Homosynaptic frequency-dependent depression by release site inactivation at neonatal hippocampal synapses in the stratum lacunosum-moleculare

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Abstract
When activated at low frequencies (0.1–1 Hz), second postnatal week synapses onto the most distal part of the apical dendritic tree (stratum lacunosum-moleculare) of rat hippocampal CA1 pyramidal cells display a frequency-dependent synaptic depression not observed for the more proximal (stratum radiatum) synapses. Depression in this frequency range is thought of as a possible contributor to behavioural habituation. In fact, in contrast to the proximal synapses, the distal synapses provide more direct sensory information from the entorhinal cortex as well as from thalamic nuclei. The use of antagonists showed that the activation of GABA_A, GABA_B, NMDA, mGlu, kainate, adenosine, or endocannabinoid receptors was not directly involved in the depression, indicating it to be intrinsic to the synapses themselves. While the depression affected paired-pulse plasticity in a manner indicating a decrease in vesicle release probability, the depression could not be explained by a stimulus-dependent decrease in calcium influx. Despite affecting the synaptic response evoked by brief high-frequency stimulation (10 impulses, 20 Hz) in a manner indicating vesicle depletion, the depression was unaffected by large variations in release probability. The depression was found not only to affect the synaptic transmission at low frequencies (0.1–1 Hz) but also to contribute to the depression evolving during brief high-frequency stimulation (10 impulses, 20 Hz). We propose that a release-independent process directly inactivating release sites with a fast onset (ms) and long duration (up to 20 s) underlies this synaptic depression.

Keywords: development, frequency depression, hippocampus, release site, synaptic plasticity

Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CHA, N-cyclohexyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; EPSP, field excitatory postsynaptic potential; KAR, kainate receptor; mGlu, metabotropic glutamate; NMDA, N-methyl-D-aspartate; PP, paired pulse; PP50, a 50-ms paired-pulse interval; SC, Schaffer collateral; SLM, stratum lacunosum-moleculare; SR, stratum radiatum.

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1 | INTRODUCTION

Glutamate synapses in the adult brain are very heterogeneous with respect to their presynaptic and postsynaptic properties in a manner likely appropriate to the function of the neuronal circuit in which they are embedded. Also, in the early developing brain, long before the synapses have acquired their adult “mature” properties, synapses may differ from each other possibly dependent on what is required of them at that age. For example, CA1 hippocampal pyramidal cells not only receive indirect excitation from entorhinal cortex stellate cells processed via the tri-synaptic pathway but also direct excitation from entorhinal cortex layer III pyramidal neurons. These entorhinal neurones (together with neurones in some thalamic nuclei) specifically project onto the most distal part of the apical dendritic tree in stratum lacunosum moleculare (SLM) (Dolleman-van der Weel et al., 2017). This distal region matures morphologically faster than the rest of the apical dendritic tree in the stratum radiatum (SR), the number of dendritic branches in the SLM region reaching its adult value already within the second postnatal week in rats (Pokorny & Yamamoto, 1981). Such differences have led to the concept of a relatively early-matured system associated with the SLM region compared with a more late-maturing system associated with the SR region (Jabes et al., 2011). When examined in the second postnatal week rats, the synapses onto this early matured distal dendritic tree (SLM-CA1 synapses) were also found to differ from those in the stratum radiatum (SR-CA1 synapses) in that they exhibited less of the activity-dependent silencing of AMPA transmission (Ma et al., 2016) thought to be involved in the on-going organization of synaptic connectivity (Hanse et al., 2009; Yasuda et al., 2011). Instead, when previously nonstimulated (naïve) SLM-CA1 synapses were exposed to low-frequency (0.1–1 Hz) stimulation, their main response was a frequency-dependent depression not observed for the SR-CA1 synapses (Ma et al., 2016).

Neonatal SLM-CA1 synapses activated at low frequencies (0.1–1 Hz) were thus depressed not only by the stimulation-dependent AMPA silencing process but also by an additional, frequency-dependent process. In fact, already the very first studies of synaptic transmission in the hippocampus using the in vitro slice preparation from rats (probably 2- to 3-month-old) noted that stimulation in this low-frequency range could result in a frequency-dependent depression. This form of depression was observed for entorhinal cortex projections onto granule cells in the dentate gyrus but not for Schaffer collateral or mossy fibre connections in the CA1 and CA3 subregions, respectively (Alger & Teyler, 1976). The entorhinal cortex brings multimodal sensory information into the hippocampus, and this form of depression was seen as a possible means for habituation towards this sensory input (Christoffersen, 1997; Teyler & Alger, 1976; White et al., 1979). The presence of this form of depression in neonatal SLM-CA1 synapses would then suggest that these synapses could have acquired such depression even before it may be required for sensory habituation, considering that spontaneous network activity rather than sensory-related activity dominates in the second postnatal week (Dawitz et al., 2020; Garaschuk et al., 1998).

