Published in final edited form as:

Eur J Neurosci. 2010 April ; 31(8): 1377–1387. doi:10.1111/j.1460-9568.2010.07180.x.

Maturation of Schaffer collateral synapses generates a phenotype of unreliable basal evoked release and very reliable facilitated release

Adrian R. B. Schiess, Chessa Scullin, and L. Donald Partridge

Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA

Abstract

Short-term synaptic plasticity undergoes important age-dependent changes that have crucial implications during the development of the nervous system. Paired-pulse facilitation is a form of short-term synaptic plasticity by which the response to the second of two temporally-paired stimuli is larger and more reliable than the response to the first stimulus. In this study, a paired-pulse minimal stimulation technique was used to measure the probability and quantal amplitude of synaptic release at hippocampal synapses from 12–16-day-old (young) and 7–9-week-old (adult) rats. In order to assess the contribution of temperature-dependent processes, we carried out experiments at both room temperature and at near physiological temperature. We report here that neither temperature nor maturation affected the low basal evoked release probability and quantal amplitude of release. However, the warmer temperature revealed a unique developmental increase in facilitated evoked release probability and quantal amplitude of release. As a result, although both basal evoked release and facilitated release are rather unreliable in synapses from young animals, the maturation process at near physiological temperature generates a phenotype with unreliable basal evoked release and highly reliable facilitated release.

Keywords

development; facilitation; hippocampus; quantal release; rat

Introduction

During the course of pre-natal and early post-natal nervous system development, synapses are formed and eliminated, transmitter phenotype is determined, signal transmission is initiated and synaptic efficacy undergoes important developmental changes that lead to mature forms of synaptic plasticity. Fast synaptic transmission at most central synapses is quantal and hence intrinsically stochastic in nature (Stevens & Wang, 1995; Dobrunz & Stevens, 1997; Huang & Stevens, 1997); thus, developmental or plastic changes in central neurotransmission generally reflect changes in the reliability of transmission at normally unreliable synapses.

Paired-pulse facilitation (PPF) is a form of short-term plasticity during which a pre-synaptic stimulus substantially enhances the response to subsequent stimuli occurring tens to hundreds of milliseconds later (Edelman *et al.*, 1987; Zucker, 1989; Zucker & Regehr, 2002). At Schaffer collateral to CA1 pyramidal neuron (Sc-CA1) synapses in the rat

hippocampus, PPF is predominantly a pre-synaptic process (Debanne *et al.*, 1996; Zucker & Regehr, 2002; Schiess *et al.*, 2006). Pre-synaptic mechanisms are very sensitive to temperature with important consequences in both developmental and plastic properties of synapses (e.g. Kushmerick *et al.*, 2006).

Considerable maturation of synaptic transmission at Sc-CA1 synapses in the rat hippocampus occurs during the first two post-natal weeks, although additional maturational processes may continue until the sixth or seventh post-natal week. Early in post-natal development, there is a high basal action potential-dependent neurotransmitter release from Schaffer collateral terminals (Bolshakov & Siegelbaum, 1995) due in part to excitatory feedback from GABA-ergic interneurons onto CA3 pyramidal neurons (Swann *et al.*, 1991b; Khazipov *et al.*, 1997). By 2 weeks of age, hippocampal GABA_A receptor-mediated neurotransmission becomes inhibitory (Garaschuk *et al.*, 1998; Ben-Ari, 2002) but a developing network of CA3-to-CA3 recurrent excitatory glutamatergic synapses causes the frequency of spontaneous action potentials in Schaffer collateral axons to remain elevated (Swann *et al.*, 1991b; Gomez-Di Cesare *et al.*, 1997). At this time, the basal probability of action potential-dependent release diminishes significantly and short-term plasticity becomes predominately PPF (Bolshakov & Siegelbaum, 1995; Wasling *et al.*, 2004). By adulthood there is a reduction in CA3 recurrent network activity and a decreased frequency of spontaneous action potentials in Schaffer collateral axons (Swann *et al.*, 1991b; Gomez-Di Cesare *et al.*, 1997). Although there is some reduction in PPF after 3 weeks of age (Dumas, 2005; Speed & Dobrunz, 2008), PPF persists as the dominant form of paired-pulse plasticity at the Sc-CA1 synapses of adult rats (Wasling *et al.*, 2004).

Previous studies have used paired-pulse minimal stimulation to study the short-term plasticity of release probability at Sc-CA1 synapses in the hippocampus of young rats generally at room temperature (Stevens & Wang, 1995; Dobrunz & Stevens, 1997; Dobrunz *et al.*, 1997; Cabezas & Buno, 2006). In this study, we compared minimal stimulation responses of Sc-CA1 hippocampal synapses from young rats with those from adult rats at both near physiological and room temperature and found important temperature-dependent developmental changes leading to the mature phenotype of a highly reliable facilitated release superimposed on a persistently unreliable basal evoked release.

Materials and methods

Slice preparation

Experiments were performed in coronal brain slices prepared from 12–16-day-old and 7–9-week-old Sprague-Dawley rats. Approximately 15 young and 60 adult animals were used for these studies. Animals were deeply anesthetized by i.p. injection of 250 mg / kg ketamine, brains were rapidly removed and slices were cut at 300 μ m with a vibroslicer (Pelco 101, St Louis, MO, USA) in an ice bath with a cutting solution containing (in mM): 220 sucrose, 3 KCl, 1.2 NaH₂PO₄, 26 NaHCO₃, 12 MgSO₄, 0.2 CaCl₂, 10 glucose and 0.01 mg / mL ketamine equilibrated with 95% O₂ / 5% CO₂. Slices were then transferred for 1 h to artificial cerebrospinal fluid with an osmolarity of 300–305 mOsm containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH₂PO₄, 1 MgSO₄, 26 NaHCO₃, 2.5 CaCl₂ and 10 glucose equilibrated with 95% O₂ / 5% CO₂ at 30°C. Slices were then maintained at room temperature until recording in a chamber (Warner Instruments, Hamden, CT, USA) maintained at 32.75–34.50°C and continuously perfused at 2 mL / min with artificial cerebrospinal fluid saturated with 95% O₂ / 5% CO₂. We studied paired-pulse plasticity at both 21.75–23.25°C (room temperature) and 32.75–34.50°C (near physiological temperature). All experiments were approved by the Institutional Animal Care and Use Committee at the University of New Mexico Health Sciences Center and conformed with NIH guidelines.

