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Developmental changes in short-term facilitation are opposite at Temporoammonic synapses compared to Schaffer Collateral synapses onto CA1 pyramidal cells

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Abstract

CA1 pyramidal neurons receive two distinct excitatory inputs that are each capable of influencing hippocampal output and learning and memory. The Schaffer collateral (SC) input from CA3 axons onto the more proximal dendrites of CA1 is part of the trisynaptic circuit, which originates in Layer II of the entorhinal cortex. The Temporoammonic (TA) pathway to CA1 provides input directly from Layer III of the entorhinal cortex onto the most distal dendrites of CA1 pyramidal cells, and is involved in spatial memory and memory consolidation. We have previously described a developmental decrease in short-term facilitation from juvenile (P13-18) to young adult (P28-42) rats at SC synapses that is due to feedback inhibition via synaptically activated mGluR1 on CA1 interneurons. It is not known how short-term changes in synaptic strength are regulated at TA synapses, nor is it known how short-term plasticity is balanced at SC and TA inputs during development. Here we describe a novel developmental increase in short-term facilitation at TA synapses which is the opposite of the decrease in facilitation occurring at SC synapses. While short-term facilitation is much lower at TA synapses compared to SC synapses in juveniles, short-term plasticity at SC and TA synapses converges at similar levels of paired-pulse facilitation in the young adult rat. However, in young adults CA3-CA1 synapses still exhibit more facilitation than TA-CA1 synapses during physiologically-relevant activity, suggesting that the two pathways are each poised to uniquely modulate CA1 output in an activity-dependent manner. Finally, we show that there is a developmental decrease in the initial release probability at TA synapses that underlies their developmental decrease in facilitation, but no developmental change in release probability at SC synapses. This represents a fundamental difference in the presynaptic function of the two major inputs to CA1, which could alter the flow of information in hippocampus during development.

Keywords

short-term plasticity; perforant path; CA1; paired-pulse facilitation; Schaffer collateral

INTRODUCTION

It is well established that hippocampus and entorhinal cortex are important for learning and memory (reviewed in Suzuki and Eichenbaum, 2000). CA1 pyramidal neurons are the major output of the hippocampus (Amaral and Witter, 1989), and activity-dependent changes at CA1 synapses are important for spatial learning and memory. CA1 pyramidal cells receive

two distinct inputs that carry information from the entorhinal cortex to hippocampus, the Schaffer collateral (SC) pathway and the temporoammonic (TA) pathway. The SC pathway is part of the trisynaptic circuit that originates in layer II pyramidal cells in entorhinal cortex (Anderson et al., 1971). SC axons extend from CA3 pyramidal cells into CA1 and form excitatory synapses onto mid and proximal dendrites of CA1 pyramidal cells in the *stratum radiatum* (SR) (Ramon y Cahal, 1968; Witter, 1993). The TA pathway, however, forms excitatory synapses directly from layer III of the entorhinal cortex onto the most distal dendrites of CA1 neurons in the *stratum lacunosum-moleculare* (SLM) (Doller and Weight, 1982; Hjorth-Simonsen and Jeune, 1972; Steward and Scoville, 1976; Witter et al., 1988). This pathway is part of the perforant path from entorhinal cortex to hippocampus; we use the terminology temporoammonic pathway (Maccaferri and McBain, 1995; Remondes and Schuman, 2003) because it makes it possible to distinguish the perforant path input to CA1 from the more widely studied perforant path input to dentate gyrus. TA and SC synapses are spatially segregated, allowing the properties of these two inputs to CA1 to be studied independently in hippocampal slices using extracellular recordings. Because TA synapses also influence the firing of CA1 pyramidal neurons (Andreasen and Lambert, 1998; Jarsky et al., 2005), yet they have different effects from SC synapses on gating the flow of information in hippocampus (Jarsky et al., 2005), it is important to understand the properties of TA synapses and how they compare to SC synapses.

The strength of excitatory synapses in the brain is not fixed, but can change depending upon the pattern of activation through short-term and long-term plasticity. Short-term plasticity refers to history-dependent changes in synaptic strength that occur on time scales of milliseconds to seconds (reviewed in Zucker, 1999; Zucker and Regehr, 2002), which can precede (Huang et al., 1992; Wexler and Stanton, 1993) or occur independently of long-term plasticity. Short-term plasticity causes the strength of synaptic transmission to be highly sensitive to the timing of inputs and shapes the information-processing characteristics of neurons (reviewed in Abbott and Regehr, 2004). While short-term changes in synaptic strength can occur via postsynaptic mechanisms, most forms of short-term plasticity occur presynaptically through changes in neurotransmitter release (reviewed in Zucker and Regehr, 2002). Multiple forms of short-term facilitation and short-term depression exist, each with a different time constant (reviewed in Zucker and Regehr, 2002). As a result, temporally rich inputs cause a complex mixture of plasticity that results in variability in synaptic strength over a wide dynamic range (Dobrunz and Stevens, 1999; Varela et al., 1997). Input patterns taken from *in vivo* recordings of action potential timing, referred to as natural stimulus patterns, provide a temporally complex stimulus that enables investigation of the interactions between the different forms of short-term plasticity in a behaviorally relevant context (Dobrunz and Stevens, 1999).

In hippocampus, extensive developmental changes occur during the period when juveniles mature into young adults, which in rodents occurs during postnatal weeks 2-5. The initial formation of place fields occurs during this period (P. Martin, personal communication) as animals first open their eyes and begin to explore their environment. Because both short- and long-term synaptic plasticity are necessary for the formation of place fields (Silva et al., 1998), it will be critically dependent upon strength and dynamics of individual synapses. Previous studies have demonstrated that short-term plasticity of SC synapses is developmentally regulated during postnatal development. There are decreases in both paired-pulse facilitation (Dekay et al., 2006; Dumas and Foster, 1995) and high frequency depression at SC synapses (Dekay et al., 2006) as juveniles mature into young adults. However, our studies using natural stimulus patterns revealed that the overall effect is a decrease in facilitation at SC synapses in young adults (Dekay et al., 2006; Speed and Dobrunz, 2008). This is caused by activation of a disynaptic feedback mechanism and mediated by the activation of both mGluR1 and GABA_B receptors, which reduces

facilitation during bursts of stimuli but not during pairs of pulses (Speed and Dobrunz, 2008). In order to understand the impact of these developmental changes for hippocampal function, we also need to know the properties of the other major input to CA1, the TA path. However, short-term plasticity of TA synapses has not been well characterized. Little is known about how short-term plasticity at TA synapses compares with that at SC synapses, and it is unknown whether short-term plasticity of TA synapses is also developmentally regulated.

In the present study, we describe a novel developmental increase in short-term facilitation at TA synapses in young adult vs. juvenile rats that is the opposite of what occurs at SC synapses. In juveniles, short-term facilitation is much lower at TA synapses compared to SC synapses. This difference is reduced in young adults as a result of the opposite developmental changes in the two pathways. However, TA synapses in young adults remain less facilitated than SC synapses in response to physiologically-relevant stimuli, consistent with a modulatory role of TA in regulating CA1 output at mature synapses. The developmental increase in short-term facilitation at TA synapses is caused by a developmental decrease in the initial probability of release, unlike at SC synapses which have no developmental change in release probability. These results indicate that developmental modulation of short-term plasticity in CA1 is input specific, which is likely to alter the flow of information through CA1 during development.

METHODS

Slice preparation

Transverse hippocampal slices were prepared from Long Evans rats as previously described (Dobrunz and Stevens, 1999; Speed and Dobrunz, 2008). Animal care and handling were carried out according to approved institutional guidelines (University of Alabama at Birmingham). Juvenile (Postnatal days 13-18) or young adult (P28-42) rats were deeply anaesthetized with isoflurane and decapitated. Brains were quickly removed and submerged in ice-cold dissecting solution containing (in mM): NaCl, 120; KCl, 3.5; CaCl₂, 0.7; MgCl₂, 4.0; NaH₂PO₄, 1.25; NaHCO₃, 26; and glucose, 10. Acute hippocampal slices (400µm) were made on a Leica VT1000s microtome, and CA3 cell bodies were physically removed. After >1hr incubation at room temperature (~25°C), the slices were placed in the recording chamber and the temperature was gradually raised from 25°C to 32.0-32.5°C.