With respect to underlying mechanism for this form of depression, these early studies on the entorhinal cortex–dentate gyrus synapses did not provide much insight into what may underlie this form of depression, except that it appeared presynaptically expressed. Later work on these synapses found such low-frequency depression (0.2 Hz) also in second postnatal week rats (Abrahamsson et al., 2005). This form of depression was associated with changes in quantal content but not in paired-pulse plasticity, and vesicle depletion was suggested as a probable underlying mechanism. With respect to the frequency-dependent component of the depression in the second postnatal week SLM-CA1 synapses (Ma et al., 2016), no attempt was made to clarify its underlying mechanism. Synaptic depression is often attributed to vesicle depletion (Fioravante & Regehr, 2011; Kavalali, 2006), but should a depression so restricted to certain synapses for a specific functional reason rely on such a common depression mechanism? Other mechanisms for depression such as decrease in vesicle release probability ($p_{ves}$), release-site inactivation, autoinhibition, and release of modulatory transmitters (Fioravante & Regehr, 2011; Zucker & Regehr, 2002) may also then have to be considered.

The present study thus aims to demonstrate which of the above possible explanations for depression may account for this frequency-dependent depression in the neonatal SLM-CA1 synapses. It also aims to disentangle the frequency-dependent depression from AMPA silencing in order to properly describe its characteristics and thus facilitate its comparison with the low-frequency-induced depressions previously described for other entorhinal projections.

2 | MATERIAL AND METHODS

2.1 | Slice preparation and solutions

Most experimental details have been described previously (Ma et al., 2016). In brief, experiments were performed on hippocampal slices from 8- to 12-day-old Wistar rats...
of either sex kept and killed in accordance with the
guidelines of the Gothenburg ethical committee for ani-
mal research. The rats were anaesthetized with isoflurane
(Abbott Scandinavia AB, Sweden) prior to decapitation.
The brain was removed, placed in an ice-cold solution
containing (in millimolar): 219 glycerol, 2.5 KCl, 1.2
CaCl₂, 7 MgCl₂, 26 NaHCO₃, 1.2 NaH₂PO₄, 11 d-glucose,
or, in some experiments, 140 cholineCl, 2.5 KCl, 0.5
CaCl₂, 7 MgCl₂, 25 NaHCO₃, 1.25 NaH₂PO₄, 0.5 ascorbic
acid, and 7 dextrose. Transverse hippocampal slices
(400 μm thick) were cut with a Vibratome (HM 650V
Microm, Germany) in the same ice-cold solution, and
they were subsequently stored in artificial cerebrospinal
fluid (ACSF) containing (in millimolar): 129 NaCl, 3 KCl,
2 CaCl₂, 4 MgCl₂, 20 NaHCO₃, 1.25 NaH₂PO₄, 0.5
ascorbic acid, 3 myo-inositol, 4 D,L-lactic acid, and
10 d-glucose. After usually 1–5 h of storage at 25°C, a
single slice was transferred to a recording chamber where
it was kept submerged in a constant flow (~2 ml min⁻¹)
at ~30°C. The perfusion artificial cerebrospinal fluid
(ACSF) contained (in millimolar): 124 NaCl, 3 KCl,
2 CaCl₂, 4 MgCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, and
10 d-glucose. Picrotoxin (100 μM) was always present in
the perfusion ACSF to block GABA_A receptor-mediated
activity. To prevent spontaneous network activity in such
disinhibited slices, a surgical cut was made between the
CA3 and CA1 regions and the higher-than-normal Ca²⁺
and Mg²⁺ were used.

