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Physiological and Anatomical Studies of Associative Learning: Convergence With Learning Studies of W.T. Greenough

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Abstract

The quest to understand how the brain is able to store information for later retrieval has been pursued by many scientists through the years. Although many have made very significant contributions to the field and our current understanding of the process, few have played as pivotal a role in advancing our understanding as William T. Greenough. The current report will utilize associative learning, a training paradigm that has greatly assisted in our understanding of memory consolidation, to demonstrate how findings emerging from the Greenough laboratory helped to not only shape our current understanding of learning induced anatomical plasticity, but to also launch future analyses into the molecular players involved in this process, especially the Fragile X Mental Retardation Protein.

Keywords

eyeblink conditioning; synapse; learning; synaptic plasticity; multiple synapse bouton; perforated; synapses

INTRODUCTION

The process by which we are able to form a memory for later retrieval has been a topic of discussion and subsequent experimentation for decades. Some of the oldest and best-characterized behavioral tasks used in laboratories for examining learning substrates are associative conditioning tasks. Typically, a neutral conditioned stimulus (CS) is paired with a salient unconditioned stimulus (US) that evokes an unconditioned response (UR). After repeated CS–US pairings, in which the CS consistently predicts the onset of the US, the CS gains the ability to elicit a learned conditioned response (CR). Studies have demonstrated that the temporal relationship between the CS and US dictates the involvement of different brain regions and neural circuits. For example, in delay eyeblink conditioning the US either immediately follows or co-terminates with the CS. Thus there is no separation in time between the two stimuli. This form of learning is mediated by brainstem-cerebellar processing (Clark, McCormick, Lavond, & Thompson, 1984; Mauk & Thompson, 1987)

and is not dependent upon neocortical processing (Norman, Buchwald, & Villablanca, 1977; Oakley & Russell, 1977; Mauk & Thompson, 1987).

TRACE EYEBLINK ASSOCIATIVE LEARNING

In trace eyeblink conditioning, where the CS and US are temporally separated by a stimulus free interval, successful learning requires not only the brainstem-cerebellum circuitry, but also hippocampal and neocortical regions (Fig. 1). The ability to learn the trace association is abolished in humans, rabbits, rats and mice after lesions in the hippocampus and specific regions of the neocortex (Clark & Squire, 1998; Han et al., 2003; Kim, Clark, & Thompson, 1995; Kronforst-Collins & Disterhoft, 1998; McGlinchey-Berroth, Carrillo, Gabrieli, Brawn, & Disterhoft, 1997; McLaughlin, Skaggs, Churchwell, & Powell, 2002; Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, & Weisz, 1986; Takehara, Kawahara, & Kirino, 2003; Takehara, Kawahara, Takatsuki, & Kirino, 2002; Tseng, Guan, Disterhoft, & Weiss, 2004; Weible, McEchron, & Disterhoft, 2000; Weiss, Bouwmeester, Power, & Disterhoft, 1999). Further evidence from our laboratory and others has suggested that the hippocampus is involved early in acquisition and plays a critical role during initial consolidation of the trace association. For example, hippocampal lesions 1 day after acquisition of the trace association abolish subsequent expression of the CR, while similar lesions 30 days after acquiring the task fail to have an impact on response (Kim et al., 1995). Analysis of hippocampal CA1 pyramidal neuronal activity in response to the CS and US during trace eyeblink conditioning demonstrated that conditioning-specific neuronal activity in CA1 precedes CR expression (McEchron & Disterhoft, 1997; Weible, O'Reilly, Weiss, & Disterhoft, 2006), suggesting that the functional properties of neurons in the hippocampus were being altered prior to expression of the behavioral output. Further characterization of hippocampal CA1 pyramidal cells revealed a peak in neuronal activity on the day the subjects began exhibiting CRs (McEchron & Disterhoft, 1997). These studies illustrate not only the hippocampal dependency of this task, but also show some of the changes that occur in the hippocampus during acquisition of a trace association.

