCI vs. LLD** Decreased FC in L-MTG[20]	**LLD vs. HC** Increased FC in R-PCC[13] and TP[25] **LLD+aMCI vs. HC** Decreased FC in left TP, PHG[15], hippo[26], cuneus **LLD+aMCI vs. LLD** Greater decrease of FC in the bilateral TP, cuneus (posterior DMN[35])	**LLD+MCI vs. LLD** Decreased FC in OG[27] **LLD vs. HC** decreased FC in PreCG[28] **LLD+aMCI vs. HC** Decreased FC in PreCG, bilat-IOG[29] and MOG[30] **LLD+aMCI vs. LLD** Decreased FC in bilat-FFG[31], IOG, MOG
Chen et al. (2016)	256 (55/114/87)	Cross-sectional	Remitted	Correlation between each pair of 36 seeds that represent five major RSNs[32]	**LLD vs. HC:** Reduced FC degree in bilat-ITG[22] **MCI vs. HC:** Trend toward increased FC degree at all nodes (except for r-ITG[22])	**LLD vs. HC:** Reduced FC strength in ECN[33]-DMN[35] *After controlling for age, sex, education and APOE genotype: reduced correlation in ECN-DMN pair in LLD > MCI vs. HC	**LLD vs. HC:** Reduced FC degree in SMA[36] **LLD vs. HC:** Reduced FC strength in SMN[37] **aMCI vs. HC:** A trend toward increased FC strength within SAL and SMN **LLD vs. aMCI:** Reduced FC strength within SAL[38] and SMN
Li et al. (2015a)	79 (23/25/18/13)	Cross-sectional	Current	Graph theory threshold range (0.03–0.5)	**LLD, aMCI, LLD+aMCI vs. HC** Combined ECN/VAN split into two distinct modules: ECN[33] and VAN[34]	**LLD and aMCI vs. HC** DMN module was split into two smaller modules	**LLD vs. HC** Disrupted FC segregation (decreased local efficiency) **LLD+aMCI > LLD > MCI** Greatest mean nodal efficiency **LLD+aMCI vs. aMCI** Decreased local efficiency **LLD+aMCI vs. all other groups** greatest disruptions in integration (diminished global efficiency) **LLD+aMCI** Had most variable modular community followed by LLD

[1]*dorsolateral prefrontal cortex (dlPFC);* [2]*thalamus (Thal);* [3]*lentiform nucleus (Lent);* [4]*dorsal striatum (caudate and putamen) d-striatum;* [5]*inferior parietal cortex (IPC);* [6]*left posterior middle temporal gyrus (pMTG);* [7]*dorsal anterior cingulate cortex (dACC);* [8]*superior parietal cortex (SPC);* [9]*anterior temporal pole (aTP);* [10]*ventrolateral prefrontal cortex (vlPFC);* [11]*inferior temporal cortex (ITC);* [12]*middle occipital gyrus (MOG);* [13]*posterior cingulate cortex (PCC);* [14]*dorsomedial prefrontal cortex (dmPFC);* [15]*parahippocampal gyrus (PHG);* [16]*ventromedial prefrontal cortex (vmPFC);* [17]*retro-splenial cortex (RSC);* [18]*post central gyrus (postCG);* [19]*superior parietal lobe (SPL);* [20]*middle frontal gyrus (MFG);* [21]*inferior frontal gyrus (IFG);* [22]*inferior temporal gyrus (ITG);* [23]*middle temporal gyrus (MTG);* [24]*inferior parietal lobule (IPL);* [25]*temporal pole (TP);* [26]*hippocampus (hippo);* [27]*occipital gyri (OG);* [28]*pre-central gyrus (PreCG);* [29]*inferior occipital gyrus (IOG);* [30]*middle occipital gyrus (MOG);* [31]*fusiform gyrus (FFG);* [32]*resting state networks (RSN);* [33]*executive control network (ECN);* [34]*ventral attention network (VAN);* [35]*default mode network (DMN);* [36]*supplemental motor area (SMA);* [37]*sensory-motor network (SMN);* [38]*salience network (SAL).*

dorsal anterior cingulate cortex (ACC)], and corticolimbic (i.e., dmPFC) regions relative to individuals with non-cognitive impairment (Xie et al., 2013) (**Table 5**).

Two other studies compared hippocampal functional connecivity in individuals with remitted LLD to a HC group. Individuals with remitted LLD showed decreased FC in the frontal-executive (parietal cortex) and corticolimbic (i.e., middle frontal gyri) regions (Shu et al., 2014; Wang et al., 2015). In addition, after 21 months of follow up, the remitted LLD had an increased FC in the frontal cortex (frontal-executive), but a greater decline in the midline posterior regions (PCC/Pcu; corticolimbic) (Wang et al., 2015) (**Table 5**).

The amygdala was used as the seed region in two studies, comparing FC between LLD individuals with current depression and a HC group. Individuals with LLD demonstrated decreased FC in both the frontal-executive regions (i.e., frontal gyri, temporoparietal cortices), and the corticolimbic regions (i.e., superior frontal gyrus) (Yue et al., 2013; Li et al., 2015b). Furthermore, individuals with LLD had increased FC in some corticolimbic regions was found the midline posterior regions (PCC) and temporal pole compared to the HC group (Li et al., 2015b) (**Tables 5**, **6**).

In addition, in the study by Li et al. (2015b) an additional comparison group (LLD+aMCI) was included. The LLD+aMCI demonstrated decreased FC in the temporoparietal, and frontal gyri and an increased FC in the parietal lobe (frontal-executive). Further, LLD+aMCI demonstrated decreased FC in the posterior midline regions (medial temporal lobe and cuneus; corticolimbic) compared to individuals with LLD and HC group (Li et al., 2015b) (**Table 5**).

rs-fMRI Studies Using ReHo (Regional Homogeneity)

Three studies used ReHo, a method that evaluates the similarity or synchronization between the time series of a given voxel and its nearest neighbors (Zang et al., 2004). Results from three studies, comparing ReHo between the LLD and the HC groups, showed ReHo differences in both frontal-executive and corticolimbic circuits. From the frontal-executive circuit, LLD individuals with current depression and individuals with remitted LLD compared to the HC group showed increased ReHo in the superior temporal gyrus (Chen et al., 2012), and putamen (Yuan et al., 2008), and decreased ReHo in the frontal gyrus (Yuan et al., 2008; Yue et al., 2013) and middle temporal gyrus (Yuan et al., 2008), respectively. From the corticolimbic regions, both individuals with current depression and remitted LLD showed lower ReHo in the superior frontal gyrus (Yuan et al., 2008; Yue et al., 2013), and precuneus (Yuan et al., 2008; Chen et al., 2012) compare to the HC group (**Table 5**).

rs-fMRI Studies Using ICA (Independent Component Analysis)

ICA is a data-driven approach that evaluates the whole brain and decomposes it into a set of independent components, each depicted as a functional map (Van Den Heuvel et al., 2009; Wang N. et al., 2012). Two studies used ICA to investigate alterations in both executive control network (ECN) and default mode network (DMN). One study found no significant difference between individuals with remitted and/or current depression LLD and the HC group (Sexton et al., 2012a). In the other study, individuals with LLD showed increased (i.e., inferior parietal) and decreased positive FC in the frontal-executive regions [i.e., dorsolateral prefrontal cortex (dlPFC)], and decreased positive FC in the corticolimbic regions (i.e., superior frontal) (Li W. et al., 2017) (**Table 5**).

rs-fMRI Studies Using Network and Graph Theory Analysis

Two studies used graph theory analysis. The first study compared four groups (LLD, aMCI, and LLD+aMCI, HC) and found alteration in small-worldness (Sigma) of all three patient groups, as well as separation of the ECN and DMN into two distinct modules in individuals with LLD and aMCI compared to HC group. In addition, individuals with LLD+aMCI had the greatest diminished global efficiency, and the most variable modular community structure compared to all other groups, as well as the greatest disruptions in the mean nodal efficiency followed by individuals with LLD and aMCI (Li et al., 2015a) (**Table 5**).

The other study compared the alteration of resting-state FC and topological organization of DMN in individuals with remitted LLD to an HC group, and found universally decreased FC and global efficiency, and increased characteristic path length of the DMN compared to the HC group (Yin et al., 2016b) (**Table 5**).

Other Methods

Five studies used other methods. One study (Yue et al., 2015) used Amplitude of Low-Frequency Fluctuations (ALFF) that measures spontaneous fluctuations in BOLD-fMRI signal intensity within the frequency range between 0.01 and 0.1 Hz. Thus, it indexes the intensity of low-frequency oscillations in rs-fMRI (Zang et al., 2007). In this study, the primary effect of disease or the interaction of disease and frequency in the LLD individuals with current depression vs. HC group was distributed over the frontal-executive (i.e., cerebellum, superior parietal) and corticolimbic (i.e., middle orbitofrontal, superior frontal gyri) regions (Yue et al., 2015).

Another study used a voxel-mirrored homotopic connectivity (VMHC) method and found a signal drop in the LLD individuals with current depression compared to the HC group, in the frontal-executive (superior temporal gyrus, posterior cerebellar) and corticolimbic (superior frontal gyrus) regions (Hou et al., 2016) (**Table 5**).

One study used a pairwise BOLD signal average correlation comparison of 87 regions in currently depressed LLD (Tadayonnejad et al., 2014) and found decreased FC in individuals with LLD compared to the HC group in frontal-executive (i.e., nucleus accumbens) and corticolimbic regions (i.e., rostral anterior cingulate cortex, superior frontal, and middle orbitofrontal gyri).

Harada et al. (2018) used a Pearson's correlation coefficient score between anterior cingulate cortex (ACC) and posterior superior temporal gyrus (pSTG). The LLD individuals with current depression showed no difference compared to the HC

group, however individuals with remitted LLD showed lower functional connectivity compared to the HC group (**Table 5**).

Finally, one study measured correlation degree and strength of functional connectivity (FC) between each pair of 36 seeds representing five major resting state networks (RSN) individuals with remitted LLD, aMCI, and HC group. Individuals with remitted LLD demonstrated reduced degree of FC in bilateral inferior temporal cortices (frontal-executive) compared to the HC group. In addition, individuals with remitted LLD and to a lesser extent individuals with aMCI showed reduced correlation in the executive control network (ECN) and default mode network (DMN) (Chen et al., 2016) (**Table 6**).

DISCUSSION

Overview

We reviewed studies measuring structural (DWI) or functional (rs-fMRI) properties of brain circuits in individuals with LDD—both early-onset depression recurring in late life and late-onset depression, as well as remitted and currently depressed—compared to a HC group. The aim of this review was to systematically investigate the evidence for structural and functional disruption in the frontal-executive and corticolimbic circuits that are implicated in dementia. We included 37 studies that met our eligibility criteria (19 DWI studies, 22 rs-fMRI studies, of which 4 studies had both DWI and rs-fMRI). Most studies but not all reported some impairment in both frontal-executive and corticolimbic circuits. We did not find any DWI or rs-fMRI studies directly comparing LLD with ADRD, however, seven DWI or rs-fMRI studies compared structural and functional alterations of brain circuits among LLD, MCI, or LLD+aMCI, and HC. All seven studies reported alterations in structure (Bai et al., 2012; Li et al., 2014; Mai et al., 2017) and function (Xie et al., 2013; Li et al., 2015a,b; Chen et al., 2016) of frontal-executive and corticolimbic circuits in those with LLD, aMCI and LLD+aMCI compared to HC individuals. Moreover, LLD+aMCI and aMCI groups were found to have more disruption in both circuits when compared to LLD (Bai et al., 2012; Li et al., 2014, 2015a,b; Mai et al., 2017). Although, five of these studies had sample sizes of less than 30 per group (Xie et al., 2013; Li et al., 2014, 2015a,b; Mai et al., 2017), these findings suggest that LLD and MCI share impairment of the same brain circuits and represent a possible continuum in disease progression toward ADRD.

Testing Our Hypothesis: Evidence and New Findings

In 2008, we proposed several models of biological mechanisms linking depression to Alzheimer's disease and related dementia (Butters et al., 2008). These models provided the insight that frontal-executive and corticolimbic circuits were potentially vulnerable in older adults with depression. We aimed to evaluate MR evidence published since 2008, for alteration in structure (DWI) and function (rs-functional MRI) of these two circuits. Despite the variability across reviewed studies with respect to study design, number of individuals, and analytic methods, some consistent results emerged. Impairment of both frontal-executive and corticolimbic circuits was present in individuals with LLD compared to HCs in some but not all studies. Some of these studies suffered from limitations of small sample sizes and heterogeneous samples (remitted vs. current, or remitted vs. non-remitting), which left them underpowered. This is important because not all individuals with LLD have MCI or develop dementia. In the few studies that included individuals with MCI or LLD plus MCI, the effects of LLD plus MCI were larger than the independent effects of these disorders, suggesting that the effects of LLD were magnified by comorbid cognitive impairment, and vice versa. This pattern is in line with our hypothesis and was implicated in previous papers as supporting the association between LLD and dementia (Butters et al., 2008; Bhalla et al., 2009; Wu et al., 2011; Andreescu et al., 2013; Boccia et al., 2015; Diniz et al., 2015, 2016; Smagula et al., 2015).

Another theme that emerged from our review is that an increasing number of studies are using network and graph theory analyses especially for comparisons among more than two groups. Four out of seven studies with additional comparison groups investigated whole brain topology in LLD, aMCI, and LLD+aMCI (Bai et al., 2012; Li et al., 2015a; Chen et al., 2016; Mai et al., 2017). These studies found disrupted network topologies—including reduced network strength, global efficiency and higher modularity- in individuals with LLD, LLD+MCI, and aMCI in comparison to HC. In addition to these differences in the global network topology, hub regions with significant diagnosis effects were mainly impaired in the frontal-executive and corticolimbic circuits. Results from network and graph theory analyses suggest that in addition to the alteration of frontal-executive and corticolimbic circuits, impairment of global brain topology is present in these disorders. Therefore, it is possible that some of the neuroimaging measurement strategies were not sufficiently addressing the underlying neurocircuit disruption, e.g., through a focus only on specific white matter tracts. Network-based analyses of brain structure and function may more directly index subtle disruptions in individuals with LLD, or LLD+MCI.

Finally, posterior DMN (posterior cingulate cortex and precuneus) appeared to have the most structural and functional alterations in LLD, and to a greater extent in aMCI and LLD+aMCI. For instance, in a DWI study, individuals with MCI had lower nodal efficiency in the posterior cingulate cortex compared to individuals with LLD (Bai et al., 2012). In rs-fMRI studies, significant ReHo reduction was detected in the posterior DMN in individuals with LLD (Yuan et al., 2008; Chen et al., 2012). In a unique longitudinal study, left hippocampal functional connectivity had a greater decline in bilateral posterior DMN in individuals with LLD compared to the HCs (Wang et al., 2015). Individuals with LLD+aMCI demonstrated lower functional connectivity in the posterior DMN relative to those with LLD (Yue et al., 2013; Li et al., 2015b). Posterior cingulate cortex is a critical brain region, and a marker of very early progression of AD as seen with T1-weighted imaging, functional MRI, postmortem, and PET studies (Rami et al., 2012; Scheff et al., 2015; Mutlu et al., 2016). These specific DMN nodes may be important as potential treatment targets in delaying the progression of LLD or LLD+MCI to dementia.

Limitations

The heterogeneity among individuals with depression as mentioned before (including early-onset depression vs. late-onset depression, and active vs. remitted, treatment-responsive vs. treatment-resistant), and medication effects including antidepressant and cognitive enhancer agents are factors that should carefully be controlled for in future studies. Hence, in the current study, we could not evaluate how different ages of onset, remission status of depression, medication types, or response to medications may influence circuit disruption. Some T1-weighted imaging studies have reported differences between early-onset depression and late-onset depression (Ballmaier et al., 2008; Sachs-Ericsson et al., 2013; Lebedeva et al., 2015), however further exploration in all imaging modalities is required. Moreover, one third of the studies have only used MMSE to measure cognition which lacks sensitivity to subtle changes in cognition and has shown to be influenced by ceiling and floor effects (Tombaugh and McIntyre, 1992). Thus, the confounding effect of cognitive impairment in individuals with LLD in these studies may contribute to the heterogeneity of results. Furthermore, it has been shown that depression increases the risk for developing other types of dementia including vascular dementia (VaD) (Diniz et al., 2013) and Dementia with Lewy bodies (DLB) (Fujishiro, 2019; Ishiguro et al., 2019). Thus, a detailed assessment of multiple types of dementia is required in future longitudinal studies.

Other MRI Modalities

There are other MRI modalities that were not addressed in this systematic review; T1-weighted image, T2-weighted Flair, and task-based fMRI. A meta-analysis assessed gray matter atrophy in 25 studies comparing AD and HC, and 6 studies comparing LLD and HC. It found gray matter volume reduction of the bilateral hippocampus in both individuals with LLD and AD, greater atrophy in frontal cortex in LLD, and greater atrophy of bilateral posterior cingulate cortex in AD. Two other meta-analyses of 17 VBM studies comparing LLD with HC (Sexton et al., 2013; Du et al., 2014). Sexton et al. (2013) found significant but small effect size of hippocampal volume reduction driven by seven studies, in addition to volume loss in the frontal cortex, putamen, and thalamus. Du et al. (2014) included 11 VBM studies and reported gray matter volume loss in parahippocampal gyrus, amygdala, and frontal cortex.

T2-weighted FLAIR imaging makes the detection of white matter hyperintensities (WMH) easier. WMH of presumed vascular origin is a common finding in individuals with cognitive impairment, stroke, or dementia (Wardlaw et al., 2015). Although a meta-analysis of 30 studies, reported significantly higher odds of WMH in LLD compared to HC, particularly in LOD vs. HC and EOD (Herrmann et al., 2008), recent studies have found no difference in WMH between older individuals with LLD and HCs (Harada et al., 2016) or between LLDs and aMCIs (Liao et al., 2017). Two longitudinal studies found no difference in WMH between individuals with remitted LLD and an HC group (Weber et al., 2012; Taylor et al., 2014), or between individuals with relapsed or non-remitted LLD, and an HC group (Taylor et al., 2014).

Finally, task fMRI has been used to study the functional activities and cognitive behaviors. With memory tasks, individuals with LLD, compared to HCs, demonstrated less activation of temporal lobe, hippocampus, amygdala; less functional connectivity between posterior cingulate cortex and medial temporal lobe; and enhanced activation in frontal cortex (inferior frontal gyrus, frontal pole, middle frontal gyrus) (Lee T. W. et al., 2013; Wu et al., 2013; Weisenbach et al., 2014). With executive function tasks, individuals with LLD showed deactivation in the dorsolateral prefrontal cortex, and lower functional connectivity between dorsolateral prefrontal cortex and dorsal anterior cingulate cortex, but higher BOLD response in frontal cortex (superior frontal cortices and left orbitofrontal cortex) (Aizenstein et al., 2009; Bobb et al., 2012). A meta-analysis of task-fMRI studies in individuals with MCI and AD found hypoactivation of the temporal lobe and compensatory hyperactivation in cingulate gyrus in both groups compared to the HC group (Li H. J. et al., 2015).

Conclusion and Future Directions

Taken together, findings of the 37 reviewed studies are partially supportive of structural and functional brain alterations in both frontal-executive and corticolimbic circuits, as well as whole brain topology in LLD. Future research should focus on investigating the longitudinal effect of depression and risk of multiple types of dementia including vascular (VaD), Lewy body (DLB), and Alzheimer's dementia (AD) using multi-imaging modalities and in large groups of individuals with LLD, never-depressed MCI and LLD+MCI. This information can help to elucidate the pattern of decline in individuals with a history of depression and the link with dementia and warrants further research. Thus, future studies should include milder and more severe depression cases, as well as cases in remission vs. cases who are actively ill, use multimodal imaging techniques and consider using sophisticated analysis methods in large enough sample sizes to determine which subgroups share risk in impaired brain circuit with MCI and dementia. Such a study is now in progress, funded by the NIMH. It aims to recruit 750 older individuals with treatment-resistant LLD, who are receiving standardized treatment as part of a pragmatic clinical trial. This longitudinal study will determine who among remitters vs. non-remitters (those with persistent depression), are at highest risk of neural circuit and cognitive change (including progression to dementia over a 24-month period). The specific aims are: (1) to test whether persistent (non-remitting) depression leads to greater cognitive decline focusing on executive and episodic memory cognitive domains and greater degradation of neural circuit crucial for effective for such cognitive functions; and (2) to test whether greater degradation of neural circuit is associated with greater cognitive decline. An exploratory aim is to build a data-driven [demographic, clinical, cognitive, imaging, senescence-associated secretory phenotype (SASP) index] model using multivariate learning methods to distinguish, at baseline, who is at greatest risk for cognitive

decline; and, to analyze latent class trajectories of depressive symptoms to go beyond the dichotomy of remission/non-remission to identify subsets of individuals at highest risk of cognitive decline, neural circuit change, and progression to dementia.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

NR-R: substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data for the work, drafting the work or revising it critically for important intellectual content, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. DM: substantial contributions to the conception or design of the work, or the acquisition of data for the work. MB and BM: substantial contributions to the conception or design of the work, revising the work critically for important intellectual content, and provide approval for publication of the content. AV: substantial contributions to the conception or design of the work, revising the work critically for important intellectual content, provide approval for publication of the content, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2020.00253/full#supplementary-material

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REVIEW
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Cortical Circuit Dysfunction as a Potential Driver of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that affects selected cortical and spinal neuronal populations, leading to progressive paralysis and death. A growing body of evidences suggests that the disease may originate in the cerebral cortex and propagate in a corticofugal manner. In particular, transcranial magnetic stimulation studies revealed that ALS patients present with early cortical hyperexcitability arising from a combination of increased excitability and decreased inhibition. Here, we discuss the possibility that initial cortical circuit dysfunction might act as the main driver of ALS onset and progression, and review recent functional, imaging and transcriptomic studies conducted on ALS patients, along with electrophysiological, pathological and transcriptomic studies on animal and cellular models of the disease, in order to evaluate the potential cellular and molecular origins of cortical hyperexcitability in ALS.

Keywords: amyotrophic lateral sclerosis, cerebral cortex, hyperexcitability, network dysfunction, intrinsic, extrinsic

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INTRODUCTION

Definition and Epidemiology of ALS

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset neurodegenerative disease of the motor neuron. It manifests as an initial focal muscular weakness and progresses into full paralysis of most of the skeletal muscles. ALS leads rapidly to death with a median survival of only 2 to 3 years of diagnosis. ALS was named after the initial histological description made by French neurologist Jean-Martin Charcot in 1869 (Charcot, 1874), who reported two pathological hallmarks in the spinal cord of patients: the degeneration of the corticospinal tract (CST), in the lateral columns (lateral sclerosis), and the disappearance of the spinal motor neurons (amyotrophic), in the ventral horns. This initial histological definition still corresponds to the current clinical definition of the disease. Indeed ALS is diagnosed when signs of corticospinal and corticobulbar neurons (CSN) degeneration (i.e., slowness of motions, hyperreflexia, spasticity), and of bulbar and spinal motor neurons (MN) degeneration (i.e., muscular weakness and atrophy, fibrillations, and fasciculations) are found in combination (Brown and Al-Chalabi, 2017; van Es et al., 2017). Above these precise histological and clinical descriptions, it is now also broadly admitted that ALS is a heterogeneous multisystem disease that also implies frequent extramotor

symptoms, and particularly behavioral and cognitive deficits, as well as defective energy metabolism (Dupuis et al., 2011).

ALS is the third most frequent neurodegenerative disease after Alzheimer's and Parkinson's diseases, with an incidence of 2.6/100,000 person-years and a prevalence of 7–9/100,000 persons (Hardiman et al., 2017b), which reflects the rapid progression of the disease even if very rare cases exist that evolve over decades. ALS affects mostly people in their late fifties, and is overall 1.5 times more frequent amongst men than women (Brown and Al-Chalabi, 2017; Rosenbohm et al., 2018). The vast majority of patients (90%) are considered sporadic, with no familial history. The remaining 10% instead are familial cases with a usually dominant transmission and a high penetrance (Brown and Al-Chalabi, 2017). More than 120 genetic mutations have been associated to ALS, and amongst those, at least 25 genes related to either familial or sporadic ALS, or both (Brown and Al-Chalabi, 2017). About 70% of familial ALS cases, and as much as 15% of sporadic ALS cases, have now been related to mutations (Chia et al., 2017). The most common causative genes are *C9ORF72* (close to 45% of familial cases), *SOD1* (close to 20% of familial cases), *FUS* and *TARDBP* (about 4% of familial cases each) (Brown and Al-Chalabi, 2017; Chia et al., 2017). These familial cases have greatly contributed to the study of the presymptomatic period, and the identification of the causative genes have and continue to inform the development of cellular and animal models of the disease.

The Debated Origin of ALS

The duality of the neuronal impairments that characterizes ALS is likely responsible for the greater severity of the disease in comparison with neurodegenerative diseases that target only the CSN (hereditary spastic paraplegia, primary lateral sclerosis) or the MN (Kennedy's disease, adult-onset spinal and muscular atrophy) (Sorenson, 2012). But this duality also raised the question of the origin of the disease, which remains controversial (Ravits and La Spada, 2009).

For Charcot, ALS was a progressive descending neurodegeneration, initiated in the motor cortex, spreading to the MN and ultimately affecting the neuromuscular junctions (Eisen et al., 1992), a view which was later called the "dying-forward" hypothesis. This view was further supported by typical symptoms revealed by deep clinical examinations, such as gait abnormalities or the split hand syndrome, which are highly suggestive of a cortical origin of the disease (Eisen et al., 1992, 2017). Two alternative scenarios were proposed later: a simultaneous and independent degeneration of CSN and MN – which was quickly abandoned, due to the somatotopic relationship that exists between CSN and MN degenerations – and a retrograde progression of the neurodegeneration, known as the "dying-back hypothesis" (for review, see Eisen and Weber, 2001). With the numerous rodent models that followed the discovery of *SOD1* (Lutz, 2018), and the more recent emergence of induced MN from ALS patients' iPSC (Guo et al., 2017), the vast majority of preclinical studies have concentrated on deciphering the mechanisms involved in MN degeneration, implicitly favoring the "dying-back" hypothesis.

Arguments Toward a Cortical Origin

Genetic and Clinical Evidences

Recent genetic data have established a clear link between ALS and frontotemporal dementia (FTD), a neurodegenerative disease that affects the frontal and temporal cortices and results in behavioral and cognitive deficits. Mutations on several common genes have been identified in ALS and FTD familial cases (Ling et al., 2013). While the clinical presentations of ALS and FTD are extremely different, about 15% of ALS patients develop over time behavioral and cognitive deficits typical of FTD, and about 15% of FTD patients develop over time motor impairments typical of ALS (Ling et al., 2013). Finally, the link between ALS and FTD is also pathologic: the vast majority of ALS patients, whether of familial or sporadic origin, and as much as 45% of FTD patients present with the so-called TDP-43 pathology, i.e., toxic intra-cytoplasmic inclusions of a phosphorylated and misfolded form of the TDP-43 protein (Neumann et al., 2006; Ling et al., 2013). Altogether, genetic, clinical and pathological data have converged to unravel a strong link between ALS and FTD. Both diseases are thus currently seen as the two extremes of a single clinical continuum, with identified common denominators among which is a shared affected region of the central nervous system, the cerebral cortex, which stresses a potential primary contribution of this structure to both diseases.

Histopathological Clues and the Corticofugal Hypothesis

Extensive examination of the TDP-43 pathology in post-mortem brains from ALS patients has shown that the motor cortex is the most affected region of the brain, together with the brain stem and spinal cord (Brettschneider et al., 2013). Importantly, by assessing the extent of the TDP-43 pathology in other brain regions, the authors proposed a "corticofugal hypothesis of ALS," with an origin of the pathology in the motor cortex, followed by a sequential corticofugal pattern of progression to the downstream targets of the motor cortex via a direct, mono-synaptic transmission, similar to a prion-like mechanism (Braak et al., 2013). Such a corticofugal propagation of neurodegeneration is supported by longitudinal diffusion tensor imaging studies with tractography or connectome analysis of ALS patients (Verstraete et al., 2013; Kassubek et al., 2014). These pathology and imaging data further support a primary impairment of the motor cortex in ALS.

Functional Studies and Early Cortical Hyperexcitability

Transcranial magnetic stimulation studies unraveled early hyperexcitability as a marker of cortical dysfunction in sporadic and familial ALS patients (Vucic and Kiernan, 2017). Importantly, longitudinal studies in pre-symptomatic *SOD1* mutation carriers revealed that cortical hyperexcitability develops prior to clinical onset of ALS (Vucic et al., 2008), and characterizes also early sporadic patients (Mills and Nithi, 1997; Zanette et al., 2002; Vucic, 2006; Hardiman et al., 2017a). The fact that cortical hyperexcitability negatively correlates with disease progression and survival (Shibuya et al., 2016) highlights the relevance of early cortical dysfunction to ALS onset and

progression. Cortical hyperexcitability has been proposed to translate into glutamatergic excitotoxicity to the downstream targets of CSN, and possibly of the whole corticofugal population (Geevasinga et al., 2016; Eisen et al., 2017; Vucic and Kiernan, 2017), contributing to the degeneration of spinal MN via an anterograde trans-synaptic mechanism (Eisen et al., 1992; Vucic and Kiernan, 2017).

First Connection Between Hyperexcitability and Pathology

In a recent article, Weskamp et al. (2019) investigated, in iPSC-derived glutamatergic neurons and in mouse primary cortical neurons, the link between hyperexcitability and TDP-43 pathology (Weskamp et al., 2019). In iPSC-derived glutamatergic neurons, neuronal activity was either induced with the potassium channel blocker tetraethylammonium (TEA) or blocked with the sodium channel blocker tetrodotoxin (TTX). In mouse primary cortical neurons, activity was induced or inhibited by glutamate or GABA, respectively. The authors elegantly demonstrated in the two models that increased neuronal activity was accompanied with increased TDP-43 immunoreactivity (Weskamp et al., 2019). They also demonstrated that hyperexcitability induced alternative splicing of *TARDBP* mRNA, leading to the up-regulation of a short isoform of the transcript. This short TDP-43 (sTDP-43) corresponded to the N-terminal part of the full-length protein, and contained a newly identified nuclear export sequence, leading to its cytoplasmic accumulation (Weskamp et al., 2019). Importantly, similar sTDP43 inclusions were identified within the spinal cord and cortex of sporadic ALS and *C9ORF72* ALS patients (Weskamp et al., 2019). In its whole, this study demonstrated for the first time a pivotal connection between neuronal hyperexcitability and TDP-43 pathology, and further supports the earliness of hyperexcitability in the cascade of pathologic events that characterize ALS.

In this review, we present a collection of studies spanning from clinical neurology to electrophysiology, pathology and transcriptomics, in order to interrogate the potential cellular and molecular origins of cortical hyperexcitability in ALS.

EVIDENCES FOR CORTICAL CIRCUITS DYSFUNCTION IN ALS

Cortical Excitability and Hyperexcitability

The terms "excitability" and "conductivity" were primarily related to the investigation of electrical properties of individual neurons and defined respectively as the capability of a structure to respond to a given electrical stimulus by generating an action potential (excitability) and to propagate it along its membrane (conductivity) (Hodgkin and Huxley, 1952). In this regard, "hyperexcitability" was thus initially considered as a decreased threshold of axonal membrane to respond to a stimulus. This initial definition evolved together with the methods to assess excitability at multiple levels of the nervous system. These span from the study of individual neuronal ion channels *ex-vivo*, to the investigation of entire brain regions with transcranial magnetic stimulation (TMS) in clinical neurology (Bakulin et al., 2016). Consequently, the term "hyperexcitability" adapted to describe more broadly an altered electrical activity of various entities, from ion channels to highly specialized neuronal networks, but still lacks a commonly accepted definition. Indeed, it varies from "the ability to respond to stimuli that normally do not evoke any response" (Bakulin et al., 2016), to "an increased or exaggerated response to a stimulus, which may usually have been expected to evoke a normal response" (Bae et al., 2013), to "the predominance of excitation over inhibition" (Bakulin et al., 2016).