Electrophysiology

All recordings were performed in a submersion chamber at 32.0-32.5°C in modified ACSF containing (in mM): NaCl, 120; KCl, 3.5; CaCl₂, 2.5; MgCl₂, 1.3; NaH₂PO₄, 1.25; NaHCO₃, 26; and glucose, 10. Nickel dichromate bipolar stimulating electrodes and recording electrodes pulled from glass pipettes (2-4µm) were placed in the SR and SLM for Schaffer collateral and temporoammonic fEPSPs, respectively. Current-source density analysis (Colbert and Levy, 1992; Empson and Heinemann, 1995b; Remondes and Schuman, 2002) was used to confirm proper electrode placement, and application of the Group II mGluR agonist DCG-IV (100µM) (Cobb et al., 2000) at the end of each experiment was used to confirm the isolation of TA synapses (Figure 1).

Stimulus intensity for extracellular field recordings was set at 50-55% of the threshold for detecting population spikes at the recording site, and baseline responses were obtained at 0.1Hz using paired-pulse stimulation at 40ms. Extracellular recording solution contained D,L-APV (100µM) to prevent induction of long-term plasticity and picrotoxin (50µM) to block fast inhibitory transmission. The natural stimulus pattern (NSP) is the spike pattern generated by an awake, freely moving rat (Fenton and Muller, 1998) as previously described

(Dekay et al., 2006; Dobrunz and Stevens, 1999; Speed and Dobrunz, 2008). The NSP is repeated 4 times per experiment with a 32 point control period at 0.1Hz separating each repetition so that each is independent of the previous repetition. For each slice, the responses are the average of the 4 repetitions for each point in the pattern. Data are normalized to the control period, so that responses >1 indicate short-term facilitation and responses <1 indicate short-term depression. Responses from different slices within a group are averaged for each point, and shown as mean $\pm$ S.E., with n indicating numbers of slices. The standard deviation of the normalized mean of the 0.1Hz control period was used to determine the percentage of responses with short-term facilitation and short-term depression. Responses more than one standard deviation above the mean were considered to be facilitated, and responses more than one standard deviation below the mean were considered to be depressed. The percentages of responses that were facilitated and depressed were then calculated for the 128 stimuli of the NSP (without the control period).

For experiments using the MK-801 method, NMDA receptor currents were isolated using whole-cell patch clamp at -40mV and intracellular recording solution containing (in mM): 100 Cs-Gluconate, 0.6 EGTA, 5.0 MgCl_2 , 10 HEPES, pH was adjusted to 7.2 with CsOH. The internal solution also contained 10 mM BAPTA, 5mM QX-314, and 10 mM ATP. To prevent postsynaptic GABA_BR-mediated inhibition, CGP55845 ($10\mu\text{M}$) was also included in all experiments. After obtaining a stable baseline in the presence of the AMPA receptor antagonist, NBQX ($20\mu\text{M}$), the use-dependent NMDAR antagonist, MK-801 ($40\mu\text{M}$) was bath applied in the absence of stimulation for 10 minutes. When stimulation was resumed, the resulting decay of the NMDAR current was fitted with a first order exponential and compared between juveniles and young adults. The access resistance, input resistance, and holding current were monitored continuously. Recordings were rejected if either access resistance or input resistance increased $>20\%$ during the experiment. To obtain MK-801 decay constants, the amplitudes of the NMDA EPSCs were normalized to baseline responses before MK-801 application and fitted with a single exponential. The decay constant (τ in number of stimuli) is reported as mean $\pm$ standard error for all slices (SE). MK-801 curves are plotted normalized to the first stimulus in the presence of MK-801 for clarity, and there was no significant decay of the NMDAR-responses at SC or TA synapses during the 10 minute wash in of MK-801 in the absence of stimulation ($p>0.05$).

Statistics

Data are presented as mean $\pm$ standard error (SE) except where indicated. Paired-pulse data were analyzed with respect to age or pathway using a one-way ANOVA with a $p=0.05$ level of significance and $\alpha>0.05$ followed by Tukey's post-hoc analysis. All other data, except where indicated, were analyzed using a two-sample unpaired Student's t-test with a significance level of $p=0.05$ and $\alpha>0.05$.

RESULTS

To investigate whether developmental changes in short-term facilitation occur at TA synapses and to compare the properties of TA vs. SC synapses, we used acute hippocampal slices from juvenile (P13-18) and young adult (P28-P42) rats. Short-term plasticity was measured using extracellular field potential recordings in response to paired-pulse stimulation and a complex, physiologically-relevant natural stimulus pattern (NSP). All recordings were done in the presence of picrotoxin to isolate excitatory synapses and APV to prevent long-term plasticity. Some of the SC data obtained using the natural stimulus pattern has been previously reported (Speed and Dobrunz, 2008).

Pure SC and TA responses are identified by current-source density analysis and are confirmed pharmacologically

TA synapses can be studied using extracellular field potential recordings where both the stimulating and recording electrodes are placed in SLM. We used source-sink analysis to verify that we were obtaining pure TA responses without contamination from SC responses (Colbert and Levy, 1992; Empson and Heinemann, 1995b; Remondes and Schuman, 2002). Stimulation of SC axons in SR results in negative field potentials (sinks) when recording in SR, but positive responses (sources) when recording in SLM (Fig. 1A, top panel). Conversely, stimulation of TA axons in SLM results in negative field potentials when recording in SLM, and positive responses when recording in SR (Fig. 1A, bottom panel). Using this method, we were able to reliably identify TA vs. SC. To confirm that responses recorded in SLM were TA synapses without contamination from SC synapses, we took advantage of the differential expression of Group II mGluRs which is high at TA synapses and not present at SC synapses (Defagot et al., 2002; Shigemoto et al., 1997). The mGluR2 agonist DCG-IV (100 μ M) had no effect on the size of the fEPSP at SC synapses (Fig. 1B2) but greatly reduced synaptic transmission at TA synapses (Fig. 1B1). Therefore, bath application of DCG-IV after each experiment reliably confirmed isolation of TA synapses, and the six experiments in which DCG-IV did not decrease the fEPSP in SLM by at least 75% were not included in our analysis of TA synapses.

TA synapses exhibit a developmental increase in short-term facilitation

Paired-pulse stimulation is a rapid, standard method of investigating changes in short-term facilitation. Because most forms of short-term plasticity occur presynaptically through changes in neurotransmitter release (reviewed in Zucker and Regehr, 2002), changes in the paired-pulse ratio are most often inversely correlated with a change in initial release probability (Dobrunz, 2002; Dobrunz and Stevens, 1997; reviewed in Zucker and Regehr, 2002). Therefore, if there is a change in the paired-pulse ratio at TA synapses from juveniles to young adults, then there may also be a change in basal transmission during development. We first tested for a developmental change in short-term plasticity of TA synapses by comparing the paired-pulse ratio (slope 2/slope1) of TA synapses in slices from juveniles (P13-P18) and young adult (P28-P42) rats.

As shown in Figure 2, juvenile responses (closed squares) demonstrate modest paired-pulse facilitation while young adults (open circles) elicit a more robust facilitation at intervals <200ms (one-way ANOVA, $p < 0.05$). This developmental increase in paired-pulse facilitation at TA synapses is the opposite of the developmental decrease in paired-pulse facilitation that occurs at SC synapses (Speed and Dobrunz, 2008). The inset of Figure 2 shows example paired-pulse traces from TA synapses, which show a large AHP in both juveniles (black trace) and young adults (red trace). However, there was no difference in the average time to peak ($35.0 \text{ ms} \pm 0.9$ juveniles, $n=7$; $33.7 \text{ ms} \pm 1.7$ young adults, $n=8$; $p > 0.05$) or the amplitude ($0.06 \text{ mV} \pm 0.01$, juveniles, $n=7$; 0.05 ± 0.01 , young adults, $n=8$, $p > 0.05$) of the AHP. Because neither the amplitude nor the time to peak of the overshoot is developmentally regulated, the AHP does not contribute to the observed developmental increase in paired-pulse facilitation at TA synapses.

Though paired-pulse facilitation is useful in the laboratory for investigating possible changes in short-term plasticity and/or basal transmission, this form of short-term plasticity is not typical of the type of activity seen at CA1 synapses *in vivo*. To further investigate changes in short-term plasticity at TA synapses, we next investigated the developmental changes in facilitation in response to a more temporally complex stimulus pattern, which we refer to as the natural stimulus pattern (NSP) (Dobrunz and Stevens, 1999). The NSP is derived from a spike pattern obtained from hippocampal place cell firing *in vivo* (Fenton and Muller, 1998),

and elicits multiple forms of short-term plasticity in a physiologically-relevant context (DeKay et al., 2006; Dobrunz and Stevens, 1999; Speed and Dobrunz, 2008). The NSP contains both low-frequency and higher-frequency interstimulus intervals and allows us to differentiate between changes in basal transmission vs. activity-dependent changes in neuromodulator activation (Speed and Dobrunz, 2008). In these experiments, the NSP is applied four times to each slice, with each repetition separated by a 0.1Hz control period. Slopes are normalized to the control period and reported as mean $\pm$ SE for all slices.