Learning Induced Biophysical Plasticity

Hippocampal neuronal biophysical properties are also changed during conditioning, in a manner that likely contributes to their enhanced firing rates early in the acquisition of conditioned responses. The post-burst afterhyperpolarization (AHP) and spike frequency accommodation are reduced in CA1 and CA3 hippocampal pyramidal neurons compared to neurons from pseudo conditioned and naive subjects (Fig. 2; Moyer, Power, Thompson, & Disterhoft, 2000; Moyer, Thompson, & Disterhoft, 1996; Thompson, Moyer, & Disterhoft, 1996a). The AHP is mediated by calcium dependent outward potassium currents that serve to regulate the discharge of action potentials during a sustained depolarization. Reducing the AHP increases the number of action potentials in response to a long depolarizing current injection, a function of both a decrease in spike frequency accommodation as well as a shorter post-action potential refractory period. Such reductions decrease the interval between action potentials and increase the total firing rate of the neuron. Several calcium and voltage dependent potassium currents are activated during single or multiple action potentials (Storm, 1990). Our laboratory has demonstrated learning-specific reductions in the voltage- and calcium-dependent BK-mediated fast AHP involved in repolarizing the action potential (Matthews, Weible, Shah, & Disterhoft, 2008; Matthews & Disterhoft, 2009), and in the SK-mediated medium AHP and the slow AHP in CA1 pyramidal neurons following both trace eyeblink conditioning and Morris water maze training (Fig. 3; Matthews, Linardakis, & Disterhoft, 2009; McKay, Matthews, Oliveira, & Disterhoft, 2009; Oh, Kuo, Wu, Sametsky, & Disterhoft, 2003). The conditioning-specific change in the slow AHP is dependent upon PKA for its expression (Oh, McKay, Power, & Disterhoft, 2009), and it is likely that these changes in intrinsic excitability are protein synthesis-dependent and involve CREB

activation (Barco, 2007; Oh et al., 2009). More complete descriptions of the intrinsic excitability changes that we and others have observed during and after learning have been reviewed elsewhere (Disterhoft & Oh, 2006).

These studies have greatly enhanced our understanding of the neural substrates of hippocampal plasticity during trace-conditioning. Interestingly, other behavioral paradigms have demonstrated similar hippocampal biophysical plasticity. For example, acquisition on a water maze task, a commonly utilized hippocampus dependent spatial learning paradigm, also results in a reduction in the hippocampal AHP (Oh et al., 2003; Tombaugh, Rowe, & Rose, 2005). Furthermore, Green and Greenough (1986) demonstrated that complex rearing, a visual and motor learning paradigm where rodents are typically reared in a large toy-filled environment, exhibited an increase in dentate granule cell population spikes and increased excitatory postsynaptic potential (EPSP) slope compared to normal caged controls. These findings parallel our observations that trace-conditioning increases the slope of the Shaffer collateral-CA1 EPSP compared to neurons from pseudo conditioned and naïve subjects (Power, Thompson, Moyer, & Disterhoft, 1997). Taken together, these studies demonstrate that acquisition of hippocampus dependent tasks alters neuronal properties, resulting in enhanced synaptic transmission and action potential probability. It would be expected that altered synaptic connectivity as reflected in physiological measures would be paralleled in the neuroanatomical characteristics of those same synaptic contacts.

Anatomical Correlates for Learning

The principal postsynaptic component of a synapse is the postsynaptic density (PSD), an electron-dense plate (or plates) lining the cytoplasmic face of the spine or dendrite immediately adjacent to the synaptic cleft and presynaptic bouton. Most synapses, when viewed in serial sections, have a PSD that is continuous, with a shape resembling a disc that can range orders of magnitude in size (Bourne & Harris, 2007; Geinisman, 1993; Nicholson & Geinisman, 2009). Perforated synapses, on the other hand, have at least one discontinuity and can occasionally be composed of multiple, physically separate PSDs synapsing with the same presynaptic bouton (Fig. 4; Bourne & Harris, 2007; Geinisman, 1993; Nicholson & Geinisman, 2009). Though both types of synapses can be found on dendrites and spines, and can be excitatory or inhibitory, most of what is known about these two synaptic subtypes has been determined by studying excitatory axospinous synapses.

In identifying perforated synapses, or subsynaptic plate perforations, as a synaptic correlate of cortical development and experience in an enriched environment, Greenough, West, and DeVogd (1978) put forth the prescient idea that such synapses may contribute to or underlie experience-dependent synaptic plasticity (reviewed in Geinisman, 2000). As they noted in 1978 (and in their initial descriptions; Peters and Kaiserman-Abramof, 1969, 1970), however, the functional significance of the perforations or subsynaptic plate perforations was less clear. Though their function is not totally understood, our recent work suggests that synapses with perforated PSDs play a central role in influencing neuronal output, regardless of their location. Additionally, our recent work indicates that when the strength of these perforated synapses is decreased, the hippocampus is unable to function normally.