Cortical excitability is critically dependent on healthy, balanced, excitatory and inhibitory components. Under physiological conditions, the ratio of excitation to inhibition is invariant and dynamically controlled by the interaction of neurotransmitters and neuromodulators with cellular receptors, leading to the final activation of neurons (Badawy et al., 2012). In the cerebral cortex, this balance is mainly equilibrated by two neurotransmitters, the excitatory glutamate that acts on N-methyl-d-aspartate (NMDA) and non-NMDA receptors, and the inhibitory gamma-aminobutyric acid (GABA) that binds $GABA_A$ and $GABA_B$ receptors (Badawy et al., 2012). Perturbation of the excitatory/inhibitory balance can lead to pathological changes in cortical excitability and to the development of neurological disease (Bozzi et al., 2017; Foss-Feig et al., 2017; Vucic and Kiernan, 2017). First attempts to assess cortical excitability were made some 40 years ago when Merton and Morton successfully stimulated the motor cortex by using transcranial electrical stimulation (TES) and delivered electrical impulses, through the scalp, to the primary motor cortex, activating underlying neurons and inducing the twitch of corresponding muscles (Merton and Morton, 1980). Despite their important achievement, the procedure was extremely painful, and Anthony T. Barker began exploring the use of magnetic fields to replace electrical stimulations. First stable transcranial magnetic stimulation devices were developed in the mid-1980s (Barker and Freeston, 1985). Using TMS, Ly et al. (2019) described cortical excitability as the strength of the response of cortical neurons to a given stimulation reflecting both neuron reactivity and response specificity and therefore constituting a fundamental aspect of human brain function.

TMS Assessment of Cortical Hyperexcitability in ALS

Commonly observed clinical features in ALS like brisk deep tendon reflexes and spasticity, that occur as a result of CSN failure, and spontaneous muscle twitching (fasciculation) and cramps that occur when MN become affected (Brown and Al-Chalabi, 2017), are considered to result from excessive electrical irritability (reviewed in Kleine et al., 2008). Caramia et al. (1991) conducted one of the first single pulse TMS paradigm (spTMS) studies to investigate excitability changes of motor responses to magnetic brain stimulation in patients with motor impairment (hyperreflexia, spasticity, and weakness) in the contexts of multiple sclerosis, ALS, spino-cerebellar ataxia, primary lateral sclerosis, and brain metastasis. ALS patients presented a particularly low threshold to induce motor evoked potential (MEP) (Caramia et al., 1991). Following spTMS studies

confirmed this lower "cortical" or "corticomotor" threshold, early in the disease and an increase of this same threshold as disease progressed (Eisen et al., 1993; Mills and Nithi, 1997; Desiato et al., 2002; Mills, 2003; Prout and Eisen, 2004). Lower cortical threshold suggested that the motor cortex of ALS patients was indeed hyperexcitable, at least at the beginning of the clinical manifestations (Caramia et al., 1991; Zanette et al., 2002).

Conventional paired pulse TMS paradigm (cppTMS), in which two stimuli, conditioning and test, are successively applied, allowed to further dissect components of motor cortex excitability (Kujirai et al., 1992; Nakamura et al., 1997) and provided evidences for the existence of early and late intracortical inhibition (ICI), as well as an intracortical facilitation (ICF) of the motor cortex (Nakamura et al., 1997).

The increasing knowledge about cortical hyperexcitability in ALS mainly comes from long and extensive work by the Australian group of researchers lead by Prof. M. Kiernan that introduced the threshold tracking variant of paired pulse (ttppTMS) (Vucic, 2006), along with several new parameters including: short interval intracortical inhibition (SICI), ICF, the long interval intracortical inhibition (LICI) and the short interval intracortical facilitation (SICF), cortical silent period duration (CSP), and index of excitation, all relevant in clinical research, diagnosis and understanding of ALS pathogenesis (reviewed in Rossini et al., 2015; Van den Bos et al., 2018). Reduction or absence of SICI, and increase of ICF have emerged as the two most robust biomarkers of cortical hyperexcitability in ALS (Vucic and Kiernan, 2017), and cortical hyperexcitability was identified as an intrinsic and early feature characterizing both sporadic and familial ALS cases (Vucic et al., 2008), thereby suggesting a similar pathophysiological process (**Figure 1**). Importantly, reduction of SICI negatively correlates with survival of ALS patients (Shibuya et al., 2016), and increased index of excitation negatively correlated with the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised score (ALS-FRS) (Van den Bos et al., 2018) highlighting the pathogenic importance of cortical hyperexcitability in ALS. In some familial cases that arise from the mutation of a known gene, cortical hyperexcitability has also been detected pre-symptomatically. This is the case of the *SOD1* mutation carriers (Vucic, 2006; Menon et al., 2015), but not the *FUS* or *C9ORF72* mutation carriers (Blair et al., 2010; Geevasinga et al., 2016).

Interestingly, cortical hyperexcitability (i.e., a combination of decreased SICI and/or CSP and/or increased ICF) was also reported in atypical forms of ALS, such as the primary lateral sclerosis (Geevasinga et al., 2016) characterized by a predominant upper motor neuron (UMN) phenotype, but also, and this is more surprising, the flail leg syndrome (Menon et al., 2016), characterized by a predominant lower motor neuron phenotype with absent or subtle UMN signs. Finally, cortical hyperexcitability has also been demonstrated in patients suffering from FTD (Bae et al., 2016). These studies indicate that cortical hyperexcitability is altogether a typical feature of ALS and closely related diseases, an early biomarker of the disease and a putative prognostic marker, highlighting a potentially important role of cortical circuits dysfunction in ALS pathogenesis.

Short interval intracortical inhibition and cortical silent period are believed to respectively reflect GABA actions on $GABA_A$ (Di Lazzaro et al., 2012, 2013) and $GABA_B$ (Chen et al., 2008) receptors, while ICF is believed to reflect activity of the glutamatergic system (Chen et al., 1998). Loss of GABAergic cortical inhibition was further confirmed by Positron Emission Tomography studies with the $GABA_A$-selective radiotracer [^{11}C] Flumazenil that showed binding reductions in sporadic or familial ALS patients compared to controls (Lloyd et al., 2000; Turner et al., 2005; Wicks et al., 2009; Yabe et al., 2012). As of today, it is admitted that dysfunction or degeneration of cortical inhibitory and excitatory components contribute together to cortical hyperexcitability in ALS, and not solely decreased inhibition or increased excitation (Nihei et al., 1993; Ziemann et al., 1996; Enterzari-Taher et al., 1997; Zanette et al., 2002; Karandreas et al., 2009).

Neuroimaging Studies in ALS

Several functional magnetic resonance imaging (fRMI) studies reported increased functional connectivity (or hyperconnectivity) in the brain of ALS patients compared to controls (Lulé et al., 2007; Douaud et al., 2011; Menke et al., 2016; Schulthess et al., 2016; Agosta et al., 2018), which was also confirmed by electroencephalography (EEG) (Iyer et al., 2015) and resting state magnetoencephalography (MEG) (Proudfoot et al., 2018).

In a longitudinal fMRI study on cortical representation of motor imagery and function during execution task, Lulé et al. (2007) reported a stronger response within premotor and primary motor areas for imagery and execution in ALS patients compared to controls at two time points, and a spread of this increased activity to the precentral gyrus and frontoparietal network at the second time point, indicating a progression over time. Cross-sectional and longitudinal analysis of resting state fMRI (RS-fMRI) data revealed increased functional connectivity successively in the motor, brainstem, ventral attention, and default mode/hippocampal networks (Schulthess et al., 2016), confirming widespread effect on connected brain networks. A recent modeling study based on MRI scans allowed predicting late stage disease burden based on early stage white matter alterations (Meier et al., 2020), further supporting disease spread from the motor cortex along white matter tracts in a spatiotemporal manner (Meier et al., 2020). Together, these studies support disease progression along the corticofugal tracts.

Two scenarios have been proposed to explain increased functional connectivity: first, recruitment of additional brain structures to compensate for the impairment between major nodes of functional networks, and second, loss of inhibitory influence resulting in increased correlation of spontaneous blood-oxygen-level-dependent signals (Douaud et al., 2011).

Increased functional connectivity upon loss of cortical inhibition in ALS is supported by PET studies (Lloyd et al., 2000) and post-mortem histological analyses (Nihei and Kowall, 1993; Nihei et al., 1993; Petri et al., 2003, 2006; Maekawa et al., 2004) (detailed in the section "Cortical Inhibitory GABAergic Interneurons in ALS"), and is in accordance with cortical

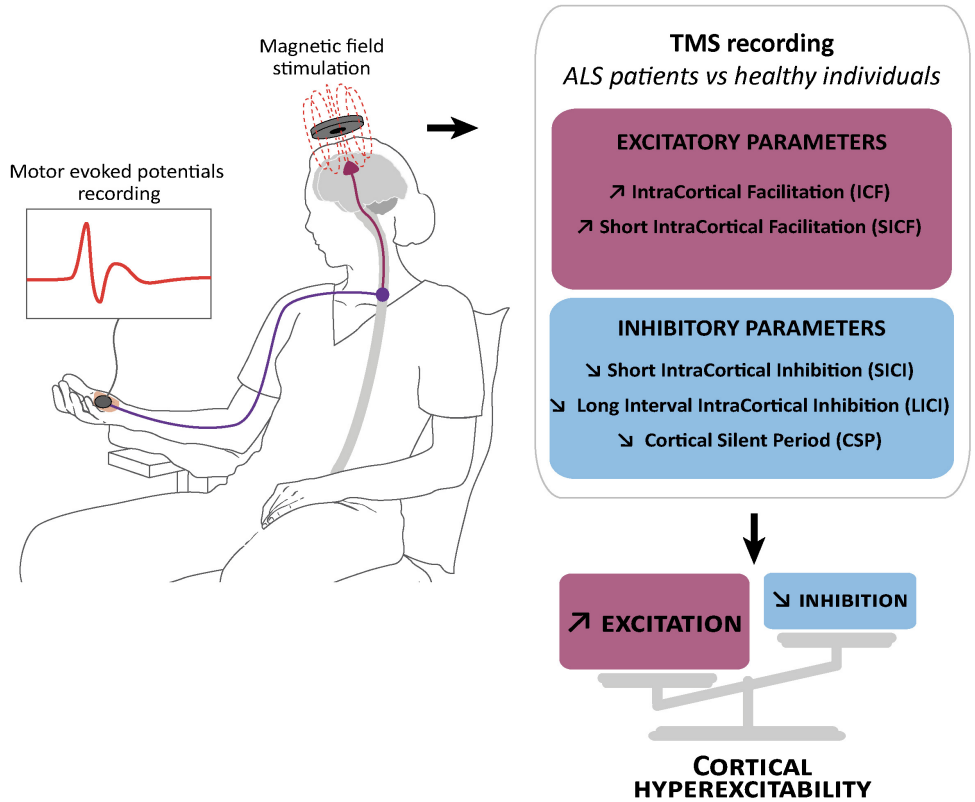


FIGURE 1 | Schematic representation of transcranial magnetic stimulation and cortical hyperexcitability in ALS. Transcranial magnetic stimulation (TMS) consists in the application of a magnetic field above the motor cortex, and the recording of motor evoked potentials in the abductor pollicis brevis muscle of the hand **(left)**. This technique showed reduction or absence of parameters related to cortical inhibition: **(right)** short interval intracortical inhibition (SICI), cortical silent period (CSP) and long interval intracortical inhibition (LICI), and an increase in parameters related to cortical excitation: intracortical facilitation (ICF) and short interval intracortical facilitation (SICF).

hyperexcitability revealed by TMS (Vucic, 2006), and further supported by a recent MEG study (Proudfoot et al., 2016).

Few studies on asymptomatic mutation carriers exist that highlight the earliness of cortical impairment in ALS. A functional MEG study reported excess beta-band desynchronization during movement execution in asymptomatic *C9ORF72* mutation carriers (Proudfoot et al., 2016). Similarly, DTI and RS-fMRI analyses revealed increased functional connectivity in asymptomatic *SOD1* or *C9ORF72* mutation carriers (Menke et al., 2016).

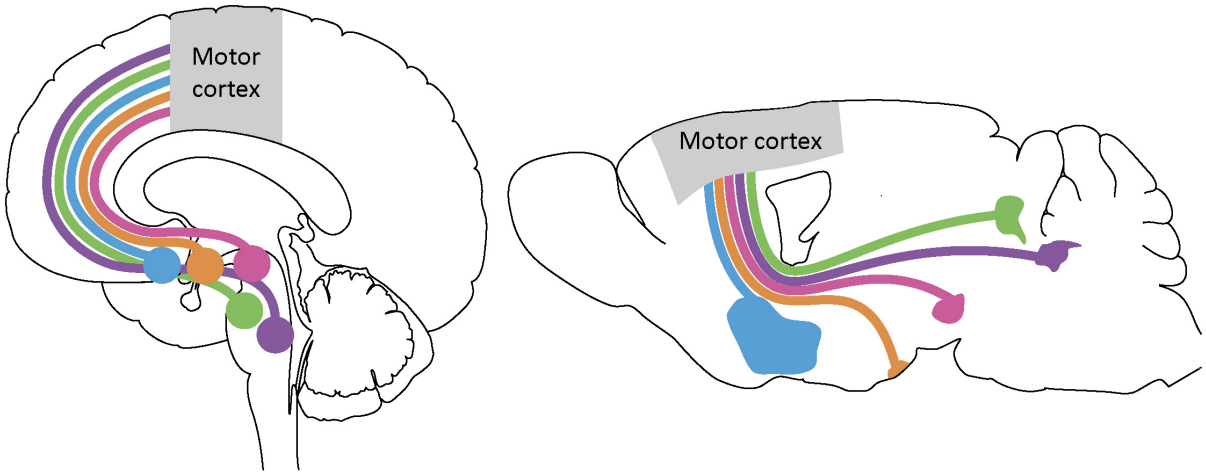
While the numerous neuroimaging studies conducted on ALS patients (further reviewed in Chiò et al., 2014; Proudfoot et al., 2019) do not formally demonstrate a cortical origin of ALS, they nevertheless confirm (i) pre-symptomatic cortical dysfunction, (ii) cortical hyperexcitability, and (iii) disease propagation along the cortical connectomes.

CORTICOPETAL MODULATIONS OF CORTICAL EXCITABILITY AND ALS

The cerebral cortex sends numerous corticofugal projections to the forebrain, midbrain and hindbrain, and as far as the spinal cord. Similarly, it receives a vast array of corticopetal projections from distant structures that influence its overall activity by the mean of various neuromodulators, the monoamines and acetylcholine (for excellent reviews see Gu, 2002; Vitrac and Benoit-Marand, 2017). This complex regulatory system includes the cholinergic neurons of the basal forebrain, the histaminergic neurons of the tuberomammillary nucleus in the hypothalamus, the serotonergic neurons of the dorsal raphe nucleus, the dopaminergic neurons of the ventral tegmental area (VTA), and the noradrenergic neurons of the locus coeruleus (**Figure 2**). ALS patients present different levels of alteration of these pathways that could all account for cortical dysfunction in the disease (**Figure 2**).

Serotonin and the Dorsal Raphe Nucleus

Serotonin is involved in numerous functions including sleep, attention or sensory information processing. Serotonergic neurons are confined to seven raphe nuclei of the brainstem. Their projections innervate a wide range of structures including the cerebral cortex, hypothalamus, amygdala, hippocampus, cerebellum, and spinal cord. The cerebral cortex is more specifically innervated by the dorsal raphe nucleus (Nestler et al., 2015). In Human, 13 serotonin receptors have been identified



	Dorsal raphe nucleus & Serotonin	Ventral tegmental area & Dopamine	Locus coeruleus & Noradrenaline	Tubero-mammillary nucleus & Histamine	Basal forebrain & Acetylcholine
ALS PATIENTS	Loss of 5-HT neurons in the brainstem	Alteration of DA signalling in the striatum (DA transporters and receptors)	Decreased NA levels in the spinal cord	Deregulation of histamine-related genes expression in the cortex TDP-43 inclusions in the TMN of 1/3 of patients	TDP-43 inclusions in the BF of 1/3 of patients
ALS MOUSE MODELS	Decreased 5-HT levels in the cortex Loss of 5-HT neurons in the brainstem	Decreased DA levels in the striatum and nucleus accumbens (VTA targets) Loss of DA neurons in the VTA	N/A	Deregulation of histamine-related genes expression in the cortex	Decreased ChAT activity in the cortex Decreased ACh neurons density in the forebrain

FIGURE 2 | Schematic representation of the monoaminergic and cholinergic systems and their impairments in ALS patients and mouse models. Some monoamine and acetylcholine nuclei send projections to the motor cortex both in human **(top left)** and mouse brain **(top right)**. Alterations in these systems have been found both in ALS patients **(table, upper part)** and ALS mouse models **(table, lower part)**. Dorsal raphe nucleus and its serotonine (5-HT) projections are represented in green; ventral tegmental area (VTA) and dopamine (DA) projections in pink; locus coeruleus (LC) and noradrenaline (NA) projections in purple; tuberomammillary nucleus (TMN) and histamine projections in orange and basal forebrain (BF) and acetylcholine (ACh) projections in blue.

amongst which only one is ionotropic and depolarizing. The 12 others are metabotropic with either excitatory or inhibitory effects. In the rodent motor cortex, serotonergic projections are found in every cortical layers (Vertes et al., 1999) and mainly act on excitatory neurons (Santana, 2004). In Human, both acute and chronic administration of paroxetine, a selective serotonergic reuptake inhibitor increased excitability of the motor cortex, as assessed by TMS (Loubinoux et al., 2005). This motor output facilitation was further confirmed by pharmacological and electrophysiological manipulations in the rat (Scullion et al., 2013). It is hypothesized that this effect is mediated by activation of the 5-HT_{1A} receptor expressed by GABAergic interneurons,

its hyperpolarizing effect on inhibitory neurons resulting in the release of inhibition on excitatory neurons (Puig et al., 2010; Vitrac and Benoit-Marand, 2017).

Serotonergic neurons also innervate the spinal cord and lower MN in particular. Serotonin action on motoneurons increases persistent calcium current, contributing to the maintenance of their excitability (Heckman et al., 2009). Dentel et al. (2013) showed the degeneration of serotonergic projections to the spinal cord and hippocampus, sometimes accompanied with the loss of serotonergic neuron cell bodies in the brainstem of ALS patients. The *Sod1*^{G86R} mouse model of ALS displayed similar serotonergic degeneration, along with a presymptomatic decrease of serotonin levels in the brainstem and spinal cord (Dentel et al., 2013). Loss of serotonin innervation of motoneurons was accompanied by up-regulation of the constitutively active 5-HT$_{2B/C}$ receptors, involved in the development of spasticity (Dentel et al., 2013). Degeneration of serotonergic neurons in *Sod1*^{G86R} mice was involved in the spasticity recorded in end-stage animals (Oussini et al., 2017), and treatment of the *Sod1*^{G86R} animals with a serotonin inverse agonist abolished end-stage spasticity (Dentel et al., 2013). Importantly, maintenance of serotonergic neurons and projection to the spinal cord in *Sod1*^{G86R} animals accelerated appearance of disease onset and worsened motoneuron degeneration without affecting the survival of the animals (Oussini et al., 2017). On the other hand, inhibition of serotonin reuptake by administration of Fluoxetine to 30 day-old presymptomatic or 70 day-old symptomatic *SOD1*^{G93A} mice did not affect their survival or motor performances, but transient postnatal Fluoxetine administration proved to be detrimental (Koschnitzky et al., 2014). In all, these studies demonstrated that early stimulation of the serotonergic system in ALS mouse models, either by preventing loss of serotonergic neurons (Oussini et al., 2017), or by treatment of young pups with serotonin reuptake inhibitor may have detrimental effects on disease onset and progression (Koschnitzky et al., 2014). It would be particularly interesting to investigate whether decreased levels of cortical serotonin observed in presymptomatic *Sod1*^{G86R} mice (Dentel et al., 2013) has an impact of cortical network function. If similar mechanisms occur in the cortex and spinal cord, it is possible that decreased levels of cortical serotonin could be compensated by the up-regulation of constitutively active serotonergic receptors. This could very well contribute to overall cortical hyperexcitability. Interestingly, decreased binding of the 5-HT$_{1A}$ receptor PET radiotracer [^{11}C]-WAY100635 was reported in several cortical areas of sporadic ALS patients compared to controls (Turner et al., 2005; **Figure 2**), but to our knowledge, binding to the constitutively active 5-HT$_{2B/C}$ receptors in the cerebral cortex of ALS patients has not been reported yet.

Dopamine and the Ventral Tegmental Area

Dopamine neurons are located in the VTA and the subtantia nigra pars compacta (SNc), both found in the brainstem. Dopaminergic projections reach different cortical areas, including the motor cortex, with a predominance in the frontal cortex, along with other structures: nucleus accumbens, striatum and hippocampus (Nestler et al., 2015). These dopaminergic projections to the motor cortex arise from the VTA and mostly target the deep layers (Vitrac and Benoit-Marand, 2017). While it is not yet clear whom of the excitatory projection neurons and inhibitory interneurons express D1 like and D2 like receptors, stimulation of the VTA of anesthetized rats induced a fast excitatory-inhibitory response of M1 neurons leading to overall facilitation of motor output to forelimb muscles (Kunori et al., 2014). However, depressions of cortical neuronal activity in response to dopamine were also reported (Gu, 2002; Vitrac and Benoit-Marand, 2017). A TMS study carried on healthy individuals showed that the receptor agonist cabergoline, thought to act mostly on D2 and D3, decreased cortical excitability (by increasing SICI) (Korchounov et al., 2006). Similarly, enhanced cortical inhibition by dopamine receptor agonists pergolide and bromocriptine, and decreased cortical inhibition by dopamine receptor antagonist haloperidol were previously reported (Ziemann et al., 1996). These data suggest that dopamine may act as a break to motor cortex excitability in humans.

This hypothesis is further supported by data obtained from Parkinson's disease patients. Parkinson's disease arises from the degeneration of dopaminergic neurons of the substantia nigra pars compact (Michel et al., 2016), but loss of neurons in the VTA has also been reported (Alberico et al., 2015). Interestingly, Parkinson's disease was also associated with cortical hyperexcitability mostly arising from decreased inhibition which can be partially reversed by dopaminergic therapies (Ridding et al., 1995; Vucic and Kiernan, 2017). Dopaminergic impairment (Martorana, 2014) and cortical hyperexcitability (Vucic and Kiernan, 2017) have also been documented in Alzheimer's disease. Similarly to the effect of dopaminergic therapies on cortical excitability in Parkinson's disease, L-Dopa restored normal intra-cortical inhibition in Alzheimer patients (Martorana, 2014). These data suggest that dopamine is involved in the modulation of cortical activity and that dopaminergic system impairment can lead to loss of cortical inhibition, and potentially to cortical hyperexcitability.

Less is known about potential dopaminergic impairment in ALS. Single photon emission computed tomography (SPECT) studies revealed a decrease of dopaminergic terminals in the putamen and caudate nucleus of ALS patients (Borasio et al., 1998), and a decrease of striatal D2 receptors (Vogels et al., 2000) without neuronal loss. Interestingly Vogels et al. (2000) attributed the loss of D2 receptor to increased glutamatergic signaling from the cerebral cortex, via the corticostriatal pathway. Fu et al. (2017) also reported on the reduced binding of ^{18}F-fallypride (D2/D3 antagonist) in different regions of the cerebral cortex. However, these studies did not investigate or report on dopaminergic signaling onto the motor cortex itself. In end-stage *SOD1*^{G93A} mice, Kostic et al., 1997 reported decreased levels of dopamine in the caudate-putamen and nucleus accumbens, together with a loss of dopaminergic neurons in both the VTA and the subtantia nigra pars compacta. In the same mouse line, MRI analysis recently confirmed VTA impairment and immunohistology revealed a loss of up to 50% of dopaminergic neurons in the VTA, and vacuolization of the remaining ones at disease end-stage

(Jouroukhin et al., 2013; **Figure 2**). Given the likely inhibitory role of dopamine on cortical networks, and the clinical and preclinical arguments indicating an impairment of the dopaminergic system in ALS patients and mouse models, it would be interesting to determine whether such impairment could also affect the motor cortex and contribute to its hyperexcitability.

Noradrenaline and the Locus Coeruleus

The large majority of noradrenaline (or norepinephrine) is produced by neurons located in the locus coeruleus, a nucleus lying on the floor of the 4th ventricle in the rostral pons. A small proportion of the noradrenaline also arises from the brainstem and the lateral tegmental regions (Nestler et al., 2015; Schwarz and Luo, 2015). The only source of cortical noradrenaline is the locus coeruleus, which also innervates other regions of the forebrain, the brainstem, the cerebellum and the spinal cord. In the motor cortex, noradrenergic projections innervate every cortical layers but a greater density is found in layer I and layer VI (Agster et al., 2013). Noradrenaline acts on adrenergic receptors, which are G-protein coupled receptors. This family is composed of nine members that belong to the α- or β-subfamilies, both types found in the cerebral cortex. Whereas all β-receptors are excitatory, α-receptors can be excitatory or inhibitory, depending on the G-protein. Treatment with selective noradrenaline reuptake inhibitor or α_2 antagonist demonstrated in TMS that noradrenaline enhanced cortical excitability in healthy humans (Plewnia et al., 2001).

Pathological studies revealed neurofibrillary tangles in the locus coeruleus of ALS patients (Orrell et al., 1995) but apparently no TDP-43 pathology (Brettschneider et al., 2013) or cell loss (Hoogendijk et al., 1995). However, liquid chromatography analysis of spinal cord samples from ALS patients revealed reduced levels of noradrenaline compared to controls (Bertel et al., 1991), suggesting that noradrenergic impairment could occur in ALS, without necessary loss of neurons in the locus coeruleus (**Figure 2**). If little is known about the state of the locus coeruleus and the noradrenergic system in ALS, this is not the case under other neurodegenerative conditions, and more particularly in PD and AD (for excellent review, see Marien et al., 2004) where neuronal loss in the locus coeruleus is more important than in the substantia nigra in PD, or the nucleus basalis in AD (Zarow et al., 2003).

Histamine and the Tuberomammillary Nucleus

In the brain, histamine is synthesized exclusively by neurons located in the tuberomammillary nucleus which lies within the posterior hypothalamus (Nestler et al., 2015), and is implicated in sleep–wake cycle, nociception, motor circuits, satiety signaling, and neuroimmune functions (Panula and Nuutinen, 2013). Histaminergic neurons project both in the brain and the spinal cord with dense projections to the cerebral cortex. Histamine acts as a neurotransmitter and activates four subfamilies of metabotropic receptors, H_{1-4} leading either to neuronal depolarization or hyperpolarization of neurons, depending on the identity of the G-protein (Haas and Panula, 2003; Shan et al., 2015). In the rodent motor cortex, histaminergic projections are found in every cortical layers (Panula et al., 1989) and can act both on excitatory and on inhibitory neurons. In addition to direct connections to the cerebral cortex, histamine may also indirectly influence cortical function via activation of cholinergic basal forebrain neurons and induction of acetylcholine release in the cortex (Cecchi et al., 2001; Zant et al., 2012). Finally, treatment with H_3 receptor inverse agonist was shown to trigger increased levels of serotonin, noradrenaline and dopamine in the rat prefrontal cortex highlighting a potential central role of histamine in regulating the function of several cortical areas (Flik et al., 2015).

Detailed analysis of the hypothalamus of ALS patients revealed the presence of TDP-43 pathology in 5/28 cases (Cykowski et al., 2014), suggesting that neurons of the tuberomammillary nucleus may not be a primary target of the TDP-43 pathology, and may maintain, in most cases their function. The group of Sebastiano Cavallaro characterized the transcription profile of the motor cortex from sporadic ALS patients compared to healthy individuals (Aronica et al., 2015). Further data mining allowed them to identify the significant up-regulation of the genes involved in histamine synthesis (histidine decarboxylase, HDC) and catabolism (histamine N-methyl transferase and diamine oxydase, HNMT) (Apolloni et al., 2017). These data were further corroborated by western blot analyses of *SOD1*^{G93A} mouse cerebral cortex showing transient symptomatic up-regulation of HDC and HNMT, along with early up-regulation of H_4 and late up-regulation of H_1 receptors (Apolloni et al., 2017). Together these studies suggest that in ALS patients and mouse models the histaminergic system may be overall up-regulated, similarly to what has been reported for Parkinson's or Huntington's diseases (Shan et al., 2015). Importantly, symptomatic treatment of *SOD1*^{G93A} mice with histidine, the brain-permeable precursor of histamine, improved motor performance and increased survival (Apolloni et al., 2019). Whether this beneficial effect can solely be attributed to an anti-inflammatory response of cortical and spinal microglia, or to additional improvement of cortical and spinal network functions remains to be determined, but this seminal study strongly highlights the histaminergic system as a new therapeutic target in ALS (Apolloni et al., 2019; **Figure 2**). In addition to its direct effect on cortical cells and networks, histamine's indirect effects via serotonin, noradrenaline, and dopamine (Flik et al., 2015), all known to also modulate motor cortex activity (Vitrac and Benoit-Marand, 2017), make this monoamine a particularly interesting neuromodulator to follow in the context of ALS cortical dysfunctions and therapeutic intervention.

Acetylcholine and the Basal Forebrain

In the human brain, the vast majority of cholinergic neurons are found clustered in eight small nuclei located in the basal forebrain and the brainstem, but scattered cholinergic neurons are also present in different brain regions (Nestler et al., 2015). Cholinergic projections to the cerebral cortex arise mostly from the nuclei of the basal forebrain, and more particularly the nucleus basalis and the substantia innominata, and follow a precise and complex topographical organization

(reviewed in Ballinger et al., 2016). In the cerebral cortex, acetylcholine acts both on excitatory pyramidal neurons and inhibitory interneurons. In addition, acetylcholine acts on two types of receptors: the ionotropic nicotinic receptors and the metabotropic muscarinic receptors. While binding of acetylcholine on nicotinic receptors induces depolarization, facilitating the excitation of the post-synaptic cell, binding of acetylcholine on muscarinic receptors induces either depolarization or hyperpolarization depending on the G-protein coupled to the receptor, and leads either to excitation or inhibition of the post-synaptic neuron (Ballinger et al., 2016). The duality of effects – excitatory or inhibitory –, along with the duality of targeted neuronal populations – excitatory or inhibitory –, and the variety of responses across cortical layers reflects the subtlety of acetylcholine action on cortical networks (Ballinger et al., 2016). Overall, if many studies converge toward a slow modulatory effect of acetylcholine resulting in an increased excitability of the targeted networks, recent work indicates that transient and faster kinetics of cholinergic signaling also exist (Sarter et al., 2014).

Basal forebrain cholinergic neurons have been mostly studied for their physiological role in sensory detection, attention, learning and memory, and their pathological contribution to cognitive decline (for review see Ballinger et al., 2016), but much less attention has been given to their effect on the motor cortex, or in the context of ALS. Kuo et al. (2007) demonstrated in the human motor cortex that acetylcholine could either increase or decrease cortical excitability, according to the basal stimulation conditions of the network, improving signal-to-noise ratios and refining information processing. Cykowski et al. (2014) reported the presence of cytoplasmic inclusions of TDP-43 in the basal forebrain and hypothalamus of one-third of ALS patients, suggesting that impairment of cholinergic neurons may occur in ALS. However, radioactive labeling of muscarinic receptors in the cerebral cortex did not show any difference between ALS patients and controls (Gredal et al., 1996), suggesting that basal forebrain alteration might not impact muscarinic receptors density in the cortex. Finally, symptomatic $SOD1^{G93A}$ mice displayed decreased numbers of cholinergic neurons in the basalis nucleus of the basal forebrain, that could account at least partly for the overall decrease in acetylcholine transferase activity measured in the cerebral cortex of these same animals (Crochemore et al., 2005; **Figure 2**). Thus, while little is known about a possible contribution of acetylcholine and basal forebrain cholinergic neurons to physiological motor cortical network function, and dysregulation in ALS, the subtle role of this neurotransmitter in other cortical areas and its implication in other neurodegenerative conditions such as Alzheimer's or Parkinson's diseases provide appealing background to push forward the investigation of the cholinergic system in the field of ALS.