Figure 3 shows that there is a developmental increase in short-term facilitation and decrease in short-term depression at TA synapses in response to the NSP (Figure 3). Figure 3A shows the group results in response to the NSP for TA synapses from juveniles (closed squares) and young adults (open circles), with the normalized fEPSP plotted against stimulus number rather than time for clarity. In response to the NSP, TA synapses in slices from juvenile rats show a mix of responses with short-term facilitation and depression while young adults have more responses that are facilitated and show very few with short-term depression (Fig. 3B). Furthermore, the percentage of responses with facilitation is higher for young adults compared to juveniles ($p < 0.05$), and the percentage of responses with depression is much lower for young adults compared to juveniles ($p < 0.05$). This is reflected in the cumulative frequency distributions of fEPSPs in Figure 3D, in which young adult responses are shifted to the right relative to juveniles, indicating a developmental decrease in the frequency of depressed responses. This causes an increase in the overall mean normalized response size during the NSP (first 128 points, not including the control period), from juveniles to young adults, indicating an overall increase in short-term facilitation of the entire pattern during development (Figure 3E unpaired Student's t-test, $p < 0.05$). These results show that there is a developmental increase in short-term facilitation in response to the temporally complex NSP, which is the opposite of the developmental decrease in facilitation previously observed at SC synapses (Speed and Dobrunz, 2008). Together with the paired-pulse results, these results indicate that developmental modulation of short-term plasticity onto CA1 pyramidal cells is input specific.

In the experiments of Figure 3 we had NMDA receptors blocked by APV to prevent postsynaptic short-term plasticity, and to prevent possible long-term plasticity that can be induced by the NSP (Dobrunz and Stevens, 1999). To verify that the developmental change in short-term plasticity also occurs at TA synapses when NMDA receptors are not blocked, we next measured the responses to the NSP in slices from juveniles and young adults in the absence of APV (Supplemental Figure 1). These experiments show that the developmental increase in short-term facilitation also occurs in the absence of APV, and that it is actually larger when NMDA receptors are active (Supplemental Figure 1B). Under these conditions, the NSP did not cause long-term plasticity in either age (Supplemental Figure 1A). Because these results indicate that the developmental increase in short-term plasticity at TA synapses occurs whether or not NMDA receptors are blocked, we returned to using APV to block NMDA receptors for the remainder of the NSP experiments.

Short-term facilitation differs between SC and TA synapses at both ages

The relative dynamics of TA and SC synapses are critical in gating the flow of information in the hippocampal circuit and enabling the proper activity-dependent wiring of neural circuits in hippocampus during neonatal development. Because short-term plasticity is developmentally regulated at both TA and SC synapses, and the changes occur in opposite directions, the relative dynamics of these pathways will change during development. In order to understand the relationship of short-term plasticity SC and TA at each developmental stage, we next compared the responses from the two pathways in slices from juveniles and in slices from young adults in response to both paired-pulse stimulation and the NSP.

Figure 4A shows that relative to TA (open circles), SC (closed squares) elicits more paired-pulse facilitation at all intervals <500 ms in juveniles (One-way ANOVA, $p < 0.05$). By young adulthood, however, paired-pulse facilitation at TA synapses is on par with facilitation at SC synapses, as seen in Figure 4B. Although the values for TA synapses are lower at every interval, the differences are no longer significant (One-way ANOVA, $p > 0.05$). The insets in Figures 4A and 4B show example traces from TA and SC at the two ages, which reveal that the AHP of TA responses is prominent at both ages, but not observed in SC responses (inset, Fig. 4A and 4B). These results show that despite a large difference in paired-pulse ratio in juveniles, the opposite developmental changes in the SC and TA pathways cause the two pathways to converge to similar levels of paired-pulse facilitation in young adults.

The differences in short-term facilitation between TA and SC synapses in juveniles are larger during stimulation with the temporally complex NSP than with paired-pulse stimulation, as shown in Figures 5A-E. Figure 5A shows the plots of normalized fEPSP vs. stimulus number during the NSP (first 128 points), which reveal a striking difference in the level of short-term facilitation between SC (closed squares) and TA (open circles) in slices from juveniles. TA demonstrates a mix of low-level short-term facilitation and short-term depression, whereas SC responses show large short-term facilitation and no depression (Figure 5B). This results in a large leftward shift of the cumulative frequency curves for TA synapses relative to SC synapses (Figure 5C). As a result, the overall mean normalized fEPSP during the NSP is significantly smaller in TA than at SC synapses ($p < 0.05$; Figure 5D). TA synapses operate over a narrower range (maximum-minimum) of responses (0.82 ± 0.08) compared to SC synapses (1.17 ± 0.13) in juveniles ($p < 0.05$), which can also be seen in the steeper slope of the cumulative frequency curve of TA responses compared to SC responses (Figure 5C). Therefore, not only do juvenile SC and TA responses differ significantly in the amount of facilitation in response to the NSP, but also in the range of responses to the NSP. Figure 5E shows the responses of juvenile SC and TA synapses as a function of the previous interstimulus interval. The response sizes are not a simple function of the previous interstimulus interval, indicating that short-term plasticity during the NSP is influenced by the timing of other previous stimuli in the pattern at both SC and TA synapses. In addition, while the magnitude of difference between short-term plasticity at SC and TA synapses is variable, the differences occur primarily at the shorter intervals. We quantified this by calculating the magnitude of the difference in the normalized fEPSP for each point and averaging them for different ranges of interstimulus intervals (Supplemental Figure 2A). There were significant differences for all interval ranges up to 1200 ms ($p < 0.05$), but not for longer interstimulus intervals.

The opposite developmental changes in short-term facilitation also cause converging levels of facilitation between SC and TA in response to the NSP, as shown in Figures 5F-J. In young adults, the difference in the level of short-term facilitation between TA (open circles) and SC (closed squares) is greatly reduced in response to the NSP (Figure 5F), but not eliminated, as was observed with paired-pulse facilitation. The TA pathway has fewer responses that are facilitated than SC, and the percentage of responses with depression is higher at TA than at SC for young adult synapses ($p < 0.5$, Figure 5G). The cumulative frequency curves of the normalized fEPSP reveal a small leftward shift of TA responses relative to SC responses (Figure 5H), although it is less pronounced than in juveniles (Figure 5C). Similarly, TA exhibits a narrower range of responses to the NSP in young adults (0.71 ± 0.07) relative to SC responses (1.25 ± 0.07 , $p < 0.05$), an effect not detected using paired-pulse facilitation alone. The mean normalized fEPSP across all 128 data points within the pattern is still significantly larger at SC synapses than TA synapses ($p < 0.05$; Figure 5I). The differences between SC and TA occur at the shorter interstimulus intervals (Figure 5J). As with juveniles, the differences are statistically significant for ranges of intervals up to 1200

ms (Supplemental Figure 2B). These results show that despite opposite developmental changes in short-term facilitation, SC demonstrates a higher level of facilitation and operates over a broader range of responses at both ages relative to TA. Thus, SC and TA are each uniquely situated to modulate postsynaptic activity in CA1 pyramidal cells depending on the pattern of synaptic activation.

The developmental decrease in short-term facilitation at TA synapses is not due to mGluR1 or GABA_B receptor activation

We next investigated possible mechanisms responsible for the developmental increase in short-term facilitation at TA synapses. Previously, we have reported that the developmental decrease in short-term facilitation in response to the NSP at SC synapses depends upon synaptic activation of both mGluR1 and GABA_B receptors (Speed and Dobrunz, 2008). High levels of glutamate released during burst activity of CA3-CA1 synapses leads to activation of mGluR1 on GABAergic interneurons that increase interneuron excitability and GABA release. Subsequent activation of GABA_B receptors on CA3 presynaptic terminals then leads to a decrease in glutamate release, an effect that is developmentally regulated and present to a greater extent at young adult synapses than at juvenile synapses (Speed and Dobrunz, 2008). The synaptic activation of mGluR1 and GABA_B receptors is primarily responsible for the developmental decrease in short-term facilitation in response to the NSP at SC synapses (Speed and Dobrunz, 2008). It is not known whether mGluR1 and/or GABA_B receptors regulate short-term plasticity at TA synapses in either juveniles or young adults, or if a similar mechanism involving both mGluR1 and GABA_B receptors contributes to the developmental change in short-term plasticity during the NSP at TA synapses, as it does at SC synapses (Speed and Dobrunz, 2008).