Patch clamping

Whole-cell voltage-clamp recordings were made from hippocampal CA1 pyramidal neurons following stimulation of the CA3 Schaffer collaterals using a Multiclamp 700B amplifier and a Digidata 1322A interfaced with pCLAMP10 software (all from Molecular Devices, Sunnyvale, CA, USA). Excitatory post-synaptic currents (EPSCs), recorded from CA1 stratum pyramidale neurons, were digitized at 500 kHz and sampled at 100 kHz. The patch electrode solution had an osmolarity of 275–278 mOsm and contained (in mM): 130 CsOH, 130 gluconic acid, 9 CsCl, 5 NaCl, 0.6 EGTA, 10 HEPES, 4 MgATP and 0.3 Na_xGTP. The patch solution was adjusted to pH 7.37 with NaOH at 21.5°C (resulting in a pH of 7.2 at 34°C), aliquoted and frozen at –80°C until use. Cells were voltage clamped at –65 mV, cell properties were monitored using the Membrane Test feature of Clampex10 and only recordings in which the access resistance changed by < 20% and the holding current was stable and less negative than –500 pA were accepted. A minimum of 15 min of stability was necessary to obtain sufficient successes for analysis. There was no significant difference in holding current [Kolmogorov–Smirnov (K–S), $D = 0.29$, $P = 0.34$] or access resistance (unpaired Student's *t*-test, $t_{38} = 1.85$, $P = 0.07$) between neurons from young and adult animals. As expected, membrane resistance was significantly greater in neurons from young than from adult animals (274.53 ± 34.81 vs. 114.49 ± 14.34 M Ω ; K–S, $D = 0.64$, $P < 0.001$) and membrane capacitance was significantly less in neurons from young than from adult animals (140.92 ± 6.78 vs. 223.62 ± 11.43 pF; K–S, $D = 0.90$, $P < 0.001$).

Paired constant-current pulses (150 μ s duration) were applied to Schaffer collateral fibers with an Iso-Flex constant-current stimulator (API Instruments, Jerusalem, Israel) and a concentric bipolar electrode (25 μ m inner pole, 125 μ m outer cylinder diameter; FHC, Bowdoinham, ME, USA) at an interpulse interval of 50 ms once every 10 s at an amplitude adjusted to produce a minimal response (see below). For simplicity, we have used the abbreviation R1 for the first response and R2 for the second response to a pair of stimuli. Data were post-filtered with a Gaussian low-pass filter at 1 kHz, which removed the majority of the high-frequency noise intrinsic to the CV-7B Headstage without affecting EPSC timing or amplitude.

Cells were selected in which success amplitudes were stable within a range of stimulus intensities of at least ± 0.5 μ A from an intensity chosen to produce a mix of successes and failures for both R1 and R2. Reducing the stimulus intensity to below the stable stimulus range resulted in only failures and stimulus intensities above this range resulted in a higher success rate, a greater range of synaptic potencies and larger EPSC amplitudes.

Terminology

As our minimal stimulation protocol may not distinguish between the response at a single synapse with one release site (see Discussion), a single synapse with multiple release sites or very few synapses, we have used the inclusive term release event to describe the response isolated by the minimal stimulus technique. For simplicity, we will define young neurons as those from 12–16-day-old rats and adult neurons as those from 7–9-week-old rats. The probability of release is the probability that a pre-synaptic action potential at one release site will result in one or more quanta being released. The success rate is the rate at which successes occur following a stimulus and is equal to or proportional to the probability of release. The synaptic potency is the peak amplitude of the EPSC resulting from a success and is proportional to the quantal amplitude of release. When a single release event is isolated, the synaptic potency is equal to the quantal amplitude of release. The synaptic efficacy is the average release in response to stimulation and can be measured as either the mean peak amplitude of all of the resulting successes and failures or as the product of the success rate $\times$ the mean synaptic potency.

Data analysis

An average template for putative successes at a fixed time interval after the stimulus was generated using a custom MatLab® (MathWorks, Natick, MA, USA) program Minimal Stimulation Analysis for pCLAMP (available at Matlab File Exchange: <http://www.mathworks.com/matlabcentral/fileexchange/22796>). This template was then used to eliminate any spontaneous and recurrent events. For experiments conducted at near physiological temperature, successes were selected if their half rise time was within 1 ms and the onset and peak were within 2 ms of the template. For experiments conducted at room temperature, these time windows were doubled to allow for the slower release kinetics. To confirm the appropriate designation of successes or failures, the current amplitude was measured for putative failures at the time when a success would otherwise have occurred and compared with the baseline noise measured at the end of the same sweep. The appropriate designation of failures was confirmed when these two measurements did not differ statistically by paired Student's *t*-test.

As the success rate and synaptic potency both vary considerably among Sc-CA1 release events (Dobrunz & Stevens, 1997; Huang & Stevens, 1997), the stability of both success rate and synaptic potency is a good indicator of recordings from a single synapse. In addition, as paired stimulation does not lead to the recruitment of additional axons during the R2 stimulus (Qian & Saggau, 1999; Schiess *et al.*, 2006), it is very probable that R1 and R2 were recorded from the same synapse. The release probability for both R1 and R2 responses was determined to be stable if the cumulative successes over time fell within two SDs of a Monte Carlo simulation of the success rate, which was calculated as follows. For each experiment, the assumed underlying probability of success was determined as the average success rate in response to the first 25% of stimuli. If the actual cumulative sum of successes differed at any point from the predicted mean cumulative sum of successes by more than two SDs (e.g. vertical line in Fig. 1A), the range of the data, and subsequently the first 25% of the recorded sequence used for prediction, was decreased until 100% of the cumulative summed successes fell within two SDs of the predicted mean.

Two criteria were used for assessing the stability of synaptic potency of successes. First, the residual differences of a least squares regression fit to the synaptic potencies of consecutive successes (Fig. 1B) were assessed for linearity by Durbin–Watson analysis. Second, stability was established if the starting and ending amplitudes differed by < 20% for the responses with the higher success rate (usually R2) and < 40% for the responses with the lower success rate (usually R1). Importantly, as there is a high degree of variability of quantal amplitude from synapse to synapse (Bekkers *et al.*, 1990; Bekkers & Stevens, 1991; Liu & Tsien, 1995; Forti *et al.*, 1997), a stable variation in quantal size is a good predictor that the same synapse or few synapses were recruited for the duration of the experiment. If the distribution of synaptic potencies over time was not significantly linear, or if the starting and ending amplitudes differed by more than 20 or 40%, for the respective responses, the range of the data was restricted until both criteria were met. A minimum of 28 successes for either R1 or R2 was required for statistical analysis so we discarded experiments that did not retain this number of successes after establishing a range with both a stable success rate and stable synaptic potency. Two recordings of release events in neurons from adult animals at near physiological temperature had very low R2 success rates, probably indicating pre-synaptically silent, or ‘mute’, synapses (Hanse & Gustafsson, 2001; Voronin & Cherubini, 2004), and were excluded because they had no R1 successes.

Statistics

Student's *t*-tests were determined with PROSTAT 4.0 (Poly Software International, Inc., Pearl River, NY, USA). Data that were not normally distributed were compared using the

non-parametric K–S test. Two-way ANOVA multiple comparison tests, linear and exponential regression, and Durbin–Watson tests for linearity were determined with SPSS 15.0 (SPSS, Inc., Chicago, IL, USA). For multiple comparisons, two-way ANOVA was used to compare the effects of: (i) age and the R1 and R2 responses; (ii) the type of paired-pulse plasticity and the R1 and R2 responses; and (iii) age and temperature. The Tukey's honestly significant difference (HSD) *post-hoc* analysis was used to determine *P* values, unless the difference in number between groups was more than twofold, in which case the Scheffe's *post-hoc* test was used to reduce the likelihood of type 1 error (Bruning & Kintz, 1987). Durbin–Watson test values provided by SPSS were determined to be statistically linear, non-linear or ambiguous with 95% confidence according to statistical tables in Draper & Smith (1998).