All above-mentioned neuromodulators can impact the activity of the motor cortex (Vitrac and Benoit-Marand, 2017). Some of them have already been associated with neurodegenerative conditions, and thus represent possible contributing factors to cortical hyperexcitability in ALS (**Figure 2**).

MORPHOLOGICAL AND FUNCTIONAL ALTERATIONS OF CORTICAL EXCITATORY AND INHIBITORY NEURONS IN ALS

Whether of extra- or intra-cortical origin, or both, cortical excitation/inhibition (E/I) imbalance results from a dysfunction of the cortical circuits, i.e., from one or several of the cellular components of the cerebral cortex: the excitatory glutamatergic projection neurons, the inhibitory GABAergic interneurons, and possibly the astrocytes, for their contribution to the tripartite synapse and role in neuronal network development and maintenance (Farhy-Tselnicker and Allen, 2018). Analysis of the contribution of these cellular populations and their numerous sub-populations is limited in patients, because the techniques that can be applied on alive persons remain at the scale of the network/structure, and because analysis of post-mortem tissues, while extremely relevant, provides the snapshot of an exhausted network, and little information about the succession of events that drove it there. This is where preclinical models, and particularly rodent models of the disease can be extremely useful, proven that they recapitulate a few essential hallmarks of the disease.

Evidences for Early Cortical Impairment in Rodent Models of ALS

If the spinal cord, the MN and the skeletal muscle occupy a prominent place in the landscape of preclinical ALS research, a few recent seminal studies contributed to shed light on a possible early role of the cerebral cortex and its neuronal populations in ALS pathophysiology. Indeed, several mouse models of the disease recapitulate CSN or subcerebral projection neuron (that comprise CNS) degeneration. This is true for several mutant *SOD1* mouse models (Zang and Cheema, 2002; Ozdinler et al., 2011; Yasvoina et al., 2013; Marques et al., 2019) but also *C9ORF72* (Liu et al., 2016) *PFN1* (Fil et al., 2016), and *TARDBP* (Wegorzewska et al., 2009; Herdewyn et al., 2014). In several of these models, CSN loss precedes motor symptoms and neuromuscular junction denervation (Zang and Cheema, 2002; Ozdinler et al., 2011; Yasvoina et al., 2013; Marques et al., 2019), indicating that cortical alterations may take place very early in these models. Recently, we ran a comprehensive spatiotemporal analysis of CSN degeneration in the $Sod1^{G86R}$ mouse model of ALS, and showed that loss of CSN preceded loss of MN but also neuromuscular junction denervation, and weight loss (Marques et al., 2019). Early loss of CSN was accompanied with pre-symptomatic occurrence of hyperreflexia, a component of the UMN syndrome (Burg et al., 2019). Finally, CSN and MN degenerations were also somatotopically related, further suggesting that early cortical impairment may negatively influence MN function and survival (Marques et al., 2019). Together, these studies indicate that loss of CSN or subcerebral projection neurons not only occurs in mouse models of ALS, but also that, when temporarily assessed, precedes MN degeneration, suggesting that alteration of cerebral cortex is a very early event on the time scale of disease progression.

First genetic manipulation in the cerebral cortex of a rat model of ALS further informed on the role of this structure in disease onset and progression. Thomsen et al. (2014) knocked-down a mutant *SOD1* transgene in the posterior motor cortex of the $SOD1^{G93A}$ rat model of ALS, using an AAV9 virus to selectively transduce neurons. AAV9-SOD1-shRNA injections delayed disease onset and extended survival without affecting disease duration (Thomsen et al., 2014). Recently, we experimentally tested the corticofugal hypothesis of ALS, by generating a mouse line ubiquitously overexpressing the murine $Sod1^{G86R}$ transgene, a condition sufficient to mimic ALS symptoms and premature death (Ripps et al., 1995), and knocked-out for the gene *Fezf2*, a condition sufficient to prevent the specification of subcerebral projection neurons (Molyneaux et al., 2005). Absence of subcerebral projection neurons, and in particular of CSN, was sufficient to delay disease onset, limit weight loss and motor decline and extend survival of the animals, providing a first experimental support to the corticofugal hypothesis (Burg et al., 2019). Together, these studies provide evidence for an early contribution of cortical dysfunctions in ALS.

Cortical Excitatory Glutamatergic Neurons, and Corticospinal Neurons in ALS

In Humans, loss of CSN, or upper motor neurons (UMN), or Betz cells, and of their projections within the CST, results in appearance of the UMN syndrome, a series of symptoms including decreased motor control, altered muscle tone and strength, hyperreflexia, spasticity, and clonus (Ivanhoe and Reistetter, 2004), and allows the diagnosis of ALS, when combined with signs of bulbar or spinal MN degeneration (muscle denervation). Quantification of the progressive loss of Betz cells can be achieved in alive patients with the triple stimulation technique which estimates the proportion of motor units that can be stimulated by TMS (Wang et al., 2019), but to our knowledge, the technique has not been used yet for longitudinal analysis of CSN loss in ALS, or on pre-symptomatic patients. Together with a "pallor of the CST" (Charcot, 1874) that runs laterally in Human spinal cord, post-mortem analyses of ALS patients revealed a depletion of the giant Betz cells (CSN) of about 50–60%, and a shrinkage of the remaining Betz cell bodies (Nihei et al., 1993; **Figure 3**).

As already mentioned, various mouse models of the disease present with a loss of CSN or of cortical layer V subcellular projection neurons at disease end stage, such as the *C9-BAC* (Liu et al., 2016), the $hPFN1^{G118V}$ (Fil et al., 2016), the $TDP\text{-}43^{A315V}$ mouse models (Wegorzewska et al., 2009; Herdewyn et al., 2014) and the $TDP\text{-}43^{G298S}$ (Müller et al., 2019). *SOD1* mouse, generated earlier and further analyzed display a progressive loss of CSN that starts pre-symptomatically (Zang and Cheema, 2002; Ozdinler et al., 2011; Yasvoina et al., 2013; Marques et al., 2019; **Figure 3**). These studies reflect early alterations of the motor cortex of mouse models of the disease. In the context of overall cortical hyperexcitability, presymptomatic loss of a subpopulation of cortical excitatory neurons may reflect the consequence of an over-active network, rather than the origin of this E/I imbalance. Thus paralleled longitudinal analyses to compare the rate of CSN loss and the progression of cortical hyperexcitability, either on patients, or in mouse models of the disease, would be particularly informative.

In mice, CSN and other layer V subcerebral projection neurons display, prior to their degeneration, morphological alterations or electrophysiological dysfunctions that reflect either their hyperexcitable state or altered excitatory and inhibitory inputs, or both. In young and presymptomatic $SOD1^{G93A}$ mice, CSN and other layer V subcerebral projection neurons present selective apical dendrite degeneration, together with a lack of spines and a vacuolation, but no impairment of the basal dendrites (Jara et al., 2015), before they start degenerating. Progressive spine loss was also reported in apical and basal dendrites of layer V subcerebral projection neurons located in the motor cortex of presymptomatic $TDP\text{-}43^{A315V}$ mice (Handley et al., 2018; **Figure 3**). Such features were also reported in the remaining Betz cells of sporadic and familial ALS cases (Hammer et al., 1979; Genç et al., 2017). In the motor cortex of $TDP\text{-}43^{Q331K}$ mice instead, Fogarty et al. (2016) reported increased spine densities along the proximal and distal compartments of apical and basal dendrites of layer V subcerebral projection neurons. While the two TDP-43 mouse models apparently display opposite spine phenotypes, it is worth mentioning that both affect the formation or maintenance of dendritic spines, in accordance with an emerging role of TDP-43 and other RNA-binding protein in synaptic integrity (Sephton and Yu, 2015).

Such morphological abnormalities are accompanied by changes in electrophysiological properties. Initial work from the team of Cristina Zona reported intrinsic hyperexcitability of primary cortical neurons from $SOD1^{G93A}$ embryos, compared to controls, as a result of increased persistent voltage-dependent Na^+ current (I_{NaP}) (Pieri et al., 2009). Patch-clamp methods on brain slices further demonstrated hyperexcitability of layer V subcerebral projection neurons from the motor cortex of presymptomatic (21 and 30 day-old) $SOD1^{G93A}$ mice (Saba et al., 2015). Kim et al. (2017) patched retrogradely labeled CSN and callosal projection neurons (CPN) present in the layer V of $SOD1^{G93A}$ motor cortex, and demonstrated increased intrinsic excitability of both neuronal types (Kim et al., 2017). Layer V subcerebral projection neurons displayed increased excitatory synaptic inputs in the presymptomatic $TDP\text{-}43^{Q331K}$ mice (Fogarty et al., 2016), but unchanged (P21) or decreased (P60) frequency of excitatory synaptic transmission in the presymptomatic $TDP\text{-}4343^{A315T}$ mice (Handley et al., 2018; **Figure 3**). However, P21 layer V subcerebral projection neurons from the $TDP\text{-}43^{A315T}$ mice presented decreased inhibitory synaptic transmission resulting in hyperexcitability that was sustained throughout disease progression (Zhang et al., 2016). Interestingly, similar observations had been made previously in the Wobbler mouse model of ALS (Nieto-Gonzalez et al., 2010).

Cortical Inhibitory GABAergic Interneurons in ALS

Cortical GABAergic interneurons (INs) can be differentiated by molecular markers, firing pattern and cortical layers organization

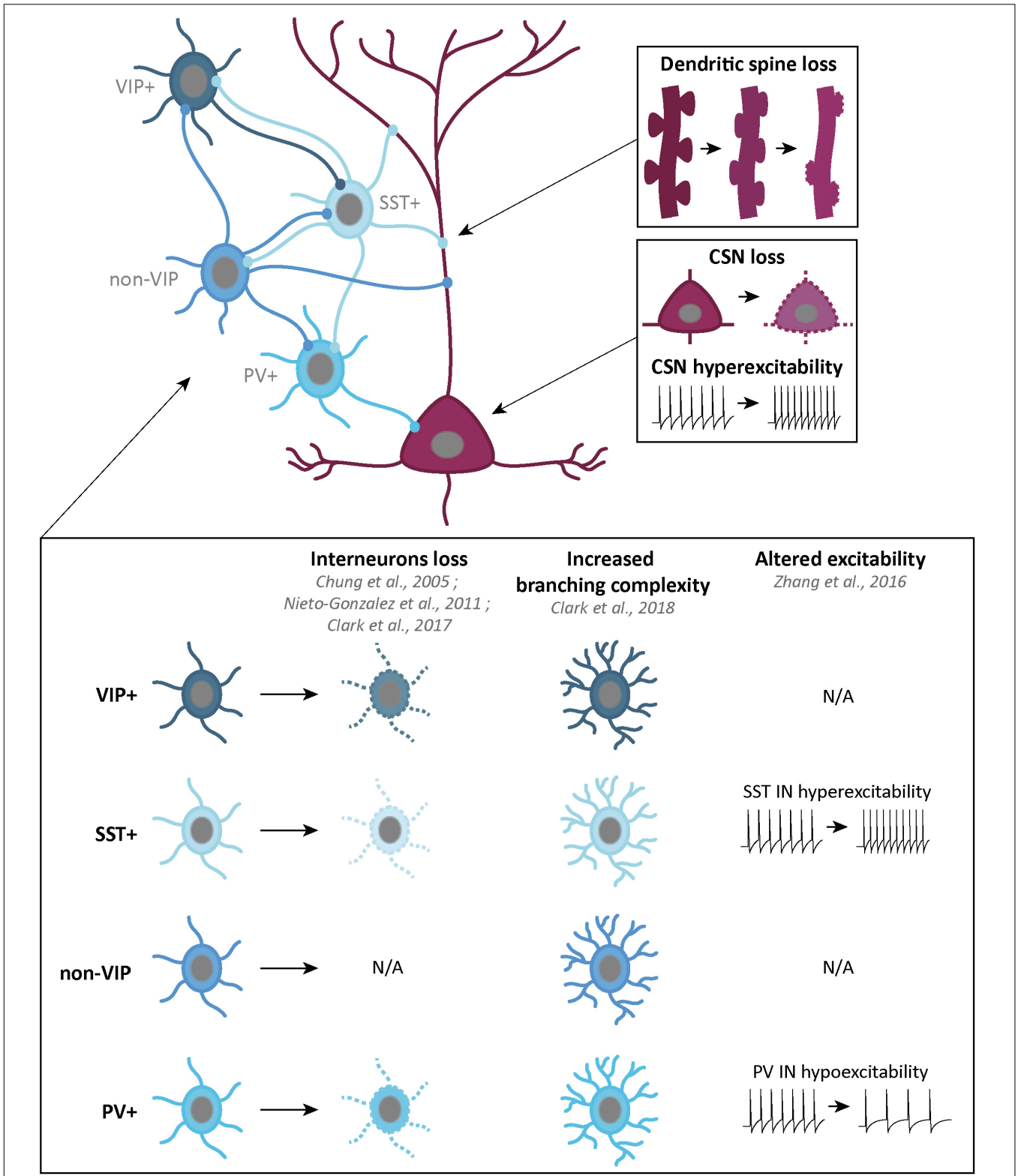


FIGURE 3 | Schematic representation of the corticospinal neurons and GABAergic interneurons, and their alterations in ALS patients or mouse models. Corticospinal neurons (CSN) are excitatory glutamatergic neurons (dark pink) which, in ALS, present progressive dendritic spine loss **(top right)**, cell loss and hyperexcitability of the remaining neurons **(middle right)**. GABAergic interneurons (IN) are inhibitory neurons (four shades of blue) which can be classified in four groups: vasointestinal peptide (VIP) IN, somatostatin (SST) IN, non-VIP IN and Parvalbumin (PV) IN. Some alterations in IN density, branching complexity and excitability have been found in different ALS mouse models and ALS patients (bottom part).

(Markram et al., 2004). Recently, a classification into three broad subgroups has been proposed: parvalbumin (PV)-positive INs (∼40%), somatostatin (SST)-positive INs (∼30%) and 5-HT_{3A}R-positive INs (∼30%) (Tremblay et al., 2016). Other markers also overlap with these groups. Neuropeptide Y (NPY), is expressed in a subpopulation of SST+ INs, and 5-HT_{3A}R+ INs group can be divided into vasointestinal peptide (VIP)-positive and non-VIP INs. Finally, while some SST+ INs and VIP+ INs share calretinin (CR) as a marker, some SST+ and non-VIP INs share both nitric oxide synthase (NOS) and Reelin markers (Tremblay et al., 2016; **Figure 3**).

Interneurons inhibit not only projection neurons but also other interneurons. Layers V projection neurons, including CSN, receive GABAergic inputs from non-VIP INs on their apical dendrite in layers I and II/III, and on their basal dendrites in layers V/VI. SST+ INs inputs only come from layers V/VI. Finally, layers V/VI PV+ INs provide somatic inhibition. VIP+ INs are specifically involved in SST+ INs inhibition (**Figure 3**).

Impairment of GABAergic inhibition, starting with the loss of cortical INs, represents a simple way to explain cortical hyperexcitability in ALS. PET studies conducted on ALS patients with [^{11}C]flumazenil revealed a decreased density of $GABA_A$ receptors (Lloyd et al., 2000). Whether this reflects a loss of cortical (excitatory and/or inhibitory) neurons, or a down-regulation of post-synaptic $GABA_A$ receptor expression, or both, is suggestive of an overall decreased cortical inhibition. Post-mortem analyses revealed decreased $GABA_A$ mRNA levels (Petri et al., 2003, 2006) significant loss of calbindin (CB) and PV immunoreactivity in the motor cortex of ALS patients, and atrophy of NPY-positive INs (Nihei and Kowall, 1993; Nihei et al., 1993; Maekawa et al., 2004). Decreased PV immunoreactivity could arise from a genuine neuronal loss, but also from decreased expression. Interestingly, decreased levels of PV modifies the dynamic of burst discharge (Albéri et al., 2013) and absence of PV increases the susceptibility to Pentylenetetrazole (PTZ)-induced seizures in mouse (Schwaller et al., 2004), suggesting that both loss of PV-positive neurons, or decreased levels of the protein may affect proper functioning of the cortical network (**Figure 3**).

Quantifications of the various IN populations in the multiple mouse models of ALS gave different results. In the primary motor cortex of pre-symptomatic Wobbler mice, Nieto-Gonzalez et al. (2010) observed decreased densities of PV-positive and SST-positive INs, together with a reduced density of GABAergic synaptic boutons. In the *SOD1^{G93A}* mouse model, classical quantification methods reported levels of PV+ INs similar to wild-type animals (Ozdinler et al., 2011; Clark et al., 2017). However, the Voronoi tessellation method revealed increased numbers of PV-positive INs, selectively in the motor and somatosensory areas, from pre-symptomatic ages to disease end-stage (Minciacchi et al., 2009). Early morphological alteration and late loss of upper layer CR-positive INs, together with a late increase of NPY immunoreactivity were also reported in *SOD1^{G93A}* mice (Clark et al., 2017). Interestingly, the authors suggested that increased NPY expression in various IN populations could reflect the need to express neuroprotectants in a context of altered and toxic cortical activity (Clark et al., 2017). In *TDP-43^{A315T}* mice, Zhang et al. (2016) reported pre-symptomatic decrease followed by symptomatic loss of SST-positive neurons (**Figure 3**). Together, the data indicate that GABAergic INs undergo modifications in various mouse models of ALS, suggesting impairments of the inhibitory circuits. Above cell numbers, levels of calcium-binding proteins, such as PV, or of neuroprotective neuropeptides, such as NPY, need to be carefully assessed to further evaluate the inhibitory component of the cortical network. Interestingly, SST-positive neurons from different cortical areas were reported to receive dopaminergic, noradrenergic, and serotonergic innervations, and SST to play the role of co-transmitter for GABA, thus involved in modulating surrounding neurons (for reviews see Liguz-Lecznar et al., 2016; Riedemann, 2019).

Kim et al. (2017) reported that PV-positive INs from neonatal and symptomatic *SOD1^{G93A}* mice were hyperexcitable, similar to excitatory layer V CSN and CPN. Contrastingly, Zhang demonstrated that PV-positive INs were hypoactive, while SST-positive INs were hyperactive in the *TDP-43^{A315T}* mouse model of ALS (Zhang et al., 2016). The authors elegantly demonstrated that hyperactivity of SST-positive INs was responsible for hypoactivity of PV-positive INs, and, in turn, for hyperexcitability of layer V subcerebral projection neurons, which could be restored to normal excitability by genetic ablation of SST-positive INs (Zhang et al., 2016; **Figure 3**). A better understanding and characterization of cortical INs families, inputs and outputs, as well as morphology and electrophysiology, will be essential in the future to better dissect the mechanisms underlying cortical hyperexcitability in ALS.

ALTERED GENE EXPRESSION RELATED TO CORTICAL CIRCUITS DYSFUNCTION IN ALS

Neuronal excitability relies first on the establishment of membrane potential, i.e., differential concentrations of cations on each side of the plasma membrane, due to the presence of ATP-dependent sodium potassium pumps (Na^+K^+ pumps). Neuronal excitability is the ability of a neuron to respond to stimuli by rapid change in membrane potential, a phenomenon that requires the selective opening of specific ion channels. Thus, amounts and compositions of ion channels are directly related to neuronal excitability, and neuronal hyperexcitability can also be seen as the result of altered expression of these protein families, along with neurotransmitter receptors and other synaptic proteins.

In order to better understand the pathologic cascade leading to the neuronal degeneration in the cerebral cortex, a few studies interrogated the transcriptomic alterations in post-mortem cortex (motor or frontal) from ALS patients (Wang et al., 2009; Lederer et al., 2007; Aronica et al., 2015; Prudencio et al., 2015; Andrés-Benito et al., 2017), or individual populations of cells purified from the mouse cerebral cortex (Kim et al., 2017; Marques et al., 2019). Given the emerging role of altered RNA metabolism – and thus altered protein expression – as a common pathological mechanism of ALS, these seminal studies may point to transcriptomic alterations that could possibly underlie cortical hyperexcitability in ALS.

Ion Homeostasis and Transportation

First microarray analysis of the motor cortex of sporadic ALS patients compared to control individuals revealed a small number of deregulated genes (Lederer et al., 2007). Amongst others, the study reported on the down-regulation of *ATP1A3* (*ATPase Na^+/K^+ transporting subunit alpha 3*), that belongs to the family of P-type cation transport ATPases, and contributes to establishing and maintaining the electrochemical gradients of Na^+ and K^+ ions across the plasma membrane. Because mutations of *ATP1A3* cause rapid-onset dystonia parkinsonism (RDP), alternating hemiplegia of childhood (AHC), or early infantile epileptic encephalopathy (EIEE), the later two being characterized by seizures (Arystarkhova et al., 2019), its down-regulation in ALS patients could have repercussions on cortical excitability. In the same study, Lederer et al. (2007) also reported on the down-regulation of *KCNC2* (*potassium voltage-gated channel subfamily C member 2*), while similar microarray analyses conducted on motor and sensory cortex (Wang et al., 2009), or on motor cortex of ALS patients (Aronica et al., 2015) revealed the up-regulation of *KCNIP2* (potassium voltage-gated channel interacting protein 2) and of *SCN7A* (sodium voltage-gated channel alpha subunit 7), respectively, further supporting altered neuronal excitability and action potential firing. Down-regulation of *SLC12A5* (*solute carrier family 12 member 5*), a K^+/Cl^- co-transporter, is also noticeable (Lederer et al., 2007). During development, increased expression of *SLC12A5*, formerly known as *KCC2*, lowers intracellular chloride concentrations below the electrochemical equilibrium potential, allowing GABA's action on postsynaptic components to switch from excitatory to inhibitory (Schulte et al., 2018). Thus, decreased expression of *SLC12A5* in the pathological context of ALS could partly reverse this effect and GABA-mediated inhibition of cortical networks.

The protein hormone *Adiponectin* (*ADIPOQ*) and its receptors R1 and R2 (*ADIPOR1* and *ADIPOR2*) were found down-regulated in the motor cortex of ALS patients compared to controls (Aronica et al., 2015). In the paraventricular nucleus of the hypothalamus, adiponectin and its receptors were shown to regulate neuronal excitability via their modulation of different potassium currents (Hoyda and Ferguson, 2010). In the hippocampus, *ADIPOR2* deletion leads to hyperexcitability of the dentate gyrus neurons (Zhang et al., 2016). Finally, adiponectin has been related to several disorders of the central nervous system, such as stroke, Alzheimer's disease, Parkinson's disease and Multiple sclerosis (for review see Baranowska-Bik and Waszkiewicz-Hanke, 2017; Bloemer et al., 2018).

Glutamatergic and GABAergic Components

Wang et al. (2009) reported up-regulations of the glutamate receptor *GRIK1* (*glutamate ionotropic receptor kainate type subunit 1*), and of the postsynaptic density scaffolding protein *HOMER 3* (*homer scaffold protein*), which binds group I metabotropic glutamate receptors, amongst numerous other proteins. Aronica et al. (2015) instead reported on the up-regulation of *GRIA1* in a subgroup of ALS patients, and down-regulation of several ionotropic and metabotropic glutamate receptors, *GRIN1*, *GRIN2A*, *GRIN2D*, *GRIA2*, and *GRIA3*, in a second subgroup of patients. This was accompanied with the down-regulation of six subunits of the $GABA_A$ receptor in one subgroup of patients (Aronica et al., 2015). These transcriptomic changes in the post-mortem motor cortex of ALS patients could reflect the broad neuronal loss that occurred prior to death. In this regard, the study by Andrés-Benito et al. (2017) is particularly interesting because it was conducted on the frontal cortex of ALS patients that showed no sign of FTD, and for which no neuronal loss in the frontal cortex was suspected. Thus, the analysis could potentially provide a molecular snapshot of cortical hyperexcitability prior to neurodegeneration. In this study, numerous glutamate receptors and transporters were found up-regulated (Andrés-Benito et al., 2017). This was the case of *GRIA1*, which codes for the ionotropic glutamate receptor AMPA 1, *GRIN2A* and *GRIN2B*, which code for NMDA receptors, and *GRM5*, which codes for the glutamate metabotropic receptor 5, along with the glutamate transporters *SLC1A2* and *SLC17A7* (Andrés-Benito et al., 2017). Interestingly, *GAD1*, that encodes the glutamate carboxylase 1, that synthesizes GABA from glutamate, and the $GABA_A$ and $GABA_B$ receptors subunits *GABRD*, *GABRB2*, and *GABBR2* were also increased (Andrés-Benito et al., 2017). Increased expression of components of the glutamatergic system are in agreement with overall increased cortical excitability, and increased expression of components of the GABAergic system could be interpreted as an attempt to counteract increased excitation.

Altered Gene Expression in Mouse Models of ALS Suggest Possible Cortical Hyperexcitability in Rodents

While to our knowledge TMS has not been employed in mouse models of ALS and broad cortical hyperexcitability has not been demonstrated in these animals, Kim et al. (2017) reported intrinsic hyperexcitability of cortical neurons, and particularly layer V subcerebral projection neurons, but also CPN and populations on interneurons. Using RNAseq on purified sub-populations of cortical neurons, they demonstrated that intrinsic hyperexcitability of corticospinal neurons (CSN) and CPN from postnatal $SOD1^{G93R}$ mice was accompanied by changes of expression of several voltage-gated Na^+ and K^+ channels, GABA and glutamate receptors (Kim et al., 2017). Interestingly, the sets of differentially regulated genes were different between the two neuronal populations, and the variations of common genes were sometimes opposite (Kim et al., 2017). This is in agreement with the different molecular identities of the two investigated populations, but may also reflect different strategies to deal with hyperexcitability, depending on the position within the cortical networks.

CONCLUDING REMARKS

A growing number of evidences point to the cerebral cortex as the origin of ALS and suggest a corticofugal propagation of the disease. In this context, the earliness of cortical hyperexcitability in ALS patients, and the recent demonstration that it is sufficient to trigger TDP-43 pathology, suggest that cortical E/I unbalance may represent *per se* a particularly relevant therapeutic target. Because numerous extrinsic (i.e., non-cortical) and intrinsic (i.e., cortical) components contribute to the fine-tuning of cortical excitability and cortical network proper functioning, the possible candidates to cortical hyperexcitability are numerous, and combined impairments are very likely. To further unravel the mechanisms behind cortical hyperexcitability in ALS, mouse models that recapitulate this typical hallmark of the disease are needed, or, more simply, current mouse models of the disease should be tested for possible cortical hyperexcitability. These will in turn allow assessing various therapeutic strategies to restore proper cortical excitability, and to determine the impact of such intervention on direct corticofugal targets, and more broadly on disease onset and progression. These first steps may in turn pave the way to a new era of treatment in the field of ALS, and potentially other neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

AB, GS-L, JS-Z, TB, and CR analyzed the data of the literature and wrote the manuscript. AB and CR designed the figures and AB elaborated them. All authors approved the publication of the manuscript.

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Impaired Phasic Discharge of Locus Coeruleus Neurons Based on Persistent High Tonic Discharge—A New Hypothesis With Potential Implications for Neurodegenerative Diseases

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The locus coeruleus (LC) is a small brainstem nucleus with widely distributed noradrenergic projections to the whole brain, and loss of LC neurons is a prominent feature of age-related neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). This article discusses the hypothesis that in early stages of neurodegenerative diseases, the discharge mode of LC neurons could be changed to a persistent high tonic discharge, which in turn might impair phasic discharge. Since phasic discharge of LC neurons is required for the release of high amounts of norepinephrine (NE) in the brain to promote anti-inflammatory and neuroprotective effects, persistent high tonic discharge of LC neurons could be a key factor in the progression of neurodegenerative diseases. Transcutaneous vagal stimulation (t-VNS), a non-invasive technique that potentially increases phasic discharge of LC neurons, could therefore provide a non-pharmacological treatment approach in specific disease stages. This article focuses on LC vulnerability in neurodegenerative diseases, discusses the hypothesis that a persistent high tonic discharge of LC neurons might affect neurodegenerative processes, and finally reflects on t-VNS as a potentially useful clinical tool in specific stages of AD and PD.

Keywords: locus coeruleus, phasic and tonic discharge, Alzheimers's disease, Parkinson's disease, norepinephrine, transcutaneous vagal stimulation, neurodegeneration, neuroprotection

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INTRODUCTION

The locus coeruleus (LC) is a small nucleus located in the brainstem near the fourth ventricle and is composed of noradrenergic (NAergic) cells. Despite comprising only roughly 30,000–50,000 neurons in the adult human brain (1–4), the LC consists of extensively branched efferent axons that project throughout the brain and spinal cord (1, 5–14). LC neurons project to all layers of the cortex (15) and have dense projections to the hippocampus (16) as well as the frontal cortex (17).

As part of the ascending reticular activating system, the LC affects consciousness, wakefulness, and attentiveness (14) by projecting axons into the whole brain and thereby activating neural networks across many brain regions synchronously. There is evidence for a functional and

topographic order within the LC. LC neurons in the dorso-rostral part project to the neocortex and the hippocampus, whereas more caudo-ventrally located neurons project to the cerebellum and the spinal cord (11, 18–21). Furthermore, tracing studies revealed that individual LC neurons receive input from 9 to 15 different brain regions indicating a largely integrative input to a single LC neuron. The release of NE locally from the soma of LC neurons activates somatodendritic α2-autoreceptors that inhibit neuronal activity via an auto-inhibitory mechanism (22, 23).

Since the loss of LC neurons is a shared feature of neurodegenerative diseases, especially Parkinson's disease (PD) and Alzheimers's disease (AD) (24–26), a better understanding of the role of the LC in AD and PD may provide important insights into the underlying mechanism of these neurodegenerative diseases.

The first part of this article focuses on the neuroprotective effects of NE. Next, it discusses potential mechanisms underlying the selective vulnerability of LC neurons to neurofibrillary tangles (NFTs)- and β-amyloid (Aβ) pathology in AD as well as α-synuclein (αSyn)-pathology in PD, respectively. Finally, a hypothesis discussing the potential relevance of changes in the discharge mode of LC neurons for neurodegeneration is presented together with t-VNS as a non-invasive technique to modulate LC activity with potentially neuroprotective effects.

LC AND NEUROPROTECTION

Besides its role as a conventional neurotransmitter in the synapse, extrasynaptically released NE has a paracrine-type of anti-inflammatory and neuroprotective effect on surrounding neurons, glia cells and microvessels (1, 27, 28). Therefore, extrasynaptically released NE decreases toxin-induced inflammatory processes [for review, see (29, 30)], endotoxin-mediated inflammation (31) and Aβ induced neuroinflammation in the brain (24, 25, 30, 32–39). Furthermore, LC neurons innervate the cerebral vasculature throughout the brain via extensive varicosities for non-synaptic release of NE (38, 40), hence playing an important role in maintaining the blood-brain barrier (3, 38, 41, 42).

Moreover, LC neurons exhibit neuroprotective properties through the secretion of brain-derived neurotrophic factor (BDNF) and nerve growth factor (24, 32, 43). BDNF is synthesized in LC neurons (44, 45) and anterogradely transported and released from axon terminals in the projection areas in an activity-dependent manner (45–47). BDNF induces neurotrophic activity, promotes the survival of NAergic neurons and increases axonal sprouting of LC neurons at the terminal sites (45, 48, 49).

N-2-Chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4), a selective neurotoxin for the LC-NAergic system in the rodent brain, is accumulated in NAergic nerve terminals, and damages them, thus resulting in rapid and long lasting loss of NE (50). DSP-4 increases the expression of proinflammatory factors, like inducible nitric oxide synthase (iNOS) (24, 30), and nitric oxide production, which in turn enhance the processing of amyloid precursor protein (APP) to Aβ (24, 51, 52). After treatment with the selective NAergic neurotoxin DSP-4, surviving LC neurons exhibit a regenerative axon sprouting in the target regions as a compensatory mechanism (45, 53), which seems to be important for the temporary maintenance of extracellular NE levels.

Thus, understanding the role of the LC in the process of neurodegeneration may start with the question: What makes LC neurons vulnerable to aging-related neuropathology?