Distance-Dependent Synaptic Scaling

Dendrites are essentially leaky cables in a salt solution. As such, voltage signals that propagate from their site of origin (i.e., the synapse) to the final integration zone in the soma/axon attenuate (roughly) in proportion to the distance between the two locations (reviewed in Rall, 1964; Sjöström, Rancz, Roth, & Häusser, 2008; Spruston, 2008; Williams & Stuart, 2003). This has long been a source of angst among neuroscientists, since it calls into question the function of excitatory synapses on the most distal dendrites, and implies

that, in the absence of compensatory mechanisms, their influence on neuronal output would be less than more proximal synapses. Using dual somatic and dendritic whole-cell patch-clamp recordings, Magee and Cook (2000) solved this long-standing issue by showing that the local unitary EPSPs from synapses on distal dendrites of CA1 pyramidal neurons were much larger than the local unitary EPSPs recorded from synapses located on more proximal dendrites of the same neuron. Remarkably, when these distally- and proximally-generated unitary EPSPs were recorded at the soma, their amplitudes were nearly indistinguishable (~ 0.2 mV). In other words, synapses increase their strength in proportion to their distance from the soma such that their influence on somatic/axonal depolarization is roughly equivalent. Through a series of elegant experiments, Magee and his group showed that the strength of AMPA-receptor (AMPA) mediated transmission is enhanced with distance from the soma, a mechanism they termed distance-dependent synaptic scaling (Andrásfalvy & Magee, 2001; Magee & Cook, 2000; Smith, Ellis-Davies, & Magee, 2003).

Whether the strength of all synapses was augmented, or whether only some synaptic subtypes increased their strength with distance from the soma was unknown. One piece of evidence in the support of the latter was the presence of unitary local EPSPs in the distal dendrites that were significantly larger than any found in the proximal recording locations. We and others had shown that perforated synapses had a higher number and density of immunogold particles for AMPARs and NMDA receptors (NMDARs; Baude, Nusser, Molnár, McIlhinney, & Somogyi, 1995; Desmond & Weinberg, 1998; Ganeshina, Berry, Petralia, Nicholson, & Geinisman, 2004a,b), and we hypothesized that a straightforward way for CA1 pyramidal neurons to implement distance-dependent synaptic scaling was to increase the strength or number of perforated synapses with distance from the soma. Using unbiased quantitative serial section electron microscopy in combination with post-embedding immunogold electron microscopy, we found that the number and proportion of perforated synapses increased progressively with distance from the soma in CA1 (Nicholson & Geinisman, 2009; Nicholson et al., 2006). Moreover, AMPAR expression was significantly higher in distal stratum radiatum than in proximal stratum radiatum, whereas NMDAR expression showed no distance-dependent differences. Remarkably, this distance-dependent regulation of (putative) synaptic strength was restricted to perforated synapses. Our colleagues, Nelson Spruston and William Kath along with their students Yael Katz, Rachel Trana, and Vilas Menon, then derived a computational model of a CA1 pyramidal neuron based on these data and showed that the distance-dependent increase in number and strength among perforated synapses could reproduce the findings of Magee and Cook (2000). They found that the amplitude of the simulated EPSPs produced by a conductance change at perforated synapses was larger locally in the distal dendrites than those produced in the proximal dendrites, but both were similar at the soma. Interestingly, the proportion of perforated synapses was highest in the most distal dendritic region, stratum lacunosum-moleculare (SLM), but their AMPAR expression was actually the lowest. The computational model indicated that these synapses, unlike those throughout stratum radiatum, may not utilize unitary EPSPs to drive somatic depolarization. Rather, the data and the computational model suggest that SLM synapses are more likely to act cooperatively to trigger a dendritic spike, which then propagates to the soma/axon to influence neuronal output (see also Ahmed & Siegelbaum, 2009; Golding, Mickus, Katz, Kath, & Spruston, 2005; Golding & Spruston, 1998; Jarsky, Roxin, Kath, & Spruston, 2005; Katz et al., 2009).

As the landmark paper by Greenough et al. (1978) suggested, these peculiar perforated synapses with the subsynaptic plate perforation may be a principal component underlying the construction of cortical circuits in an experience-expectant manner (Greenough, 1984; Grossman, Churchill, Bates, Kleim, & Greenough, 2002; Markham, Black, & Greenough, 2007) in that they permit location-independent information processing to occur in cortical pyramidal neurons, essentially allowing a dendritic democracy to operate where each

synapse can contribute to neuronal output (Häusser, 2001). It will be important for future studies to determine how critical this compensatory mechanism is for brain function (e.g., Sanderson et al., 2008), what its developmental timescale is and whether it co-emerges with the ontogeny of hippocampus-dependent learning, whether its establishment and maintenance is experience-dependent, and whether the distance-dependent changes in synaptic morphology are a general rule for cortical pyramidal neurons even in regions that lack electrophysiological evidence for such scaling (e.g., Williams & Stuart, 2002). Though perforated synapses captured the attention of the Greenough laboratory long ago, much remains to be discovered about their function in neuronal integration and their role in supporting behavior and cognition.