Numerical values for data measurements are presented as the mean $\pm$ SE, unless otherwise specified.

Results

The adult phenotype of synaptic facilitation results from developmental changes in vesicular release mechanisms. As the parameters of quantal release are highly dependent on active processes, such as those responsible for Ca^{2+} flux and vesicle cycling, they are likely to be dependent on temperature. We thus used the minimal stimulation technique in order to assess developmental- and temperature-dependent changes in unitary synaptic release events. We measured release events in young (12–16-day-old) and adult (7–9-week-old) rats at both room (21.7–23.3°C) and near physiological (32.8–34.5°C) temperatures. We took advantage of the fact that minimal stimulation provides an independent measurement of success rate and synaptic potency (e.g. Fig. 1) to access contributions of release probability and quantal amplitude in the development of the adult phenotype of short-term facilitation.

Age-dependent effects of temperature

As previous stimulation studies have generally examined immature Sc-CA1 synapses at room temperature, we first assessed basic age- and temperature-dependent changes before focusing on the developmental changes in short-term plasticity at near physiological temperature. As shown in Fig. 2, there were neither any significant temperature-dependent changes in R1, R2 and % PPF in young animals nor any age- or temperature-dependent changes in success rate (see figure legend for statistical analyses). However, two important changes are clearly apparent in this figure. First, there is a significant developmental increase in the facilitation of synaptic efficacy at near physiological temperature (interaction effect of age $\times$ temperature, $F_{1,23} = 7.50$, $P = 0.008$), which results from a significant age-dependent increase in the facilitation of synaptic potency (interaction effect of age $\times$ temperature, $F_{1,23} = 10.77$, $P = 0.002$) and an age-dependent trend toward an increase in the facilitation of success rate. Second, facilitation of synaptic efficacy of release events in adult animals is rather sensitive to temperature (interaction effect of age $\times$ temperature, $F_{1,23} = 7.50$, $P = 0.008$), which results from a significant temperature-dependent increase in the facilitation of synaptic potency (interaction effect of age $\times$ temperature, $F_{1,23} = 10.77$, $P = 0.002$) and a temperature-dependent trend toward an increase in the facilitation of success rate (Fig. 2).

These observations underscore the development of the mature synaptic phenotype with a low reliability of basal release and high probability of facilitated release. In addition, they place in context our observations at near physiological temperature with previously reported observations that were carried out at room temperature where, interestingly, the developmental changes are not apparent. Importantly, these temperature-dependent effects are consistent with, and may underlie, the strengthening of facilitation seen at higher

stimulus intensities at these synapses (Klyachko & Stevens, 2006). We will thus focus on two elemental parameters of pre-synaptic vesicle release (success rate and synaptic potency) and will then consider implications of their interactions in the development of the adult phenotype of synaptic transmission at near physiological temperature.

Success rate

Because basal evoked success rate, which reflects the probability of release, has been shown to play a role in controlling facilitation (Dobrunz & Stevens, 1997), we compared the success rate of R1 with the subsequent success rate of R2. Interestingly, although we found consistent facilitation of success rate in release events of adults, this was not always the case in those of young animals (Fig. 3A and B). Although the overall success rate at near physiological temperature for release events from both adults and young animals showed facilitation (main effect of R1 vs. R2, $F_{1,74} = 46.84$, $P < 0.001$) (Fig. 3C), those from young animals fell into two categories. There was significant facilitation of the mean R2 success rate over that of R1 in 84% of these release events (interaction effect of type of plasticity $\times$ R1 vs. R2, $F_{1,34} = 13.41$, $P < 0.005$) but in the remaining 16% of events there was paired-pulse depression (Fig. 3D). Furthermore, the R1 success rate was significantly greater during depressed than during facilitated release events of young animals (interaction effect of type of plasticity $\times$ R1 vs. R2, $F_{1,34} = 13.412$, $P < 0.005$) (Fig. 3D), consistent with pre-synaptic depression caused by either vesicle depletion or Ca^{2+} channel inactivation (Zucker & Regehr, 2002). In adults, we never observed depression so the success rate for R2 was always greater than that of R1. However, as in young neurons, we found that there was an inverse relationship between the facilitation of the R2 success rate and the R1 success rate (immature: slope = -4.59 ± 0.14 , $R^2 = 0.79$, $P < 0.0001$; mature: slope = -5.58 ± 0.34 , $R^2 = 0.49$, $P < 0.0001$). Interestingly, we even observed two instances in adult neurons in which there were R2 successes without any R1 successes. Overall, although the success rate was more consistently facilitated in release events from adult neurons, there was not a significant difference in the facilitation of success rate between the two age groups (unpaired Student's t -test, $t_{27.77} = 2.09$, $P = 0.051$) (Fig. 3E).

Synaptic potency

Independent of the success rate, a change in synaptic potency, which reflects quantal amplitude, could provide a mechanism for synaptic plasticity. There was a significant difference in mean synaptic potency between R1 and R2 in release events of mature neurons but not in immature neurons (Fig. 4B). However, on closer scrutiny of individual experiments, immature neurons revealed more complexity. For each of these experiments, the individual R1 synaptic potencies were compared with the individual R2 synaptic potencies as previously described (Dobrunz & Stevens, 1997) by using the K-S test for non-parametric analysis of distribution of two sample sets. This allowed us to determine whether R1 and R2 synaptic potencies were samples from two biologically similar or different populations. As a result, each individual experiment was assigned to one of two groups: 37% with a significantly different distribution of R2 to R1 synaptic potency resulting in facilitation (Fig. 4D and G) and 63% without a significantly different distribution of R2 to R1 synaptic potency resulting in no facilitation (Fig. 4D and F). Interestingly, those release events from young neurons that were facilitated exhibited an equivalent amount of facilitation to that seen in adult neurons (unpaired Student's t -test, $t_{9.48} = 0.17$, $P = 0.88$) (Fig. 4E).

In the adult hippocampus, the synaptic potency of R2 was consistently greater than that of R1 (main effect of R1 vs. R2, $F_{1,70} = 23.15$, $P < 0.001$) (Fig. 4B) and, moreover, facilitation of the R2 synaptic potency in adult neurons was significantly greater than that of young neurons (main effect of age, $F_{1,70} = 7.21$, $P < 0.01$) (Fig. 4B and C). Finally, we found PPF

of synaptic potency far more frequently (89%) than instances where there was no facilitation (11%).

The mature phenotype for short-term plasticity of synaptic potency is clearly that of consistent and robust facilitation. The development of this phenotype was due solely to an increase in the synaptic potency of R2 between release events of young and adult neurons, whereas the R1 synaptic potency remained virtually unchanged during development (Fig. 4B).