We first investigated whether the mechanism underlying the developmental increase in facilitation at TA synapses is also mGluR1 sensitive. To test whether synaptic activation of mGluR1s alters short-term facilitation at TA synapses, we compared the responses before and during addition of the mGluR1 antagonist LY367385 (10 μ M), as shown in Figure 6. Figure 6A shows that there was little or no difference in the responses of TA synapses in slices from juveniles to the NSP before (closed squares) and during bath application of the mGluR1 antagonist LY367385 (open circles). We found no difference in the cumulative frequency distribution of juvenile responses (Figure 6B), and there was no significant difference in the overall normalized mean response size between control and drug ($p>0.05$; Figure 6C) indicating that synaptic activation of mGluR1 receptors does not regulate short-term facilitation during the NSP at juvenile TA synapses. Similarly, there was little or no change in short-term facilitation in the presence of mGluR1 antagonist in young adults (Figure 6D), though LY367385 did cause a small rightward shift in the cumulative frequency curve (Figure 6E). However, there was no difference in mean response size in the presence of LY367385 relative to control ($p>0.05$; Figure 6F). Therefore, synaptic activation of mGluR1 does not play a significant role in short-term plasticity at TA synapses and does not contribute to the developmental increase in short-term facilitation.

Although mGluR1 receptors are not involved, and thus the mechanism of the developmental change in facilitation is different from that at SC synapses, it is still possible that a change in the activation of GABA_B receptors could underlie the developmental increase in short-term facilitation at TA synapses. GABA_B receptor function is developmentally regulated and increases from 2-4 weeks (Dumas and Foster, 1998; Nurse and Lacaille, 1999), and an increase in tonic activation of GABA_B receptors could cause an increase in short-term facilitation. However, we found no evidence for tonic activation of GABA_B receptors; application of the GABA_B receptor agonist CGP55845 (10 μ M) did not alter the basal synaptic response size (juvenile $106.2 \pm 4.2\%$ of control, $p>0.05$, $n=5$; young adult $97.2 \pm 3.0\%$ of control, $p>0.05$, $n=5$) or the paired-pulse ratio (juvenile $102.4 \pm 1.7\%$ of control,

$p > 0.05$, $n = 5$; young adult $102.7 \pm 4.6\%$ of control, $p > 0.05$, $n = 5$) in either juveniles or young adults. In addition to possible presynaptic effects on glutamate release, activation of postsynaptic GABA_B receptors can cause a hyperpolarizing postsynaptic current (Benardo, 1995; Gahwiler and Brown, 1985; Otmakhova and Lisman, 2004) that could underlie the characteristic AHP of the TA response. However, CGP55845 did not alter the amplitude or time to peak of the AHP at TA synapses in slices from either juveniles (amplitude $0.06 \text{ mV} \pm 0.01$, control; $0.06 \text{ mV} \pm 0.01$, drug; $n = 3$, $p > 0.05$; time to peak $33.2 \text{ ms} \pm 1.7$, control; $35.4 \text{ ms} \pm 2.6$, drug; $n = 3$, $p > 0.05$) or young adults (amplitude $0.08 \text{ mV} \pm 0.01$, control; $0.06 \text{ mV} \pm 0.01$, drug; $n = 4$, $p > 0.05$; time to peak ($34.7 \text{ ms} \pm 0.8$, control; $35.4 \text{ ms} \pm 0.6$, drug; $n = 4$, $p > 0.05$), indicating that GABA_B receptors are not involved in the AHP of field EPSPs at TA synapses at our stimulus intensity. Our stimulus intensity is relatively low given the hyperexcitability of CA1 synapses in the absence of fast inhibitory transmission, and it is possible that higher stimulus intensities could evoke significant GABA_B receptor-mediated contributions to the AHP (Pitler and Alger, 1994).

We next tested whether activity-dependent activation of GABA_B receptors modulates short-term plasticity at TA synapses during the NSP by comparing the responses before and during application of CGP55845 (Figure 7). At TA synapses in slices from juveniles, there was no change in the responses to the NSP before and during application of CGP55845. There was no change in the cumulative probability distribution (Figure 7A), or in mean response size between control and drug at juvenile TA synapses ($p > 0.5$; Figure 7B). In contrast, we found that in slices from young adults TA responses to the NSP were slightly more facilitated in the presence of the GABA_B receptor antagonist, causing a rightward shift of the cumulative frequency curve (Figure 7C), and an increase in the overall mean response size with CGP55845 as compared to control ($p < 0.05$; Figure 7D). This means that GABA_B receptors are synaptically activated and decrease short-term facilitation at TA synapses in slices from young adults but not juveniles. Because this decreases the amount of short-term facilitation in young adults, this effect does not contribute to the developmental increase in short-term facilitation at TA synapses, but instead reduces it.

A decrease in initial release probability underlies developmental changes in short-term plasticity at TA, but not SC synapses

So far, we have shown that the developmental changes in short-term facilitation at TA synapses occur in the opposite direction and are not regulated by the same mechanism as at SC synapses. Because the level of short-term facilitation is generally inversely related to initial release probability (Dobrunz, 2002; Dobrunz et al., 1997; Sun and Dobrunz, 2006; Sun et al., 2005; reviewed in Zucker and Regehr, 2002), we next investigated whether changes in initial release probability could underlie the developmental changes in short-term plasticity at either TA or SC synapses using the MK-801 method (Huang and Stevens, 1997; Rosenmund et al., 1993). If the developmental increase in paired-pulse ratio is due to a change in initial release probability, then we would expect the initial release probability to be higher in young adults compared to juveniles. For these experiments we used whole cell patch clamp recordings from CA1 pyramidal cells and recorded NMDA receptor currents in the presence of NBQX ($20 \mu\text{M}$) to block AMPA receptor responses. MK-801 ($40 \mu\text{M}$) was bath applied, and the rate of block of the NMDA EPSC amplitude by MK-801 was quantified by fitting the decay curves with a single exponential. In these experiments, a faster MK-801 decay curve is representative of a higher initial release probability.

The results from the MK-801 experiments are shown in Figure 8. The rate of block of the NMDAR-mediated EPSC by MK-801 is much slower at young adult TA synapses ($\tau = 21.7 \pm 3.1$) than at juvenile TA synapses ($\tau = 8.1 \pm 1.7$, $p < 0.05$; Figure 8A). This indicates that the initial release probability is lower at TA synapses from young adults compared to juveniles. In contrast, we found no significant change in the rates of EPSC block by MK-801

at SC synapses between juveniles ($\tau = 14.7 \pm 3.0$) and young adults ($\tau = 18.5 \pm 2.3$, $p > 0.05$; Figure 8B), indicating that the release probability of SC synapses does not change during this period of postnatal development. These results suggest that the developmental change in short-term plasticity at TA synapses, but not SC synapses, is due to a change in initial release probability. This demonstrates that SC and TA employ independent methods of regulating short-term facilitation during development, as well as opposite trends in short-term facilitation during development.

One issue in interpreting the MK-801 experiments is whether there is a change in NMDA receptor subunit composition that could itself alter the rate of block of NMDA EPSCs by MK-801. In addition to depending upon the probability of glutamate release and the concentration of MK-801, the rate of block of NMDA EPSCs by MK-801 also depends on the open probability of NMDA receptors and the fraction of open receptors that are blocked (Huang and Stevens, 1997). The peak open probability of NMDA receptors, in turn, depends upon the NMDA receptor subunit composition and is greater at receptors containing NR2A than NR2B (Chen et al., 1999). If the NMDAR subunit composition can change during development (Williams et al., 1993), there could potentially be differences in the relationship between release probability and the MK-801 blocking rate between juveniles and young adults. We tested whether there is a change in the percentage of NMDA receptors containing the NR2B subunit by measuring the effects of the specific NR2B antagonist RO25-6981 (1 μ M) on the NMDA EPSC amplitude (Fig. 8C). Blocking NR2B-containing receptors caused a significant decrease in EPSC amplitude at TA synapses in both juveniles and young adults ($p < 0.05$, Figure 8D), however the percent of block was not different between the two ages ($p > 0.05$). RO25-6981 also decreased the NMDA EPSC amplitude at SC synapses ($p < 0.05$, Figure 8E), again with no difference between juveniles and young adults ($p > 0.05$). These results indicate that the proportion of NR2B-containing receptors is not changing during this developmental time period (juvenile to young adult) in either pathway, and that the MK-801 blocking rate can be used to test for differences in release probability between juveniles and young adults without the potential complication of changes in NR2B subunits. Our results from Figures 8A and 8B can therefore be interpreted as showing that TA synapses have a developmental decrease in release probability, consistent with the developmental increase in paired-pulse facilitation we observe at these synapses. In contrast, SC synapses have no developmental change in release probability between juveniles and young adults. Therefore, the developmental decrease in paired-pulse facilitation at SC synapses is not caused by a developmental increase in release probability.