Biophysical and Anatomical Age-Related Correlates of Learning Impairments

As mammals age, many of them are able to retain their ability to acquire hippocampus-dependent behaviors like maze learning or trace eyeblink conditioning, but a substantial proportion of them show marked impairments (reviewed in Burke & Barnes, 2010; Disterhoft & Oh, 2006; Gallagher & Rapp, 1997; Wilson, Gallagher, Eichenbaum, & Tanila, 2006). Acquisition of trace-associations are impaired in aged rabbits (Deyo, Straube, & Disterhoft, 1989; Graves & Solomon, 1985; Powell, Buchanan, & Hernandez, 1981; Straube, Deyo, Moyer, & Disterhoft, 1990; Thompson et al., 1996a), aged rats (Knuttinen, Gamelli, Weiss, Power, & Disterhoft, 2001), aged mice (Kishimoto, Suzuki, Kawahara, & Kirino, 2001), and aged humans (Finkbiner & Woodruff-Pak, 1991; Knuttinen, Power, Preston, & Disterhoft, 2001).

Our work suggests that a combination of biophysical and structural synaptic abnormalities contribute to age-related impairments, both of which are consistent with reduced excitability and/or excitation in the hippocampus of aged, cognitively impaired animals. For example, the post-burst afterhyperpolarization (AHP) of CA1 pyramidal neurons, which is reduced in response to trace-conditioning (Fig. 2; Moyer et al., 1996, 2000; Thompson, Moyer, & Disterhoft, 1996b), is increased in aged subjects (Fig. 5; Landfield & Pitler, 1984; Matthews et al., 2009; Moyer, Thompson, Black, & Disterhoft, 1992; Moyer et al., 2000; Oh, Power, Thompson, Moriearty, & Disterhoft, 1999). This increase is evidence of decreased cellular excitability that may contribute to age-related deficits in learning trace-eyeblink associations. Consistent with this notion, we have shown that pharmacological treatments that reduce the post-burst AHP in CA1 pyramidal neurons enhance trace conditioning rate in aging but not young animals (nimodipine, an L-type calcium channel blocker (Deyo et al., 1989; Moyer et al., 1992); metrifonate, a cholinesterase inhibitor (Kronforst-Collins et al., 1997; Oh et al., 1999)). Interestingly, aging animals that are trained and learn have reduced post-burst AHPs as compared to learning impaired animals (Moyer et al., 2000; Tombaugh et al., 2005), which suggests that the ability to modify the channels underlying the AHP contributes in an important manner to the functional reorganization of the hippocampal neural circuit that, presumably, allows learning to occur in young animals and some of their aged counterparts. In this regard, it is important to note that there is specificity to the age-related changes in channel function/plasticity. The BK-mediated voltage- and calcium-dependent fast AHP is changed by learning but not by aging; in the same neurons, the slow post-burst AHP, a calcium-dependent outward potassium current, is affected by both (Matthews et al., 2008).

It is likely that intrinsic biophysical (i.e., non-synaptic) and synaptic deregulation collude to render the aged hippocampus dysfunctional in some animals. Though much is known regarding the abnormal encoding for spatial and cue-related stimuli in aged rats with learning impairments, as well as the age-related biophysical differences, the synaptic substrates underlying the degraded neuronal processing remain unknown. In collaboration with Michela Gallagher's laboratory, we behaviorally characterized aged rats on the spatial

water maze task (~28 months) and then, 1 month later, analyzed synapse number and size using conventional quantitative serial section electron microscopy (Geinisman et al., 2004; Nicholson, Yoshida, Berry, Gallagher, & Geinisman, 2004). Based on previous electrophysiological studies (reviewed in Barnes, 1994), we predicted that aged rats with impairments in Morris water maze learning would show a loss of axospinous synapses in CA1 stratum radiatum, whereas those with preserved learning would have numbers comparable to young adults. Remarkably, we found that the numbers of both perforated and non-perforated synapses in CA1 stratum radiatum remained constant regardless of age or cognitive ability (Geinisman et al., 2004). Though we found that synaptic numerical density was lower in the aged impaired rats, this was offset by a reduction in tissue shrinkage due to fixation and processing, yielding a total number estimate similar to both the aged unimpaired group as well as the young adult rats.