Pre-synaptic origin of paired-pulse facilitation

The synaptic potency data provide a means to evaluate the potential for a post-synaptic contribution to short-term plasticity. Excluding a role for a very rapid, vesicle-independent anterograde messenger, an R1 success should be necessary for any short-term alteration of R2 synaptic potency that is the result of a change in post-synaptic receptor sensitivity. Thus, a post-synaptic contribution to short-term plasticity should be reflected as a difference in R2 synaptic potency between when R1 was present and when R1 was absent. We therefore analyzed separately the two populations of young neurons that either did or did not exhibit facilitation of R2. We considered those events that exhibited no change between the R1 and R2 synaptic potency (Fig. 5B) to be a negative control. There was no overall difference in release events from the young facilitated (unpaired Student's *t*-test, $t_{11,99} = 0.59$, $P = 0.57$) or non-facilitated (unpaired Student's *t*-test, $t_{21,99} = 0.40$, $P = 0.90$) (Fig. 5A and B) or the adult (unpaired Student's *t*-test, $t_{32,39} = 0.14$, $P = 0.70$) (Fig. 5C) Sc-CA1 synapses between the R2 synaptic potencies when R1 succeeded and when R1 failed. However, in two instances (11%) of release events from young neurons and in one instance (6%) of release events from adult neurons, the R2 synaptic potency was reduced in those cases when R1 failed. In one instance (5%) of a young neuron and in two instances (11%) of adult neurons, the R2 synaptic potency was actually greater when R1 failed. Thus, in all of our experiments, there was a potential contribution from post-synaptic sensitization during R2 in 8% of release events and a potential contribution from post-synaptic desensitization during R2 in another 8% of release events. Based on this evidence, we conclude that paired-pulse plasticity of the synaptic potency was most frequently due to pre-synaptic changes in quantal release that remain invariant during the development.

Developmental changes in synaptic efficacy

Both success rate and synaptic potency contribute to synaptic efficacy and this should reflect the average post-synaptic response and therefore be a measure of the reliability of synaptic signaling. Although the R1 synaptic efficacy was similar between release events in young and adult neurons, the facilitation of R2 synaptic efficacy was a more robust property of release events of adult than of young neurons (interaction effect of age $\times$ R1 vs. R2, $F_{1,74} = 5.72$, $P < 0.05$) (Fig. 6A). Thus, release events from adult neurons exhibited a greater facilitation of synaptic efficacy than did release events in young neurons (unpaired Student's *t*-test, $t_{23,38} = -2.72$, $P < 0.05$) (Fig. 6B). This suggested that the mature phenotype of adult Sc-CA1 synapses is one in which short-term plasticity is very efficacious in increasing synaptic reliability.

Half-width and onset latency

In release events from young neurons, we found no difference in EPSC half-width between R1 and R2 (8.33 ± 0.78 vs. 9.03 ± 0.72 ms; paired Student's *t*-test, $t_{18} = 1.92$, $P = 0.07$). However, a difference was evident in adult neurons such that R2 half-width was significantly longer than that of R1 (10.99 ± 0.95 vs. 12.60 ± 1.02 ms; paired Student's *t*-test, $t_{17} = 3.48$, $P < 0.005$). In release events from adult neurons, the R2 synaptic potency was greater than that of R1 as was the R2 EPSC half-width because it had a slower decay

than the R1 EPSC (Fig. 7A). To qualitatively assess this relationship, we compared the facilitation of the synaptic potency with the facilitation of the EPSC half-width (Fig. 7B). Although there was a significant positive correlation in release events from adult neurons ($F_{1,16} = 7.93$, $P = 0.012$) (Fig. 7B), there was only a trend towards a correlation in release events from young neurons ($F_{1,17} = 1.58$, $P = 0.23$) (Fig. 7B). This suggests that the developmental process by which synaptic potency is facilitated is either causative of, or coupled to, a mechanism that leads to an increase of R2 EPSC half-width over that of R1.

In release events from young neurons, there was no difference in onset latency from the stimulus to the R1 or to the R2 EPSC (3.74 ± 0.24 vs. 3.72 ± 0.24 ms; paired Student's t -test, $t_{18} = 0.40$, $P = 0.69$) but, in adults, the R2 EPSP latency was significantly shorter than that of R1 (3.38 ± 0.26 vs. 3.23 ± 0.26 ms; paired Student's t -test, $t_{17} = 2.15$, $P = 0.046$). This resulted in a significant negative correlation between the facilitation of synaptic potency and the difference in EPSC onset latencies in release events from adult neurons ($F_{1,16} = 8.79$, $P < 0.01$) (Fig. 7D). Thus, there is an age-dependent mechanism for the shortening of the R2 onset latency that is concurrent with facilitation of the synaptic potency, which develops between 16 days and 7 weeks of age.

Discussion

We have shown that at near physiological temperature, release events from both young and adult neurons exhibit a predominantly low probability and small quantal amplitude of basal evoked release, indicating that these parameters change only minimally during development. In addition, we found a significant increase in the probability of facilitated release at both ages. However, although there was little facilitation of the quantal amplitude in release events from young neurons, there was consistent facilitation in adult neurons. As a result, although both young and adult neurons exhibit unreliable basal evoked release, maturation of the synaptic facilitation process established a very highly reliable facilitated release. As all Sc-CA1 release events from adult neurons, at near physiological temperature, show short-term facilitation of the success rate at an interpulse interval of 50 ms (Fig. 3C), it is apparent that the mature phenotype of release probability is that of facilitation. Coincident with the development of this adult phenotype are changes in synaptic potency and release timing, which will be considered next.

Developmental changes in synaptic potency

At near physiological temperature, there was no developmental change in the R1 synaptic potency but there was a dramatic developmental change of R2 synaptic potency from little or no facilitation to the mature phenotype of significant facilitation. There was, however, a similar facilitation of R2 synaptic potency in both age groups when restricted to the subpopulation of release events from young neurons that did exhibit facilitation (Fig. 4). Thus, one component of pre-synaptic development is an increase in short-term plasticity that is due to a large age-dependent increase in the percent of synapses exhibiting facilitation of R2 quantal release. Importantly, those release events in both age groups that showed significant facilitation of the R2 synaptic potency over that of R1 exhibited facilitation of the R2 synaptic potency that was independent of R1 release and therefore predominantly due to a pre-synaptic mechanism of facilitation.

We observed an increase in R2 EPSC half-width of facilitated release events from both young and adult neurons. As the facilitation of synaptic potency is correlated with the facilitation half-width (Fig. 7B), the charge delivered during R2 is greater than that during R1 because of an increase in both the amplitude and duration of the EPSC. The increase in R2 EPSC half-width is more prevalent in release events from adult neurons than in those

from young neurons thereby contributing to the increase in the reliability of facilitated synaptic transmission in these adult neurons.

