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Synaptic strength and postsynaptically silent synapses through advanced aging in rat hippocampal CA1 pyramidal neurons

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Abstract

Synaptic dysfunction is thought to contribute to age-related learning impairments. Detailed information regarding the presence of silent synapses and the strength of functional ones through advanced aging, however, is lacking. Here we used paired-pulse minimal stimulation techniques in CA1 stratum radiatum to determine whether the amplitude of spontaneous and evoked miniature excitatory postsynaptic currents (sEPSCs and eEPSCs, respectively) changes over the lifespan of rats in hippocampal CA1 pyramidal neurons, and whether silent synapses are present in adult and aged rats. The amplitudes of both sEPSCs and eEPSCs at resting membrane potential (i.e., clamped at −65 mV) initially increased between 2 weeks and 3 months, but then remained constant through 36 months of age. The potency of the eEPSCs at depolarized membrane potentials (i.e., clamped at +40 mV), however, was highest among 36-month old rats. Additionally, presynaptically silent synapses in CA1 stratum radiatum disappeared between 2 weeks and 3 months, but postsynaptically silent synapses were present through advanced aging. The similarity of silent and functional synapses in CA1 hippocampus at resting membrane potentials throughout adulthood in rats may indicate that impairments in the mechanisms of synaptic plasticity and its subsequent stabilization, rather than deficient synaptic transmission, underlie age-related cognitive decline. Such a notion is consistent with the increased amplitude of synaptic currents at depolarized potentials, perhaps suggesting an upregulation in the expression of synaptic NMDA receptors once rats reach advanced age.

Keywords: Synapse; Hippocampus; CA1; Aging; EPSC; Silent synapse; Minimal stimulation; Whole-cell

1. Introduction

Synaptic transmission is the primary means of communication between neurons and is thought to be targeted during normal aging. One of the most-studied regions of the brain with regard to synapses is the hippocampal CA1 region (Harris and Kater, 1994; Geinisman, 2000; Spruston and McBain, 2007). The detailed information regarding the functional and structural properties of synapses in this region have proven critical for guiding research aimed at identifying the cellular substrates of individual variability in aged animals (Toescu et al., 2004; Burke and Barnes, 2006; Disterhoft and Oh, 2006; Wilson et al., 2006; Foster, 2007). A major question is why some aged animals show severe impairments in hippocampus-dependent behavioral tasks, whereas others the same age learn as well as young adults. Multiple processes probably collude to disrupt neuronal function (e.g., synaptic transmission) and plasticity (e.g., activity-dependent changes in synaptic strength or intrinsic excitability), but it is likely that age-related changes in one parameter have a feed-forward effect on other parameters.

For example, both intrinsic and synaptic plasticity in CA1 pyramidal neurons are impaired in aged rats (reviewed in Toescu et al., 2004; Burke and Barnes, 2006; Disterhoft and Oh, 2006; Wilson et al., 2006; Foster, 2007). These forms of plasticity depend on postsynaptic depolarization and could therefore be a consequence of reductions in synapse number or synapse strength. Indeed, previous work using electron microscopy has found evidence that some synapses may be weaker in aged rats with impaired hippocampus-dependent memory (Nicholson et al., 2004; but see Barnes et al., 1992),

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even though synapse number does not predict cognitive status (Geinisman et al., 2004; Calhoun et al., 2007). Therefore, the possibility remains that age-related changes in behavioral, synaptic and intrinsic plasticity derive from a dysregulation of cellular processes responsible for maintaining or altering synaptic strength.

One working model that could account for many of these findings posits that the dyshomeostasis of intracellular Ca^{2+} changes the rules that govern cellular plasticity (Khachaturian, 1987; Landfield, 1987; Toescu et al., 2004; Foster, 2007; Toescu and Verkhratsky, 2007). Though not traditionally considered a form of plasticity, the maintenance of synaptic strength is in fact an active process that involves Ca^{2+}-mediated cascades (Malinow and Malenka, 2002; Nicoll, 2003; Kennedy and Ehlers, 2006). Probing synapses at steady state could thus provide snapshots of the integrity of synapses throughout the lifespan of rats, and reveal crucial insight into what is and is not changing as animals transition from young adults with functional hippocampal neurons to aged animals with dysfunctional ones. For instance, determining the strength of individual synapses throughout the lifespan of rats could help clarify whether synapses are weakened throughout the aging process (e.g., Barnes et al., 1992; Hsia et al., 1998; Nicholson et al., 2004). If such evidence is found, one could posit that synaptic potentials in aged rats result in depolarizations that are unable to the recruit the processes that support physiological forms of plasticity like long-term potentiation (LTP) or increases in neuronal excitability. Additionally, determining whether synapses that are especially sensitive to LTP-induction protocols are absent in very old animals is important, as such a loss would make it more difficult to induce plasticity at aged synapses.