Given the correlation between synapse size and AMPAR and NMDAR expression (Ganeshina et al., 2004b; Katz et al., 2009; Kharazia & Weinberg, 1999; Nusser et al., 1998; Racca, Stephenson, Streit, Roberts, & Somogyi, 2000), one possible synaptic substrate of hippocampal dysfunction in aged learning-impaired rats, in the absence of frank synapse loss, would be a reduction in size of the PSD of axospinous synapses in CA1 stratum radiatum. Since synapse number was maintained across the lifespan in rats, a reduction in the size of their PSDs would suggest a loss of synaptic receptors. Though subtle in its morphological change, a loss of receptors would reduce EPSP amplitude and perhaps reflect a conversion of functional synapses into silent or very weak ones. Using unbiased serial section electron microscopy, we measured the length of each PSD profile in serial sections for perforated and non-perforated axospinous synapses, and estimated their areas as the product of the cumulative PSD length and section thickness (Nicholson et al., 2004). This approach revealed a remarkably specific correlate of cognitive dysfunction in aged rats: a reduction in the size of perforated axospinous synapses. Though the size and distribution of sizes among the non-perforated synapses for young adult, aged learning-unimpaired, and aged learning-impaired rats were not significantly different from each other, PSD sizes for perforated synapses from aged rats with spatial learning impairments showed a reduction in the prevalence of larger PSDs relative to their age-matched cognitively intact counterparts as well as the young adult group. Interestingly, segmented, completely partitioned (SCP) synapses, a perforated synaptic subtype closely linked to long-term potentiation (Geinisman, 1993; Toni et al., 2001) which show an unusually high expression of synaptic AMPARs (Ganeshina et al., 2004b; Nicholson & Geinisman, 2009), showed reductions in size in both aged groups. Learning impairments were only present, however, when the size reduction among SCP synapses was accompanied by a reduction in the size of the other perforated synaptic subtypes. These data suggest that, rather than a loss of synapses, a reduction in their strength is just as disruptive to hippocampal function, particularly when the hypothesized weakening affects the perforated synapses.

Taken together, our work suggests that perforated synapses are important for maintaining lines of communication between the dendritic arborizations, where inputs are integrated initially, and the soma/axon where these integrated voltage signals are summed into the final output of the neuron in the form of an action potential. Additionally, when these synapses are reduced in strength, but not necessarily number, it appears that hippocampal function and hippocampus-dependent behaviors are impaired. As shown by Greenough et al. (1978), this synaptic subtype emerges with development and increases in number when rats are exposed to an enriched environment in a manner similar to that found after induction of long-term potentiation (Geinisman, 2000). An intriguing possibility is that the emergence and maintenance of perforated synapses are linked tightly to brain function and, ultimately, learning and memory across the lifespan of rats.

STRUCTURAL SYNAPTIC CORRELATES OF LEARNING

Greenough and Bailey (1988) surveyed the literature on structural synaptic correlates of learning and memory in vertebrates and invertebrates (Greenough & Bailey, 1988). Though the acquisition time, the brain region, the species, and the learned behaviors themselves differed, Greenough and Bailey (1988) suggested that there are two main structural synaptic substrates of memory. Short-term memories, they proposed, involve a rearrangement of existing connections in the form of the number or location of neurotransmitter vesicles and/or structural modifications in the morphology of the presynaptic active zone or PSD size/curvature. Long-term memories, they surmised, were associated with both morphological changes and synaptogenesis. As shown by both the previous and ensuing work from the Greenough laboratory, learning a complex motor skill is associated with both synaptogenesis and a proliferation of multiple synapse boutons (MSBs; Black, Isaacs, Anderson, Alcantara, & Greenough, 1990; Federmeier, Kleim, & Greenough, 2002; Kleim, Lussnig, Schwarz, Comery, & Greenough, 1996; Kleim, Vij, Ballard, & Greenough, 1997; Kleim et al., 1998), whereas exposure to an enriched environment is associated with morphological changes in the size or shape of the PSD (e.g., Wesa, Chang, Greenough, & West, 1982; Sirevaag & Greenough, 1985) as well as a proliferation of MSBs (Jones, Klintsova, Kilman, Sirevaag, & Greenough, 1997). Our own work within CA1 stratum radiatum corroborates this pattern, but extends it to the acquisition of another complex motor skill: trace eyeblink conditioning (Geinisman et al., 2000; Geinisman, Berry, Disterhoft, Power, & Van der Zee, 2001).