At both ages, the R2 synaptic efficacy was significantly facilitated over that of R1, although this effect was more pronounced during release events from adult than from young neurons (Fig. 6). In release events from adult neurons, the large facilitation of synaptic efficacy results from the conjunction of a low release probability and a low synaptic potency of R1, and a high release probability and a high synaptic potency of R2. For trains of action potentials in the adult hippocampus, this facilitated synaptic efficacy would be expected to generate a high signal-to-noise ratio for physiologically-relevant signals at frequencies around 20 Hz (50 ms interspike interval).

Release timing in the adult synaptic phenotype

Coincident with increased facilitation of R2 synaptic potency, the R2 EPSC onset latency was shorter than that of R1 (Fig. 7C and D). During release events in adult neurons, this decrease in onset latency occurred despite an expectation that the R2 pre-synaptic conduction velocity should be slightly slower than that of R1 (Meeks & Mennerick, 2007). Furthermore, there was a significant correlation in adult neurons between the shortening of the R2 onset latency and the facilitation of the R2 synaptic potency, suggesting a common mechanism for these two phenomena. To determine whether the shortening of the R2 onset latency was mediated by a pre-synaptic or post-synaptic mechanism, we compared this latency when R2 was preceded by R1 with that when R1 failed in release events that had the largest difference in onset latency ($540 \pm 80 \mu\text{s}$; $n = 5$). The difference in R2 onset latency of $60 \pm 150 \mu\text{s}$ between these two subgroups was not significant (paired Student's *t*-test, $t_4 = 0.54$, $P = 0.69$), indicating that the R2 onset latency did not depend on successful R1 release. This supports the presence of a pre-synaptic mechanism of shortening of the R2 onset latency and suggests a common mechanism for the shortening of R2 onset latency and the facilitation of R2 synaptic potency in mature synapses.

Likelihood of single sites

Previous studies (Dobrunz & Stevens, 1997) made a distinction between compound and single synapses. The former are synapses with multiple release loci capable of releasing multiple quanta following a single action potential. In these synapses, the synaptic potency of R2 is greater than that of R1 with no change in either the latency or shape of the response. By contrast, the single synapses reflect only one release locus with no significant change in synaptic potency between R1 and R2.

A total of 63% of our release events from young animals met the criteria for single synapses and the remaining 37% were classified as compound synapses that were either single synapses with multiple release loci or a few synapses. The R2 synaptic potency was consistently greater than that of R1 in our release events from adult neurons (Fig. 4) and only 11% of these qualified as single synapses by the above criteria. Because the stimulus technique was identical in experiments with both young and adult neurons, the reliable increase in the R2 synaptic potency of release events from adult neurons must have been due primarily to: (i) consistently recruiting more than one synapse, (ii) recruiting multiple release loci at one synapse or (iii) stimulating a single release locus synapse in which the R2 release was somehow facilitated. If multiple synapses were recruited or if release events from adult synapses occurred at more release loci than those from young neurons, the R1 success rate and R1 synaptic potency should then have been greater in release sites of adult than in young neurons but they were not. It is also unlikely that more synapses or release loci were recruited in release events from adult neurons because this would require exactly compensatory changes in R1 success rate and R1 synaptic potency. Interestingly, structural

evidence (Sorra & Harris, 1993) predicts that about 60% of Schaffer collateral axons form only a single release locus synapse with one dendritic spine on a given CA1 pyramidal neuron. This is similar to the 63% single release loci determined for our young neurons, further suggesting that the minimal stimulus technique should be equally likely to isolate a single release locus synapse during release events in both young and adult neurons. As the R1 success rate, synaptic potency and synaptic efficacy were all similar during release events in young and adult neurons, but the R2 synaptic potency and synaptic efficacy differed with age at near physiological temperature, the most straightforward conclusion is that R2 release mechanisms undergo more marked developmental change than do R1 release mechanisms. This suggests that approximately the same number of active synapses and release loci were recruited in the experiments from young and adult neurons because: (i) the same minimal stimulus technique was employed in both groups, (ii) the R1 release mechanism was similar for experiments from both ages and (iii) mature synapses have fewer Schaffer collateral branches (Swann *et al.*, 1991a,b). We thus infer that our protocol effectively isolated one release locus per neuron in experiments from the majority of both young and adult rats. This implies that the developmental transition to consistent facilitation of the R2 synaptic potency in adult neurons is probably due to the maturation of a temperature-sensitive mechanism for the short-term facilitation of quantal release. Interestingly, this occurs in the presence of a temperature-dependent decrease in R1 synaptic potency of mature release sites (Fig. 2), which perhaps results from a decreased pre-synaptic Ca^{2+} entry at the higher temperature (Borst & Sakmann, 1998).

Mechanisms for facilitation of synaptic potency

One interpretation of our data is that R2 successes occur from a more energetically favorable state than those during R1. The R1 event can fail, succeed as a release event through a transient fusion pore or succeed as a full fusion event. During facilitation, the R2 event can also respond in these three ways but it can also succeed as a transient fusion pore that opens more quickly with a larger open diameter or succeeds as a full fusion event that is initiated more quickly. During release events in adult neurons, facilitation of both the success rate and the synaptic potency could result from an increased likelihood of these latter two mechanisms, which would allow for a higher probability of transmitter release during R2. In addition, a prevalence of these latter two mechanisms would lead to a shortening of the R2 onset latency. Both of these mechanisms could be caused by changes in vesicle and plasma membrane interactions during the interval between R1 and R2, such as through the presence of a hemi-fused state. A potential contributory factor in the maintenance of a hemi-fused state could be a role of residual $[\text{Ca}^{2+}]_i$, the regulation of which undergoes developmental changes during this period (Scullin *et al.*, 2010). Consistent with this suggestion is the observation that there is a delay of vesicle reacidification following transient fusion pore events, which implies that a hemi-fused state may persist for 400–860 ms (Gandhi & Stevens, 2003). Interestingly, this time range is consistent with the time-course of decay of PPF, which decays exponentially over a few 100 ms (Edelman *et al.*, 1987; Zucker, 1989; Zucker & Regehr, 2002).

Development of the mature phenotype of short-term facilitation

We have shown here that release events from both young and adult animals have unreliable basal evoked release and that synaptic maturation at near physiological temperature leads to a phenotype of highly reliable facilitated release. Although the methodology of minimal stimulation does not allow for the exact determination of the number of Sc-CA1 release loci isolated at the recorded neuron in a given experiment, our findings suggest that only a single release locus was recruited in the majority of experiments from both young and adult animals at near physiological temperature. The evidence is even stronger that the minimal stimulus paradigm consistently recruited the same small number of release loci in the