Our results also indicate that SC synapses have a larger proportion of NR2B containing receptors than TA synapses in juveniles ($p < 0.5$, Figure 9A) and in young adults ($p < 0.5$, Figure 9B). To directly test the effect of subunit composition on the MK-801 blocking rate, we next measured the MK-801 blocking rate with NR2B-containing receptors blocked by RO25-6981, using SC synapses from young adults where the block by RO25-6981 was the largest. Despite the fact that RO25-6981 blocked over 50% of the NMDA EPSC, we found no difference in rate of block by MK-801 in the presence of RO25-6981 compared to control ($p > 0.05$, Figure 9C). These results indicate that the rate of block of NMDA EPSCs by MK-801 is unaffected by the composition of NR2B vs. NR2A subunits, consistent with a previous report (Chavis and Westbrook, 2001), and show that the rate of block by MK-801 can be used to test for differences in the synaptic release probability even when there are differences in the NR2B subunit composition. When we compared MK-801 decay constants between TA and SC synapses we found a small but significant difference in slices from juveniles (Figure 9D, $p < 0.05$) but not young adults (Figure 9E, $p > 0.05$). These results suggest that the initial release probability is higher at TA synapses compared to SC synapses in slices from juveniles but not in slices from young adults.

DISCUSSION

The main finding of our paper is that TA synapses have a developmental increase in short-term facilitation, which is the opposite of what occurs at SC synapses. Paired-pulse facilitation at TA synapses increases from juveniles to young adults, as does short-term facilitation in response to a complex, physiologically-relevant natural stimulus pattern. This developmental increase in short-term facilitation is opposite of the developmental decrease in short-term facilitation we previously reported at SC synapses (Speed and Dobrunz, 2008). In juveniles, TA synapses have less paired-pulse facilitation and facilitation in response to the NSP than SC synapses. In young adults, although the levels of paired-pulse facilitation are not different between TA and SC synapses, TA synapses remain less facilitated than SC synapses in response to the NSP. Finally, we determined that this developmental increase in short-term facilitation at TA synapses is due to a decrease in initial release probability, rather than differential activation of mGluR1 or GABA_B receptors during development as occurs at SC synapses (Speed and Dobrunz, 2008). Our results therefore show that opposite developmental changes occur in short-term facilitation at TA synapses compared to SC synapses, and that the mechanisms underlying these developmental changes are different between the two synapses.

Developmental changes in short-term plasticity at TA and SC synapses

The developmental increase in paired-pulse facilitation at TA synapses is similar to the developmental increases in the paired-pulse ratio found at other non-hippocampal excitatory synapses, including synapses onto layer 2/3 and layer 5 pyramidal cells in cortex (Reyes & Sakmann, 1999), synapses onto striatal neurons (Choi and Lovinger, 1997), calyx of Held synapses (Taschenberger and von Gersdorff, 2000), and synapses onto motor neurons in the spinal cord (Li and Burke, 2002). Because the developmental increase in paired-pulse facilitation at TA synapses occurs over the same developmental period that there is a decrease in paired-pulse facilitation at SC synapses, this indicates that there is pathway specific developmental regulation of short-term plasticity at the two inputs to CA1 pyramidal cells.

While a fast and useful means of measuring short-term plasticity, paired-pulse and constant-frequency stimulation are not representative of the firing patterns that hippocampal synapses receive *in vivo*. When we investigated changes in short-term plasticity at TA synapses in response to temporally complex stimulation, we found that the developmental changes at TA synapses in response to the NSP appear to be caused as much by the loss of short-term depression as by the increase in short-term facilitation. Similar decreases in short-term depression during late postnatal development have been reported at Layer 5 pyramidal neurons in the neocortex in which there is a shift from paired-pulse depression to paired-pulse facilitation (Frick et al., 2007), and from high-frequency depression to high-frequency facilitation with age (Chen and Roper, 2004). As with paired-pulse facilitation, the developmental increase in short-term facilitation during the NSP at TA synapses is the opposite of the developmental decrease in short-term facilitation during the NSP that occurs at SC synapses.

In juveniles, TA synapses have less paired-pulse facilitation and less facilitation in response to the NSP as compared to SC synapses. These differences in short-term plasticity will cause the relative strengths of the two input pathways to CA1 to be dynamically changing during temporally complex firing patterns that occur *in vivo*. In particular, high frequency bursts will cause a decrease in the strength of the TA excitatory input to CA1 as compared to the SC input. While the overall effects on CA1 firing will also be influenced by the dynamics of SC and TA excitatory synapses onto inhibitory interneurons and by inhibitory synapses, the

difference between TA and SC synapses is likely to alter the flow of information through hippocampus.

The opposite developmental trends in short-term plasticity cause SC and TA synapses to converge to similar levels of paired-pulse facilitation in young adults. However, stimulation with the NSP revealed that there are still large differences in young adults in how the two pathways respond to physiologically relevant patterns of activation. The majority of the SC responses to the NSP were facilitated to a large degree, whereas the majority of TA responses were not facilitated or exhibited much lower levels of facilitation even during burst activity. This may indicate a weaker role of TA relative to SC in determining CA1 output, with the relative balance between the two pathways being frequency-dependent. The responses of SC and TA during the NSP were not a simple function of the previous interstimulus interval at either age. This indicates that the history of other prior stimuli also affect short-term plasticity, and that the temporal complexity of the pattern (caused by variability in the interstimulus intervals) is important in addition to frequency in determining the differences between SC and TA responses. Therefore, although SC and TA develop through opposite developmental trends in short-term plasticity to reach similar levels of paired-pulse facilitation, each pathway remains able to uniquely modulate postsynaptic activity in CA1 cells in response to complex, physiologically relevant synaptic activity. Our results using the NSP form the foundation for further study into the relative influences of SC and TA inputs on CA1 output and on the induction of long-term plasticity, and how this might differ between both juveniles and young adults.

While using the NSP provides a more physiologically relevant stimulus pattern in its temporal variability and mixture of frequencies, our experiments differ from activity *in vivo* in several respects. One potential difference is the number of synapses activated synchronously during the NSP. The synchronous firing of a small number of afferent fibers is consistent with endogenous activity at Schaffer collateral synapses because CA3 pyramidal cells *in vivo* fire in groups (Buzsaki et al., 1983; Csicsvari et al, 2000). Entorhinal cortex neurons also can fire synchronously, and the ability of TA synapses to fire CA1 neurons is dependent on the synchrony of network activity (Soltesz and Jones, 1995). Because we had GABA_A receptors blocked, our stimulus intensity was necessarily low in order to avoid overexcitation of the hippocampal circuitry, resulting in a small population of synchronously activated fibers in our experiments that is likely to be comparable to what occurs *in vivo*. We blocked GABA_A receptors in our experiments in order to isolate changes in excitatory transmission at synapses onto CA1 pyramidal cells. With inhibition intact, the output of CA1 cells will also be influenced by the dynamics of inhibitory synaptic transmission. Future experiments will be needed to test whether there are also developmental changes in short-term plasticity at inhibitory synapses made by interneurons in SR and/or SLM onto CA1 pyramidal cells, or at the excitatory SC and TA inputs onto these interneurons.

In most of our experiments recordings were done in the presence of the NMDAR antagonist APV in order to isolate presynaptic changes in short-term facilitation without contamination from postsynaptic short-term plasticity at NMDA receptors. In addition, blocking NMDA receptors prevents the possible induction of NMDA receptor dependent long-term plasticity. However, we find that the developmental difference in short-term facilitation was actually greater in the absence of APV (Supplemental Figure 1), indicating that is not dependent on the blockade of NMDARs.

In our experiments, we used the same NSP to stimulate both pathways in order to directly compare the responses of TA and SC synapses, and our results indicate that the presynaptic properties of TA synapses are different from those of SC synapses in both juveniles and

young adults. *In vivo* these two pathways receive different inputs, and it is possible that the two pathways are differentially tuned based on differences of their input firing patterns. Future studies comparing the responses to NSPs derived from CA3 vs. entorhinal cortex layer III neurons will be useful for further investigation into the mechanisms and effects of short-term facilitation at TA vs. SC synapses.