Experience-Dependent Changes in Synapse Size

Previous work from our laboratory had shown that the field EPSP generated in CA1 stratum radiatum, by stimulating the axons of CA3 pyramidal neurons, was larger 1 hr and 1 day after the last trace eyeblink conditioning session in rabbits (Power et al., 1997). Using unbiased, quantitative serial section electron microscopy, we asked whether the acquisition of this hippocampus-dependent task was associated with a learning-related increase in synapse number and size in CA1 stratum radiatum (Geinisman et al., 2000; Figure 6). In contrast to our own expectations, synapse number was not higher in the trace conditioned group relative to a pseudoconditioned yoked control group, or naïve rabbits. Additionally, there were no changes in size among perforated synapses. Rather, a subtle morphological change restricted to the smallest non-perforated synapses emerged as the synaptic change associated with the acquisition of the trace eyeblink conditioned response. Specifically, we found that trace conditioning resulted in a ~15% reduction in the number of the smallest non-perforated synapses (Fig. 7).

In CA1, many non-perforated synapses are smaller than even the smallest perforated synapse (Ganeshina et al., 2004b; Nicholson & Geinisman, 2009). Moreover, the expression level for AMPARs and NMDARs is much lower among the non-perforated synapses compared to their perforated counterparts, even when their PSD sizes are identical (Ganeshina et al., 2004a,b). Lastly, many (30–50%) non-perforated synapses lack AMPAR immunoreactivity, and this AMPAR immuno-negativity is most common among the smallest synapses (Ganeshina et al., 2004a,b; Nicholson et al., 2006; Nicholson & Geinisman, 2009; Nusser et al., 1998; Petralia et al., 1999; Racca et al., 2000; Takumi, Ramirez-León, Laake, Rinvik, & Ottersen, 1999). Therefore, one possibility is that the conditioning-specific decrease in the proportion of the smallest non-perforated synapses reflects a transformation of many of the smallest and presumably AMPAR immunonegative synapses into larger ones that express AMPARs as a result of experience (i.e., trace eyeblink conditioning). This subtle change would allow more synapses to contribute unitary potentials to the field EPSP, and could therefore underlie the conditioning-specific enhancement of synaptic transmission found after trace eyeblink conditioning (Power et al.,

1997). Thus, as discussed in Greenough and Bailey (1988), this putative functional change in the absence of net synaptogenesis suggests that a remodeling of existing connections may accompany, even transiently, the acquisition of a complex behavior.

Proliferation of MSBs

Most axonal boutons that make excitatory axospinous synapses in hippocampal area CA1 “contact” one spine. Such single-synapse boutons contrast with MSBs, which can make synapses with 2–5 different spines (Nicholson & Geinisman, 2009; Sorra & Harris, 1993). MSBs have been found to increase in number under a variety of conditions that induce synaptic plasticity or reorganization, including reactive or recuperative synaptogenesis (Hatton, 1990; Jones, 1999; Kirov, Sorra, & Harris, 1999; Meshul et al., 2000; Steward, Vinsant, & Davis, 1988), visual deprivation (Friedlander, Martin, & Wassenhove-McCarthy, 1991), hormonal fluctuations (Woolley, Wenzel, & Schwartzkroin, 1996; Yankova, Hart, & Woolley, 2001), motor skill learning (Federmeier et al., 2002), exposure to an enriched environment (Jones et al., 1997), induction of long-term potentiation (Toni, Buchs, Nikonenko, Bron, & Muller, 1999), and neurogenesis (Toni et al., 2007). We examined whether the acquisition of trace eyeblink conditioning in rabbits, which did not result in a net synaptogenesis but did produce morphological changes in (presumably) pre-existing connections, was associated with an increase in the number of MSBs (Fig. 7; Geinisman et al., 2001).

We found that trace eyeblink conditioning increased the number of MSBs relative to a pseudoconditioned group as well as naïve controls by ~20%. Interestingly, however, neither the number of synapses per MSB nor the number of perforated synapses per MSB was changed. Nonetheless, the increase in PSD size among non-perforated synapses (Geinisman et al., 2000) combined with the proliferation of MSBs (Geinisman et al., 2001) does suggest that more synapses, some of which may have been strengthened by trace eyeblink conditioning, will be activated simultaneously (Fig. 6).