hippocampus of adult as in young animals, as the R1 success rate, R1 synaptic potency and R1 synaptic efficacy were similar at both ages. In contrast, however, the R2 synaptic potency was greater during release events in adult neurons than in the majority of young neurons, which had a bimodal distribution. This age-dependent increase in R2 synaptic potency was predominantly due to a pre-synaptic mechanism of facilitation. In addition, the similarity of the R2 success rate at both ages suggests that the increase in R2 synaptic potency was not due to a compounding of simultaneous R2 successes from multiple release loci but due to facilitation of quantal release at individual synapses. Thus, at near physiological temperature, the age-dependent increase in short-term facilitation of mature synapses is due to a greater pre-synaptic reliability during facilitated release without a change in basal release properties. Interestingly, we have found that residual pre-synaptic $[Ca^{2+}]_i$ decays more rapidly in mature than in immature synapses (Scullin *et al.*, 2010) and this appears to contradict the establishment of the adult phenotype of more reliable facilitation. It has been shown that there is an approximately 2.5-fold increase in the readily releasable pool size during developmental tuning of the calyx of Held synapse (Taschenberger & von Gersdorff, 2000). As release probability in individual SC-CA1 synapses is determined in part by the readily releasable pool size (Dobrunz, 2002), a developmental increase in this pool size could underlie more reliable release even in the presence of a more rapid decay of residual $[Ca^{2+}]_i$. Furthermore, we have found that the correlation of PPF to residual $[Ca^{2+}]_i$ during paired pulses in adult pre-synaptic terminals is not fully established in young animals (Scullin *et al.*, 2010) and development of this relationship could contribute to the establishment of the adult phenotype of reliable facilitation. Importantly and consistent with previous reports of short-term plasticity (Klyachko & Stevens, 2006), the processes underlying this adult phenotype of facilitation are strongly temperature dependent.

Acknowledgments

This work was supported by grant R01-MH07386 from the National Institutes of Health. The authors thank Fernando Valenzuela and Michael Wilson for critically reading the manuscript.

Abbreviations

EPSC	excitatory post-synaptic current
HSD	honestly significant difference
K-S	Kolmogorov–Smirnov
PPF	paired-pulse facilitation
PPR	paired pulse ratio
R1 and R2	responses to the first and second of paired pulses, respectively
Sc-CA1	Schaffer collateral to CA1 pyramidal neuron

References

- Bekkers JM, Stevens CF. Excitatory and inhibitory autaptic currents in isolated hippocampal neurons maintained in cell culture. *Proc Natl Acad Sci USA*. 1991; 88:7834–7838. [PubMed: 1679238]
- Bekkers JM, Richerson GB, Stevens CF. Origin of variability in quantal size in cultured hippocampal neurons and hippocampal slices. *Proc Natl Acad Sci USA*. 1990; 87:5359–5362. [PubMed: 2371276]
- Ben-Ari Y. Excitatory actions of gaba during development: the nature of the nurture. *Nat Rev Neurosci*. 2002; 3:728–739. [PubMed: 12209121]

- Bolshakov VY, Siegelbaum SA. Regulation of hippocampal transmitter release during development and long-term potentiation. *Science*. 1995; 269:1730–1734. [PubMed: 7569903]
- Borst JG, Sakmann B. Calcium current during a single action potential in a large presynaptic terminal of the rat brainstem. *J Physiol*. 1998; 506(Pt 1):143–157. [PubMed: 9481678]
- Bruning, JL.; Kintz, BL. *Computational Handbook of Statistics*. Scott, Foresman; Glenview, IL: 1987.
- Cabezas C, Buno W. Distinct transmitter release properties determine differences in short-term plasticity at functional and silent synapses. *J Neurophysiol*. 2006; 95:3024–3034. [PubMed: 16436482]
- Debanne D, Guerineau NC, Gahwiler BH, Thompson SM. Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation affects subsequent release. *J Physiol*. 1996; 491(Pt 1):163–176. [PubMed: 9011608]
- Dobrunz LE. Release probability is regulated by the size of the readily releasable vesicle pool at excitatory synapses in hippocampus. *Int J Dev Neurosci*. 2002; 20:225–236. [PubMed: 12175858]
- Dobrunz LE, Stevens CF. Heterogeneity of release probability, facilitation, and depletion at central synapses. *Neuron*. 1997; 18:995–1008. [PubMed: 9208866]
- Dobrunz LE, Huang EP, Stevens CF. Very short-term plasticity in hippocampal synapses. *Proc Natl Acad Sci USA*. 1997; 94:14843–14847. [PubMed: 9405701]
- Draper, NR.; Smith, H. *Applied Regression Analysis*. Wiley; New York: 1998.
- Dumas TC. Late postnatal maturation of excitatory synaptic transmission permits adult-like expression of hippocampal-dependent behaviors. *Hippocampus*. 2005; 15:562–578. [PubMed: 15884034]
- Edelman, GM.; Gall, WE.; Cowan, WM. *Neurosciences Institute (New York NY). Synaptic Function*. Wiley; New York: 1987.
- Forti L, Bossi M, Bergamaschi A, Villa A, Malgaroli A. Loose-patch recordings of single quanta at individual hippocampal synapses. *Nature*. 1997; 388:874–878. [PubMed: 9278048]
- Gandhi SP, Stevens CF. Three modes of synaptic vesicular recycling revealed by single-vesicle imaging. *Nature*. 2003; 423:607–613. [PubMed: 12789331]
- Garaschuk O, Hanse E, Konnerth A. Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J Physiol*. 1998; 507(Pt 1):219–236. [PubMed: 9490842]
- Gomez-Di Cesare CM, Smith KL, Rice FL, Swann JW. Axonal remodeling during postnatal maturation of CA3 hippocampal pyramidal neurons. *J Comp Neurol*. 1997; 384:165–180. [PubMed: 9215716]
- Hanse E, Gustafsson B. Quantal variability at glutamatergic synapses in area CA1 of the rat neonatal hippocampus. *J Physiol*. 2001; 531:467–480. [PubMed: 11230519]
- Huang EP, Stevens CF. Estimating the distribution of synaptic reliabilities. *J Neurophysiol*. 1997; 78:2870–2880. [PubMed: 9405507]
- Khazipov R, Leinekugel X, Khalilov I, Gaiarsa JL, Ben-Ari Y. Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices. *J Physiol*. 1997; 498(Pt 3):763–772. [PubMed: 9051587]
- Klyachko VA, Stevens CF. Temperature-dependent shift of balance among the components of short-term plasticity in hippocampal synapses. *J Neurosci*. 2006; 26:6945–6957. [PubMed: 16807324]
- Kushmerick C, Renden R, von Gersdorff H. Physiological temperatures reduce the rate of vesicle pool depletion and short-term depression via an acceleration of vesicle recruitment. *J Neurosci*. 2006; 26:1366–1377. [PubMed: 16452660]
- Liu G, Tsien RW. Properties of synaptic transmission at single hippocampal synaptic boutons. *Nature*. 1995; 375:404–408. [PubMed: 7760934]
- Meeks JP, Mennerick S. Action potential initiation and propagation in CA3 pyramidal axons. *J Neurophysiol*. 2007; 97:3460–3472. [PubMed: 17314237]
- Qian J, Saggau P. Modulation of transmitter release by action potential duration at the hippocampal CA3-CA1 synapse. *J Neurophysiol*. 1999; 81:288–298. [PubMed: 9914289]
- Schiess AR, Scullin CS, Partridge LD. Neurosteroid-induced enhancement of short-term facilitation involves a component downstream from presynaptic calcium in hippocampal slices. *J Physiol*. 2006; 576:833–847. [PubMed: 16931546]