POTENTIAL FACTORS FOR THE VULNERABILITY OF LC NEURONS

Why LC neurons are vulnerable to aging and aging-related neurodegenerative diseases, such as PD and AD, is not completely understood. One potential reason for the vulnerability of LC neurons might be intense mitochondrial demand caused by sustained cellular excitability attributable to autonomous pacemaking activity in these neurons, which results in mitochondrial dysfunction and cumulative oxidant stress (54–56).

A common feature of LC neurons is autonomous pacemaking. Small-conductance Ca^{2+}-activated K^+ (SK) channels are essential regulators of the intrinsic pacemaking of LC neurons and the activation of SK channels is primarily coupled to Ca^{2+} influx via the opening of L- and T-type calcium (Ca^{2+}) channels (55, 57, 58). Activity-dependent Ca^{2+} entry through L-type Ca^{2+} channels enables feed-forward stimulation of mitochondrial oxidative phosphorylation, and thereby helps to prevent bioenergetic shortage when activity needs to be sustained, but in turn leads to basal mitochondrial oxidant stress (59). Hence, autonomous pacemaking caused by Ca^{2+} signaling in LC neurons requires elevated mitochondrial activity, leading to oxidative stress under basal conditions (55, 59–62), and thus resulting in elevated susceptibility to mitochondrial impairment.

Another potential factor for the vulnerability of LC neurons are their highly branched, long and thinly myelinated or unmyelinated axons that cause high energy demand, because ATP requirements for propagation of axon potentials grow exponentially with the level of branching (63, 64).

Oxidative stress together with required mitochondrial oxidative phosphorylation to sustain neurotransmitter release and cellular excitability, could interfere with key cellular functions, such as degradation of damaged and misfolded proteins (59), promoting protein aggregation and finally resulting in cell death. Subsequently, the brain is deprived of its NAergic innervation which may be a key step in the early stages of neurodegenerative diseases, such as AD and PD (64, 65).

Abbreviations: AD, Alzheimers's disease; Aβ, amyloid-beta; ACC, anterior cingulate cortex; αSyn, α-synuclein; BDNF, brain derived neurotrophic factor; CRF, corticotrophin-releasing hormone; LC, locus coeruleus; NA, noradrenaline; NE, norepinephrine; NFT, neurofibrillary tangles; NTS, nucleus of the solitary tract; OFC, orbitofrontal cortex; PD, Parkinson's disease; PFC, prefrontal cortex; RBD, rapid eye movement sleep behavioral disorder; SNc, substantia nigra pars compacta; SubC, subcoeruleus; t-VNS, transcutaneous vagal stimulation.

PHYSIOLOGY OF LC NEURONS

LC neurons show two different discharge modes, tonic and phasic. In the tonic mode, LC neurons show irregular but continuous firing patterns at 1–6 Hz whereas during the phasic mode, LC neurons fire in short (<300 ms) bursts of higher frequencies (10–15 Hz) that can occur spontaneously or associated with salient stimuli (1, 66, 67). Tonic discharge is high during stress and agitation, moderate during active wakefulness, low during drowsiness and completely absent during REM sleep (1, 66, 68, 69). Complete silencing of LC neurons during REM sleep may be due to elevated inhibitory GABAergic input from the ventral medulla (70–72). There is an inverted U-shaped correlation between tonic and phasic discharge, in such a way that phasic discharge to salient stimuli in the environment is optimal at a moderate tonic discharge level (1).

LC neurons are electrotonically coupled through gap junctions between dendrites outside of the nucleus, in the peri-coerulear region (73, 74). The strength of coupling changes between both discharges modes with increased coupling during phasic activation and decreased coupling in the tonic mode. The shift between the two discharge modes is thought to be modulated by the anterior cingulate (ACC) and the orbitofrontal cortices (OFC) of the prefrontal cortex (PFC) (1).

The PFC is important for a number of cognitive and executive functions (75), and strongly innervated and modulated by NAergic ascending projections from the LC. Aston-Jones and Cohen proposed that glutamatergic projections from the OFC and the ACC back to the LC are important in generating the patterns of LC activity (1). Besides, corticotrophin-releasing hormone (CRF)-containing afferences from the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala, increase tonic firing (74). Furthermore, LC neuronal activity is inhibited by local GABAergic interneurons, located dorsomedial to the LC nucleus, which hyperpolarize LC cells and reset their spontaneous activity (76).

STRESS-INDUCED CHANGES IN LC ACTIVITY

Sustained tonic activity during waking is metabolically demanding and may render LC neurons a vulnerable target to stress (77). Particularly, stress-induced high tonic activity, mediated in part by the stress-related neuropeptide CRF, causes vulnerability to damage induced by high energy demands (33, 78) and makes LC neurons stress-sensitive. Stressful stimuli activate the hypothalamic-pituitary-adrenal axis and cause a release of CRF. CRF-immunoreactive fibers densely innervate the pericoerulear region that contains the dendrites of LC neurons (79) and CRF increases the tonic discharge of LC neurons (80–84). CRF peptide promotes the tonic discharge mode of LC neurons with a decreased maximum magnitude and slower onset, but a much longer duration of activity (74). Furthermore, stress seems to cause long-lasting changes in the LC that directly impact LC function and induce morphological alterations in LC neurons, such as proliferation of dendrites and axons (80, 85–94).

As a protection against these changes, stress-induced increase of NE release triggers auto-inhibitory mechanisms via α2-autoreceptors on LC neurons (78, 95, 96), which induce hyperpolarization and decrease the sensitivity of LC neurons to stimulation. This negative feedback mechanism protects LC neurons against stress-induced changes and damage of these autoregulatory mechanisms may contribute to neurodegenerative diseases like AD and PD (74, 78).

In conclusion, LC neurons are metabolically demanding and highly vulnerable to stress. Given that death of LC neurons is a shared feature of PD and AD (24–26), the damage of autoregulatory mechanisms that protect LC neurons from stress-induced changes might be involved in neurodegenerative processes of both diseases.

LC and AD Pathology

Neurofibrillary tangles (NFTs) are aggregates of the microtubule-associated protein tau and increasing levels of tau pathology characterize the advancing stages in the development of AD. The LC is the first brainstem structure that displays pretangle material [for details see (97–100)], and thus axonal projections from the LC to the transentorhinal region could be important for the anterograde induction of tau pathology (97). Since aggregation of tau in the LC is one of the first pathological hallmarks of AD and precedes cortical tau pathology, it may act as a seed for subsequent spreading of tau pathology throughout the brain (38, 97, 98, 100–103). As hyperphosphorylated tau levels in the LC increase, the volume of the LC decreases in early stages of AD (102, 104). While total numbers of LC neurons are relatively stable until Braak stage II, they are significantly reduced in Braak stages III-VI, and analyses in the human brain revealed that as the Braak stage increased by one unit, the average LC volume decreased by 8.4% (38, 104). A loss of 30% characterizes the transition to MCI and a 55% reduction represents AD (105).

Since NE released from LC neurons is needed to maintain Aβ clearance, the progression of LC degeneration contributes to Aβ pathology in AD (36, 106), and further degeneration of LC neurons might be triggered by an Aβ-mediated failure in the anterograde and retrograde transport of neurotrophic factors like BDNF in LC axons (99, 107, 108). The bidirectional relationship between Aβ pathology and LC degeneration may lead to an exponential progression of the disease, because increased Aβ-levels exaggerate LC degeneration, which in turn reduces NE levels in the terminal fields that diminish the internalization of Aβ by microglia, adding to increased Aβ pathology (35). Progressive loss of LC neurons and the concomitant decrease of NE levels in the brain diminish anti-inflammatory and neuroprotective mechanisms and finally result in an exacerbation of Aβ-induced pro-inflammatory processes and neurotoxicity (31, 109), as well as tau pathology (101, 110).

NE deficiency in the cortex impairs the activation of microglia, the induction of their migration toward amyloid plaques and the stimulation of the internalization and clearance of Aβ (35). Furthermore, NE deficiency results in an increased tau phosphorylation and compromises neurogenesis in the dentate

gyrus, dendritic arborization of new neurons and synaptic plasticity (111).

Smaller fusiform cells located in the dorsal part of the LC (23, 112, 113) that project to forebrain regions such as the PFC (23), are characterized by a high density of voltage-gated Ca^{2+} channels, which enables higher spontaneous firing frequencies (57). Furthermore, this subpopulation of PFC-projecting LC neurons is more excitable and responsive to glutamate than LC neurons projecting to other cortical circuitries (114). This subpopulation of LC neurons that can be distinguished from other LC neurons on the basis of their anatomical projections, molecular phenotypes, and electrophysiological properties, may be more vulnerable to activity-dependent cellular dysfunctions and the neurodegenerative processes in AD because of their higher basal discharge rates (114).

In summary, it can be concluded that NFT pathology causes dysfunction of LC neurons resulting in decreased NE levels in target regions, which contributes to Aβ pathology. This, in turn, accelerates LC degeneration and diminishes anti-inflammatory and neuroprotective effects of NE, resulting in increased Aβ plaque load. Therefore, LC degeneration and Aβ pathology synergistically interact to generate neurodegeneration in AD.

LC and PD Pathology

In PD, α-synuclein (αSyn)-positive deposits, called Lewy bodies, can be found in the LC in Braak stage II, and thus earlier than in the substantia nigra pars compacta (SNc) (115, 116). It is believed that αSyn burden may lead to neuronal dysfunction and impaired neurotransmitter release, but αSyn pathology does not correlate well with cell death (2, 117, 118). Accordingly, αSyn may contribute to neurodegeneration in PD, but is not likely to be the sole reason (119). Furthermore, there is evidence that αSyn can be transferred across synapses and spread within postsynaptic cells in a prion-like fashion (3, 120).

A loss of LC neurons can be found throughout the rostral-caudal extent of the nucleus (20), earlier and in a greater magnitude as compared to the SNc (20, 121), with the onset of LC pathology occurring more than 10 years before the clinical diagnose of PD (122, 123).

Neuromelanin (NM) is an autophagic product synthesized via oxidation of catecholamines and subsequent reactions, and it is the main iron storage mechanism in neurons that protects them from iron-mediated neurotoxicity caused by superoxide free radicals (124–127). Although intraneuronal NM is neuroprotective, NM released by dying neurons can trigger neuroinflammation via activation of microglia (128). Postmortem studies have shown NM accumulation in LC neurons with increasing age (129). Several studies found an inverted U-shaped correlation between NM accumulation in LC cells and age with peak levels around 60 years, followed by an age-related decline related to loss of LC neurons (130, 131). However, some studies reported a linear age-related increase of NM in LC neurons without age-related decline (132). Nevertheless, under pathological conditions involving LC degeneration, the pigment is diminished (132, 133).

NM-sensitive Magnetic resonance imaging (NM-MRI) allows for *in vivo* visualization of the LC by exploiting the presence of NM (125, 134–138). Studies indicate that NM-MRI can detect structural alterations in the LC in early disease stages (139), even in patients with rapid eye movement (REM) sleep behavioral disorder (RBD) (140, 141), thus indicating NM-MRI as a potential biomarker in prodromal stages of neurodegenerative diseases [(138, 142) for a review see (143, 144)].

RBD is a prodrome of α-synucleinopathies, like PD, that appears 10 or more years before the first motor symptoms occur (145–147). Patients with RBD show fully developed αSyn pathology in the LC, equivalent to the pathology found in patients diagnosed with PD, while exhibiting normal nigrostriatal dopaminergic innervation (148). This begs the question if there are special characteristics of LC neurons, which are relevant to early αSyn pathology and Lewy body burden (106, 149–151).

One feature of LC neurons that may affect their sensitivity for PD-pathology is the presence of elevated Ca^{2+} concentrations in the cytosol (119). Autonomous pacemaking of LC neurons caused by voltage-sensitive Ca^{2+} channels (57) requires extensive Ca^{2+} entry to stimulate oxidative phosphorylation, which promotes high levels of reactive oxygen species (ROS) production, thus elevating oxidative stress (55, 152). Thus, sustained neurotransmitter release and neuronal excitability of LC neurons require high energetic demands that could impair other key cellular functions, such as degradation of misfolded proteins and promote their accumulation in intracellular inclusions (59). αSyn is widely expressed in the nervous system and located in presynaptic terminals, where it is involved in the regulation of synaptic vesicle exocytosis (153–157). Furthermore, αSyn interferes with Ca^{2+} homeostasis (59, 158–160), for instance by increasing ion permeability of the lysosomal and plasma membrane (161–163). Extracellularly accumulated αSyn increases the permeability of Ca^{2+} channels resulting in increased cytoplasmatic Ca^{2+} (119). Hence, αSyn oligomers are able to increase internal Ca^{2+} concentrations and Ca^{2+}, in turn, increases αSyn oligomerization with cytotoxic effects (164–166). This positive feedback cycle of αSyn oligomerization and increased internal Ca^{2+} concentration could make LC neurons more vulnerable to PD pathology, because of Ca^{2+} channel-mediated pacemaking and the cellular burden associated with it (57). NE stabilizes αSyn in a soluble, monomeric form, thereby preventing the formation of toxic oligomers and enabling the disaggregation of existing fibrils (167). Consequently, decreased NE levels increase αSyn oligomerization, and therefore, LC degeneration and αSyn pathology synergistically interact to induce neurodegeneration in PD.

DISCUSSION

Persistent High Tonic Discharge of LC Neurons and Potential Implications for Neurodegenerative Diseases

The findings mentioned above suggest that AD and PD are both characterized by a significant degeneration of LC neurons, despite having distinct pathologies (22, 106, 121). Postmortem studies reveal disease-specific patterns of LC cell loss, affecting the whole LC in PD. In AD, particularly the rostral and dorsal

parts of the LC are affected, along with cortical-projecting LC neurons, while the caudal and ventral parts that contain non-cortical-projecting neurons are spared (20, 104). Slight differences in LC pathology in AD and PD could be a result of different underlying neuropathological mechanisms that may depend on the internal organization of the LC nucleus, the modulation of neuronal activity and the complexity of axonal projections of LC cells. The LC is not a single functional entity of neurons that function as a whole in order to control global arousal. Instead, LC neurons appear to be a collection of NAergic cells with sub-specializations according to their anatomical projections (114, 168–170), electrical properties (75, 168) and co-transmitter content, with distinctive roles in regulating brain function (168, 171).

The loss of LC neurons occurs earlier and in a greater magnitude than the atrophy of the hippocampus in AD and the loss of dopaminergic cells in the SNc in PD (25, 26, 104, 106, 121, 150, 172–175). However, LC pathology starts much earlier than evidence of cell loss. Grinberg and colleagues showed tau pathology in the LC in Braak stage 0, but no significantly decreased number of LC neurons until Braak stage III (104), indicating that LC neurons may survive substantial tau burden even with impaired NAergic neurotransmission (176). Moreover, αSyn pathology in the LC is evident early in the premotor phase of PD (Braak stage II), prior to the involvement of the dopaminergic SNc (Braak stage III) (177), but LC neurons can survive with αSyn pathology for years before significant cell loss is evident (20, 26, 121). Thus, tau- and αSyn pathology is verifiable in LC neurons many years before a significant loss of LC neurons is detectable in AD and PD, respectively (45, 106, 150, 174, 178–180).

Previous studies suggest that early damage to the LC in preclinical or prodromal AD may result in a persistent state of high tonic activity (181), which might be detrimental to brain functions that require phasic responses. Wang et al. (182) have shown that the mean firing rate of LC neurons increases significantly 2 and 4 weeks after unilateral lesion of the nigrostriatal pathway in the rat by local injection of 6-hydroxydopamine (6-OHDA) into the right substantia nigra pars compacta (SNc). Furthermore, the percentage of neurons with irregular firing patterns increased significantly. The authors postulated that 6-OHDA lesions of the SNc caused loss of LC neurons and decreased NE concentration in the LC of 6-OHDA-lesioned rats, which in turn resulted in overactivity of residual LC neurons. Moreover, patch-clamp data from Parkin-knockout mice also showed increased spontaneous firing of aged LC neurons caused by alterations in calcium-dependent excitability (183). In contrast, however, Miguelez et al. (184) found no effects of 6-OHDA infusion into the right medial forebrain bundle on the number of spontaneously active LC neurons, but in turn reported a significantly lower basal firing rate after 6-OHDA infusion and postulated that changes in firing rate may be attributed to dopaminergic degeneration. Another study showed an increase in α2-adrenoceptor mRNA in the LC in 6-OHDA-lesiond rats (185), and α2-adrenoceptors modulate the firing rate of the LC by inhibiting neuronal activity (186). With respect to these apparently opposing results, new mouse models, which overexpress αSyn in the LC seem to be a promising approach, since these models capture some cardinal morphological changes in human PD more closely (187, 188). Unfortunately, no electrophysiological LC data based on these models have been reported to date. The fundamental problem, however, off all the reported electrophysiological findings is that in all studies, recordings have been conducted under anesthesia, which impacts spontaneous LC activity (66, 189–192), and thus limits any conclusions about tonic LC discharge in awake animals, let alone patients.

Since recording from LC neurons is a challenging and invasive task, human data are lacking to date. Therefore, to make inferences about LC activity in humans, one has to rely on more indirect measures, like event-related potentials (ERPs), for instance the P300. The P300 is an event-related potential (ERP) that can be recorded in humans from the scalp in an auditory oddball paradigm. In contrast to former reviews that discuss glutamate as the most important neurotransmitter for the generation of the P300 as well as the cholinergic system and GABAergic influences as important modulators (193), more recent reviews suggest that dopamine and NE are the most important modulators for the generation of the P300 (194–196). The P300 can be devided into two subcomponents, the P3a and the P3b, respectively (196). P3a seems to depend on dopaminergic (DAergic) activity and is thought to reflect a novelty-driven orienting response to distractors that can be recorded more frontally on the scalp (194). On the other hand, P3b seems to be related to memory and decision making driven by phasic NAergic LC activity and can be recorded from more temporal-parietal areas (195, 196). Hence, a dual-transmitter P300 hypothesis was assumed that associated DAergic neurotransmission with P3a and LC-NAergic neurotransmission with P3b (194). Furthermore, Nieuwenhuis et al. (195) suggest that the P300 reflects phasic activity of the LC-NAergic system, and recent studies suggest that phasic LC activity depends on background tonic discharge of LC neurons in an inverted U-shaped manner, with highest phasic discharge rates at moderate levels of tonic LC activity, which would in turn create the largest P3b amplitudes (1, 197).

Also, animal studies suggest that the P300 can be interpreted as a cortical correlate of the phasic LC response (195, 197–199), and the hypothesis that phasic LC activity contributes to P300 generation during a target detection task is consistent with the fact that both phasic LC activity and the P300 depend on the motivational significance of the eliciting stimulus as well as underlying attentional mechanisms and show congruent latencies in response to target stimuli [for review see (195)]. Hence, it was suggested that the P300 reflects NE mediated enhancement of gain in the cerebral cortex induced by phasic LC activity and thus enhances cortical encoding of salient stimuli (1, 67, 195, 198).

In addition, studies in PD patients have shown a reduced P300 amplitude (200–206) or an increase in P300 latency (207, 208), whereas in AD patients an increase in P300 latency was found (209–213). If the hypothesis given above is correct and an optimal rate of phasic LC activity is contingent upon a moderate level of tonic activity (1), then a reduction in P300 amplitude and/or

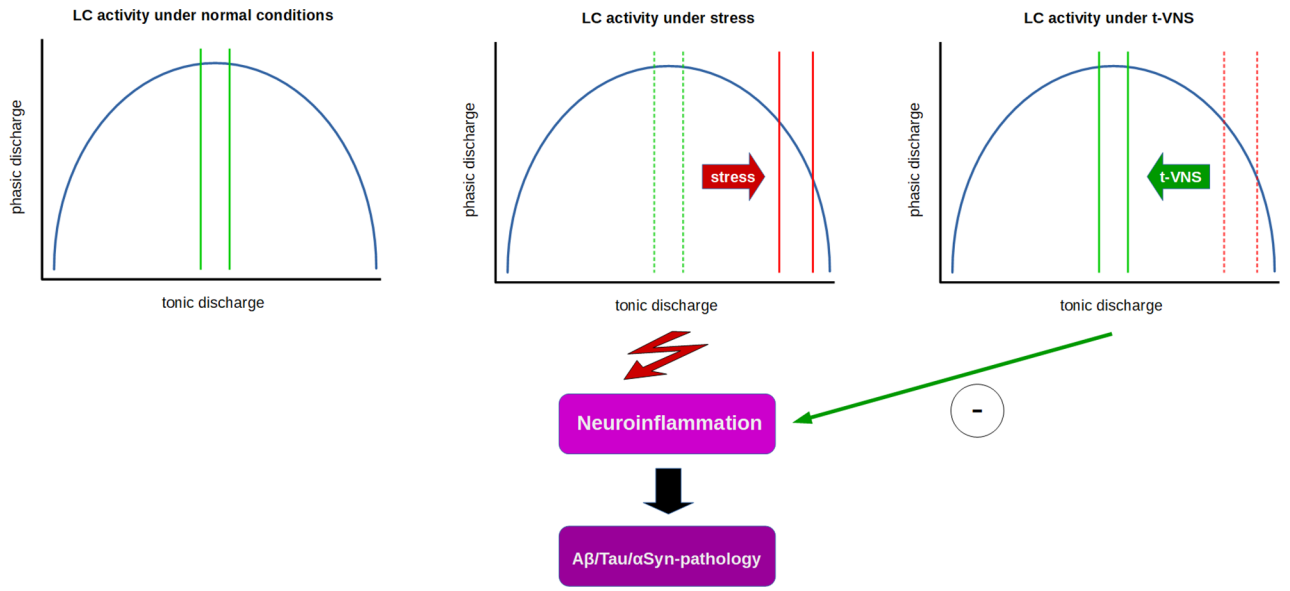

FIGURE 1 | Relation between tonic and phasic LC discharge (blue curve) and proposed effects of t-VNS treatment. Under normal conditions **(left)**, a moderate range of tonic activity (indicated by green lines) allows for an optimal level of phasic activity in response to salient stimuli. Under constant stress **(middle)**, LC tonic activity is thought to shift from moderate to high tonic discharge (solid red lines) which in turn impairs phasic discharge, presumably facilitating neuroinflammatory processes and contributing to neurodegeneration. t-VNS treatment **(right)** is proposed to restore tonic discharge to a moderate range, thus enabling an optimal level of phasic discharge, and presumably inhibiting neuroinflammatory/neurodegenerative processes by neuroprotective effects of NE.

increase in P300 latency might reflect impaired phasic activity caused by persistent high tonic LC activity.

Furthermore, persistent high tonic discharge of LC neurons during REM sleep could explain RBD, an early feature of neurodegenerative disorders, including PD (214). RBD is characterized by REM sleep without atonia (RSWA), leading an individual to "act out" dreams, and lesions of the LC and subcoeruleus (SubC) nucleus complex have been theorized to be one possible cause of RBD (215, 216). Glutamatergic neurons in the ventral part of the SubC project to the ventromedial medulla and the spinal cord, who's GABA and glycine neurons inhibit motoneurons and initiate REM sleep atonia (214). These glutamatergic neurons in the SubC get inhibitory afferents from NAergic LC neurons (217, 218). Because normally, tonic discharge of LC neurons is completely absent during REM sleep (66), the SubC is not inhibited by the LC during that state and can thus promote REM sleep muscle paralysis. Therefore, a constantly high tonic discharge of LC neurons during REM sleep could result in an over-inhibition of the SubC and thus explain RSWA.

Moreover, persistent high tonic LC activity during sleep could contribute to the accumulation of protein aggregation and promote neurodegeneration by an inhibition of the glymphatic system (219). The glymphatic system is a macroscopic waste clearance system formed by astroglial cells (220). It is turned on during sleep and enables the elimination of potentially neurotoxic waste products, including Aβ [for details see (219)]. Hence, dysfunction of the glymphatic system could thus contribute to the accumulation of misfolded or hyperphosphorylated proteins and could thereby render the brain more vulnerable to developing a neurodegenerative pathology, because all neurodegenerative diseases are characterized by accumulation of aggregated proteins (221), e.g., misfolded Aβ and NFT in AD or misfolded αSyn in PD, respectively.

A distinct subpopulation of LC neurons in the dorsal part of the nucleus that innervates the PFC (168, 222) and the hippocampus (170), seems to be particularly vulnerable to the pathophysiological processes in AD. Since these LC cells are characterized by greater synaptic excitability, higher spontaneous firing frequencies, and higher susceptibility to glutamate (75), they could be more vulnerable to stress and hyperactivity-dependent cell death. If LC neurons in the dorsal part of the nucleus are indeed the first set of neurons affected by the pathophysiological processes, then this might result in persistent high tonic activity and impaired task-related phasic activity in those cells (181), which in turn may result in compromised PFC and hippocampal functions (64, 176).

Transient silence of LC neurons during REM sleep and prior to each non-REM sleep spindle seems to be important for synaptic plasticity and essential for hippocampus-dependent memory consolidation (223). Swift and colleagues could show that increased LC activity during sleep has no effect on the stability and duration of sleep states, but impairs learning related signatures of non-REM and REM sleep (223). Since glutamatergic projections from the SubC to the medial septum innervate the hippocampus and promote the generation of the theta electroencephalographic activity characterizing REM sleep

(218), a persistent high tonic discharge of LC neurons could impair theta oscillation in the hippocampus during REM sleep, which has been shown to be important for consolidation of hippocampal-dependent memories (224, 225).

Since LC neurons in the dorsal part of the nucleus show minimally overlapping projections to the OFC, the medial PFC, and anterior cingulate (ACC) cortex (75), respectively, neuronal activity in individual prefrontal subregions could also be impaired, resulting in compromised cognitive and executive functions, e.g. shifting of the attentional focus and behavioral adaptation in a changing environment. NAergic projections of LC neurons to the PFC are critical for the ability to rapidly switch attention between stimuli and stimulus categories (226–229), and therefore tests of cognitive flexibility could possibly be used to determine LC dysfunction in early stages of AD (230) and PD (231).

Consequences of Persistent and Abnormally High Tonic Discharge of LC Neurons

Neuronal plasticity is essential to adapt to a changing environment through strengthening, weakening or adding of synaptic connections or by promoting neurogenesis (232). Alteration in synaptic plasticity is an early feature in AD with abnormally suppressed efficacy of neuronal plasticity linked to cognitive decline (232). Since deprivation of cortical NAergic innervation is associated with reduced expression of genes important for synaptic plasticity in the cerebral cortex, the NAergic system seems to have a gating function for neuronal plasticity (233). Oberman and Pascual-Leone (232) hypothesize that cortical "hyperplasticity" in autism spectrum disorder (ASD) may provide protection for this population against the development of age-related cognitive decline and AD, and it was assumed that in autism, tonic LC discharge is affected, which in turn has a protective effect against later development of AD (234).

Assuming a persistent and abnormally high tonic discharge of LC neurons in the dorsal part of the nucleus in early stages of AD (20, 64, 104, 170, 181), phasic discharge of these cells—which requires a moderate tonic discharge level—could be impaired as a result. Because the release of high amounts of NE requires phasic spiking of LC neurons (32, 65, 235–238), NE levels in the cortex might not be high enough in this scenario to activate low-affinity β-adrenoceptors that facilitate the anti-inflammatory and neuroprotective effects of NE (32, 35, 65). It can be assumed that sustained high tonic activity would induce compensatory mechanisms that could initially help to maintain homeostasis (85, 89, 92–94), such as axon sprouting in the terminal fields, augmented synthesis of NE by increased activity of tyrosine hydroxylase and dopamine beta-hydroxylase, and decreased NE transporter activity (174, 180, 239–242). All these compensatory mechanisms, however, would further serve to increase the energy demand and oxidative stress of LC neurons. As a protection against these changes, NE could trigger auto-inhibitory mechanisms via α2-autoreceptors on LC neurons (78, 95, 96), which induce hyperpolarization and decrease the sensitivity of LC neurons to dendritic stimulation. These autoregulatory mechanisms may protect LC neurons, but could on the other hand interfere with LC functions, because they further impair the ability of LC neurons to create selective phasic responses, as described above. Subsequently, sustained increase of basal mitochondrial oxidant stress in tonically discharging LC neurons could contribute to an impaired capability to maintain other key cellular functions, such as the degradation of damaged or misfolded proteins (59), giving rise to axon terminal degeneration as an adaptation to excessively high metabolic demand. The consequences of decreased NE levels in the PFC and hippocampus are increased neuro-inflammation and neurodegeneration, and finally, increased amounts of damaged axonal proteins, such as αSyn and tau-protein may promote aggregation and accelerate cell death of LC neurons. If a dysbalance between tonic and phasic discharge of LC neurons is part of the problem—due to the fact that sustained high tonic discharge impairs phasic discharge to salient stimuli—then modulation of afferent stimulation may be an option to normalize LC functions.

t-VNS, a Potential Non-invasive Technique to Increase Phasic Discharge of LC Neurons

Transcutaneous vagal stimulation (t-VNS) is a novel non-invasive brain stimulation technique that increases activation of the LC and NE release (243), via activation of the auricular branch of the vagal nerve at the external ear (244), and fMRI studies in humans show that t-VNS and invasive vagal nerve stimulation (i-VNS) activate the same afferent vagal projection sites (245). The vagal nerve innervates the nucleus of the solitary tract (NTS), which directly modulates the activity of LC neurons via monosynaptic excitatory projections (246, 247) and indirectly excites the LC via the nucleus paragigantocellularis, providing pathways by which VNS could directly drive short latency spiking in the LC (248, 249). Stronger activation of LC dendrites in the peri-coerulear area via t-VNS may significantly influence the activity of LC neurons, for instance via changes in their electrotonic coupling (250) due to gap junctions between dendrites in the peri-coerulear region (73, 74). Previous studies show that when LC neurons are isolated from the peri-coerulear dendritic region, synchronous activity is reduced or abolished (73). This suggests that the modulation of electrical interactions between dendrites in the peri-coerulear area can stimulate synchronous activity within the LC. Hence, t-VNS may increase electrotonic coupling, and in that way may promote phasic activation and decrease tonic activation of LC neurons, which in turn could normalize the dysbalance in LC activity and facilitate phasic spiking of LC neurons required for the release of NE levels high enough to activate low-affinity β-adrenoceptors to promote anti-inflammatory and neuroprotective mechanisms (32, 35, 65).

Electrophysiological studies in rats show that i-VNS increases the firing rate of LC neurons above their baseline activity (246, 251–253). Moreover, VNS causes a significant increase in the percentage of LC neurons firing in bursts (251–253), which in turn leads to a greater NE release in terminal fields as compared to single pulses (254). Short trains of VNS drive rapid, phasic

neural activity in the LC (248), and 30 second trains of VNS increase firing rates of LC neurons and NE concentrations in the cortex and hippocampus on the order of minutes to hours (246, 248, 251, 253, 255).

Since direct measurements would require invasive procedures there is a lack of human data on LC activity. However, a recent review of Burger and colleagues discusses the P300 as a potential biomarker for t-VNS effects (256) that seems to represent the phasic activity of the LC-NAergic system. Studies investigating t-VNS effects on P3b in an oddball paradigm in healthy subjects found increased P300/P3b amplitudes compared to sham stimulation (257, 258). Furthermore, consistent with the hypothesis that P3b reflects the activation of the LC-NA system, no effects of t-VNS stimulation on P3a were found (257, 259). However, there are other studies that could not confirm those data (260, 261).

If phasic LC activity in humans could be assessed by P3b measurements, a reduction in P3b amplitude and/or increase in latency in PD or AD patients could possibly indicate t-VNS as a valid non-invasive treatment option. Indeed, we have shown that t-VNS in healthy subjects leads to an increase in P3b amplitude and a reduction in P3b latency (258). Based on the literature cited above, these findings might be interpreted as t-VNS having a positive influence on the imbalance between tonic and phasic discharge by shifting LC activity toward increased phasic activation. If this presumption can stand the test of further experimental scrutiny, then t-VNS may have potential as a clinical tool used to normalize the imbalance between tonic and phasic LC activity in patients in certain stages of neurodegenerative diseases, and possibly have additional benefits by promoting anti-inflammatory and neuroprotective effects which require sufficiently large NE levels related to phasic LC discharge (32, 35, 65, 262). Recently, auricular t-VNS has been shown to have neuroprotective effects on dopaminergic neurons in 6-OHDA-treated rats and the authors suggested that these effects might be related to the inhibition of neuroinflammation (262). Thus, these results indicate t-VNS as a prospectively useful tool with potential anti-inflammatory and neuroprotective effects in early stages of neurodegenerative diseases, like AD and PD (**Figure 1**).