All excitatory synapses on hippocampal CA1 pyramidal neurons contain NMDA receptors, but a subpopulation of these synapses lacks AMPA receptor immunoreactivity (Nusser et al., 1998; Petralia et al., 1999; Takumi et al., 1999; Racca et al., 2000; Ganeshina et al., 2004; Nicholson et al., 2006; Nicholson and Geinisman, in press). These electron microscopic findings corroborate previous descriptions of "postsynaptically silent" synapses that lack AMPA receptors, contain NMDA receptors, and are rendered functional only by the voltage-dependent removal of Mg^{2+} from their NMDA receptor channel pore (Liao et al., 1995; Isaac et al., 1995; Durand et al., 1996). These postsynaptically silent synapses, which are small and located on small thin spines (Takumi et al., 1999; Nicholson et al., 2006; Nicholson and Geinisman, in press), are thought to be especially sensitive to LTP-induction protocols (Kasai et al., 2003; Bourne and Harris, 2007). One possibility is that such synapses are absent or relatively infrequent in aged rats, which has the consequence of reducing the occurrence of plasticity by virtue of the unavailability of postsynaptically silent synapses. Importantly, there also exist "presynaptically silent" synapses whose transmission failures derive from the absence of neurotransmitter release, or from the release of neurotransmitter in an amount that is sufficient to activate only high-affinity NMDA receptors (Kullmann and Asztely, 1998; Gasparini et al., 2000; Voronin et al., 2004; Voronin and Cherubini, 2004).

Previous studies have postulated that postsynaptically silent synapses may be involved in activity-dependent synaptic plasticity, such as that involved in the early development of neuronal microcircuits (Durand et al., 1996; Isaac et al., 1997; Petralia et al., 1999). This notion is consistent with studies in infant rat tissue (<3 weeks old) showing that LTP converts postsynaptically silent synapses into functional ones via the insertion of AMPA receptors into their postsynaptic membrane (Liao et al., 1995; Isaac et al., 1995; Durand et al., 1996; Malinow and Malenka, 2002; Nicoll, 2003). If silent synapses in the hippocampus are present primarily during early development, they should disappear or decrease in frequency with age. If, however, silent synapses are a constant substrate upon which mechanisms of activity-dependent plasticity act (Malinow and Malenka, 2002; Nicoll, 2003), then they should mirror the persistence of LTP and other forms of synaptic plasticity in the hippocampus throughout life in rats (Burke and Barnes, 2006; Disterhoft and Oh, 2006; Gallagher et al., 2006; Foster, 2007).

Here we combine whole-cell patch-clamp recordings from CA1 pyramidal neurons with paired-pulse minimal stimulation techniques (McNaughton et al., 1981; Dumas and Foster, 1995; Stevens and Wang, 1995; Isaac et al., 1996; Hsia et al., 1998) and show that postsynaptically silent synapses and the strength of functional ones at resting membrane potentials persist through life in rats, even in rats too old to learn hippocampus-dependent tasks. However, when synapse strength is probed at depolarized potentials (+40 mV), synapses in the oldest rats are strongest. Such voltage-dependent differences in synaptic strength may indicate that advanced aging is associated with an increased expression of synaptic NMDA receptors, which may increase intracellular Ca^{2+}, and possibly exacerbate Ca^{2+} dyshomeostasis following synaptic activation that is strong enough to remove the Mg^{2+}-blockade of NMDA receptor channel pores.

2. Methods

All experiments were performed in accordance with the animal care and handling guidelines set forth by Northwestern University.

2.1. Slice preparation and recordings

Transverse hippocampal slices (300 μm) were prepared in ice-cold oxygenated artificial cerebral spinal fluid (aCSF) from 10 to 16 day, 3 month, 16 month and 32–36 month old Fisher 344 × BN F1 rats. Slices were quickly transferred to an oxygenated holding chamber and incubated for 25 min at 35 °C, after which they were held at room temperature until recordings were made. The recording chamber was continuously superfused with solution heated to 32–34 °C and saturated with 95% O_2/5% CO_2. The standard extra-