A caveat for this interpretation is necessary, however, based on our recent work (Nicholson and Geinisman, 2009). MSBs in hippocampal region CA1 are comprised of three main subtypes (1) those involving only non-perforated synapses, (2) those involving only perforated synapses, and (3) those involving a mix of these two synaptic subtypes. When the AMPAR and NMDAR expression of synapses involved in a MSB was compared with that found among PSDs apposed with a single-synapse bouton, we found that the vast majority of synapses involved in a MSB either all show an unusually low level of receptor expression, or include a synapse with an unusually high level of receptor expression and one or more synapses with unusually low levels of receptor expression. Consequently, a presynaptic action potential that invades a MSB is likely to produce significant depolarization in, at most, only one of the spines. Unfortunately, a re-analysis of the MSB data from the Geinisman et al. (2001) study is not possible, but an intriguing idea for future research is that the strength or size of synapses involved in MSBs is increased as a result of conditioning, and that the experience-dependent proliferation of MSBs is associated with a selective retention of relevant connections and a pruning of redundant or irrelevant ones (Greenough, 1984). In other words, trace eyeblink conditioning may increase the number of MSBs as well as increase the strength of their synapses, perhaps reflecting both a rapid experience-dependent morphological change as well as a more enduring synaptic reorganization (Greenough & Bailey, 1988).

NEOCORTICAL LEARNING INDUCED PLASTICITY

These and other studies alike have elegantly described how the hippocampus, involved in the earliest stages of acquisition of the CR and in its initial consolidation or storage in other

brain regions, is altered during this process; however, the final storage site for the trace eyeblink CR does not reside in the hippocampus. Hippocampal lesions 30 days after acquisition of a trace association do not hinder subsequent performance (Kim et al., 1995; Takehara et al., 2002). These and other studies, along with various learning theories, have suggested that one of the most likely candidate locations for long-term-memory storage is the neocortex. To facilitate examining age-related learning induced sensory cortical plasticity, recent work from our laboratory utilizing either delay- or trace-conditioning paradigms have taken advantage of the simple, well characterized whisker somatosensory system as a suitable conditioning stimulus (Das, Weiss, & Disterhoft, 2001; Galvez, Weiss, Weible, & Disterhoft, 2006). In the whisker somatosensory system each whisker on the face relays input via a tri-synaptic pathway from the trigeminal nerve to medullary barrelets, then to thalamic barreloids, and finally to layer IV of the somatosensory barrel cortex in a topographically-organized map of the whisker pad (Gould, 1986; Killackey & Leshin, 1975; Woolsey & Van der Loos, 1970; Woolsey, Welker, & Schwartz, 1975). Greenough and Chang (1988) were the first to characterize the developmental growth and retraction of neurons in somatosensory barrel cortex. This highly organized yet simple neuronal pathway development, facilitated examination of neuronal plasticity and the molecular players involved. In further characterization of the somatosensory barrel cortex Greenough and coworkers determined that this dendritic development was dependent upon 6-hydroxydopamine (Loeb, Chang, & Greenough, 1987) and a protein that played a major role in shaping a large portion of William Greenough's scientific career, the Fragile X Mental Retardation Protein (FMRP; Galvez, Gopal, & Greenough, 2003). Subsequent analyses determined that FMRP, in addition to regulating dendritic growth and development also played a role in spine maturation in somatosensory barrel cortex (Fig. 8; Galvez & Greenough, 2005), occipital cortex (Comery et al., 1997; Irwin, Galvez, & Greenough, 2000; Irwin et al., 2001, 2002; McKinney, Elisseou, & Greenough, 2002) and hippocampus (Grossman, Elisseou, McKinney, & Greenough, 2006).

Utilizing this simple well characterized pathway, with whisker stimulation as a CS, we have recently demonstrated in young rabbits and mice that trace-eyeblink conditioning results in a metabolic expansion of the conditioned barrels compared to non-conditioned barrels on the adjacent hemisphere and to pseudo-conditioned barrels in layer IV of the somatosensory cortex (Fig. 9; Galvez et al., 2006; Galvez, Cua, & Disterhoft, 2009). Importantly, there was no such expansion in barrel size for animals that learned non-forebrain dependent delay eyeblink conditioning (Galvez et al., 2009). These results indicate that the expansion is not merely due to sensory activation of the barrel cortex, but is a result of learning an association mediated by input to this region and thus could be a site of storage for the trace association.