Concluding Remarks

Studies suggest that early damage to the LC in preclinical or prodromal phases of neurodegenerative diseases, such as AD and PD, may result in an abnormally persistent state of high tonic activity of the LC (181) that impairs phasic discharge, which requires a moderate tonic activity level. Since phasic LC discharge is essential for optimization of cognitive and neural network function (67), as well as anti-inflammatory and neuroprotective effects, a potential facilitation of phasic LC activity by t-VNS might be a useful clinical tool in early stages of neurodegenerative diseases, like AD and PD (**Figure 1**).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

KJ developed the presented hypotheses and wrote the manuscript.

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REVIEW
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Exciting Complexity: The Role of Motor Circuit Elements in ALS Pathophysiology

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Amyotrophic lateral sclerosis (ALS) is a fatal disease, characterized by the degeneration of both upper and lower motor neurons. Despite decades of research, we still to date lack a cure or disease modifying treatment, emphasizing the need for a much-improved insight into disease mechanisms and cell type vulnerability. Altered neuronal excitability is a common phenomenon reported in ALS patients, as well as in animal models of the disease, but the cellular and circuit processes involved, as well as the causal relevance of those observations to molecular alterations and final cell death, remain poorly understood. Here, we review evidence from clinical studies, cell type-specific electrophysiology, genetic manipulations and molecular characterizations in animal models and culture experiments, which argue for a causal involvement of complex alterations of structure, function and connectivity of different neuronal subtypes within the cortical and spinal cord motor circuitries. We also summarize the current knowledge regarding the detrimental role of astrocytes and reassess the frequently proposed hypothesis of glutamate-mediated excitotoxicity with respect to changes in neuronal excitability. Together, these findings suggest multifaceted cell type-, brain area- and disease stage- specific disturbances of the excitation/inhibition balance as a cardinal aspect of ALS pathophysiology.

Keywords: Amyotrophic lateral sclerosis, excitability, upper motor neurons, lower motor neurons, interneurons, astrocytes, neural circuits, excitotoxicity

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease primarily characterized by the death of upper motor neurons (UMN) and lower motor neurons (LMN) (Kiernan et al., 2011). Upon diagnosis patients only live up to 3–5 years, suffering from progressive paralysis and eventually die from respiratory failure (Cervenakova et al., 2000; Taylor et al., 2016). ALS is the most common form of adult motor neuron diseases with an incidence of ∼2/100,000 per year (Marin et al., 2017) and a prevalence of 2–5/100,000 people (Chiò et al., 2013). The majority of ALS cases (90–95%) occur sporadically (sALS) with unknown etiology, while a mere 5–10% are classified as familial (fALS), out of which only about 40–60% are related to already known mutations (Chen S. et al., 2013; Mejzini et al., 2019). To date, more than 50 genes and

gene variants have already been identified in ALS patients (Taylor et al., 2016; Mejzini et al., 2019). The most common forms of fALS are caused by either a hexanucleotide repeat expansion in the chromosome 9 open reading frame 72 (*C9orf72*, ∼40% of all fALS cases), mutations in the superoxide dismutase 1 (*SOD1*, ∼20%), in the TAR DNA-binding protein 43 (*TARDBP*, ∼4%) or in the fused in sarcoma (*FUS*, ∼3%) gene (Renton et al., 2014; Mejzini et al., 2019). One key pathological hallmark of ALS is intracellular protein aggregation, largely as a result of protein misfolding and/or cytosolic mislocalization (Blokhuis et al., 2013; Tyzack et al., 2019). Mechanistically, a number of molecular processes has been proposed to be causally related protein aggregation and motor neuron (MN) death, such as impaired RNA processing (Polymenidou et al., 2012) and proteostasis (Ruegsegger and Saxena, 2016), intracellular Ca^{2+} dyshomeostasis and reduced Ca^{2+} buffering capacity (Grosskreutz et al., 2010; Kawamata and Manfredi, 2010; Leal and Gomes, 2015), cytoskeletal derangements/axonal transport deficits (Xiao et al., 2006; Marinković et al., 2012), mitochondrial dysfunction (Kawamata and Manfredi, 2010), oxidative stress (Barber and Shaw, 2010), and excitotoxicity (Van Den Bosch et al., 2006) to name a few. In addition to those cell autonomous processes, i.e., processes that occur within the affected population of MN, there is also ample evidence pointing toward non-cell autonomous processes, conferred e.g., by glia cells, which strongly regulate disease onset and progression (Boillée et al., 2006; Philips and Rothstein, 2014; Serio and Patani, 2018). These research findings have spurred the development of numerous potential therapeutic substances with anti-glutamatergic, anti-inflammatory, anti-oxidative or neuroprotective effects, which have been tested for the treatment of ALS (Petrov et al., 2017). Amongst these compounds, only Riluzole (anti-glutamatergic) (Bensimon et al., 1994) and recently Edaravone (anti-oxidative) were approved by the FDA (Abe et al., 2014). However, these two compounds offer only very moderate benefits (Petrov et al., 2017; Jaiswal, 2019). A much-improved mechanistic insight into disease pathophysiology is thus urgently needed in order to identify novel, effective therapeutic approaches to combat ALS. One intriguing and consistent finding in both mouse models of the disease (Kim et al., 2017; Martínez-Silva et al., 2018) as well as in human patients (Kanai et al., 2006; Tamura et al., 2006; Vucic et al., 2008; Menon et al., 2015, 2017; Cengiz et al., 2019) are changes in neuronal excitability, which have been proposed to represent one of the earliest modifications in a cascade of pathological events leading to eventual MN death. The mechanisms underlying these excitability changes, as well as their downstream consequences are still incompletely understood, but hold the great promise for an early diagnosis and the identification of novel treatment options. In this review, we will thus summarize the current knowledge regarding alterations of excitability and activity of affected upper and lower motor neurons in humans and rodent models of the disease. Next, we will recapitulate molecular, structural and functional changes found in direct or indirect neuronal input partners of affected MNs and we will address alterations found in astrocytes, which also play an important role in the regulation of MN excitability and health.

WHAT IS "*EXCITABILITY*" AND HOW IS IT ASSESSED IN HUMANS AND RODENT MODELS OF ALS?

The term *excitability* refers to a neuron's propensity to generate an output [change in membrane potential, typically in the form of an action potential (AP), **Box 1**] in response to an input exceeding a certain threshold. This intrinsic property of a neuron ("intrinsic excitability") is determined by a number of factors that define biophysical properties of the cell, such as the composition, affinity and quantity of receptors (Hou and Zhang, 2017; Terunuma, 2018), pores or channels (e.g., K^+ and Na^+ channels) (Rutecki, 1992; Edwards and Weston, 1995; Schulz et al., 2006; Lin and Baines, 2015). When investigating the excitability or activity of individual neurons or neuronal populations, different methodological approaches and read-outs can be employed. The excitability of individual neurons can be assessed e.g., by intracellular recordings or patch-clamping (Cowan and Wilson, 1994; Hutcheon et al., 1996; Uusisaari et al., 2007; Gentet et al., 2010; Segev et al., 2016) (see **Box 2**). The excitability of multiple neurons or neuronal populations, on the other hand, can be monitored by extracellular field recordings (Jun et al., 2017; De Franceschi and Solomon, 2018) or optical means, such as voltage sensitive dyes (Kuhn et al., 2008; Akemann et al., 2013) or calcium indicators (Stosiek et al., 2003; Chen J.L. et al., 2013). To probe the excitability of neurons in humans, more indirect measures are typically employed, such as a combination of transcranial magnetic stimulation (TMS) together with electroencephalography (EEG) (Miniussi et al., 2012; Hill et al., 2016; Gonzalez-Escamilla et al., 2018) or for the motor system TMS stimulation of the motor cortex and simultaneous recording of the motor-evoked potential (MEP) of the respective innervated muscle. The actual activity of neurons (that is the frequency of APs) hinges on a number of factors, including the intrinsic excitability of a neuron, strength of individual synapses, as well as on the quantity and timing of excitatory synaptic input and its regulation by inhibition, a phenomenon called excitation-inhibition (E/I)

BOX 1 | Definitions of neuronal hyperexcitability and hyperactivity.
Hyperexcitability: Excitability is an electrophysiological property of a neuron, referring to its propensity to depolarize its membrane potential upon a given stimulus. It can be assessed e.g., by measuring the current needed to cause an AP (rheobase) or the frequency of fired APs in response to a defined input (frequency-current curve, F-I curve). Hyperexcitability is used to characterize a neuron, which is capable of producing a significantly larger AP frequency upon a defined input/stimulus compared to the majority of neurons of the same type under control conditions.
Hyperactivity: Neuronal activity refers to the frequency of fired AP (typically spontaneously elicited APs – that is without a defined stimulation or in case of awake mice in the absence of any behavior), which can be measured either by electrophysiological recordings or optical means employing Ca^{2+} indicators to probe changes in intracellular calcium in the form of fluorescent signals (transients) as a proxy for neuronal activity. A cell is considered hyperactive if it fires more APs (or Ca^{2+} transients) than the majority of neurons of the same type under identical conditions. The effective activity cut off defining hyperactivity is so far applied heuristically (Busche et al., 2015, 2008; Lerdkrai et al., 2018; Burgold et al., 2019).

BOX 2 | Techniques and terminologies used in clinical studies measuring cortical excitability in ALS patients.

Transcranial magnetic stimulation (TMS): A non-invasive procedure of applying a local time-varying magnetic field using a stimulation coil to depolarize neurons beyond their AP firing threshold. For the assessment of motor cortex excitability it is coupled with the measurement of motor evoked potentials (MEP), recorded from a contralateral innervated muscle (e.g., abductor pollicis brevis muscle) (Vucic et al., 2013).

Threshold-tracking TMS: The electromagnetic stimulus intensity required to maintain a target MEP response of 200 μV (motor threshold) is measured, reflecting the excitability of UMN (Fisher et al., 2002; Vucic et al., 2018; Cengiz and Kuruoğlu, 2020).

Resting motor threshold (RMT): The lowest electromagnetic stimulus intensity required to produce an MEP of at least 50 μV at rest (Rothwell et al., 1999; Rosso and Lamy, 2018).

Intracortical facilitation (ICF): Increased excitability primed by a conditioning stimulus. The MEP is measured upon the application of a paired pulse electromagnetic stimulation at an interstimulus interval of 7−30 ms (Wagle-Shukla et al., 2009).

Short-interval intracortical inhibition (SICI): Inhibition of the MEP response upon a conditioning stimulus applied at a short latency (7−10 ms) prior to the actual stimulation. It compares the MEP amplitude with and without a preceding subthreshold conditioning stimulus (Wagle-Shukla et al., 2009; Cengiz and Kuruoğlu, 2020).

Cortical silent-period (CSP) duration: Assessment of the duration a voluntary muscle contraction (as measured be EMG) is interrupted by a previous TMS stimulation (typically 100−300 ms upon TMS stimulation). It is considered a functional assessment of intracortical inhibition, elicited by the activation of GABAergic interneurons (Cantello et al., 1992; Vucic et al., 2008; Poston et al., 2012).

balance (He and Cline, 2019; Kiernan et al., 2019). It is important to note that altered excitability is not necessarily reflected in altered neuronal activity. Alterations in intrinsic excitability can be compensatory in response to reduced input to re-establish former activity levels (e.g., after sensory deprivation) (Hengen et al., 2013; Lambo and Turrigiano, 2013). Under pathological conditions, changes in excitability can be concordant or discordant with changes in neuronal activity (Le Feber et al., 2014; Busche and Konnerth, 2015; Kim et al., 2017). To get to the heart of the matter, it is thus important to assess not only intrinsic properties of a neuron, but to also characterize and quantify synaptic inputs and measure effective activity levels – ideally *in vivo* in the intact CNS.

Excitability Changes of UMN

Research of the past decade has identified altered excitability of UMN and LMN in ALS, both in human patients as well as in rodent models of the disease or cell culture systems. Notably, it has been suggested that the pathology is initiated in the motor cortex and propagates further to the spinal cord, forming the basis of the "dying forward hypothesis" (Eisen and Weber, 2001; Braak et al., 2013). Alternatively, the "dying-backward hypothesis," proposed by others, posits that the disease is initiated in the muscle or the neuromuscular junction (NMJ), from where it retrogradely affects LMNs and subsequently UMNs in cortex (Kiernan et al., 2011; Baker, 2014). Whether or not there is one common mode of disease-initiation shared by all forms of ALS, remains unanswered to date. Nonetheless, changes in excitability have been reported for both systems and shall be summarized here.

What is the evidence for excitability changes of UMN? In ALS patients, there is compelling evidence that motor cortex (M1) is hyperexcitable (Ziemann et al., 1997; Zanette et al., 2002; Vucic et al., 2008, 2009; Menon et al., 2015, 2017; Shibuya et al., 2017; Van den Bos et al., 2018; Cengiz et al., 2019; **Table 1**). A common method to assess cortical excitability in humans is threshold-tracking transcranial magnetic stimulation (TMS, see **Box 2**). The approach is based on the application of a local time- varying magnetic field of increasing intensities to gauge the intensity needed to depolarize neurons beyond their firing threshold (Barker et al., 1985; Hess and Ludin, 1988; Hallett, 1996; Oliveri et al., 2000, Oliviero et al., 2011). Although the mechanisms underlying the TMS-triggered depolarization of pyramidal neurons (PN) are still incompletely understood, it is a widely used approach to investigate the excitability of neuronal populations (Kim et al., 2005), such as UMN in humans (Ziemann et al., 1997; Siciliano et al., 1999; Zanette et al., 2002; Turner et al., 2005b; Vucic et al., 2008; Vucic and Kiernan, 2009; Menon et al., 2015, 2017; Shibuya et al., 2017; Van den Bos et al., 2018; Cengiz et al., 2019; Cengiz and Kuruoğlu, 2020). To verify the activation of UMN [or corticospinal excitability; (Cortes et al., 2012)], the motor evoked potential (MEP) of the innervated muscle is recorded (e.g., the abductor pollicis brevis muscle) (Vucic and Kiernan, 2006; Van den Bos et al., 2018). Alterations in cortical excitability could either be caused by enhanced intrinsic excitability and/or excitation or decreased inhibition. To differentiate those two options, different TMS stimulation protocols were developed to selectively investigate excitatory and inhibitory circuit function. A phenomenon reflecting inhibitory network function is short interval intracortical inhibition (SICI), which is probed by pairing a subthreshold TMS stimulus with a suprathreshold stimulus within a time window of 7−10 ms. The suprathreshold stimulus, needed to evoke a defined MEP, is much higher compared to a stimulation without a preconditioning stimulus (Vucic and Kiernan, 2006; Vucic et al., 2008; Wagle-Shukla et al., 2009; Shirota et al., 2010). Excitatory network function, on the other hand, is tested by assessing intracortical facilitation (ICF). In this case a subthreshold conditioning stimulus is followed by a test stimulus within 10−30 ms (Oliveri et al., 2000; Vucic and Kiernan, 2006). The intensity of the test stimulus, necessary to evoke a defined MEP response, is lower compared to an unconditioned stimulus (Ziemann et al., 1998; Vucic et al., 2008; **Box 2**). Employing variations of those TMS stimulation protocols studies have identified a reduction in the threshold needed to generate a MEP (Zanette et al., 2002; Menon et al., 2015, 2017), as well as a reduction in the intracortical inhibition (Ziemann et al., 1997; Zanette et al., 2002; Turner et al., 2005a,b; Vucic et al., 2008, 2009; Vucic and Kiernan, 2009; Menon et al., 2015, 2017; Shibuya et al., 2017; Van den Bos et al., 2018; Cengiz et al., 2019; Cengiz and Kuruoğlu, 2020) and an increase in the intracortical facilitation (Zanette et al., 2002; Vucic et al., 2008; Vucic and Kiernan, 2009; Menon et al., 2017; Van den Bos et al., 2018), thereby establishing that M1 E/I imbalance in ALS is based on a combination of increased excitability and decreased inhibition (**Table 1**).

TABLE 1 | Summary of excitability changes reported in motor cortex of ALS patients.

ALS type	Age (average)	Method	Finding	References
sALS	63.8 years	paired-pulse TMS	hyperexcitability & inhibition ↓: MEP_{max} ↓, ICI ↓	(Ziemann et al., 1997)
	65.3 years	threshold-tracking TMS	compromised inhibition: MEP onset latency ↑, CSP duration ↑/↓, early stages: CSP duration ↑, later stages: CSP duration ↓	(Siciliano et al., 1999)
	61.4 years	single pulse and paired-pulse threshold tracking TMS	inhibition ↓: MEP amplitude ↔, RMT ↔, ICF ↔, ICI ↓, CSP duration ↓	(Zanette et al., 2002)
	56.3 years	microarray, RT-qPCR of post-mortem tissue	various transcriptional alterations, including NMDA and AMPA receptors	(Aronica et al., 2015)
	62.5 years	threshold-tracking TMS	hyperexcitability in cortex (effect more prominent contralateral to site of disease onset) & inhibition ↓: MEP amplitude ↑, RMT ↓, SICI ↓, CSP duration ↓	(Menon et al., 2017)
sALS and fALS (hom $SOD1^{D90A}$)	57 years (sALS), 53 years (fALS)	single pulse and paired-pulse threshold tracking TMS; [^{11}C]flumazenil PET ($GABA_A$ receptor ligand)	inhibition ↓: SICI ↓, [^{11}C]flumazenil binding ↓, neuronal loss/dysfunction ↑	(Turner et al., 2005a,b)
fALS (SOD1, asymp. and symp.)	40 years (asymp.), 58.7 years (symp.)	threshold-tracking TMS	hyperexcitability and inhibition ↓: MEP amplitude ↑, ICF ↑, SICI ↓, stimulus-response curve ↑	(Vucic et al., 2008)
n.s.	67 years	ISH histochemistry on human postmortem M1	inhibition ↓: $GABA_A$ α1 subunit mRNA ↓, $GABA_A$ β1-subunit mRNA ↑	(Petri et al., 2003)
	59.5 years	3-T proton magnetic resonance spectroscopy	excitatory & inhibitory NT imbalance: GABA ↓, Glu ↓ (in Riluzole-treated group)	(Foerster et al., 2013)
	60.2 years	single pulse and paired-pulse threshold tracking TMS	hyperexcitability & inhibition ↓: MEP amplitude ↑, RMT↓, ICF ↑, SICI ↓, CSP duration ↓	(Zanette et al., 2002; Menon et al., 2015)
	63 years	single pulse and paired-pulse threshold tracking TMS	hyperexcitability & inhibition ↓: MEP amplitude ↑, RMT ↔, SICF ↑, SICI ↓, CSP duration ↓	(Van den Bos et al., 2018)
	59.8 years	threshold-tracking TMS	hyperexcitability & inhibition ↓: mean SAI and LAI values ↓, SICI ↓, MEP/CMAP amplitude ratio ↑	(Cengiz et al., 2019)
	61.3 years			(Shibuya et al., 2017)
	57.1 years	threshold-tracking TMS	inhibition ↓: RMT ↔, ICF ↔, SICF ↓ (for ISIs 1–1.8 ms & 2–3 ms), SICF ↔ (for ISI 4–4.6 ms), SICI ↓	(Cengiz and Kuruoğlu, 2020)
fALS	58 years	threshold-tracking TMS	hyperexcitability & inhibition ↓: SICI ↓, ICF ↑	(Vucic and Kiernan, 2009)

AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; asymp., asymptomatic; CMAP, compound muscle action potential; CSP, cortical-silent period; fALS, familial ALS; GABA, γ-aminobutyric acid; $GABA_A$, γ-aminobutyric acid receptor subtype A; Glu, glutamate; hom, homozygous; SOD1, superoxide dismutase 1; ICF, intracortical facilitation; ISH, in situ hybridization; ISI, interstimulus interval; LAI, long latency afferent inhibition; M1, primary motor cortex; MEP, motor evoked potential; MEP_{max}, maximum motor evoked potential; mRNA, messenger RNA; n.s., not specified; NMDA, N-methyl-D-aspartate; NT, neurotransmitter; PET, positron emission tomography; RT-qPCR, Real-Time quantitative polymerase chain reaction; RMT, resting motor threshold; SAI, short latency afferent inhibition; sALS, sporadic ALS; SICF, short interval intracortical facilitation; SICI, short interval intracortical inhibition; symp., symptomatic; TMS, transcranial magnetic stimulation.

Remarkably, there is evidence that cortical hyperexcitability precedes the actual onset of UMN and LMN symptoms, thus arguing in favor of a cortical origin of ALS (Vucic et al., 2008). What is known about the underlying molecular mechanisms causing cortical hyperexcitability? Studies employing proton magnetic resonance spectroscopy, positron emission tomography (PET) imaging or postmortem immunohistochemistry and whole-genome sequencing unraveled increased tissue levels of glutamate-glutamine and reduced levels of GABA (Foerster et al., 2012; Foerster et al., 2013; Khademullah et al., 2020), lower PV-expressing interneuron count (Khademullah et al., 2020), decreased $GABA_A$ receptor densities, changes in $GABA_A$ receptor composition (reduced α1 subunit and increased β1 subunit expression) (Petri et al., 2003), downregulation of NMDA receptor subunits and dysregulation of AMPA receptors in M1 (Aronica et al., 2015). What are the cellular or circuit mechanisms underlying the observed changes in cortical excitability? Transgenic (tg) mouse models of ALS are a valuable tool to address this question (**Table 2**). The most frequently used model in ALS research is the $SOD1^{G93A}$ tg mouse

TABLE 2 | Excitability related alterations of upper motor neurons (UMN) in rodent ALS models.

Disease stage	ALS model	Age	Method of investigation	Finding	References
presymptomatic	SOD1^{G93A}	11–12 DIV	whole-cell patch clamp recordings in neonatal mouse-derived cortical culture	RMP ↔, input resistance ↔, hyperexcitability: spiking frequency ↑, persistent Na^+ current ↑	(Pieri et al., 2009)
		P5–P6	whole-cell patch clamp recordings in neonatal brain slice	RMP ↔, input resistance ↔, hyperexcitability: rheobase ↓, max. AP frequency ↑	(Kim et al., 2017)
		P26–P40	whole-cell patch clamp recordings in brain slice	RMP for CSN ↓, RMP for CCN↔, input resistance, ↔, spiking frequency ↔, rheobase ↔, max. AP frequency ↔	
		P21–P40	whole-cell patch clamp recordings in brain slice and dye filling	EPSC ↑, IPSC ↔, dendritic arbor length ↓, apical & basal dendritic spine density ↓	(Fogarty et al., 2015, 2016b)
		P26–P31	whole-cell patch clamp recordings in brain slice, RT-qPCR, WB and IHC	RMP ↔, input resistance ↔, hyperexcitability: F-I gain ↑, rheobase ↓, sEPSC & mEPSC ↑, basal dendritic arborization ↑, basal dendritic spine density ↔, VGLUT2 mRNA & protein expression ↑	(Saba et al., 2016)
		P28–P35	Golgi-Cox staining	cortical thickness ↓, soma volume ↔, dendritic arbor length, ↓ apical & basal dendritic spine density ↓	(Fogarty et al., 2016b)
		P60	*in vivo* ^{1}H-MRS of brain	Gln ↔, Glu ↔, GABA ↓, Gly ↔, Glx ↔, Gln/Glu ↔	(Lei et al., 2019)
	TDP-43^{Q331K}	P26–P35	whole-cell patch clamp recordings in brain slice and morphology assessment	EPSC ↑, IPSC ↔, apical & basal dendritic spine densities ↑	(Fogarty et al., 2016a)
symptomatic	SOD1^{G93A}	P6–P75	Golgi-Cox staining	cortical thickness ↓, soma volume ↔, dendritic arbor length ↓, apical & basal dendritic spine density ↓	(Fogarty et al., 2016b)
		P90–P129	whole-cell patch clamp in brain slice	RMP ↔, hyperexcitability: rheobase ↓, max. AP frequency ↑, input resistance ↑	(Kim et al., 2017)
		P92–P134	*in vivo* two-photon Ca^{2+} imaging in awake mice	neuronal activity ↔	(Kim et al., 2017)
		P100	*in vivo* ^{1}H-MRS of brain	Glu ↓, GABA, Gly, Glx, Gln and Gln/Glu ↔	(Lei et al., 2019)
		P115		Glu ↓, GABA ↓, Gln/Glu ↑, Gly, Glx and Gln ↔	(Lei et al., 2019)
		P120–P122		Glu ↓, Glx ↓, Gln ↑, Gln/Glu ↑, GABA and Gly ↔	(Lei et al., 2019)
		P120	Golgi-Cox staining	cortical thickness ↓, soma volume ↔, dendritic arbor length ↓, basal dendritic spine density ↓	(Fogarty et al., 2016b)
		P120–P165	WB, intracerebral dialysis and HPLC of dialysate	[Glu & Asp] ↑, extracellular Glu clearance (Glu extraction fraction) ↓, GLT-1, GLAST and EAAC1 expression ↔	(Alexander et al., 2000; Deitch et al., 2002)

AP, action potential; Asp, aspartate; CCN, corticocortical neuron; CSN, corticospinal neuron; DIV, days in vivo; EAAC1, excitatory amino acid transporter 3; EPSC, excitatory postsynaptic currents; F-I, frequency-current; GABA, γ-aminobutyric acid; GLAST, glutamate–aspartate transporter; Gln, glutamine; Gln/Glu, ratio of glutamine and glutamate; GLT-1, glutamate transporter 1; Glu, glutamate; Glx, sum of glutamate and glutamine; Gly, glycine; HPLC, high performance liquid chromatography; IHC, immunohistochemistry; IPSC, inhibitory post synaptic currents; mEPSC, miniature excitatory postsynaptic currents; motor neuron; mRNA, messenger ribonucleic acid; RT-qPCR, real-time quantitative polymerase chain reaction; RMP, resting membrane potential; sEPSC, spontaneous excitatory postsynaptic currents; SOD1, superoxide dismutase 1; TARDBP, TAR DNA binding protein; tg, transgenic; VGLUT2, vesicular glutamate transporter 2; WB, western blotting.

(Gurney et al., 1994), which has been extensively characterized in the past decades. However, studying UMN pathology in rodent models is hampered by the fact that the motor system is wired up differently, such that UMN do not monosynaptically impinge on LMN (Anderson et al., 2010; Kaneko, 2013; Shepherd, 2013) and mouse models of the disease only partly mimic the degeneration of UMN. Thus, for quite some time it had been questioned as to whether UMN degeneration is recapitulated rodent models of the disease at all. In order to demonstrate UMN involvement in rodent models, Zang and Cheema (2002) and Özdinler et al. (2011) characterized the abundance and signs of UMN degeneration in the SOD1^{G93A} mouse model. They found a reduction of UMN quantity (identified upon retrograde labeling) early during the presymptomatic stage, coinciding with evidence for UMN apoptosis and UMN somata size reduction, even a month prior to changes in overall numbers (**Figure 1**). Furthermore, layer V PN in the SOD1^{G93A} mouse model are also affected structurally (Fogarty et al., 2015), as seen in a regression of apical dendrites and a reduction in the density of dendritic spines, the structural correlates of post-synapses, on apical and basal dendrites of layer V neurons (Fogarty et al., 2015, 2016b; **Figure 1**). Importantly, these abnormalities

occurred early presymptomatically (P21−P30) and persisted until late in life in this mouse model (Fogarty et al., 2015, 2016b). Similar findings were observed in another model, namely UCHL1 -/- mice, which carry an intragenic deletion within the ubiquitin carboxy-terminal hydrolase L1 gene. These mice display clear signs of UMN degeneration, including vacuolated apical dendrites, dendritic regression and spine loss (Jara et al., 2015). Contrary to those reports TDP-43^{Q331K} tg mice, expressing TDP-43 with a Q331K mutation, seem to possess even a greater spine density of layer V PN than the WT controls already early presymptomatically (P30) (Fogarty et al., 2016a; **Figure 1**). The investigation of other, novel or less frequently studied, mouse models of ALS has yielded mixed results. While most of them present with motor symptoms, coinciding with neuronal degeneration in the spinal cord, assessment of cortical pathology has either not been conducted in detail yet or yielded variable results. As such, Alsin^{KO} (Gautam et al., 2016), hPFN1 ^{G118V} (Fil et al., 2017), Prp-TDP43^{A315T} (Wegorzewska et al., 2009) or transgenic models of C9orf72 hexanucleotide repeat expansion (Batra and Lee, 2017) show signs of UMN degeneration, but a detailed morphological investigation has not been performed yet (see **Supplementary Table 1** for overview of mouse models).