Mechanism of developmental changes in short-term plasticity

The type of mechanism underlying the developmental change in short-term plasticity at TA synapses is different from that at SC synapses. Short-term plasticity can be altered by activity-dependent changes in the probability of glutamate release through the activation of neuromodulatory receptors, such as has been observed during trains of stimuli at SC synapses (Speed and Dobrunz, 2008; Sun and Dobrunz, 2006). We have previously shown that the developmental decrease in short-term facilitation at SC synapses occurs through an increase in neuromodulatory effects, mediated by activity-dependent activation of mGluR1 and GABA_B receptors (Speed and Dobrunz, 2008). Using the NSP, we found that glutamate released during burst activity at SC synapses activates mGluR1 on interneurons, most likely located in the SR, and increases GABA release. This activates GABA_B receptors on SC presynaptic terminals and leads to a decrease in the probability of glutamate release, which reduces the amount of short-term facilitation during the burst. This effect is greater in at SC synapses in slices from young adults than in juveniles, and blocking either mGluR1 receptors or GABA_B receptors largely eliminates the observed decrease in short-term facilitation in young adults in response to the NSP (Speed and Dobrunz, 2008).

In contrast, our results here show that this mechanism does not appear to operate at TA synapses, because blocking mGluR1 receptors has no effect on short-term plasticity at TA synapses from either age. While immunoreactivity for mGluR1 has been detected in interneurons in SLM, the level of expression is low (Ferraguti et al., 2004). It is therefore not clear whether the stimulation of TA synapses by the NSP is not sufficient to activate mGluR1 receptors on interneurons and/or whether these interneurons do not modulate short-term plasticity of TA synapses in the same way as SC synapses. In either case, it is clear that the developmental increase in short-term facilitation at TA synapses is not simply due to a reversal of the mechanism underlying the developmental decrease in facilitation at SC synapses.

The activation of presynaptic GABA_B receptors that reduce glutamate release is neuromodulatory mechanism that could potentially have a significant impact on short-term plasticity at TA synapses, independent of mGluR1 activation. However, we found no evidence for tonic activation of GABA_B receptors, which would decrease initial release probability and increase short-term facilitation. In addition, blocking GABA_B receptors had no effect on the responses to the NSP in juveniles and caused a modest increase in short-term facilitation during the NSP in young adults. These results show that activity-dependent activation of GABA_B receptors does not contribute to the observed developmental increase in facilitation at TA synapses, but in fact may counteract it by decreasing facilitation during burst stimulation in young adults.

Our results show that there is a developmental change in the initial release probability at TA synapses, which decreases in young adults compared to juveniles. Because the amount of facilitation is inversely related to the initial release probability of the synapse (Dobrunz, 2002; Dobrunz et al., 1997; reviewed in Zucker and Regehr, 2002), it is likely that the developmental increase in short-term facilitation at TA synapses is secondary to this decrease in initial release probability. This developmental decrease in release probability is similar to what has been previously reported at other synapses in the rat brain (Taschenberger et al., 2002). In contrast, our results show no change in the initial release

probability at SC between juveniles and young adults. This indicates that the developmental decrease in paired-pulse facilitation at SC synapses is not caused by an increase in release probability, and must be due to another mechanism. While changes in the amount of paired-pulse facilitation often are caused by changes in the initial release probability, it is important to note that it is not always the case (Kokaia et al., 1998; Markram, 1997). Together, our results show that the opposite developmental trends in short-term facilitation at SC and TA synapses occur through different mechanisms: SC via an increase in activity-dependent mGluR1 and GABA_B receptor activation and TA via a decrease in initial release probability.

In choosing MK-801 as our method of measuring synaptic efficacy, we took several factors into account. First, these experiments were performed in the presence of the GABA_B receptor antagonist CGP55845 to prevent stimulus intensity-dependent activation of postsynaptic GABA_B receptors which could cause an artificial decrease in the amplitude and duration of the NMDAR-mediated EPSCs. This is particularly important because the stimulus intensity required to elicit sufficiently large NMDAR-mediated responses for the MK-801 experiments is much greater than that used to measure the AMPAR-mediated responses during the NSP and paired-pulse stimulation. However, addition of the GABA_B antagonist during the NSP experiments did not change the baseline response size or paired-pulse ratio of the AMPAR-mediated fEPSPs, indicating that the initial release probability is not affected by tonic activation of GABA_B receptors. Another potential concern is whether the MK-801 method is sensitive enough to detect small changes in initial release probability. However, we successfully observed the developmental decrease in initial release probability at TA synapses, and the MK-801 method has been used to successfully detect differences in release probability between SC synapses onto different types of interneurons (Sun and Dobrunz, 2006; Sun et al., 2005), and between thalamocortical and hippocampal synapses (Castro-Alamancos and Connors, 1997). Therefore, we interpret the lack of change in the MK-801 blocking rate as indicating that there is no overall difference in synaptic efficacy between juvenile and young adult SC synapses.

One factor to consider when studying developmental changes in release probability using the MK-801 method is whether the results are influenced by developmental changes in the subunit composition of NMDARs. NMDARs consist of a mandatory NR1 subunit (Forrest et al., 1994) along with NR2A-D subunits (Dingledine et al., 1999; McBain and Mayer, 1994) in order to form a functioning receptor. Though rare, heterotrimers consisting of NR1/NR2A/NR2B subunits have been reported in the hippocampus (Al-Hallaq et al., 2007) that exhibit channel kinetics intermediate between those of NR1/NR2A and NR1/NR2B receptors (Brimecombe et al., 1997). Although total NMDAR expression is stable in late postnatal development (Arrigoni and Greene, 2004; Nicholson et al., 2006), there is a developmental increase in the NR2A/NR2B ratio in early postnatal development (Kirson et al., 1999). Because previous reports have found that NR2B subunits have a lower peak open probability than do NR2A subunits (Erreger et al., 2005), a difference in the subunit composition could potentially contribute to a difference in MK-801 blocking rates between juveniles and young adults and complicate the interpretation of these experiments. We tested for possible changes in NMDAR subunit composition using RO25-6981, an antagonist specific for receptors containing the NR2B subunit. Because RO25-6981 can bind to heterotrimers of NR1/NR2A/NR2B (Hawkins et al., 1999), they are also likely to be blocked, in addition to NR1/NR2B receptors. We found no significant difference between juveniles and young adults in either pathway in the fraction of NMDAR current blocked by the antagonist, consistent with earlier reports showing no developmental difference in NR2B subunit expression over this age range (Kirson et al., 1999). As a result, the slower rate block by MK-801 of TA synapses in young adults vs. juveniles indicates a developmental decrease in release probability, rather than a change in the fraction of NR2B receptors.

We did see a greater effect of RO25-6981 in SC at both ages compared to TA, consistent with an earlier study (Arrigoni and Greene, 2004), indicating that SC synapses have a higher percentage of NR2B-containing receptors than TA. Importantly, we saw no significant difference in MK-801 blocking rate in the presence vs. absence of the NR2B-containing NMDAR receptor antagonist, RO25-6981, using young adult SC synapses where the % block by RO25-6981 was largest. This is in agreement with a previous report that differences in the NMDA receptor subunit composition did not alter the overall relationship between release probability and the MK-801 blocking rate for cultured neurons (Chavis and Westbrook, 2001).

Because we find that NR2A/NR2B subunit composition does not affect rate of MK-801 blockade, our data show that TA synapses have a higher initial release probability than SC synapses in juveniles, consistent with their lower paired-pulse ratio. However, both the difference in release probability and the difference in paired-pulse ratio between TA and SC have largely disappeared in young adults. Because a difference still persists in the response to the NSP, this means that another mechanism must also contribute to the differences in short-term plasticity between TA and SC synapses in young adults. Our results show that here is less short-term facilitation at TA synapses compared to SC synapses in young adults at short interstimulus intervals (up to 1200 ms) during the NSP. Although short-term plasticity for each stimulus is determined by the recent history of stimulation and not just the previous interstimulus interval, this time frame suggests the possibility of stimulus-dependent activation of neuromodulatory receptors. There is a wide variety of G-protein coupled receptors that are capable of altering the probability of neurotransmitter release, including mGluRs, GABA_B receptors, muscarinic acetylcholine receptors, and others. Because TA synapses are different from SC synapses in having a high level of presynaptic Group II mGluR receptors that decrease release probability in response to synaptic activation (Kew et al., 2001; Kew et al., 2002; Price et al., 2005), one possible mechanism is the activation of these neuromodulatory receptors. Future experiments will be needed to determine the mechanism(s) underlying the differences in short-term plasticity during the NSP between TA and SC synapses in young adults, and to see if other mechanism(s) (in addition to a difference in release probability) contribute to the difference between TA and SC in juveniles.