Expanding upon these observations, we subsequently lesioned the barrel cortex, either before or following acquisition of the trace association. These analyses determined that pre- and post-conditioning barrel cortical lesions impaired whisker-trace-eyeblink conditioning acquisition and retrieval respectively (Fig. 9; Galvez, Weible, & Disterhoft, 2007). Further analysis of the eyeblink behavior revealed a higher occurrence of non-appropriately timed blinks in the post-conditioning barrel cortical lesion group, suggesting that the subjects knew they needed to blink, however they had lost the ability to appropriately time the blink (Galvez et al., 2007). These results along with the observed cytochrome oxidase barrel cortical expansion suggest that the barrel cortex (1) is a site of storage for an aspect of the trace association and (2) plays a role in CR timing or signaling. These hypotheses are further supported by subsequent analysis of neuronal activity in layer IV of the barrel cortex during conditioning. Such analyses have demonstrated an increase in CS neuronal activity during acquisition (Galvez et al., unpublished work). Further characterization of these neurons and their neuronal activity during and after acquisition of the trace association will assist in our

understanding of the neuronal mechanism involved in memory consolidation. This is a process William Greenough has spent his entire career examining.

The scientific contributions that Professor William Greenough has made and continues to make have been pivotal for our understanding of the process by which our brains are able to learn new information. Furthermore, as evident from the small sampling of studies mentioned in this chapter, there are very few in the scientific community who can claim to have had as active a role in shaping and defining the current theories for learning induced anatomical, electrophysiological, and molecular neuronal plasticity such as William Greenough has done through the years.

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FIGURE 1.

Simplified schematic of the connections necessary for trace eyeblink conditioning. The dotted box delineates the structures necessary for delay eyeblink conditioning. Note arrows do not necessary indicate monosynaptic connections.

FIGURE 2. Trace eyeblink conditioning reduces the after-hyperpolarization (AHP) compared to pseudo conditioned subjects. Figure modified from Figure 3 in Matthews and Disterhoft (2009).

FIGURE 3.

Acquisition on a water maze task reduces hippocampal afterhyperpolarization (AHP). Learners exhibited significantly reduced AHP compared to subjects that were not able to acquire the task (Nonlearners) and Controls. Figure modified from Figure 3 in Oh et al. (2003).

FIGURE 4.

Synapses in CA1 stratum radiatum of rat hippocampus. (a1–a3) Serial section conventional electron micrographs showing a multiple synapse bouton (msb) forming two non-perforated axospinous synapses (sp1 and sp2), as well as a single synapse bouton (at) forming a perforated axospinous synapse (sp3). (b1–b4) Serial sections from tissue prepared for post-embedding immunogold electron microscopy for AMPA-type receptors, showing the characteristically high immunoreactivity of perforated synapses. (c1–c3) Serial sections from tissue prepared for post-embedding immunogold electron microscopy for AMPA-type receptors, showing a multiple synapse bouton (msb) that forms two non-perforated axospinous synapses (sp1 and sp2) exhibiting characteristically weak immunoreactivity (white arrowheads) for AMPA-type receptors. In all panels, black arrows delineate the presence of a perforation in the postsynaptic density; black arrowhead delineate the edges of the postsynaptic density; sp, spine; at, axon terminal. Scale bar is 0.5 μ m.

FIGURE 5.

Age related changes in hippocampal afterhyperpolarization (AHP). Top: The slow AHP in CA1 of aged subjects is significantly larger than in young subjects. Bottom: The fast AHP in CA1 of aged subjects is not significantly different from young subjects Modified from Figure 3 in Matthews and Disterhoft (2009).

FIGURE 6.

Scenarios in which MSB proliferation might support activity-dependent synaptic reorganization. (A) Experience leads to synaptogenesis by supporting the formation of new axospinous synapses onto axonal terminals that already synapse with a dendritic spine. (B) Synaptic pruning leads to the elimination of some synapses concomitantly with the formation of new ones. (C) Motile spines change their presynaptic terminal to one already synapsing with a dendritic spine.

FIGURE 7.

Analysis of trace eyeblink conditioning on post-synaptic density size and the number of multiple synapse boutons in CA1 of the hippocampus. Figures are modified from figures in Geinisman et al. (2000, 2001).

FIGURE 8.

Mice with the fragile X mental retardation syndrome have more immature appearing spines compared to controls. Photomicrographs of apical dendrites from wildtype (WT) and a mouse model of the fragile X mental retardation syndrome (FraX) mice. Modified from Figure 1 in Galvez and Greenough (2005).

FIGURE 9.

Schematic of the metabolic neocortical barrel representation and trace acquisition curves during normal and barrel neocortical lesion conditions. Gray circles delineate conditioned barrels. During normal learning the number of percent conditioned response (% CR) increases with subsequent days of training. With acquisition of the task, the metabolic neocortical barrel representation increases (large gray circles). When the barrels are lesioned (black shape) prior to training subjects cannot learn the association (non-learner; Galvez et al., 2006, 2007, 2009).