Importantly, do these structural alterations translate into functional deficits? To this end, patch-clamp recordings of layer V PN were performed in acute brain slices of early presymptomatic SOD1^{G93A} and TDP-43^{Q331K} tg mice and indeed, an increase in the frequency of excitatory synaptic currents (Fogarty et al., 2015, 2016a,b; Saba et al., 2016), a decrease in rheobase (Saba et al., 2016), but no change in inhibitory synaptic frequency (Fogarty et al., 2015, 2016a,b) was found (**Figure 1**). Cortical layer V comprises several different populations of PN, which can be classified based on

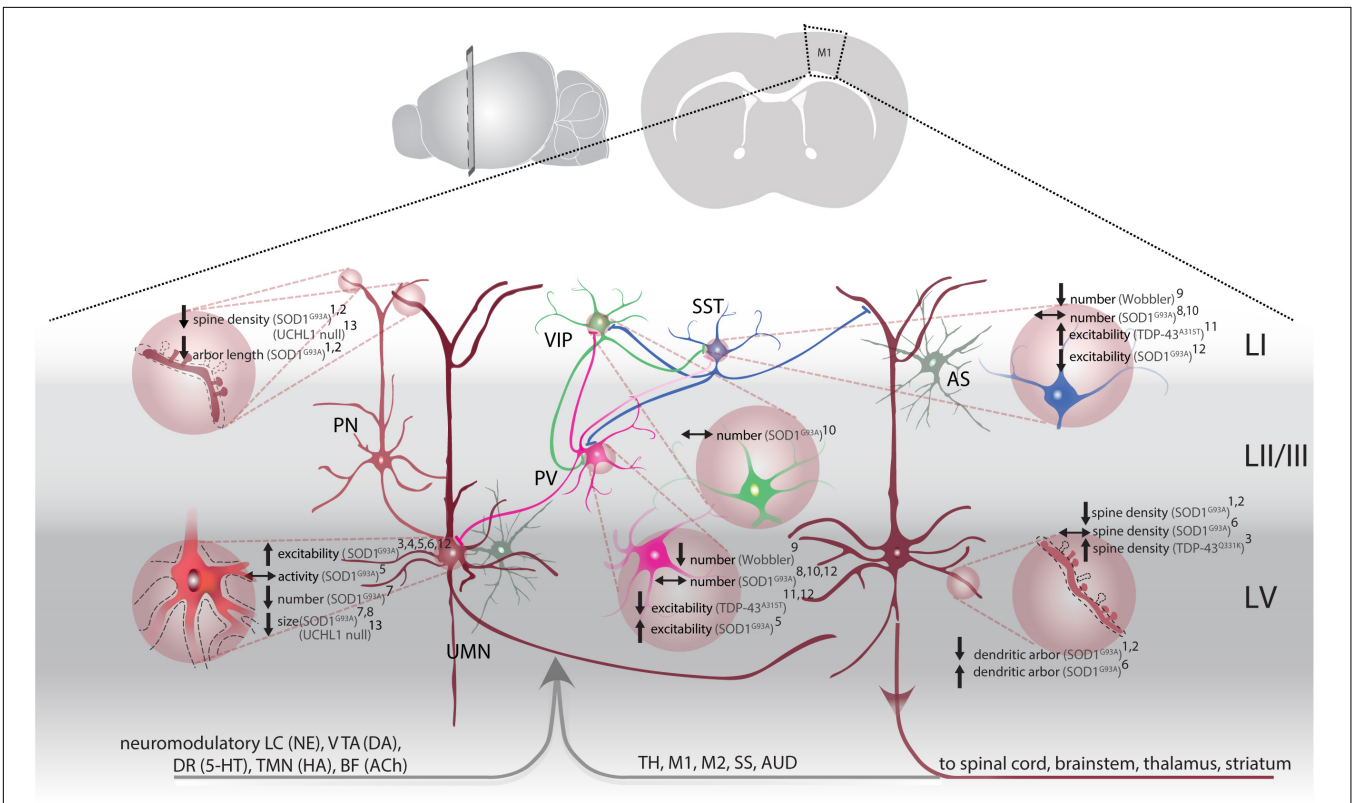


FIGURE 1 | M1 circuitry and pathophysiological changes in ALS. Local (glutamatergic, excitatory) input to upper motor neurons (UMN, brown) is mainly provided by upstream LII/III pyramidal neurons (PN, light brown) and modulated by astrocytes (AS, gray). Parvalbumin (PV, magenta), somatostatin (SST, blue) and vasoactive intestinal peptide (VIP, green) interneurons provide GABAergic input within M1. PV and SST target PN, including UMNs, as well as inhibit each other (inhibition of PV by SST more frequent). VIP are disinhibitory by synapsing on PV and SST. Long-range input to M1 originates from cortical and subcortical structures: thalamus (TH), primary motor cortex (M1), secondary motor cortex (M2), somatosensory cortex (SS), auditory cortex (AUD). Neuromodulatory input stems from: the locus coeruleus [LC, releasing norepinephrine (NE)], ventral tegmental area [VTA, releasing dopamine (DA)], dorsal raphe [DR, releasing serotonin (5-HT)], tuberomammillary nucleus [TMN, releasing histamine (HA)] and basal forebrain [BF, acetylcholine (ACh)]. UMN project to the spinal cord, brainstem and send axon collaterals to the thalamus and striatum. Pathological changes (light brown filled circles) are identified throughout the M1 microcircuitry: Structural changes, e.g., altered spine density and dendritic regression are observed on apical dendrites of LII/III PN[1,2] and on apical and basal dendrites of UMN[1,2], along with a reduction in overall number[7] and soma size[7,8,12]. UMN are hyperexcitable[3,4,5,6], but don't display overall activity changes[5]. Interneuron density was affected: while PV and SST are reduced in Wobbler mice[9], density of all three interneuron subtypes remained unchanged in SOD1^{G93A} mice[8,10]. Excitability of PV and SST was altered differentially. While hyperexcitability was observed in PV of SOD1^{G93A} mice[5], hypoexcitable PV were accompanied by hyperexcitable SST in TDP-43^{A315T} mice[11]. [1](Fogarty et al., 2016b); [2](Fogarty et al., 2015); [3](Fogarty et al., 2016a); [4](Pieri et al., 2009); [5](Kim et al., 2017); [6](Saba et al., 2016); [7](Zang and Cheema, 2002); [8](Özdinler et al., 2011); [9](Nieto-Gonzalez et al., 2011); [10](Clark et al., 2017); [11](Zhang et al., 2016); [12](Gautam et al., 2016).

their projection areas. Kim et al. used retrograde labeling to identify UMN amongst other populations and characterized electrophysiological properties at different disease stages in $SOD1^{G93A}$ tg mice (Kim et al., 2017). These experiments revealed disease stage − specific changes: already in neonatal mice UMN were hyperexcitable, seen in a lower rheobase and increased maximal firing frequency. In presymptomatic mice, these changes were normalized and did not differ from WT UMN anymore. In symptomatic mice a hyperexcitable phenotype was observed again (Kim et al., 2017; Buskila et al., 2019), which, however, did not reflect in an actual change in neuronal activity *in vivo* as measured by means of two-photon calcium imaging (Kim et al., 2017; **Figure 1**). Moreover, RNA-sequencing analysis in $SOD1^{G93A}$ tg mice revealed differential expression of over 300 genes in UMN early postnatally, such as the downregulation of CACNB4 (voltage-dependent L-type calcium channel subunit beta-4) and GABAR4 [γ-aminobutyric acid (GABA) receptor subunit alpha-4] (Kim et al., 2017). Together, these findings indicate that both intrinsic excitability as well as excitatory synaptic input is increased in UMN in mouse models of the disease and that these changes occur very early during the presymptomatic stage of the disease. Changes in excitability, however, do not seem to alter the activity of UMN *in vivo* (**Figure 1**).

BOX 3 | Techniques used to assess LMN excitability in ALS patients and common terminology.

Electromyography (EMG): Measurement of electrical activity (voltage change) in a muscle. Parameters assessed are the frequency, amplitude and shape of signals, and whether they occur spontaneously. EMG recordings are decisive for the differentiation between neurogenic or myogenic lesions. Signs typical of a neurogenic lesion (as in ALS) are abnormal spontaneous activity, presence of fasciculation potentials and fibrillations, reduced motor unit recruitment and motor unit potentials with greater amplitude or duration to name a few (Joyce and Carter, 2013). These parameters set diagnostic criteria for ALS defined by the revised El Escorial criteria (Ludolph et al., 2015).

Nerve conduction studies (NCS): Synonymous to nerve conduction velocity (NCV). NCS assess the velocity of an applied electrical signal propagating along a peripheral nerve. It is often combined with EMG to also measure the compound muscle action potential (CMAP). While ALS patients do not exhibit demyelination, thus have normal nerve conduction velocity, decreased CMAP is commonly seen (Mogyoros et al., 1998; Kanai et al., 2006; Tamura et al., 2006; Vucic and Kiernan, 2006; Vucic and Kiernan, 2009), which primarily indicates a reduction in intact motor axons innervating the respective muscle (Mallik and Weir, 2005).

Excitability Changes of LMN

What is known about the electrophysiological properties of LMN in ALS patients? The investigation of LMN activity/excitability in humans largely relies on indirect measures, such as nerve conduction studies (NCS) and electromyography (EMG) (Joyce and Carter, 2013; **Box 3** and **Supplementary Table 2**). These studies revealed an increase in motor unit excitability, evidenced by the increased presence of fasciculation potentials, double discharges of the motor unit (Kostera-Pruszczyk et al., 2002; Piotrkiewicz et al., 2008) and aberrant single motor unit firing (Piotrkiewicz et al., 2008) and increased axonal excitability (Bostock et al., 1995; Kanai et al., 2006; Nakata et al., 2006; **Figure 2**). Increased axonal excitability in ALS is likely due to enhanced persistent axonal Na^+ conductance and impairments in axonal K^+ conductance (Bostock et al., 1995; Horn et al., 1996; Mogyoros et al., 1998; Kanai et al., 2006; Nakata et al., 2006; Tamura et al., 2006; Vucic and Kiernan, 2006, 2009; **Box 3**) and was suggested to contribute to fasciculation potentials typical of ALS (de Carvalho and Swash, 2013; Howells et al., 2018; **Figure 2** and **Supplementary Table 2**). However, others showed that fasciculations cannot be solely explained by increased Na^+ conductance, but must rely on impairments of all ion channels, including reduced inward and outward rectifying K^+ channels (Howells et al., 2018). Nevertheless, these studies can only provide indirect measures of overall neuronal excitability. Indeed, Nakata et al. reported that distal parts of the axon display more prominent K^+ channel dysfunction than the nerve trunk, thus hyperexcitability is more evident in nerve terminals (Nakata et al., 2006).

As direct access to LMN in humans is impeded for obvious reasons, a large part of studies addressing LMN electrophysiological alterations is conducted *in vitro*, employing induced pluripotent stem cell (IPSC)- derived MN from patient fibroblasts (Sareen et al., 2013; Wainger et al., 2014; Devlin et al., 2015; Naujock et al., 2016), cultured MN derived from spinal cord of early postnatal or embryonic mice (Pieri, 2003; Kuo et al., 2004; Kuo et al., 2005; Martin et al., 2013) or acutely isolated spinal cord/brain stem slices (Kuo et al., 2004; Bories et al., 2007; van Zundert et al., 2008; Pambo-Pambo et al., 2009; Quinlan et al., 2011; Martin et al., 2013; Leroy et al., 2014; Jiang et al., 2017; **Figure 3** and **Tables 3**, **4**). Thanks to recent advances in stem cell research, it is now possible to study the excitability and activity of MN derived from human patients' fibroblasts through the generation of IPSC. These studies revealed that IPSC-derived MN from ALS patients are hyperexcitable and hyperactive in early cultures (2−6 weeks) (Wainger et al., 2014; Devlin et al., 2015), while they become hypoexcitable and hypoactive, as evidenced by a lower firing rate – input (F-I) gain and reduced spontaneous activity compared to control MN, when maintained longer (7−10 weeks old cultures) (Sareen et al., 2013; Zhang et al., 2013; Devlin et al., 2015; Naujock et al., 2016; **Figure 3**). The discrepancy between studies could potentially be explained by differences in the proportion of mature MN in cultures, as features like repetitive firing requires complete functional maturation (Devlin et al., 2015). Another important point to take into account is that while the majority of these cultures consist of neurons (80%), only ∼50% of which are classified as MNs while rest are potentially spinal interneurons (Devlin et al., 2015). Mirroring *early* hyperexcitability observed in rodent and human UMN as well as IPSC-derived LMN, cultured LMN derived from spinal cord of embryonic or neonatal $SOD1^{G93A}$ tg mice were also shown to be hyperexcitable as seen in an increased firing frequency upon current injection (Pieri, 2003), increased maximum firing rate and F-I gain (Kuo et al., 2004), increased persistent Na^+ current and decreased spiking threshold (Kuo et al., 2005; **Figure 3**). Similarly, hyperexcitability was also reported in slice culture studies obtained from embryonic/neonatal $SOD1^{G93A}$ or $SOD1^{G93A\text{-}low}$ tg mice (**Figure 3**). Whole-cell patch-clamp

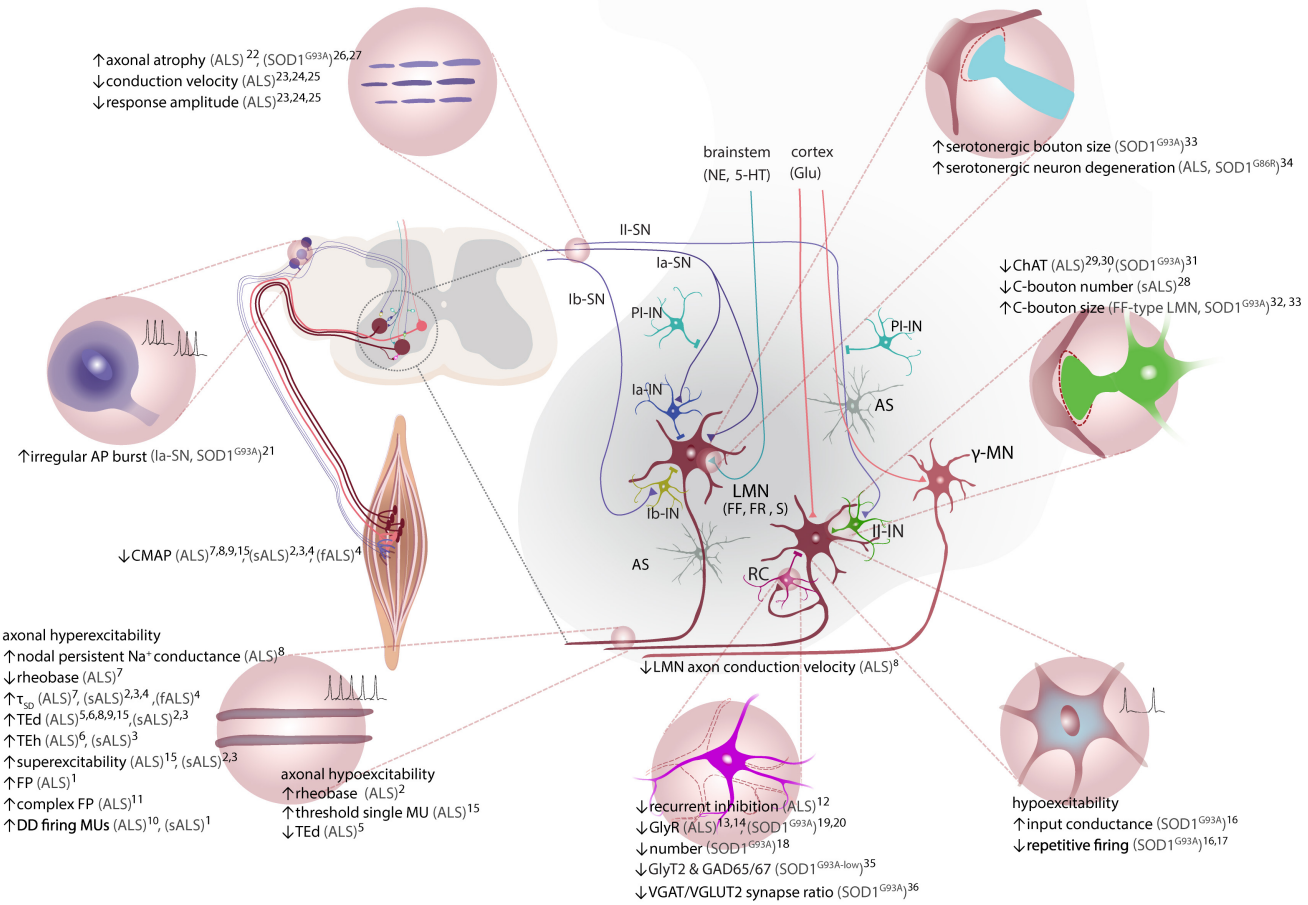
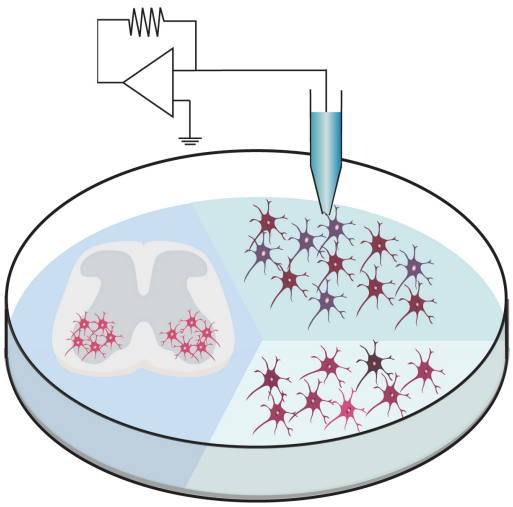


FIGURE 2 | ALS-associated alterations in ventral spinal cord circuitry. LMN receive inhibitory input via Ia-IN, Ib-IN, and RC, and excitatory inputs from cortiospinal tract (UMN), II-IN and SN. γ-motor neurons, which are spared in ALS, do not receive direct inputs from Ia-SN. Excitatory inputs to LMNs via Ia afferent terminals are controlled by PI-IN. Both excitatory and inhibitory inputs are tightly regulated by the proprioceptive feedback provided by sensory afferents (Ia, Ib and II-SN) and astrocytes. Axonal hyperexcitability and hypoexcitability are reported in ALS patients. Decreased RC synapses on LMN and lower number of RC is reported. LMN hypoexcitability is present *in vivo* in SOD1^{G93A} tg mouse model. Ia-SN neurons exhibit irregular firing patterns as an indication of their altered excitability/activity. Cholinergic C-bouton number is decreased in sALS patients, but C-boutons are enlarged especially onto vulnerable FF LMN in SOD1^{G93A} tg mice. Protein and mRNA levels of ChAT are decreased in spinal cord of ALS patients. Reduced ChAT expression is reported in II-IN and C-boutons on MN of SOD1^{G93A} tg mice. Serotonergic boutons on LMN are increased in low-copy SOD1^{G93A} tg mice, whereas serotonergic neurons in brainstem degenerate in both ALS patients and SOD1^{G86R} tg (not shown). Please note that monosynaptic connections between UMN and LMN are only present in humans. Neuromodulatory synapses are depicted as one (somata located in brainstem). CPGs and descending reticulospinal tract projections to LMN via commissural INs are not depicted for simplicity. Studies with unspecified type of ALS are referred to as (ALS). AP, action potential; AS, astrocytes; CMAP, compound muscle action potential; ChAT, choline acetyltransferase; CPG, central pattern generator; DD, double-discharge; fALS, familial ALS; FF, fast-fatigable; FP, fasciculation potential; FR, fast-resistant; gamma (γ)-motor neuron, γ-MN; GAD65/67, glutamic acid decarboxylase 65/67; Glu, glutamate; GlyT2, glycine transporter 2; Ia-/Ib-IN, class Ia/Ib spinal interneuron; II-IN, class II spinal interneuron; LMN, lower motor neuron; MU, motor unit; NE, norepinephrine; Ia-/Ib-SN, class Ia/Ib sensory neuron; II-SN, class II sensory neuron; PI-IN, presynaptic inhibitory interneuron; RC, Renshaw cell; sALS, sporadic ALS; SOD1, superoxide dismutase 1; S, slow; TEd, depolarizing threshold electrotonus; TEh, hyperpolarizing threshold electrotonus; τ_{SD}, strength-duration constant, UMN, upper motor neuron; VGAT, vesicular GABA transporter; VGLUT, vesicular glutamate transporter 2; 5-HT, serotonin. [1](Kostera-Pruszczyk et al., 2002); [2](Kanai et al., 2006); [3](Vucic and Kiernan, 2006); [4](Vucic et al., 2009); [5](Bostock et al., 1995); [6](Horn et al., 1996); [7](Mogyoros et al., 1998); [8](Tamura et al., 2006); [9](Nakata et al., 2006); [10](Piotrkiewicz et al., 2008); [11](de Carvalho and Swash, 2013); [12](Raynor and Shefner, 1994); [13](Hayashi et al., 1981); [14](Whitehouse et al., 1983); [15](Howells et al., 2018); [16](Delestrée et al., 2014); [17](Martínez-Silva et al., 2018); [18](Chang and Martin, 2009); [19](Chang and Martin, 2011a); [20](Chang and Martin, 2011b); [21](Seki et al., 2019); [22](Heads et al., 1991); [23](Hammad et al., 2007); [24](Pugdahl et al., 2007); [25](Sangari et al., 2016); [26](Guo et al., 2009); [27](Sassone et al., 2016); [28](Nagao et al., 1998); [29](Oda et al., 1995); [30](Virgo et al., 1992); [31](Casas et al., 2013); [32](Pullen and Athanasiou, 2009); [33](Saxena et al., 2013); [34](Dentel et al., 2012); [35](Hossaini et al., 2011); [36](Sunico et al., 2011).



embryonic/neonatal LMN cultures

hyperexcitability:

↓spiking threshold (SOD1^{G93A})[7]
↑persistent Na$^+$ current (SOD1^{G93A})[7]
↓glycinergic current (SOD1^{G93A})[15]
↓glycinergic mIPSCs (SOD1^{G93A})[15]
↑F-I gain (SOD1^{G93A})[6]
↑firing frequency (SOD1^{G93A})[5]

brainstem/spinal cord slices

hyperexcitability:

↓rheobase (S-type LMN, SOD1^{G93A})[12], (SOD1$^{G93A\text{-}low}$)[13]
↑RMP (SOD1^{G93A})[13]
↓spiking threshold (S-type LMN, SOD1^{G93A})[12]
↑persistent Na$^+$ current (SOD1^{G93A})[9]
↑persistent inward currents (SOD1^{G93A})[10]
↑F-I gain (SOD1^{G93A})[6]
↑F-I gain to current pulses (SOD1$^{G93A\text{-}low}$)[13]
↑maximum firing rate (SOD1^{G93A})[6]
↑firing frequency (SOD1^{G93A})[11]
↑repetitive firing (SOD1^{G93A})[14]
↑spontaneous activity (SOD1^{G93A})[9]

hypoexcitability:

↓input resistance (SOD1^{G85R})[8]
↓F-I gain to ramp current (SOD1^{G85R}, SOD1$^{G93A\text{-}low}$)[13]

normoexcitability:

↔F-I gain (SOD1^{G93A})[16]

IPSC-derived LMN cultures

hyperexcitability:

↓DR K$^+$ peak current (SOD1^{A4V})[1]
↑F-I gain (TARDBP, C9orf72)[2]
↑total/mean firing rate (C9orf72)[1]
↑spontaneous activity (SOD1, FUS, C9orf72)[1]
↑synaptic activity (SOD1, FUS)[4]

hypoexcitability:

↓membrane capacitance (SOD1, FUS)[4]
↑FI and DR K$^+$ peak currents (FUS)[4]
↓persistent VG K$^+$ peak current (TARDBP, C9orf72)[2]
↓FI Na$^+$ peak current (TARDBP, C9orf72)[2]
↓Na$^+$ peak current (SOD1, FUS)[4]
↓F-I gain (C9orf72)[3]
↓repetitive firing (TARDBP, C9orf72)[2], (C9orf72)[3], (SOD1, FUS)[4]
↓single/adaptive firing (SOD1, FUS)[2]
↓synaptic activity (TARDBP, C9orf72)[2], (SOD1, FUS)[4]

normoexcitability:

↔single/repetitive firing (SOD1, FUS)[4]
↔spontaneous activity (SOD1, FUS)[4]
↔VG ion channel currents (SOD1, FUS)[4]

FIGURE 3 | ALS-associated changes in LMN excitability assessed *in vitro*. Findings obtained through recordings of LMN (brown) in embryonic/neonatal cell culture, IPSC-derived LMN culture or spinal cord slice culture are shown. Data indicative of hyperexcitability is shown in red, hypoexcitability in blue and no change in green. Abbreviations: C9orf72, chromosome 9 open reading frame 72; DR, delayed-rectifying; FI, fast -inactivating; F-I, frequency-current; FUS, fused in sarcoma; IPSC, induced pluripotent stem cell; LMN, lower motor neuron; RMP, resting membrane potential; SOD1, superoxide dismutase 1; TDP-43, TAR DNA-binding protein 43; VG, voltage-gated. [1](Wainger et al., 2014); [2](Devlin et al., 2015); [3](Sareen et al., 2013); [4](Naujock et al., 2016); [5](Pieri, 2003); [6](Kuo et al., 2004); [7](Kuo et al., 2005); [8](Bories et al., 2007); [9](van Zundert et al., 2008); [10](Quinlan et al., 2011); [11](Martin et al., 2013); [12](Leroy et al., 2014); [13](Pambo-Pambo et al., 2009); [14](Jiang et al., 2017); [15](Chang and Martin, 2011a); [16](Delestrée et al., 2014).

recordings made in lumbar/sacral spinal cord or brainstem slices demonstrated a more depolarized resting membrane potential and lower rheobase (Pambo-Pambo et al., 2009; Leroy et al., 2014), increased F-I gain (Kuo et al., 2004; Pambo-Pambo et al., 2009), firing frequency (Martin et al., 2013), persistent inward currents (Quinlan et al., 2011), and persistent Na$^+$ current accompanied by enhanced spontaneous activity (van Zundert et al., 2008; **Figure 3**). However, Pambo-Pambo et al. (2009) demonstrated that the application of a different stimulation protocol (slow ramp current injection designed to test slow persistent currents), LMN from low copy SOD1^{G93A} as well as SOD1^{G85R} tg mice are hypoexcitable. These studies collectively report alterations of LMN excitability, observed mainly as initial hyperexcitability, which later changes into hypoexcitability. To investigate LMN in a more physiological setting, Delestrée et al. (2014) recorded from lumbar LMN in presymptomatic (P40−P50) SOD1^{G93A} anesthetized mice and found that their excitability was unchanged (current needed to trigger an AP), but their input conductance was increased (**Figure 3**). Furthermore, a substantial fraction of those had lost their ability to fire repetitively, arguing for the hypoexcitability of LMN in early stages *in vivo* (Delestrée et al., 2014). Seemingly at odds with these findings, are data pointing toward increased excitability of LMN (prolonged repetitive firing and higher frequency of spontaneous EPSPs) upon the stimulation of the dorsal root in acutely isolated sacral spinal cords obtained from presymptomatic − early symptomatic (P50−P90) SOD1^{G93A} tg mice (Jiang et al., 2017). Interestingly, based on pharmacological testing Jiang et al. propose that the observed changes in spontaneous depolarization originate at least in part from spinal network inputs rather than through the peripheral afferents (Jiang et al., 2017). Given the differences in sample preparation, recording technique, stimulation procedure and age of the animals, a decisive conclusion remains unattainable at the moment. Another important aspect to address, concerns the fact that LMN constitute distinct subtypes, which differ in their intrinsic excitability (Delestrée et al., 2014; Leroy et al., 2014; Martínez-Silva et al., 2018) and vulnerability (Pun et al., 2006; Hegedus et al., 2008; Saxena et al., 2009). As such, three main LMN subtypes can be distinguished. LMN innervating fast-fatigable (FF) extrafusal muscle fibers are the most vulnerable and degenerate early in the disease course. LMN innervating fast-resistant (FR) muscle fibers are less vulnerable and degenerate later in the disease, while those innervating slow (S) muscle fibers are resistant and persist until the end stage of the disease (Pun et al., 2006; Hegedus et al., 2008). In order to address whether the cell type specific vulnerability is reflected in different intrinsic properties of those subtypes, Martínez-Silva et al. performed intracellular recordings of LMN in deeply anesthetized mice *in vivo*. They discerned motor units (FF, FR, and S) based on their contractile properties by applying a pulse stimulation to the recorded LMN and measuring the force

TABLE 3 | Excitability related alterations of lower motor neurons (LMN) in rodent ALS models.

Disease stage	ALS model	Age	Method of investigation	Finding	References
Presymptomatic	$SOD1^{G93A}$	neonatal	whole-cell patch-clamp recordings MNs cultured from spinal cord	hyperexcitability: firing frequency ↑	(Pieri, 2003)
		neonatal and embryonic	intracellular recordings in neonatal organotypic spinal cord slice cultures	hyperexcitability: F-I gain ↑, maximum firing rate ↑	(Kuo et al., 2004)
			patch-clamp recordings in embryonic primary MN culture	hyperexcitability: F-I gain ↑	
		embryonic	whole-cell patch-clamp recordings of MNs cultured from spinal cord	hyperexcitability: persistent Na^+ currents ↑, spiking threshold ↓	(Kuo et al., 2005)
		P4–P10	whole-cell patch-clamp recordings in brainstem slices	hyperexcitability: persistent Na^+ currents ↑, spontaneous activity ↑	(van Zundert et al., 2008)
		P0–P12	whole-cell patch-clamp recordings in isolated lumbar and sacral spinal cord	hyperexcitability: persistent inward currents ↑	(Quinlan et al., 2011)
		E17.5	whole-cell patch-clamp recordings in isolated lumbar spinal cord	hyperexcitability: firing frequency ↑	(Martin et al., 2013)
		P6–P10	whole-cell patch-clamp recordings in lumbar spinal cord, current stimulation of the ventral rootlet	S-type LMN hyperexcitability: rheobase ↓, spiking threshold ↓	(Leroy et al., 2014)
		P40–P50	intracellular recordings of LMNs in sacral spinal cord	normoexcitability: F-I gain ↔, input conductance↑	(Delestrée et al., 2014)
		P34–P82	current clamp recordings of lumbar LMNs in anesthetized mice *in vivo*	normoexcitability: F-I gain ↔, input conductance ↑ hypoexcitability: repetitive firing ↓	
		P50–P90	intracellular recordings of LMNs and ventral root of isolated sacral spinal cord	hyperexcitability: repetitive firing ↑	(Jiang et al., 2017)
		E12–P14	whole-cell patch-clamp recordings of MNs cultured from spinal cord	inhibition ↓: glycinergic currents ↓, glycinergic mIPSCs ↓	(Chang and Martin, 2011a)
	$SOD1^{G93A}$ and FUS^{P525L}	P46–P60	current clamp recordings of lumbar LMNs in anesthetized mice *in vivo*	FF- and FR-type LMN hypoexcitability: repetitive firing ↓	(Martínez-Silva et al., 2018)
	$SOD1^{G85R}$	P6–P10	Intracellular recordings in isolated lumbar spinal cord	hypoexcitability: input resistance ↓	(Bories et al., 2007)
		P6–P10	whole-cell patch-clamp recordings in isolated lumbar spinal cord	hypoexcitability: F-I gain to ramp current ↓	(Pambo-Pambo et al., 2009)

F-I, frequency-current; FUS, fused in sarcoma; LMN, lower motor neuron; mIPSCs, miniature inhibitory postsynaptic currents; MN, motor neuron; RMP, resting membrane potential; SOD1, superoxide dismutase 1.

developed at the muscle tendon (Martínez-Silva et al., 2018). Investigations were performed in presymptomatic $SOD1^{G93A}$ (P46–P60) and FUS^{P525L} tg mice (P30). They found that the most vulnerable FF type and a fraction of FR type LMN lost the ability to fire repetitively, thus became hypoexcitable, while S type LMN did not exhibit any changes in excitability (Martínez-Silva et al., 2018). The phenotype worsened with advancing disease, seen in an increase in the fraction of non-repetitively firing neurons in older FUS^{P525L} mice. These studies unraveled yet another level of complexity in ALS pathogenesis indicating large LMN subtype differences. Notably, S type LMN were shown to be hyperexcitable early postnatally (P6–P10), suggesting that hyperexcitability might even serve a neuroprotective role (Leroy et al., 2014). This notion is in concordance with an earlier publication, which demonstrated that treating low copy $SOD1^{G93A}$ tg mice with a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) agonist during the presymptomatic phase (P80), decreased misfolded SOD1 aggregates and ER stress and delayed muscle denervation by FR and S type LMN (Saxena et al., 2013). It also prevented the decline in muscle force, when administered slightly later (P145). Prolonged treatment (beginning $\leq$ P145) with AMPA receptor agonist even increased survival rates (Saxena et al., 2013). Notwithstanding, systemic application of the AMPA agonist cannot delineate the cellular/circuit mechanisms underlying the observed protective effect. To this end, LMN-selective, chemogenetic approaches were used to demonstrate that the effects were cell-autonomous (Saxena et al., 2009). Of note, the inverse experiment, that is blocking AMPA receptors by the application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), aggravated disease progression and shortened life span, thereby further substantiating the notion of neuroprotection by elevated excitation. These intriguing data contradict the dogma of glutamate-mediated excitotoxicity and the current therapeutic standards, which aim at reducing neuronal excitation (Bensimon et al., 1994). Despite not being fully conclusive, these findings collectively argue for more complex alterations of LMN excitability that are likely LMN subtype – and disease stage – dependent, which could explain why increased activation is protective for some while detrimental for others. Taken together, while findings of UMN excitability are more consistent, LMN excitability/activity in ALS warrants further scrutiny.

TABLE 4 | Excitability changes detected on ALS patient IPSC-derived MN.