Importance of differences in TA and SC synapses during development

Early postnatal life is a critical period in the development of the mammalian nervous system, at a time when inherited disorders or environmental insults can have dramatic consequences and cause lasting cognitive impairment and developmental disabilities (Rice and Barone, 2000). Extensive changes occur in neuronal connectivity during neonatal development as a result of synapse consolidation and elimination (reviewed in Feldman and Knudsen, 1998). This activity-dependent remodeling of synaptic connections contributes to the formation of functional neural circuits early in development and is critical for the proper refinement of these early connections into the fine-tuned patterns of adult neural circuits (reviewed in Goodman and Shatz, 1993). Since the activity dependence of synaptic remodeling results from the coincidence of postsynaptic depolarization with presynaptic activity, this process will be critically dependent upon the strength and dynamics of individual synapses (reviewed in Goodman and Shatz, 1993). Developmental changes in short-term plasticity and the initial release probability such as we report here are therefore likely to be very important for normal development of hippocampal circuits.

In CA1, there is an overall increase in the strength of the SC input pathway in young adults as compared to juveniles (Dumas and Foster, 1995). Because there is no increase in the average strength of the postsynaptic response to the neurotransmitter glutamate (Hsia et al., 1998), this indicates that the extensive enhancement in SC synaptic transmission that occurs

during neonatal development is due to increases in presynaptic function and/or synapse number. There is a large increase in synapse number during postnatal development due to synaptogenesis, such that the density of excitatory synapses is twice as large in adults as compared to juveniles (Harris et al., 1992). Previously it was not known whether there was also a developmental increase in release probability that also contributed to the increase in synaptic strength of SC synapses. Our results show that this is not the case, suggesting that the developmental increase in the SC pathway is due only to an increase in synapse number. In contrast, the developmental decrease in release probability at TA synapses serves to decrease the strength of this pathway in young adults vs. juveniles, and will decrease the relative strength of TA compared to SC in young adults.

TA is traditionally considered to be a weaker excitatory input to CA1 as compared to SC because of its smaller number of synapses (Megias et al., 2001), greater distance from the soma, and smaller AMPA/NMDA receptor ratio (Arrigoni and Greene, 2004; Nicholson et al., 2006; Otmakhova et al., 2002). In addition, TA synapses terminate on a large number of inhibitory interneurons in the SR and the SLM (Empson and Heinemann, 1995a) positioning it for a more modulatory role in CA1 output than SC (Nolan et al., 2004). The distal dendrites of CA1 pyramidal cells also exhibit a different array of receptors and ion channels than the proximal dendrites, including Ca^{2+} -sensitive K^{+} channels (Magee et al., 1998) and HCN^{-} channels (Nolan et al., 2004; Shin and Chetkovich, 2007), which aid in dendritic integration of TA inputs and enable these distant synapses to influence CA1 output. Strong stimulation of TA can directly inhibit (Colbert and Levy, 1992; Remondes and Schuman, 2002) excitatory transmission at SC synapses while less intense activity similar to that seen *In vivo* (Frank et al., 2001) enhances SC activity (Buzsaki, 2002; Dudman et al., 2007; Judge and Hasselmo, 2004; Levy et al., 1998). Our results suggest that TA synapses have a higher release probability than SC synapses in juveniles, which will help to increase the influence of TA synapses compared to SC synapses, but that this difference has largely disappeared in young adults. Because TA exhibits significantly lower levels of short-term facilitation than SC synapses, the relative strength of these two input pathways is also frequency dependent at both ages.

Strong activation of TA synapses has also been shown to initiate dendritic action potentials in CA1 cells (Dudman et al., 2007; Levy et al., 1998; Remondes and Schuman, 2002) and drive action potential firing in CA1 pyramidal cell somas (Andreasen and Lambert, 1998; Jarsky et al., 2005). Interestingly, the propagation of dendritic action potentials to the CA1 cell bodies is gated by SC synaptic activity (Jarsky et al., 2005), which indicates the importance of interactions between TA and SC. In addition, because short-term plasticity influences cell spiking, the difference in short-term plasticity between TA and SC will dynamically alter the gating of information between these two pathways, an effect that is developmentally regulated.

Though the SC input has been the most widely studied excitatory CA1 pathway in the hippocampus due to its strong input and role in spatial and associative learning and memory (Gruart and Delgado-Garcia, 2007; Martinez and Derrick, 1996), recent studies have shown that the TA pathway is also important for hippocampal dependent learning and memory (Sybirska et al., 2000). TA provides the main source of specific sensory input to CA1 (McNaughton et al., 1989; Vinogradova, 2001). The entorhinal cortex is involved in spatial recognition (Fyhn et al., 2004), and the TA path carries positional information from entorhinal cortex that is sufficient to establish CA1 place cells (Brun et al., 2002). In addition the TA path can maintain place recognition if SC inputs are lesioned (Brun et al., 2002). Furthermore, the function of the trisynaptic circuit is not required for some forms of spatial memory that are instead impaired by damage to entorhinal cortex (Jarrard et al., 1984). The TA pathway has been shown to be important for the consolidation of spatial

memory (Remondes and Schuman, 2004), and interactions between the SC and TA pathways have been shown to be important for spatial learning and memory (Axmacher et al., 2006; Brun et al., 2002; Nolan et al., 2004), associative memory (Lisman, 1999), and declarative memory (Axmacher et al., 2006).

Long-term potentiation (LTP) at SC synapses has been strongly implicated as a cellular correlate of learning and memory (Gruart and Delgado-Garcia, 2007). Like SC synapses, TA synapses are also capable of LTP (Colbert and Levy, 1993; Remondes and Schuman, 2003) and long-term depression (LTD) (Dvorak-Carbone and Schuman, 1999). Activation of TA synapses can enhance SC synaptic activity (Dudman et al., 2007) and can modulate the induction of LTP at SC synapses (Dudman et al., 2007; Levy et al., 1998; Remondes and Schuman, 2002). We have previously reported that application of the NSP to SC synapses in the presence of NMDAR activation induces long-term plasticity (Dobrunz and Stevens, 1999), further emphasizing the physiological significance of the NSP. However, when the same experiment was performed in the presence of NMDAR activation at TA synapses, no long-term plasticity was induced (Supplemental Figure 1). This could be due to the presence of the GABA_A receptor antagonist, picrotoxin, or to the pattern of activation since the NSP was recorded from a place cell rather than from an EC III neuron. However, our results comparing SC and TA responses to the same NSP reveal important differences in the developmental regulation of short-term plasticity between TA and SC synapses, including each pathway's unique ability to respond to complex trains of stimuli in both juveniles and young adults. Because short-term plasticity can influence which patterns of activation result in induction of LTP and LTD, these developmental changes in short-term plasticity are likely to be a mechanism that influences long-term plasticity in both pathways. The data presented here emphasizes the need for future studies comparing NSPs derived from CA3 vs. entorhinal cortex layer III neurons to study the effects of short-term facilitation at TA vs. SC synapses on CA1 firing and long-term plasticity.

The entorhinal cortex and TA pathway have been implicated in several neurological diseases, including developmental disorders that cause mental retardation (MRDD). Neurons in layer III of entorhinal cortex, where the TA path originates (Steward and Scoville, 1976), have been shown to be destroyed in Rett Syndrome (Leontovich et al., 1999), a developmental disorder that strikes girls during infancy and childhood. TA neurons are also preferentially susceptible to damage in temporal lobe epilepsy (Du et al., 1993), leading to memory impairment (Schwarcz and Witter, 2002). Entorhinal cortex shows reduced volume and neuronal loss in Down Syndrome (Sadowski et al., 1999), and morphological abnormalities in patients with schizophrenia (Arnold, 2000). Because of the importance of the TA pathway to learning and memory, there may be alterations in the functional properties of TA synapses that contribute to cognitive impairment in these or other MRDD syndromes. Our experiments have shown that the influence of TA synapses on CA1 output is developmentally regulated and changes between juveniles and young adults. It will be important to determine if alterations in the balance of short-term plasticity between SC and TA synapses during development contribute to developmental abnormalities in learning and memory that occur in disorders such as epilepsy (Ang et al., 2006; Wozny et al., 2005), Rett Syndrome (Nelson et al., 2006), and Fetal Alcohol Syndrome (Mameli et al., 2005) in which CA1 short-term plasticity is altered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Isolating Schaffer collateral (SC) and Temporoammonic (TA) synapses

(A) Average of 10 traces illustrating the use of the source/sink properties of CA1 pyramidal neurons to isolate SC vs. TA synapses. (A) Top panels: Recording in *stratum radiatum* (SR) yields negative field potentials to stimulation in SR and positive field potentials to stimulation in *stratum lacunosum moleculare* (SLM). Bottom Panel: Recording in SLM yields negative field potentials to stimulation in SLM and positive potentials to stimulation in SR. (B, C) Bath application of the mGluR2 agonist DCG-IV (100 μ M) selectively decreases synaptic transmission at TA synapses (B) but not at SC synapses (C).