- Scullin CS, Wilson M, Partridge LD. Developmental changes in presynaptic calcium clearance kinetics and synaptic plasticity in mouse Schaffer collateral terminals. *Eur J Neurosci.* 2010; 31:817–826. [PubMed: 20374283]
- Sorra KE, Harris KM. Occurrence and three-dimensional structure of multiple synapses between individual radiatum axons and their target pyramidal cells in hippocampal area CA1. *J Neurosci.* 1993; 13:3736–3748. [PubMed: 8366344]
- Speed HE, Dobrunz LE. Developmental decrease in short-term facilitation at Schaffer collateral synapses in hippocampus is mGluR1 sensitive. *J Neurophysiol.* 2008; 99:799–813. [PubMed: 18032567]
- Stevens CF, Wang Y. Facilitation and depression at single central synapses. *Neuron.* 1995; 14:795–802. [PubMed: 7718241]
- Swann JW, Gomez CM, Rice FL, Smith KL, Turner JN. Anatomical study of CA3 hippocampal neurons and networks during postnatal development. *Soc Neurosci Abstr.* 1991a; 17:450.13.
- Swann JW, Smith KL, Brady RJ. Age-dependent alterations in the operations of hippocampal neural networks. *Ann NY Acad Sci.* 1991b; 627:264–276. [PubMed: 1652915]
- Taschenberger H, von Gersdorff H. Fine-tuning an auditory synapse for speed and fidelity: developmental changes in presynaptic waveform, EPSC kinetics, and synaptic plasticity. *J Neurosci.* 2000; 20:9162–9173. [PubMed: 11124994]
- Voronin LL, Cherubini E. ‘Deaf, mute and whispering’ silent synapses: their role in synaptic plasticity. *J Physiol.* 2004; 557:3–12. [PubMed: 15034124]
- Wasling P, Hanse E, Gustafsson B. Developmental changes in release properties of the CA3-CA1 glutamate synapse in rat hippocampus. *J Neurophysiol.* 2004; 92:2714–2724. [PubMed: 15295011]
- Zucker RS. Short-term synaptic plasticity. *Annu Rev Neurosci.* 1989; 12:13–31. [PubMed: 2648947]
- Zucker RS, Regehr WG. Short-term synaptic plasticity. *Annu Rev Physiol.* 2002; 64:355–405. [PubMed: 11826273]

Fig. 1.

Criteria for release event selection. (A) Representative example of an R1 cumulative success plot, which exceeds two SDs from the mean at the time of the black vertical line. Mean value of 500 Monte Carlo simulations of a Gaussian distribution (gray dotted line) based on the actual average success rate during the first 25% of the experiment (broad black line) and two SDs from the mean (dashed gray lines). The actual cumulative successes over the duration of the experiment are shown by the narrow black line. (B) An example of stable R2 synaptic potency over the duration of an experiment. Best fit line: synaptic potency (pA) = $(-0.0054) \times (\text{success number}) + 9.9775$. Across the sample period, the best fit line decreases by 6.18%. The Durbin–Watson test yields a significantly linear value of 1.88. Near physiological temperature.

Fig. 2.

Age and temperature effects on success rate, synaptic potency and synaptic efficacy as measured in R1 and R2 EPSCs and as % PPF. Vertical bar labels in left panel apply to all panels: young room temperature (black, rm), young near physiological temperature (white, phys), adult room temperature (dark gray, rm) and adult near physiological temperature (light gray, phys) (young room temperature, $n = 10$; adult room temperature, $n = 13$; young near physiological temperature, $n = 19$; adult near physiological temperature, $n = 18$). Two-way ANOVA for interaction effects between ages and temperatures; Tukey's HSD *post-hoc* test. Success rate: R1 main effect of age on R1 success rate, $F_{1,56} = 0.57$, $P = 0.45$; temperature on R1 success rate, $F_{1,56} = 0.11$, $P = 0.74$; (age $\times$ temperature) on R1 success rate, $F_{1,23} = 0.18$, $P = 0.67$; age on R2 success rate, $F_{1,56} = 0.43$, $P = 0.52$; temperature on R2 success rate, $F_{1,56} = 5.29$, $P = 0.025$; (age $\times$ temperature) on R2 success rate, $F_{1,23} = 2.05$, $P = 0.16$; age on PPF success rate, $F_{1,56} = 1.38$, $P = 0.24$; temperature on PPF success rate, $F_{1,56} = 3.34$, $P = 0.073$; (age $\times$ temperature) on PPF success rate, $F_{1,23} = 2.58$, $P = 0.11$. Synaptic potency: age on R1 synaptic potency, $F_{1,56} = 13.54$, $P = 0.001$; temperature on R1 synaptic potency, $F_{1,56} = 7.00$, $P = 0.011$; (age $\times$ temperature) on R1 synaptic potency, $F_{1,23} = 12.08$, $P = 0.001$; age on R2 synaptic potency, $F_{1,56} = 16.58$, $P < 0.001$; temperature on R2 synaptic potency, $F_{1,56} = 1.32$, $P = 0.25$; (age $\times$ temperature) on R2 synaptic potency, $F_{1,23} = 0.62$, $P = 0.44$; age on PPF synaptic potency, $F_{1,56} = 0.025$, $P = 0.88$; temperature on PPF synaptic potency, $F_{1,56} = 0.98$, $P = 0.33$; (age $\times$ temperature) on PPF synaptic potency, $F_{1,23} = 10.77$, $P = 0.002$. Synaptic efficacy: age on R1 synaptic efficacy, $F_{1,56} = 2.78$, $P = 0.10$; temperature on R1 synaptic efficacy, $F_{1,56} = 1.86$, $P = 0.18$; (age $\times$ temperature) on R1 synaptic efficacy, $F_{1,23} = 5.36$, $P = 0.024$; age on R2 synaptic efficacy, $F_{1,56} = 9.74$, $P = 0.003$; temperature on R2 synaptic efficacy, $F_{1,56} = 1.23$, $P = 0.27$; (age $\times$ temperature) on R2 synaptic efficacy, $F_{1,23} = 0.41$, $P = 0.53$; age on PPF synaptic efficacy, $F_{1,56} = 1.11$, $P = 0.30$; temperature on PPF synaptic efficacy, $F_{1,56} = 3.40$, $P = 0.071$; (age $\times$ temperature) on synaptic efficacy, $F_{1,23} = 7.50$, $P = 0.008$. Statistical P values are represented as follows: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Fig. 3.