ALS model	Age	Method of investigation	Finding	References
fALS (*SOD1*$^{A4V, D90A, G85S}$, *FUS*^{MGH5b,H517Q}, *C9orf72*)	4 WIV	multielectrode array and whole-cell patch-clamp recordings in IPSC- derived MNs	hyperexcitability and hyperactivity: spontaneous activity ↑, total/mean firing rate ↑ (*C9orf72*), DR K^+ peak currents ↓ (*SOD1*^{A4V})	(Wainger et al., 2014)
fALS (*C9orf72*)	9–11 WIV	whole-cell patch-clamp recordings in IPSC-derived MNs	hypoexcitability: F-I gain ↓, repetitive firing ↓	(Sareen et al., 2013)
fALS (*TDP-43* ^{M337V} or *C9orf72*)	2–6 WIV		early hyperexcitability: F-I gain ↑	(Devlin et al., 2015)
	7–10 WIV		switch to late hypoexcitability: single/adaptive/repetitive firing ↓, synaptic activity ↓, persistent VG K^+ peak currents ↓, FI Na^+ peak currents ↓	
fALS (SOD1 $^{D90A, R115G}$ *FUS*^{R521L,R521C}, ^{R495QfsX527})	3–4 WIV		normoexcitability: single/repetitive firings ↔, spontaneous activity ↔, VG ion channel currents↔	(Naujock et al., 2016)
			early hyperexcitability: synaptic activity ↑	
	7–10 WIV		switch to late hypoexcitability at ∼7–10 WIV in fALS cultures: repetitive firing ↓, membrane capacitance ↓, FI and DR K^+ peak currents ↑ (FUS only), Na^+ peak currents ↓	
	13–14 WIV		hypoexcitability: synaptic activity ↓	
fALS (*TDP-43*^{A90V})	4–5 WIV		hypoactivity: spontaneous activity ↓	(Zhang et al., 2013)

C9orf72, chromosome 9 open reading frame 72 gene; fALS, familial ALS; sALS, sporadic ALS, sALS; DR, delayed-rectifying; FI, fast -inactivating; F-I, frequency-current; FUS, fused in sarcoma gene; RMP, resting membrane potential; SOD1, superoxide dismutase 1 gene; TDP-43, TAR DNA-binding protein 43; tg, transgenic; VG, voltage-gated; WIV, weeks in vitro.

THE ROLE OF CIRCUIT ELEMENTS IN MOTOR NEURON EXCITABILITY AND DEGENERATION IN ALS

Despite ample evidence indicating changes in intrinsic excitability of motor neurons (likely via cell autonomous mechanisms), there is also data arguing for altered synaptic inputs (non-cell autonomous processes) (Fogarty et al., 2015; Jiang et al., 2017). As neuronal activity hinges on both parameters, we will summarize the current knowledge of sources providing synaptic inputs and how these might be affected by disease pathology. To obtain a more complete picture of these mechanisms, we will first delineate the cortical and spinal cord circuitries facilitating and regulating UMN and LMN activity.

Cortical Circuits and Drivers of Cortical Neurodegeneration in ALS

Upper motor neurons (a.k.a. Betz cells in humans) are PN that reside in cortical layer VB. They receive local excitatory input from intratelencephalic neurons (IT) within M1. IT neurons constitute neurons that project transcallosally (corticocortical, CC) and those projecting to the striatum (corticostriatal, CStr). UMN largely receive input from upstream layer II/III IT (CC) and intralaminar input within layer V, from both layer VA (CC, CStr) and VB (UMN, also referred to as pyramidal tract neurons, PT) (**Figure 1**). Remote input to UMN (PT) is provided by the contralateral motor cortex, secondary motor cortex, somatosensory cortices, sensory and motor thalamus, parietal – and frontal cortex (Anderson et al., 2010; Hooks et al., 2013; Shepherd, 2013; Yamawaki and Shepherd, 2015; Commisso et al., 2018; **Figure 1**). UMN (PT) are highly multiprojectional, known to innervate multiple targets through collaterals, branching off from the main axon that is projecting to its caudal destination in the spinal cord or brain stem (Shepherd, 2013; **Figure 1**). In addition to excitatory input, GABAergic interneurons provide inhibition within the local microcircuitry and across brain areas to modulate UMN activity (Isaacson and Scanziani, 2011; Tatti et al., 2017; Swanson and Maffei, 2019). Interneurons can be classified based on their morphology, physiological properties, postsynaptic target(s) and surface markers expressed (Markram et al., 2004; Tremblay et al., 2016). Three major, largely non-overlapping GABAergic interneuron subtypes in rodents and humans are parvalbumin (PV), somatostatin (SST), and ionotropic serotonin receptor 5-HT3a expressing interneurons [majority of which express vasoactive intestinal peptide (VIP)] (Rudy et al., 2011; Tremblay et al., 2016; Wood et al., 2017; **Figure 1**). Together they constitute 10–20% of the cortical neuronal population (Nigro et al., 2018; Swanson and Maffei, 2019). PV-expressing cells comprise ∼40% of the cortical GABAergic population and are known to be fast-spiking, with low input resistance, providing strong inhibition on PN (Bartos and Elgueta, 2012; Tremblay et al., 2016; Safari et al., 2017; Wood et al., 2017; Yu et al., 2019). They are typically basket cells, which synaptically target the soma and proximal dendrites of PN (Bartos and Elgueta, 2012; Safari et al., 2017; Veres et al., 2017). The second most common type are SST-expressing interneurons (30% of all interneurons), most of which are Martinotti cells that

target the dendrites of PN (Nigro et al., 2018). Their somata are distributed throughout layers II to VI, but are most abundant in layer V (Scheyltjens and Arckens, 2016; Nigro et al., 2018). The remaining less well-characterized 5-HT3a subtype composes 30% of the interneuronal population (Lee et al., 2010; Rudy et al., 2011). Vasoactive intestinal polypeptide (VIP)-expressing interneurons are the most commonly seen within this subtype. Notably, VIPs mainly serve a disinhibitory role within the network as they inhibit SST- and PV-expressing interneurons (Lee et al., 2013; Yu et al., 2019). These excitatory and inhibitory inputs to UMN are fine-tuned, based on the behavioral context and attentional state, by multiple neuromodulatory systems, such as dopamine (DA), norepinephrine (NE, also known as noradrenaline), serotonin (5-HT), histamine (HA) and acetylcholine (ACh) (Gu, 2002; Conner et al., 2010; Shepherd, 2013; Vitrac and Benoit-Marand, 2017; **Figure 1**). Together, these different circuit elements are involved in directing UMN activity by regulating synaptic inputs. Functional deficits of one of these elements or compromised connectivity within the network can affect activity levels of UMN and impair information processing of UMN. Non-cell-autonomous processes have been shown to contribute to the reported cortical hyperexcitability. As such neurons and glia cells that shape and regulate the activity of MN need to be considered in the pathogenesis as well. The three main putative non-cell autonomous sources (excluding glia cells, which will be addressed in a separate paragraph), causing increased MN excitation, are thus: (1) increased excitatory input, (2) decreased inhibition, and (3) reduced neuromodulation. What is the evidence for a potential contribution of those factors?

Increased Excitatory Input

Electrophysiological recordings of LV PN in mouse models of ALS ($SOD1^{G93A}$, $TDP\text{-}43^{Q331K}$) demonstrated increased synaptic excitation, occurring already in early pre-symptomatic stages (Fogarty et al., 2016a,b; Saba et al., 2016). These electrophysiological changes were accompanied by structural changes seen in a lower cell complexity (reduction of dendritic arbor length) and a reduction of spines (sites of excitatory input) on apical and basal dendrites in the $SOD1^{G93A}$ model, but an increase in spine density in the TDP-43 model (Fogarty et al., 2015, 2016a,b). Furthermore, an increase in the expression of vesicular glutamate transporter VGLU2 has been shown selectively in M1 of presymptomatic $SOD1^{G93A}$ tg mice (Saba et al., 2016). This finding strongly indicates that presynaptic input to M1, including UMN, is increased either quantitatively or qualitatively. The source or cellular origin of this increased input, however, remains elusive thus far. Notably, a recent publication argues for aberrant connectivity and thus increased synaptic input to M1 provided by S1 and contralateral M2 already in juvenile (very early presymptomatic) $SOD1^{G93A}$ tg mice (Commisso et al., 2018). This aberrant connectivity aggravated with disease progression, resulting in larger input from areas, such as the thalamus, contralateral M1, auditory cortex and the caudoputamen in later disease stages (Commisso et al., 2018). Of note, hyperconnectivity is also observed in ALS patients, thus providing a possible mechanism of cortical hyperexcitability (Commisso et al., 2018).

Compromised Inhibition

Increased excitation or a shift in the balance between excitation/inhibition (E/I) can also result from defective inhibition. Indeed, functional clinical studies employing TMS indicate that cortical hyperexcitability is at least in part based on a reduction of cortical inhibition (Prout and Eisen, 1994; Zanette et al., 2002; Vucic et al., 2008; Menon et al., 2015; Cengiz et al., 2019). Moreover, a reduction in GABA levels was found in motor cortex of ALS patients using proton magnetic resonance spectroscopy (MRS) (Foerster et al., 2012). This effect was only partially rescued by treatment with Riluzole (Foerster et al., 2012). Along these lines, an altered molecular composition of the $GABA_A$ receptor has been shown, evidenced by a reduction of α1-subunit mRNA and an increase in the β1-subunit mRNA levels in postmortem motor cortex, which could indicate altered receptor function (Petri et al., 2003). Mouse models of the disease have further substantiated the notion of compromised inhibition in ALS pathophysiology. Whole-cell patch-clamp recordings of cultured interneurons from embryonic Gad67-GFP:$SOD1^{G93A}$ mice revealed that interneurons (subtype not specified) were morphologically less complex and less excitable (Clark et al., 2018). Subtype-specific investigations have furthermore unraveled a selective impairment of different interneuron populations. The largest interneuron population in the cortex, PV-expressing interneurons, was shown to undergo disease-stage specific changes. While PV interneurons in presymptomatic (P26−P35) $SOD1^{G93A}$ tg mice did not differ from WT controls, they turned hyperexcitable in symptomatic (P90−P129) mice (Kim et al., 2017), indicating a possible compensatory mechanism (**Figure 1**). The effects, however, might also be mutation specific, as in the $TDP\text{-}43^{A315T}$ mouse model, PV interneurons were found to be hypoexcitable in presymptomatic mice and received more inhibitory synaptic input compared to the WT controls (Zhang et al., 2016). In the same model, the authors found a striking increase in the excitability of SST interneurons, which led them to propose a microcircuit model of UMN hyperexcitability, in which hyperexcitable SST interneurons depress PV interneurons, thereby releasing the break on UMN (Zhang et al., 2016; **Figure 1**). In addition to structural and electrophysiological changes, there are also contradictory findings regarding interneuron subtype-specific density in motor cortex. While the density of PV, SST and VIP interneurons remain unchanged in $SOD1^{G93A}$ mice (Özdinler et al., 2011; Clark et al., 2017), there is a decrease in PV and SST density in Wobbler mice (Nieto-Gonzalez et al., 2011). Remarkably, a SOD1 zebrafish model of the disease even suggests that interneuron dysfunction precedes MN degeneration (McGown et al., 2013). It is relevant to stress that also interneurons express glutamatergic synapses, some of which even carry AMPA receptors lacking the GluA2 subunit, which renders them highly Ca^{2+} permeable akin to MN (Akgul and McBain, 2016), thus potentially also putting interneurons at risk of receiving large Ca^{2+} influx. Most importantly, one needs to acknowledge that a global anti-glutamatergic treatment will

also affect the excitability and activity-dependent recruitment of inhibitory neurons, which could further exacerbate a potential inhibitory deficit. Though these are all intriguing novel aspects of cortical hyperexcitability, it remains to be clarified whether these findings are generalizable to other forms/mutations of the disease, whether the effect is disease-stage dependent and of course whether similar cell type specific changes are at play in human patients.

Impaired Neuromodulation

As neuromodulation further tunes neuronal activity, it could also modify E/I balance in M1. In ALS patients, there are data demonstrating a dopaminergic as well as a serotonergic deficit (Takahashi et al., 1993; Borasio et al., 1998; Sandyk, 2006; Vermeiren et al., 2018). Serotonin exerts amongst others inhibitory control of glutamatergic release via 5-HT_{1B} presynaptic receptors (Muramatsu et al., 1998), the lack of which could contribute to excitotoxicity. Moreover, ALS patients have increased levels of norepinephrine in blood and the cerebrospinal fluid (CSF) (Ziegler et al., 1980), which could synergize with glutamate and increase neuronal excitation (Mather et al., 2016). For a more detailed description on the impact of altered neuromodulation in motor cortex in ALS see Brunet et al. (2020). Together, these studies emphasize that circuit elements other than the mainly affected UMN are strongly altered likely already early in the disease. Whether these precede or follow UMN excitability changes remains to be resolved. Therefore, non-cell autonomous mechanisms warrant more attention and further investigation.

Spinal Circuit Elements Contributing to LMN Degeneration

In analogy to evidence indicating a casual involvement of circuit mechanisms in the degeneration of UMN, also LMN degeneration is likely a result of more complex alterations within spinal circuits. The majority of inputs LMN receive are provided by descending tracts from supraspinal regions e.g., cortex and brainstem. In humans, UMN-LMN connections are mainly monosynaptic, while in rodents this connection is polysynaptic, involving interposed interneurons (Lemon, 2008). Nevertheless, LMN activity is regulated by spinal interneurons in all mammals (Bikoff et al., 2016; Bikoff, 2019). For instance, the execution of a motor command is tightly controlled via class I interneurons (Ia-IN and Ib-IN, inhibitory: glycinergic), Renshaw cells (inhibitory: glycinergic/GABAergic), and class II interneurons (II-IN, excitatory: glutamatergic/cholinergic) (Côté et al., 2018; **Figure 2**). While class II-IN are the sole source of cholinergic inputs (Rozani et al., 2019), they are not the only neuromodulatory input LMN receive (Heckman et al., 2003). Descending tracts primarily from brainstem nuclei modulate LMN excitability via noradrenergic (from locus coeruleus) and rich serotonergic (from raphe nucleus) inputs, mainly facilitating their excitation (Heckman et al., 2003; Bruinstroop et al., 2012; **Figure 2**). Noradrenergic inputs project to the ventral horn (Jones and Yang, 1985; Bruinstroop et al., 2012), where multiple noradrenergic receptors are expressed, however, whether they form a direct monosynaptic connection to LMN is not clear (Smith et al., 1995, 1999; Day et al., 1997; Mizukami, 2004; Tartas et al., 2010; **Figure 2**). Furthermore, descending serotonergic inputs form both monosynaptic synapses with LMN (Sławińska and Jordan, 2019), as well as indirect synapses via spinal interneurons, comprising the central pattern generator (CPG). CPGs are neural networks in the spinal cord that generate rhythmic movements, which are largely independent of descending inputs from higher motor areas and sensory inputs from sensory afferents (Grillner, 2003). Spinal interneurons are proposed to provide their input to CPGs or form part of the network, which are regulated by neuromodulatory inputs (Gerasimenko et al., 2016; Laliberte et al., 2019). Moreover, LMN receive proprioceptive feedback from muscles via three major sensory afferent subtypes, namely Ia, Ib and II afferents (excitatory: glutamatergic) (Brown, 1981; **Figure 2**). An additional spinal interneuron population, presynaptic inhibitory interneurons (PI-IN, inhibitory: GABAergic), synapsing on Ia afferent terminals also tune final sensory input on the LMN (Stein, 1995; Fink et al., 2014; **Figure 2**). Furthermore, LMN regulate their own activity by activating Renshaw cells, which in turn inhibit LMN, a phenomenon called recurrent inhibition (Eccles et al., 1954; Cullheim et al., 1977; Nishimaru et al., 2005; **Figure 2**).

Excitatory Inputs

Excitatory glutamatergic input to the ventral horn is provided mainly by descending projections from motor cortex (corticospinal tract, UMN), pontine and medulla (reticulospinal tract, commissural interneuron), sensory afferents and partially by excitatory II-IN and LMN-Renshaw cell synapses (Brownstone and Bui, 2010; Côté et al., 2018; **Figure 2**). There have been reports of affected excitatory input to LMN in ALS (Menon et al., 2019; Seki et al., 2019). Modified excitatory input is also recapitulated in mouse models of ALS. Genetic reduction of vesicular-glutamate transporter 2 (VGLUT2) expression in $SOD1^{G93A}$ tg mice reduced motor neuron loss in lumbar spinal cord and brainstem, but had no impact on disease onset or life span, suggesting a partial rescue of degenerative processes (Wootz et al., 2010). Another main glutamatergic input is provided by proprioceptive afferents, which also seem to be affected in the disease. Seki et al. (2019) have reported the occurrence of irregular action potential bursts selectively in Ia proprioceptive sensory neurons located in the brainstem of presymptomatic (P8−P14) $SOD1^{G93A}$ tg mice. To address a potential impact of impaired sensory input on LMN function and health, Lalancette-Hebert et al. eliminated Ia inputs on LMN. To this end, they crossed $SOD1^{G93A}$ tg mice with the $Egr3^{KO}$ (early growth response 3 knockout) mutant mouse, which develops muscle spindle degeneration and thus lack proper Ia sensory feedback. The reduction of excitatory inputs from Ia afferents in $SOD1^{G93A}/Egr3^{KO}$ double mutants slowed down LMN loss, but again did not alter disease progression (Lalancette-Hebert et al., 2016). However, when in addition genetically ablating γ-MNs, which innervate muscle spindles, disease onset was delayed and lifespan increased (Lalancette-Hebert et al., 2016). Thus, alterations of excitatory sensory inputs seem to negatively affect LMN health in the disease.

Compromised Inhibition

As in cortex, impaired inhibition is also a feature of circuit deficits in the spinal cord in ALS. In the early 90s, an abnormal reduction of recurrent inhibition was reported in spastic ALS patients (Raynor and Shefner, 1994) and decreased glycinergic receptor expression has been found (Hayashi et al., 1981; Whitehouse et al., 1983; **Figure 2**). Moreover, a reduction in glycine levels in the serum was shown (Malessa et al., 1991), while others demonstrated the opposite (Kostera-Pruszczyk et al., 2002). Mouse models recapitulated the finding of compromised inhibition as evidenced by a progressive loss of glycinergic synapses on LMN presymptomatically and of Renshaw cells during the early symptomatic phase in $SOD1^{G93A}$ tg mice (Chang and Martin, 2009) and decreased glycine transporter 2 (GlyT2) and glutamic acid decarboxylase (GAD65/67) expression in the ventral horn of symptomatic low-copy $SOD1^{G93A}$ tg mice (Hossaini et al., 2011; **Figure 2**). The loss of inhibitory spinal interneurons (primarily but not exclusively Renshaw cells) was apparent in the late symptomatic stage, whereas motor neuron loss was reported at earlier time point, suggesting that motor neuron degeneration may trigger interneuronal pathology (Hossaini et al., 2011). In line with these findings, another study also reported a reduction of the inhibitory (vesicular GABA transporter, VGAT)/excitatory (VGLUT2) synapse ratio of hypoglossal motor neurons occurring already at presymptomatic stages in the same mouse model (Sunico et al., 2011). Of note, the imbalance resulted from an increase in excitatory synapses and a decrease in inhibitory contacts (Sunico et al., 2011). Cardinal aspects were also confirmed in cell culture models, where a decrease in postsynaptic glycine receptor expression was found (Chang and Martin, 2011a,b). Moreover, whole-cell patch clamp recordings in motor neuron cultures obtained from embryonic $SOD1^{G93A}$ tg mice revealed decreased glycine-induced currents and glycine receptor expression on LMN, whereas no change in $GABA_A$-induced currents (Chang and Martin, 2011a). Whereas, Carunchio et al. described an increased affinity of the $GABA_A$ receptor subtype, expressed on cultured embryonic MN from $SOD1^{G93A}$ tg mice, indicating a potential compensatory effect in response to reduced glycinergic inhibition (Carunchio et al., 2008).

Sensory Circuits

Sensory systems are considered less or not affected in ALS (Kawamura et al., 1981). However, there is also evidence for compromised sensory feedback onto LMN, the role of which in ALS pathology currently warrants further scrutiny. Sensory feedback from muscles to LMN is provided by afferents of proprioceptive sensory neurons, whose somata are located in the dorsal root ganglion (DRG) (**Figure 2**). This sensory feedback is regulated by spinal interneurons (Ia, Ib, II, and PI-IN). Early studies have revealed peripheral sensory nerve pathology, seen as axonal atrophy and increased remyelination in ALS patients (Heads et al., 1991; **Figure 2**). Further studies in symptomatic patients revealed reduced sensory nerve response amplitudes and conduction velocities (Hammad et al., 2007; Pugdahl et al., 2007; Sangari et al., 2016) and impaired dorsal column integrity, even in the absence of sensory deficits (Cohen-Adad et al., 2013; **Figure 2**). Mouse models of the disease partially recapitulate these findings, e.g., the degeneration of dorsal root axons was detected in presymptomatic $SOD1^{G93A}$ tg mice, without obvious sensory deficits (Guo et al., 2009; Sassone et al., 2016). Moreover, sensory neurons (in particular Ia afferents) are also susceptible to cellular stress, misfSOD1 accumulation and mitochondrial damage at early disease stages (Guo et al., 2009; Sábado et al., 2014; Vaughan et al., 2018).

Altered Neuromodulation

Neuromodulatory transmitters, such as acetylcholine, serotonin, dopamine or noradrenaline play a prominent role in the adjustment of excitatory inputs to LMN (Zagoraiou et al., 2009; Tartas et al., 2010; Sharples et al., 2014; Sławińska and Jordan, 2019). There is ample evidence indicating profound alterations of neuromodulators in ALS pathophysiology. For instance cholinergic input provided by C-boutons was decreased in sporadic ALS patients (Nagao et al., 1998; **Figure 2**). Moreover, a reduction in ChAT expression was observed both at the protein (Oda et al., 1995) and the mRNA level in spinal cord of ALS patients (Virgo et al., 1992). Concordantly, the expression of choline acetyltransferase (ChAT) in C-boutons and in cholinergic interneurons of presymptomatic $SOD1^{G93A}$ tg mice was also reduced compared to wild-type mice (Casas et al., 2013; **Figure 2**). In contrast, Pullen and Athanasiou (2009) reported an increased LMN C-bouton coverage in both presymptomatic and late-symptomatic $SOD1^{G93A}$ tg mice. This discrepancy could be due to a LMN subtype – specific modulation of cholinergic inputs. Indeed, a disease-associated enlargement of C-boutons was confined to FF subtype LMN in the $SOD1^{G93A}$ mouse model during the presymptomatic phase, while it affected the majority of LMN as disease progressed (Saxena et al., 2013). In order to test the hypothesis that C-bouton enlargement served a compensatory and neuroprotective role, Saxena et al. used a pharmacological approach to modify neuronal excitability. As proposed, treatment with the AMPA antagonist CNQX caused an increase of C-bouton size, while the AMPA agonist caused a decrease in size of those synapses on LMN (Saxena et al., 2013). These findings demonstrate that enhancement of LMN excitability through cholinergic input could serve as a compensatory mechanism in response to LMN activity reduction (Saxena et al., 2013). Behavioral support for this notion is provided by a study, in which the silencing of these premotor cholinergic interneurons compromised the compensation of impaired locomotor behavior at a much younger age in $SOD1^{G93A}$ tg mice (Landoni et al., 2019). Notably, restoring C-boutons number and function in presymptomatic $SOD1^{G93A}$ tg mice by viral-mediated delivery of type III-Neuregulin-1 (a trophic factor regulating neurotransmission and synaptic plasticity) extended survival (Lasiene et al., 2016). Together, these findings highlight the relevance of compensatory cholinergic input for LMN function and health. In addition to cholinergic input, LMN are also regulated by serotonergic and noradrenergic inputs. Earlier reports demonstrated increased levels of serotonin and noradrenaline in the ventrolateral lumbar spinal cords obtained post-mortem from ALS patients (Bertel et al., 1991). Corroborating these findings, serotonergic boutons

were shown to be substantially increased around LMN in presymptomatic (P50) low-copy SOD1^{G93A} tg mice (Saxena et al., 2013; **Figure 2**). In contrast, serotonergic neuron degeneration was reported in ALS patients and SOD1^{G86R} tg mice, contributing to spasticity in the latter (Dentel et al., 2012). While serotonin mainly modulates LMN activity positively, depending on the specific receptor subtypes expressed on MN, it can also inhibit neuronal activity (Perrier et al., 2013). In addition to LMN also spinal interneurons, sensory afferent terminals, and astrocytes are modulated by serotonin via a large set of different serotonergic receptors (Bardoni, 2019). Therefore, the net effect of serotonin on LMN is also dependent on the activation of the particular interneuron population (inhibitory/excitatory) and the receptor types expressed on those neurons (Ciranna, 2006), further complicating the interpretation of those findings with respect to ALS pathophysiology. Overall, the data strongly argues for a compensatory increase of facilitatory neuromodulatory inputs to LMN in ALS. Collectively, it can be argued that a more complex dysregulation of spinal circuitries accompanies and potentially triggers LMN dysfunction and degeneration in ALS. The underlying sequence of events establishing cause and consequence and the nature (i.e., compensatory or detrimental) of the observed alterations remains to be elucidated.

ROLE OF GLIAL CELLS IN NEURONAL DEGENERATION AND EXCITABILITY

Neuronal communication is strongly regulated and modulated by glial cells. In particular, astrocytes represent a vital component of neural circuits (Ben Achour and Pascual, 2012; Allen and Eroglu, 2017; Durkee and Araque, 2019). One of the core tasks is the effective clearance of glutamate from the synaptic cleft, which is a prerequisite for a spatiotemporal confined synaptic transmission and should protect neurons from detrimental "overstimulation" (Rothstein et al., 1996; Trotti et al., 1999). Since the early 2000s, glial cells have gained increasing attention as an integral part of ALS pathophysiology, but their role in the disease is still incompletely understood. Several molecular, genetic and transplantation studies argue for both a toxic gain-of-function (Nagai et al., 2007; Marchetto et al., 2008; Re et al., 2014; Rojas et al., 2015) as well as a loss-of-physiological function in astrocytes (Rothstein et al., 1995; Howland et al., 2002; Kaiser et al., 2006; Ferraiuolo et al., 2011; Bataveljic et al., 2012; Kawamata et al., 2014) in ALS (**Figure 4** and **Supplementary Table 3**), which we will summarize here.

Altered Astrocytic Function and Molecular Composition

Astrocytes as Regulators of Extracellular Glutamate

One of the earliest piece of evidence arguing for a causal involvement of astrocytes in ALS pathophysiology was the downregulation of the astrocytic glutamate transporter EAAT2 (a.k.a. GLT1 in rodents) in motor cortex and spinal cord of both familial and sporadic end-stage ALS patients (Rothstein et al., 1995; **Figure 4** and **Supplementary Table 3**). Similar findings were made in mouse models of the disease both in spinal cord (SOD1^{G85R} and SOD1^{G93A} tg mice) (Bruijn et al., 1997; Howland et al., 2002; Wilson et al., 2003; Bendotti et al., 2008) and in motor cortex (ALS-PDC tg mice) (Wilson et al., 2003). This reduction was specific to the EAAT2, as the expression of the EAAT1 (GLAST) or the neuronal EAAT3 (EAAC-1) was not affected (Rothstein et al., 1995). The decrease in EAAT2 expression was suggestive of impaired glutamate uptake and was hence proposed to cause glutamate-mediated excitotoxicity on MN (Rothstein et al., 1995). This notion resonated within the field of neurodegeneration research, as astrocytic loss of EAAT2 expression was also observed in other neurodegenerative diseases, such as Alzheimer's disease (Li et al., 1997; Jacob et al., 2007), Huntington's disease (Arzberger et al., 1997; Shin et al., 2005; Faideau et al., 2010), and epilepsy (Binder and Steinhauser, 2006; Wetherington et al., 2008), arguing for a convergence of cellular mechanisms shared by these diseases. However, we still lack proof of a direct causal link between EAAT2 reduction and MN death. Manipulation of EAAT2 expression in rodent models of ALS thus far yielded mixed results. Heterozygous knock out of the glutamate transporter (SOD1^{G93A}/GLT1$^{\pm}$ tg mice) caused a faster disease progression and decreased survival slightly (Pardo et al., 2006). To address the question whether the reduction of GLT1 is sufficient to cause neuronal degeneration, in a seminal manuscript Rothstein et al. (1996) knocked out GLT1 in the CNS of rats by chronic intraventricular application of antisense oligonucleotides. As hypothesized, the consecutive decrease in GLT1 expression resulted in an increase in extracellular glutamate (30-fold increase – 16 μM), which was accompanied by ultrastructural changes such as cytoplasmic vacuolization, dilated Golgi apparatus and lamellated intracytoplasmic structures. Phenotypically, these mice exhibited progressive deficits in motor function, including ataxia, dystonia and hindlimb paralysis (Rothstein et al., 1996). One caveat of the study is that GLT1 levels in the spinal cord were not reduced upon the intraventricular infusion of antisense oligonucleotides. Thus, the observed motor deficits are likely mainly a result of damaged supraspinal motor areas at least in the initial phase. These studies confirm a toxic role of elevated glutamate levels. However, the resulting extracellular glutamate levels exceeded those seen in ALS patients (Plaitakis and Constantakakis, 1993; Shaw et al., 1995; Cid et al., 2003). Does overexpression of reuptake transporters mitigate disease symptoms? While this seems a conceivable approach, the hitherto available data is inconclusive. One early study reported a delay in muscle force loss, but no effect on disease onset or life span in SOD1^{G93A} tg mice crossed to a transgenic mouse line expressing the hEAAT2 under the GFAP promoter (Guo et al., 2003). Intriguingly, in a slice culture screening approach β-lactam antibiotics were identified to stimulate EAAT2 expression and were subsequently tested *in vivo* for its impact on disease progression in SOD1^{G93A} tg mice (Rothstein et al., 2005). Indeed, chronic treatment with Ceftriaxon, a β-lactam antibiotic, initiated at symptom onset (12 weeks) delayed neuronal loss, muscle weakening and increased mouse survival (Rothstein et al., 2005). These highly promising findings initiated a clinical trial probing the efficacy of Ceftriaxon to alleviate disease progression