Figure 2. Paired-pulse facilitation increases during development at TA synapses

The paired-pulse ratio of juvenile (closed squares, $n=7$) synapses is significantly less than at young adult (open circles, $n=7$) synapses at intervals 30 ms-100 ms (one-way ANOVA, $p<0.05$). Inset: Average of 20 traces at juvenile (gray) and young adult (black) TA synapses at 50 ms paired-pulse interval.

Figure 3. TA synapses demonstrate a developmental increase in short-term facilitation and decrease in short-term depression in response to a natural stimulus pattern

(A) Responses to the natural stimulus pattern (NSP) are normalized to a control period (0.1 Hz, points 129-160) in which there is no short-term plasticity, and plotted against stimulus number. Juvenile TA synapses (A1, $n=7$) demonstrate a mix of facilitation and depression in response to the NSP, whereas young adult synapses (A2, $n=8$) show less short-term depression as well as more short-term facilitation. Insets: Example traces from juvenile TA synapses (A1) and young adult TA synapses (A2) during a 2.5 s region of the NSP. Scale bars: 150 ms, 0.12 mV. (B) The percent of responses that are facilitated vs. depressed is different in both juveniles and young adults ($p < 0.05$). (C) Responses of TA synapses from juveniles (closed squares, from A1) and young adults (open circles, from A2) are plotted on the same graph to illustrate the differences in the responses. (D) There is a rightward shift in the cumulative frequency curves of young adults (open circles) versus juveniles (closed squares). (E) The mean response size to the NSP, excluding the control period, is larger in young adult compared to juvenile TA synapses, also indicating the increase in short-term facilitation at TA synapses during development ($p < 0.05$).

Figure 4. Differences in paired-pulse ratio between SC and TA synapses diminish with age (A) Juvenile TA synapses (open circles, $n=7$) demonstrate much lower paired-pulse facilitation than do SC synapses (closed squares, $n=7$) at intervals less than 500 ms (one-way ANOVA, $p<0.05$). Inset: Average of 20 traces from juvenile SC (black) and TA (gray) synapses at 50 ms paired-pulse interval. (B) Paired-pulse facilitation is not significantly different between young adult SC (closed squares, $n=6$) and TA (open circles, $n=7$) synapses (one-way ANOVA, $p>0.05$). Inset: Average of 20 traces from young adult SC (black) and TA (gray) synapses.

Figure 5. Responses to the natural stimulus pattern are less facilitated at TA synapses compared to SC synapses in both juveniles (A-E) and young adults (F-J)

(A) Averaged data from juvenile TA synapses (open circles, $n=7$) show significantly less short-term facilitation in response to the natural stimulus pattern compared to SC synapses (closed squares, $n=6$). Inset: Example traces from juvenile SC synapses (top) and TA synapses (bottom) during a 2.5 s region of the NSP. Scale bars: 150 ms, 0.12 mV. (B) In juveniles, the percent of responses that are facilitated during the NSP is smaller at TA synapses as compared to SC synapses ($p < 0.05$), and the percent of responses that are depressed is greater at TA synapses as compared to SC synapses ($p < 0.05$). (C) Cumulative frequency curves of normalized responses reveal a large shift toward less facilitated responses at TA synapses (open circles) compared to SC synapses (closed squares). (D) The mean normalized fEPSP of juvenile responses to the NSP is smaller at TA synapses compared to SC synapses ($p < 0.05$). (E) The responses of SC synapses (closed squares) and TA synapses (open circles) (from Figure 5A) plotted against interstimulus interval in ms. (F) Averaged data from young adults plotted against stimulus number reveal that TA responses (open circles, $n=8$) are still less facilitated than SC responses (closed squares, $n=6$). Inset:

Example traces from young adult SC synapses (top) and TA synapses (bottom) during a 2.5 s region of the NSP. Scale bars: 150 ms, 0.105 mV (SC), 0.12 mV (TA). (G) In young adults, the percent of responses that are facilitated during the NSP is smaller at TA synapses as compared to SC synapses ($p < 0.5$), and the percent of responses that are depressed is greater at TA synapses as compared to SC synapses ($p < 0.5$). (H) The cumulative frequency plot for TA synapses (open circles) remains shifted leftward toward less facilitated responses compared to SC synapses (closed squares) in young adults. (I) The mean normalized fEPSP of responses to the NSP is also smaller at TA synapses than at SC synapses in young adults ($p < 0.05$). (J) The responses of SC synapses (closed squares) and TA synapses (open circles) (from Figure 5F) plotted against interstimulus interval in ms.

Figure 6. The developmental increase in short-term facilitation at TA synapses is not due to mGluR1 activation

(A) Normalized mean response sizes to the NSP are plotted against stimulus number for juvenile TA synapses before (closed squares) and after the addition of 10 μ M LY367385 (open circles, n=5). Bath application of LY367385 did not shift the cumulative frequency of fEPSPs (B), and there is no significant difference in the mean normalized fEPSPs in juveniles (C) (paired t-test, $p>0.5$). Young adult (n=4) fEPSPs in response to the NSP show a slight increase in facilitation at points already highly facilitated in control (D). LY367385 did not significantly alter the cumulative frequency distribution of young adult responses (E), nor did LY367385 cause a significant change in the average response size (F) in young adults (paired t-test, $p>0.5$).

Figure 7. The developmental increase in short-term facilitation at TA synapses is not GABA_BR-sensitive

(A) Normalized mean response sizes to the NSP are plotted against stimulus number for juvenile TA synapses before (closed squares) and after the addition of 10 μ M CGP55845 (open circles, n=4). (B) The cumulative frequency of juvenile TA responses to the NSP is unchanged from control (closed squares) after bath application of 10 μ M CGP55845 (open circles), as is the mean normalized fEPSP (C) (paired t-test, p>0.05). Young adult responses to the NSP (D, n=5) are slightly facilitated in the presence of CGP55845 as evidenced by the modest rightward shift in the cumulative frequency of fEPSPs (E) and by an increase in the mean response size (F) (paired t-test, p<0.05).

Figure 8. A decrease in initial release probability underlies the developmental increase in facilitation at TA synapses

(A) At TA synapses, the blockade of whole-cell NMDAR-mediated EPSCs by MK-801 (40 μ M) is faster in juveniles (closed squares, $n=5$) than young adults (open circles, $n=8$) ($p<0.05$). (B) At SC synapses, the rates of MK-801 blockade are similar between juveniles (black squares, $n=6$) and young adults (open circles, $n=6$) ($p>0.05$). (C) Example traces showing that RO25-6981 (1 μ M), which is an NMDA receptor antagonist specific for receptors containing the NR2B subunit, decreases the amplitude of the NMDA EPSC. (D) RO25-6981 significantly decreases the NMDA EPSC amplitude in slices from juveniles and young adults at TA synapses (TA juvenile = $29.6\% \pm 2.5$, $n=5$, $p<0.5$; TA young adult = $31.9\% \pm 6.1$, $n=3$, $p<0.5$), but there is no difference at TA synapses between the amount of block in juveniles vs. young adults ($p>0.05$). (E) RO25-6981 significantly decreases the NMDA EPSC amplitude in slices from juveniles and young adults at SC synapses (SC juvenile = $48.3\% \pm 4.7$, $n=3$, $p<0.05$; SC young adult = $53.3\% \pm 3.9$, $n=5$, $p<0.5$), but there is no difference at SC synapses between the amount of block in juveniles vs. young adults ($p>0.05$).

Figure 9. Initial release probability is greater at TA vs. SC synapses in slices from juveniles, but not different in slices from young adults

(A) The % block of the NMDA EPSC by RO25-6981 (1 μ M) is greater at SC synapses than TA synapses in juveniles (A) and young adults (B) (*, $p < 0.05$). (C) The rates of block of the NMDA EPSC by MK-801 (40 μ M) are similar in the presence and absence of RO25-6981 at young adult SC synapses (RO25-6981 $\tau = 13.8 \pm 1.9$, $n=4$; control $\tau = 18.5 \pm 2.3$, $n=6$, $p > 0.05$). (D) In juveniles, the MK-801 decay constant is smaller at TA synapses compared to SC synapses ($p < 0.05$), indicating a faster rate of block and therefore a higher release probability. (E) In young adults, there is no significant difference between the MK-801 decay constants from TA and SC synapses ($p > 0.05$), indicating no difference in release probability.