The relationship of success rate and age. (A) Representative examples of success and failures for release events in young neurons showing depression (A1), for young neurons showing facilitation (A2) and for adult neurons showing facilitation (A3) (failures, gray; successes, black; 50 superimposed traces for each condition). (B) Cumulative success rates for representative examples in A (R1, black line; R2, gray line). (C) Facilitation of the R2 success rate during release events from young ($n = 19$) and adult ($n = 18$) neurons. Two-way ANOVA; Tukey's HSD *post-hoc* test. Main effect of age on success rate, $F_{1,74} = 0.241$, $P = 0.625$; main effects of R1 vs. R2 on success rate, $F_{1,74} = 46.84$, $P < 0.001$; interaction effect between the age and R1 vs. R2 on success rate, $F_{1,74} = 2.531$, $P = 0.116$. (D) Two subpopulations of release events from young neurons, one that exhibited depression ($n = 3$) and one that exhibited facilitation ($n = 16$). Two-way ANOVA; Scheffe's *post-hoc* test. Main effect of type of plasticity on success rate, $F_{1,34} = 0.45$, $P = 0.51$; main effect of R1 vs. R2 on success rate, $F_{1,34} = 1.846$, $P = 0.183$; interaction effect between the type of plasticity and the R1 vs. R2 on success rate, $F_{1,34} = 13.412$, $P < 0.005$. (E) Success rate paired pulse ratio (PPR) during release events in young ($n = 19$) and adult ($n = 18$) neurons (unpaired Student's *t*-test, $t_{27.77} = 2.09$, $P = 0.051$). The white dotted line represents no plasticity. Near physiological temperature. Statistical *P* values are represented as follows: * $P < 0.05$, *** $P < 0.001$ and **** $P < 0.0005$.

Fig. 4.

The relationship of synaptic potency and age. (A) Representative examples of averaged successes of release events from young and adult neurons. R1, black line; R2, gray line. (B) R1 and R2 synaptic potency of release events in young ($n = 19$) and adult ($n = 18$) neurons. Two-way ANOVA; Tukey's HSD *post-hoc* test. Main effect of age on synaptic potency, $F_{1,70} = 7.21$, $P < 0.01$; main effect of R1 vs. R2 on synaptic potency, $F_{1,70} = 23.15$, $P < 0.001$; interaction effect between age and R1 vs. R2 on synaptic potency, $F_{1,70} = 5.80$, $P < 0.05$. (C) Mean synaptic potency PPR of release events in young ($n = 19$) and adult ($n = 18$) neurons. K-S test, $D = 0.63$, $P = 0.001$. The white dotted line represents no plasticity. (D) Two subpopulations of release events in young neurons, as determined by a K-S test, that exhibit either no change ($n = 12$) or facilitation ($n = 7$) of the synaptic potency. Two-way

ANOVA; Scheffe's *post-hoc* test. Main effect of type of plasticity on success rate, $F_{1,34} = 6.00$, $P < 0.05$; main effect of R1 vs. R2 on synaptic potency, $F_{1,34} = 9.87$, $P < 0.005$; interaction effect between the type of plasticity and R1 vs. R2 on synaptic potency, $F_{1,34} = 8.85$, $P = 0.005$. (E) Mean synaptic potency PPR during only facilitated release events in young ($n = 7$) and adult ($n = 16$) neurons. Unpaired Student's *t*-test, $t_{9,48} = 0.17$, $P = 0.88$. The white dotted line represents no change in plasticity. (F and G) Representative examples of cumulative R1 (solid circles) and R2 (open squares) synaptic potencies. (F) Example with no difference between R1 and R2 synaptic potencies. K-S test, $D = 0.17$, $P = 0.21$. (G) Example with R2 synaptic potencies greater than R1. K-S test, $D = 0.31$, $P < 0.01$. Near physiological temperature. Statistical *P* values are represented as follows: *** $P < 0.001$ and **** $P < 0.0005$.

Fig. 5. Comparison of the R2 synaptic potency when R1 succeeded vs. when R1 failed. (A–C) Mean R2 synaptic potencies when R1 succeeded (R1) vs. when R1 failed (no R1). (A) Release events in young neurons that showed facilitation of the mean R2 synaptic potency. Unpaired Student's *t*-test, $t_{11.99} = 0.60$, $P = 0.57$, $n = 7$. (B) Release events in young neurons that showed no change between the R1 and R2 mean synaptic potency. Unpaired Student's *t*-test, $t_{21.99} = -0.40$, $P = 0.90$, $n = 12$. (C) Release events in all experiments from adult neurons. Unpaired Student's *t*-test, $t_{32.39} = 0.13$, $P = 0.70$, $n = 18$. (D and E) Representative examples of cumulative R2 synaptic potencies when R1 succeeded (black circles) and when R1 failed (gray circles). (D) Example with no difference in R2 when R1 succeeded or failed. K–S test, $D = 0.16$, $P = 0.79$. (E) Example in which the R2 synaptic potency was greater when R1 failed than when R1 succeeded. K–S test, $D = 0.32$, $P < 0.01$. Near physiological temperature.

Fig. 6.

The effects of age on synaptic efficacy. (A) Mean synaptic efficacy. Two-way ANOVA; Tukey's HSD *post-hoc* test. Main effect of age on synaptic efficacy, $F_{1,74} = 2.31$, $P = 0.13$; main effect of R1 vs. R2 on synaptic efficacy, $F_{1,74} = 40.35$, $P < 0.001$; interaction effect between the age and R1 vs. R2 on synaptic efficacy, $F_{1,74} = 5.72$, $P < 0.05$. (B) Mean synaptic efficacy PPR. Unpaired Student's *t*-test, $t_{23,38} = -2.72$, $P = 0.012$. The white dotted line represents no plasticity. Near physiological temperature. Statistical *P* values are represented as follows: * $P < 0.05$ and **** $P < 0.0005$.

Fig. 7.

Age-dependent changes in correlations between the mean synaptic potency PPR and differences in the timing of R1 and R2 mean EPSCs. (A) Representative traces of mean EPSCs during a release event in an adult neuron showing the half-width (dotted line) of R1 (black) and R2 (gray) with the R1 amplitude scaled to equal that of R2. (B) Mean synaptic potency PPR vs. half-width PPR. No significant correlation in release events from young neurons (black squares, black regression line). One-way ANOVA, $F_{1,17} = 1.58$, $P = 0.23$, $n = 19$. Significant positive correlation in release events from adult neurons (gray circles, gray regression line). One-way ANOVA, $F_{1,16} = 7.93$, $P = 0.012$, $n = 18$. (C) Representative traces of mean EPSCs of a release event from an adult neuron showing the onset latencies between the stimulus artifact (arrow) and the onset of R1 (black dotted line) and R2 (gray dotted line). Onset latency was determined as the time from the stimulus artifact (vertical arrow) to a threshold where the EPSP reached 10% of its total mean amplitude (vertical dotted line). (D) Mean synaptic potency PPR vs. R2–R1 onset latency. No significant correlation in release events from young neurons (black squares, black regression line). One-way ANOVA, $F_{1,17} = 0.003$, $P = 0.96$, $n = 19$. Significant negative correlation in release events from adult neurons (gray circles, gray regression line). One-way ANOVA, $F_{1,16} = 8.79$, $P < 0.01$, $n = 18$. Black dotted lines in B and D represent no change in half-width or onset latency, respectively (horizontal lines), and no change in mean synaptic potency (vertical line). Near physiological temperature.