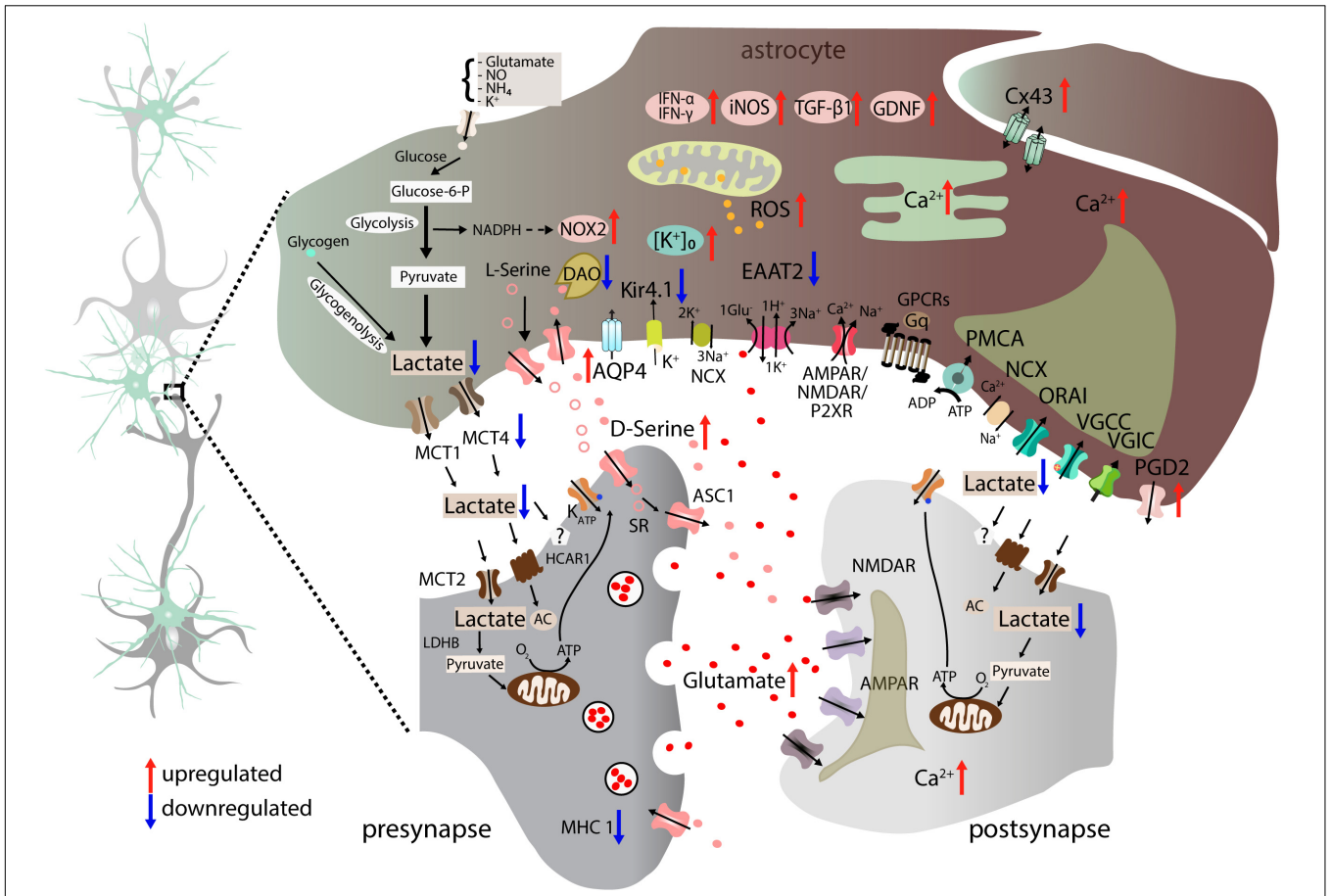


FIGURE 4 | Altered neuron-astrocyte interaction in ALS. Astrocytes lose homeostatic function through multiple proposed molecular dysregulations. Astrocytic EAAT2 downregulation results in increased synaptic glutamate (red dots) and enhanced postsynaptic NMDAR and AMPAR activation. Moreover, astrocyte-mediated K^+ clearance is disrupted due to downregulation of Kir4.1 and upregulation of co-localized AQP4 channels. Astrocyte-neuron lactate shuttle is impaired as a result of reduced MCT4 expression leading to lower extracellular lactate concentration (Ferraiuolo et al., 2011). Astrocytes gain toxic properties by generating factors such as ROS, NOX2, and iNOS (Marchetto et al., 2008), NOS or TGF-β1 (Endo and Yamanaka, 2015). In addition, they secrete proinflammatory cytokines IFN-γ and IFN-α/β (Aebischer et al., 2011) and upregulate PGD2 receptor expression. Glutamate-excitotoxicity is aggravated by increased levels of D-Serine (pink dots) due to reduced degradation caused by DAO downregulation in astrocytes. Furthermore, astrocytes also display Ca^{2+} dyshomeostasis in the cytosol and ER. Connectivity between astrocytes is altered by elevated expression of the gap junction protein Cx43. Blue arrows indicate downregulation, whereas red arrows show upregulation. AC, adenylyl cyclase; ADP, adenosine diphosphate; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; AQP4, aquaporin-4; ASC1, astrocytic transporter SLC7A10; ATM, ataxia telangiectasia mutated; Ca^{2+}, calcium ion; Cx43, connexin 43; EAAT2, excitatory amino acid transporter 2; glucose-6-P, glucose-6-phophate; GDNF, glial cell-derived neurotrophic factor; GPCRs, G-protein-coupled receptors; Gq, q subunit of heterotrimeric G protein; HCAR1, hydroxycarboxylic acid receptor 1; IFN-α, interferon alpha; IFN-β, interferon beta; IFN- γ, interferon gamma; iNOS, inducible nitric oxide synthase; K^+, potassium ion; Kir4.1, inward-rectifying potassium channel 4.1; LDHB, lactate dehydrogenase B; MCT1, monocarboxylate transporter 1; MCT2, monocarboxylate transporter 2; MCT4, monocarboxylate transporter 4; MHC1, major histocompatibility complex class I; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NCX, sodium-calcium exchanger; NH_4, ammonium; NMDAR, N-methyl-D-aspartate receptor; NO, nitric oxide; NOX2, NADPH oxidase 2; NOS, nitric oxide synthase; O_2, oxygen; ORAI: Ca^{2+} release-activated Ca^{2+} channel protein 1; P2XR, ionotropic receptors; PDG2, prostaglandin D_2 receptor; PMCA, plasma membrane Ca^{2+}-ATPase; VGIC, voltage-gated ion channels.

in ALS. Unfortunately, a stage-3 clinical study could not show any beneficial impact on symptom progression or survival in ALS patients, deeming the substance ineffective (Cudkowicz et al., 2014). Apart from a mere passive role provided by synaptic glutamate uptake, astrocytes have recently also been acknowledged as active partners regulating neurotransmission and synaptic function (reviewed by Chung et al., 2015). Amongst others, astrocytes were shown to instruct the composition of neuronal glutamate receptors, thereby determining neuronal vulnerability (Van Damme et al., 2007). To demonstrate this astrocytic property Van Damme et al. employed a MN-astrocyte co-culture system to investigate the interaction of both populations between two different rat strains. Both strains differed in the vulnerability of AMPA-mediated excitotoxicity due to differential expression of the GluR2 subunit of the AMPA receptor in MN (low level GluR2 AMPA receptors facilitate high Ca^{2+} permeability). Interchanging the astrocyte and MN population from both strains in the co-culture system revealed that astrocytes determine MN GluR2 abundance thereby regulating MN vulnerability (Van Damme et al.,

2007). Importantly, the expression of $SOD1^{G93A}$ abolished the capacity of astrocytes to up-regulate the GluR2 subunit and thus reduce the vulnerability of MN to excitotoxicity (Van Damme et al., 2007). Another interesting aspect of EAAT2 function is its potential detrimental role due to posttranslational modification. Earlier work has demonstrated that the EAAT2 can be cleaved by caspase 3 and the resulting sumoylated c-terminal fragment subsequently accumulates within astrocytes, conveying neurotoxic effects (Foran et al., 2014). A number of other posttranslational modifications of the EAAT2, such as palmitoylation, nitrosylation or ubiquitination have been identified (Peterson and Binder, 2019), but their putative impact on ALS pathophysiology remains to be clarified.

Astrocytes as Regulators of Extracellular Potassium

In addition to impaired glutamate re-uptake, disrupted astrocyte-mediated clearance of K^+ (termed spatial K^+ buffering) and H_2O was suggested to promote neuronal hyperexcitability (Haj-Yasein et al., 2011; Florence et al., 2012; Devinsky et al., 2013; Bellot-Saez et al., 2017), and therefore could contribute to glutamate-mediated excitotoxicity (**Figure 4** and **Supplementary Table 3**). Extracellular K^+ is taken up into astrocytes via Na^+/K^+ ATPases (Hertz and Chen, 2016) and inward-rectifying Kir4.1 channels, the latter of which is also important for K^+ re-release from astrocytes (Bay and Butt, 2012). Kir4.1 channels can co-localize with the water channel, aquaporin 4 (AQP4), indicating functional synergy (Nagelhus et al., 1999; **Figure 4**). Previously, a decrease in Kir4.1 expression was observed in the ventral horn of the spinal cord in pre-symptomatic $SOD1^{G93A}$ mice (Kaiser et al., 2006; **Figure 4**). In addition, a reduction of Kir4.1 channel sensitivity and number, along with an increased expression of AQP4 was observed in both motor cortex and brain stem of symptomatic $SOD1^{G93A}$ tg rats (Bataveljic et al., 2012). Corroborating these results, iPSC-derived astrocytes obtained from ALS patients with a SOD1 mutation also showed decreased Kir4.1 expression (Kelley et al., 2018). These findings indicate a lowered K^+ buffering capacity of mutant astrocytes. Indeed, conditional knock out of Kir4.1 caused premature death, seizures and ataxia and was shown to cause membrane depolarization as well as an impairment of K^+ and glutamate uptake by astrocytes (Djukic et al., 2007). To determine the pathological role of the reported Kir4.1 downregulation in ALS, Kelley et al. (2018) knocked out astrocytic Kir4.1 channels in spinal cord of $SOD1^{G93A}$ tg mice, but found no further acceleration of MN loss. The pathophysiological relevance of Kir4.1 reduction in ALS thus remains unresolved.

Astrocytes as Providers of Energy

Lactate is one of the main energy substrates for neurons and is predominately produced in astrocytes from glucose or glycogen. Lactate has gained increasing attention in the modulation of neuronal excitability, plasticity and neuroprotection (Magistretti and Allaman, 2018). Notably, lactate generation in astrocytes hinges on extracellular glutamate, thus neuronal activity, which stimulates the uptake of glucose into astrocytes (Pellerin and Magistretti, 1994; Magistretti and Allaman, 2018). The astrocyte-neuron lactate shuttle (ANLS) model (Pellerin and Magistretti, 1994) proposes that astrocytes produce lactate and release it through transmembrane monocarboxylate transporters (MCTs, in particular MCT1 and MCT4), high-capacity cation channels and pannexins into the extracellular space, from where neurons take it up through transmembrane transport by MCT2 or G-protein coupled receptors [hydrocarboxylic acid receptor 1 (HCAR1) – reducing cAMP and thus reducing neuronal activity (Bozzo et al., 2013) and through a still unidentified Gs-coupled receptor] (**Figure 4**). Within the neuron, lactate is metabolized to pyruvate and NADH, stimulating a plethora of intracellular signaling cascades (Magistretti and Allaman, 2018; **Figure 4**). Lactate was suggested to serve a neuroprotective role against glutamate-mediated excitotoxicity (Magistretti and Allaman, 2018). Supporting this notion, treatment with L-lactate reduced the lesion size and neuronal death induced by high doses of glutamate (Ros et al., 2001). What is known about lactate and the ANLS in ALS? Cell culture and animal experiments indicate a lactate deficiency in ALS. Using *in vivo* ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) approach Lei et al. (2019) observed a reduction of lactate in $SOD1^{G93A}$ tg mice most prominently seen in motor cortex, starting at the early symptomatic phase in motor cortex and in the late symptomatic stage also in brainstem. In an astrocyte-MN co-culture system of tg astrocytes with WT MN, lactate was reduced, when compared to cultures containing WT astrocytes, indicating defects in the generation or release of lactate by astrocytes (Ferraiuolo et al., 2011; Madji Hounoum et al., 2017; **Figure 4** and **Supplementary Table 3**). Treating those co-cultures in addition with glutamate further aggravated the reduction of lactate, along with a strong modification of diverse metabolic pathways (Madji Hounoum et al., 2017). Taken together, the aforementioned studies suggest a lactate deficiency in ALS, which could cause metabolic dysfunction and compromised neuroprotection.

Toxic Gain of Function of Astrocytes

As opposed to the loss of physiological function, there is compelling *in vitro* and *in vivo* evidence for astrocytes secreting toxic factors causing MN degeneration and death in ALS (Nagai et al., 2007; Re et al., 2014). The majority of studies rely on the application of conditioned media derived from cultured astrocytes of transgenic mice or IPSC-derived from ALS patients (sALS and diverse fALS cases) or astrocyte-MN co-culture systems, which can cause dysfunction and/or death of cultured MN (Nagai et al., 2007; Re et al., 2014; Rojas et al., 2014; Zhao et al., 2020). In *in vivo* settings, in which conditioned media from cultured astrocytes expressing $SOD1^{G93A}$ was infused into the spinal cord of rats, motor dysfunction was observed upon 8 days of infusion, accompanied by MN death and reactive astrogliosis (Ramirez-Jarquin et al., 2017). Transplantation studies, in which $SOD1^{G93A}$ glial-restricted precursor cells (Papadeas et al., 2011) or astrocytes differentiated from patient derived IPSC (Chen et al., 2015) were injected into the spinal cord, revealed the development of motor deficits. However, the effect might be mutation-specific, as astrocytes carrying the $TDP\text{-}43^{A315T}$ mutation did neither cause MN degeneration in co-culture nor upon transplantation into rat spinal cord *in vivo* (Haidet-Phillips et al., 2013).

A corresponding tg rat model, based on the astrocyte-selective expression of the TDP-43^{M337V}, on the other hand, developed a severe phenotype consisting of MN loss, progressive paralysis and premature death (P70−P80) (Tong et al., 2013). Notably, the inverse experiment, that is the focal transplantation of WT astrocytes (glial-restricted precursors) into the cervical spinal cord of SOD1^{G93A} tg rats, exerted protective effects, thus mitigating fore-limb motor deficits and the corresponding motor neuron loss, and even prolonged life span (Lepore et al., 2008). Surprisingly, a similar approach employing human glial-restricted progenitors transplanted into spinal cord of SOD1^{G93A} tg mice was ineffective (Lepore et al., 2011), potentially indicating species differences. Is the toxic effect conveyed by astrocytes mediated through changes in MN excitability? Again, the results are somewhat inconclusive: Conditioned media from cultured astrocytes expressing SOD1^{G93A} was sufficient to cause acute hyperexcitability on cultured MN as evidenced by increased persistent Na^+ inward currents, repetitive firing and calcium transients followed by MN death days later (Fritz et al., 2013). On the other hand, co-culture of IPSC-derived astrocytes from *C9orf72* mutation carriers caused hypoexcitability and actual activity loss in MN due to impaired activity of voltage-activated Na^+ and K^+ currents (Zhao et al., 2020). What is known about those putative toxic factor(s) released by astrocytes? Although multiple molecular candidates were proposed, a final conclusive answer to that question is still pending. Obvious candidates, such as glutamate, TNFα or interleukin-1β, interleukin-6 and interferon-γ could readily be excluded (Nagai et al., 2007). While the identification of a single toxic molecule proves highly difficult, a number of pathways involved were successfully unraveled. As such, it has been shown that the detrimental effects of conditioned astrocytic media involved the activation of Na^+ channels causing hyperexcitability, concomitant Ca^{2+} influx, mitochondrial damage and the generation of reactive oxygen species (ROS), triggering apoptosis (Rojas et al., 2014, 2015). Others also found evidence for an impact on astrocytic health, including increased levels of ROS and NADPH oxidase 2 (NOX2) within astrocytes derived from spinal cord and brain of SOD1^{G93A} and SOD1^{G37R} tg mice, respectively (Cassina et al., 2008; Marchetto et al., 2008; **Figure 4**). Incubating SOD1^{G93A} expressing astrocytes with antioxidants and nitric oxide synthase inhibitors, prevented MN loss, indicating that the release of potential toxic factors involves mitochondrial damage and ROS generation within astrocytes (Marchetto et al., 2008). Another candidate is the prostaglandin D2 (PGD2) receptor, which is upregulated in SOD1^{G93A} expressing astrocytes. Pharmacological blockade of the PGD2 receptor ameliorated the toxic affects conveyed by tg astrocytes onto WT MN (Di Giorgio et al., 2008), due to hitherto unknown mechanisms. Furthermore, the pro-inflammatory cytokine interferon-γ (IFN-γ) was found to play a role in astrocyte-mediated neurotoxicity. At odds with the initial study by Nagai et al. (2007) interferon signaling pathways, most prominently involving IFN-γ (Aebischer et al., 2011) and IFN-α/β (Wang et al., 2011) were shown to be upregulated in astrocytes of SOD1^{G93A} tg mice (**Figure 4**). Inhibition of IFN signaling by either knock-out of the IFNα receptor 1 or knock out of the LIGHT-LT-βR protein (downstream of IFN-γ) prolonged survival of SOD1^{G93A} tg mice (Aebischer et al., 2011; Wang et al., 2011). The discrepancy between studies regarding the change in IFN- γ concentration was assigned to species differences (rat vs. mouse) and the source, MN were derived from (primary cell culture vs. embryonic stem cell derived). In addition to the molecules mentioned above, transforming growth factor β (TGF-β), a cytokine important for immune response regulation (Butovsky et al., 2014; Yamanaka and Komine, 2018) has also been implicated in astrocyte-mediated toxicity. In various mouse models of the disease (SOD1^{G93A}, SOD1^{G85R}, SOD1^{G37R}) as well as in sALS patients, TGF-β1 expression was increased (Endo et al., 2015; Endo and Yamanaka, 2015) (**Figure 4**). Selective overexpression of TGF-β1 in astrocytes in the SOD1^{G93A} model (crosses of SOD1^{G93A} and GFAP-TGF-β1 tg mice) accelerated disease progress, while pharmacological inhibition of TGF-β signaling prolonged survival of SOD1^{G93A} tg mice (Endo et al., 2015). Yet another molecule suggested to be involved in astrocyte-triggered MN dysfunction is D-Serine. D-Serine is an endogenous co-activator of NMDA receptors (Mothet et al., 2000; Panatier et al., 2006), and thus a prime candidate molecule involved in the observed excitability changes in ALS. Notably, mutations in the gene encoding the D-amino acid oxidase (DAO), a D-Serine degrading enzyme, is causing fALS (Mitchell et al., 2010; Kondori et al., 2018) and D-Serine expression is increased in the spinal cord (mainly located to glia cells) of fALS and sALS patients, as well as in symptomatic SOD1^{G93A} tg mice (Sasabe et al., 2007; **Figure 4**). Genetic knock out of DAO causes MN degeneration and abnormal locomotor behavioral (but not a full ALS-like phenotype) (Sasabe et al., 2012). DAO activity was also markedly reduced in SOD1^{G93A} tg mice, particularly in the anterior column of the spinal cord, a region where descending motor tracts impinge on LMN, and could be localized to astrocytes (**Figure 4**). These data suggest that astrocytes actively contribute to increased D-Serine levels, which promotes glutamate-mediated excitotoxicity through NMDA receptors. This hypothesis, however, took a slight turn as a recent publication unraveled that L-Serine instead of D-Serine was released by astrocytes (Wolosker et al., 2016). L-Serine is converted into D-Serine within neurons by the serine racemase (SR) and consecutively released by antiporters in a non-vesicular-dependent manner (Wolosker et al., 2016; **Figure 4**). Released D-Serine can be taken up by neurons and astrocytes, that latter of which degrade D-Serine through the DAO (Wolosker et al., 2016). Despite evidence arguing against gliotransmission of D-Serine by astrocytes, major findings, indicating increased D-Serine activity, still hold true and underscore a potential causal involvement in ALS pathophysiology. Most studies to date view astrocytes in ALS as a homogenous population. However, there is evidence arguing for a prominent role of only a subset of these in ALS pathophysiology (Diaz-Amarilla et al., 2011; Jiménez-Riani et al., 2017; Liddelow et al., 2017). This notion first arose as astrocytes with an aberrant phenotype were detected in primary spinal cord cultures of SOD1^{G93A} tg rats (Diaz-Amarilla et al., 2011). Those astrocytes (termed AbAs) possessed a high proliferative capacity and their supernatant was even more toxic to motor neurons than that of mixed astrocyte cultures. Recent work now indicates that

these neurotoxic reactive astrocytes (termed A1 astrocytes) in fact represent a common feature of CNS diseases (Liddelow et al., 2017). A1 astrocytes lose a number of physiological functions and instead gain neurotoxic properties. These highly intriguing recent findings even suggest a general causative role of reactive astrocytes in CNS disorders.

GLUTAMATE-MEDIATED EXCITOTOXICITY

One of the central tenets in ALS research, the "glutamate-mediated excitotoxicity" (and also in other neurodegenerative diseases), posits that neuronal degeneration occurs due to excessive glutamatergic stimulation as a result of compromised glutamate uptake and/or increased presynaptic release (Heath and Shaw, 2002). How do recent findings of altered intrinsic excitability/activity of and synaptic inputs to MNs reconcile with this prevailing dogma? Being the main excitatory neurotransmitter in the CNS, glutamate is released from the presynapse upon AP firing and binds to high-affinity Ca^{2+} permeable ionotropic receptors, namely NMDA -, AMPA -, kainate -, and metabotropic glutamate receptors (Blanke and VanDongen, 2009; Wright and Vissel, 2012). Glutamate is subsequently removed from the synaptic cleft by Na^{+}-dependent excitatory amino-acid transporters expressed on both neurons and astrocytes (EAATs in humans, and GLAST-1 and GLT1-1 in rodents) (O'Donovan et al., 2017) and recycled into the presynaptic terminal. When glutamate removal from the synaptic cleft is compromised, the extended presence or larger quantities of glutamate could cause an increased Ca^{2+} influx into the postsynapse, resulting in "hyperexcitation." A prolonged increase in intracellular Ca^{2+} is suggested to exhaust the Ca^{2+} buffering capacity of the cell, provided by calcium binding proteins, the endoplasmic reticulum (ER) and mitochondria (Grosskreutz et al., 2010; Lautenschlaeger et al., 2012). Furthermore, a potential disruption of Ca^{2+} handling by the ER/mitochondria has been suggested to cause the generation of reactive oxygen species (ROS) (Heath and Shaw, 2002; Grosskreutz et al., 2010). Since vulnerable MNs express low levels of $GABA_A$ and glycine receptors (Lorenzo et al., 2006), high levels of Ca^{2+}-permeable GluR2-deficient AMPA receptors, but low levels of Ca^{2+} buffering proteins, such as parvalbumin and calbindin (Ince et al., 1993; Leal and Gomes, 2015), rendering them susceptible to excess neuronal stimulation and Ca^{2+} influx. Yet, what is the actual evidence for excess glutamate in ALS pathophysiology? In the early 90s, elevated levels of glutamate in the blood (Babu et al., 1998; Kostera-Pruszczyk et al., 2002) and CSF of ALS patients were reported (Rothstein et al., 1990). Others, however, could not confirm these findings (Perry et al., 1990), found only an increase of glutamate in the serum and not in the CSF of sALS patients (Kostera-Pruszczyk et al., 2002) or even observed decreased glutamate levels in the CSF of fALS patients (primarily SOD1 fALS) (Wuolikainen et al., 2011). Mechanistically, an impaired glutamate uptake had been proposed based on the observation of reduced GLT-1 expression in postmortem cortex and spinal cords obtained from ALS patients (Rothstein et al., 1995). But to what extent do glutamate levels in the CSF or serum reflect synaptic concentration? Under physiological conditions, the glutamate concentration is ∼20−50 μM in serum plasma and ∼0.2−4 μM in CSF (Perry et al., 1990; Kostera-Pruszczyk et al., 2002; Cid et al., 2003). In ALS patients a range from ∼40 to 100 μM in plasma and ∼6–10 μM in the CFS was observed (Perry et al., 1990; Rothstein et al., 1990; Plaitakis and Constantakakis, 1993; Shaw et al., 1995; Kostera-Pruszczyk et al., 2002; Cid et al., 2003). In order to probe a potential toxic effect on MN, Cid et al. tested glutamate levels reminiscent of CSF concentrations in ALS patients (∼5.8 μM) and healthy controls (<2.8 μM) on cultured MNs and reported MN death when treated with glutamate levels found in ALS patients (Cid et al., 2003). Furthermore, the toxicity conveyed by the CSF from ALS patients could be prevented by the application of AMPA and kainate receptor antagonists in cell culture (Couratier et al., 1993; Sen et al., 2005). Chronic delivery of 3 and 5 mM kainic acid (kainate and AMPA receptor agonist) via intrathecal pumps for 4 weeks resulted in MN death in the spinal cord of mice and rats (Sun et al., 2006; Blizzard et al., 2016). While the latter experiments seem to support the glutamate-mediated excitotoxicity hypothesis, it is important to realize that the concentrations applied are two orders of magnitude higher than the glutamate levels observed in the CSF of ALS patients. Notably, chronic non-selective pharmacological blockade of glutamate uptake transporters via osmotic minipumps in the rat spinal cord did not cause any LMN degeneration or motor deficits, although the extracellular glutamate levels were ∼4-5 μM, being 3−4 times higher than in the control group (Tovar-y-Romo et al., 2009). Additional *in vivo* studies reported a particular vulnerability of dorsal horn neurons upon chronic AMPA administration (Nakamura et al., 1994; Hirata et al., 1997) or combined blockade of glutamate reuptake transporters and glutamate (Hirata et al., 1997). However, the authors did not observe any degeneration in the ventral horn, where LMN are located (Hirata et al., 1997). These studies indicate that AMPA receptor activation, but not elevated extracellular glutamate *per se*, can drive MN degeneration. Indeed, Corona and Tapia (2004) showed that microdialysis of a glutamate transport inhibitor (for ∼1 hour) into the rat spinal cord does not cause MN loss, despite increased glutamate levels (∼6−7 μM). When they, however, enhanced AMPA receptor activation, by perfusing 6−12 mM AMPA (for ∼25 min), 90−100% of MNs were lost. These *in vivo* studies have one point in common: the application of high doses of glutamate agonists (mainly AMPA) that are orders of magnitude higher than the synaptic glutamate levels (Moussawi et al., 2011). Extracellular glutamate concentrations range from 0.02 to 30 μM. This wide range of concentrations reflects the variation of synaptic, perisynaptic and non-synaptic glutamate levels, which needs to be taken into account to understand the effect of increased extracellular glutamate. Under resting/baseline conditions, the extracellular glutamate concentration varies from 0.02 to 2.0 μM (Moussawi et al., 2011). This concentration can even drop to nanomolar levels as assessed in acute hippocampal slices (Herman and Jahr, 2007). *In vivo*, the synaptic glutamate levels can increase to millimolar levels during neurotransmission, returning to 0.02 μM in less than 10 ms via glial and neuronal

reuptake (Dzubay and Jahr, 1999). Is there a link between hyperexcitability and glutamate excess? Assuming a reduction of glutamate reuptake was the initial driver of increased glutamate levels, enhanced excitatory synaptic input could be a consequence. However, homeostatic mechanisms are expected to cause a change in synaptic scaling and intrinsic excitability to compensate for increased excitatory drive (Turrigiano et al., 1998; Joseph and Turrigiano, 2017), in which case a reduction of excitability would occur. Is the observed hyperexcitability of MN thus unrelated to glutamate levels, and even further boosts glutamatergic release, causing a vicious cycle? Which factors could be involved in disabling homeostasis in MN? Or is hyperexcitability (at least convincingly demonstrated for motor cortex) rather a result of altered E/I balance within the network instead of a cell autonomous process? Is it possible that UMN and LMN degeneration are disparate processes caused by different mechanisms? The apparent discrepancy of excitability changes and proposed molecular alterations thus open up many questions. Taken together, findings on glutamate-mediated excitotoxicity described above are circumstantial due to a number of reasons including the following: (i) The actual synaptic glutamate exposure time and concentration under physiological conditions and in ALS patients is yet to be disclosed (Moussawi et al., 2011), thus hampering proper *in vitro* and *in vivo* modeling of this disease aspect. (ii) Increased levels of glutamate in CSF or serum do not necessarily reflect the synaptic concentrations a MN is exposed to, mainly due to very fast glial clearance mechanisms. (iii) Cell type-specific effects of excess glutamate need to be addressed *in vivo*. Inhibitory interneurons in cortex and spinal cord also express glutamate receptors, some of which even highly Ca^{2+}-permeable AMPA receptors lacking the GluA2 subunit (Akgul and McBain, 2016), thus potentially also putting interneurons at risk in ALS. Transcriptomic and proteomic datasets of these distinct populations have only recently been gathered, leaving many aspects of neuronal activity regulations still unanswered (D'Erchia et al., 2017; Maniatis et al., 2019; Marques et al., 2019). (iv) Homeostatic and compensatory mechanisms or the failure thereof have not been addressed yet. Healthy neurons are endowed with the necessary molecular machinery to compensate for altered input by adjusting their intrinsic excitability or synaptic strength in order to maintain output, a phenomenon called homeostatic plasticity (Hengen et al., 2013, 2016). One of the molecules involved in sensing altered neuronal activity is the Calcium/Calmodulin-dependent protein kinase type IV (CaMKIV), which senses Ca^{2+} influx in response to AP firing of a neuron (Hengen et al., 2013; Joseph and Turrigiano, 2017). Importantly, many CNS disorders are accompanied by an increase in intracellular Ca^{2+}, including MN in ALS (Leal and Gomes, 2015). Pathological Ca^{2+} elevations could thus undermine the activity sensing machinery of a neuron. However, if this was the case one would expect to find hypoexcitability in affected neurons, which is in fact the case for LMN when recorded *in vivo* (Leroy et al., 2014; Martínez-Silva et al., 2018). While this might be a too simplistic model to explain MN degeneration in ALS, it yet emphasized the question as to how and why altered excitability occurs in the first place, indicating a failure of the homeostatic machinery.

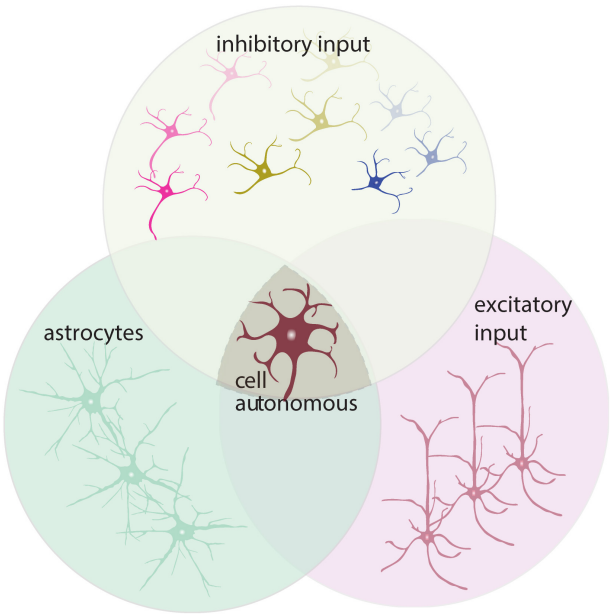


FIGURE 5 | Interaction and synergy of proposed cell-autonomous vs. non-cell-autonomous mechanisms contributing to MN degeneration. Schematic depicting interdependency of proposed mechanisms. Cell-autonomous mechanisms refer to changes that take place within the affected MN, e.g., altered receptor expression. Non-cell-autonomous mechanisms refer to changes occurring on cell types other than MN, causally involved in the degenerative process, e.g., increased excitation (purple), lack of inhibition (yellow) and dysfunctional glutamate-reuptake system involving astrocytes (green).

CONCLUDING REMARKS

We here summarized the current knowledge regarding structural and functional deficits of different elements of cortical and spinal cord motor circuitries in ALS. In summary, there is compelling evidence that motor cortex in ALS is hyperexcitable, the underlying mechanisms of which remain to be elucidated, but likely involve increased excitation and decreased inhibition simultaneously. The current data available for LMN and spinal circuits, however, appear less conclusive, but most recent *in vivo* recordings along with IPSC-derived MN approaches rather argue for a hypoexcitability of LMN. Changes in neuronal excitability and/or activity of different cell types and the consecutive impairment of neural circuit function have been reported in a number of CNS disorders, such as ALS-FTD (Zhang et al., 2016), Alzheimer's disease (Busche et al., 2008; Grienberger et al., 2012; Liebscher et al., 2016; Schmid et al., 2016), Parkinson's disease (Taverna et al., 2008), spinal muscular atrophy (Mentis et al., 2011), Huntington's disease (Burgold et al., 2019), multiple sclerosis (Ellwardt et al., 2018), schizophrenia (Mukherjee et al., 2019), and glioblastoma (Venkatesh et al., 2019). What is the basis and what are the consequences of altered neuronal excitability? There are likely different mechanisms at play in the different diseases. In this review we have delineated a plethora of molecular and cellular mechanisms suggested to play a role

(**Figure 5**). To conclusively answer that question, it is important to also acknowledge that "no neuron is an island." In other words, neurons are embedded within complex local and global circuits, in which their activity is in addition to cell autonomous processes, regulated by numerous feedforward and feedback loops (**Figure 5**). Consequently, even "pure" cell autonomous defects will very likely leave a mark on the connected neurons. As changes in structure, function (excitability/activity/response properties) and connectivity develop gradually over time (as far as we know) and are paralleled by compensatory or even maladaptive processes of diverse network elements, the identification of the initial perturbation remains challenging. Are excitability changes relevant after all or rather represent an epiphenomenon? The current data convincingly shows that excitability and activity alterations of diverse neuronal subtypes are linked to markers of degeneration, such as the accumulation of intracellular protein aggregates and ER stress, and importantly that the manipulation of neuronal excitability can exert pronounced beneficial effects including a prolongation of life span (Saxena et al., 2013; Zhang et al., 2016). But these studies also highlight that the excitability modulation needs to be cell type and disease stage dependent, which might explain the failure of hitherto clinical trials. The enormous challenge for the future will thus be to piece together the jigsaw puzzle of the wealth of factors and processes identified thus far and determine their causal relevance and sequence of events in human ALS pathophysiology. A successful therapeutic strategy, in particular so as patients are almost exclusively diagnosed, when major tissue damage has occurred, will likely have to consist of a combinatorial treatment taking into account cell type-, brain area- and disease stage- specific alterations.

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All authors wrote and edited the manuscript.

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SUPPLEMENTARY MATERIAL

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