2.2 | Recording and analysis

Electrical stimulations and recordings of synaptic
responses were carried out in either stratum lacunosum/
moleculare (SLM) or in stratum radiatum (SR), these
regions visually identified by using ECLIPSE
E600 microscopy (Nikon, Japan) and a Color LCD
Monitor (FlexScan L365, EIZO, USA; see Figure 1; Ma
et al., 2016). Stimuli consisted of biphasic constant
current pulses (200 + 200 μs, 20–50 μA) delivered
through an insulated tungsten microelectrode (resistance:
~0.1 MΩ). Only a single stimulating electrode was used
to provide a synaptic input either in the SLM or in the SR
region. Field excitatory postsynaptic potential (fEPSP)
recordings were made by means of a glass micropipette
(filled with 1-M NaCl, resistance ~2 MΩ); fEPSPs were
sampled at 10 kHz with an EPC-9 amplifier (HEKA
Elektronik, Lambrecht, Germany) and filtered at 1 kHz.

Since this study, as well as some earlier studies from
our lab (e.g., Abrahamsson et al., 2005; Ma et al., 2016;
Xiao et al., 2004) that included synaptic depression that is
induced by stimulation frequencies normally used as test
stimulus frequencies (0.05–0.2 Hz), no stimulation was
applied prior to the initiation of an experimental run.
That is, the depression observed is that of a previously
nonstimulated (naïve) synaptic input. This also means
that for each slice only a single experimental run was
made. For a single experiment, usually only two to three

![FIGURE 1](image-url)

**FIGURE 1** Time course of paired-pulse (PP) plasticity at second postnatal week synapses. (a) PP (second/first fEPSP amplitude) ratio is plotted against inter-stimulus interval for SLM-CA1 synapses (n = 5). Below are shown example SLM-CA1 fEPSPs (n = 5 sweeps) taken at three different inter-stimulus intervals. (b) PP ratio is plotted against inter-stimulus interval for SR-CA1 synapses (n = 6). (c) Example SLM-CA1 fEPSPs where the first and second fEPSPs (1-s interstimulus interval) are shown superimposed before and after normalization to the same peak amplitude. The fEPSPs are also shown expanded to illustrate the afferent volley. For the construction of the graphs in (a) and (b), each experiment was started with the longest inter-stimulus interval and ending with the shortest, and five sweeps were given for each interval. Experiments were performed using control solution (4:4 Ca:Mg) and in the presence of 100-μM PTX, 50-μM d-AP5, and 1-μM CGP.
slices were used, and these slices were exposed to different forms of experimental protocol. During the second postnatal week, synapses significantly alter their properties, for example, with respect to magnitude of short-term synaptic plasticity (Ma et al., 2016). Thus, for comparison of data sets of depression, care was taken to have age-matched data sets. To achieve such matching, experiments using a certain protocol were spread out during the week. This means that the \( n \) values given in the text refer to the number of separate experiments.

fEPSPs were analysed offline using custom-made IGOR Pro (WaveMetrics, Lake Oswego, OR) software. fEPSP amplitude was measured from the average of an 8-ms segment immediately preceding the stimulus artefact (see example fEPSPs in Figure 1a) to the mean amplitude during a 2-ms window around the negative peak. With the stimulation intensities used (20–50 \( \mu \)A, depending on animal age), the naive fEPSPs were \( \sim 0.5–1 \) mV in amplitude and subthreshold for spike initiation (Ma et al., 2016). Synaptic depression in the individual experiments was calculated as the percentage decrease with respect to the very first evoked fEPSP. The presynaptic volley was measured from its peak value. If this value, averaged from the last five records in an experimental run, differed \( >3\% \) from that of the first five records, the experiment was discarded (see Strandberg et al., 2009). In six experiments, the relation between this measure of the volley and that of the fEPSP was examined by decreasing the stimulation strength in three to four steps creating fEPSPs down to 30–40\% of baseline value. A regression line through these values \( (n=19) \), setting the value of the baseline fEPSPs and volleys to 100, had a slope of 1.04.

2.3 | fEPSP responses during brief train activation

When a brief high-frequency train was used as a test synaptic response GABA\(_A\), GABA\(_B\), NMDA and mGlu receptor antagonists were always present in the bath to ascertain that the synaptic response during the train did not contain components related to the activation of these receptors as well as components arising because of synaptic plasticity processes induced by the activation of these receptors. Because our measure of fEPSP magnitude could be affected in a nonlinear manner by build-up of local depolarization when using such brief train stimulation as test stimulation, the fEPSP responses during such trains were in some experiments compared before and after lowering the stimulation strength. A reduction of the first fEPSP in the train by \( \sim 25\% \), that is, to an extent comparable with that of the observed depression, had no significant effect on the synaptic response during such trains, the average amplitude of the last three fEPSPs being \( 20.6 \pm 1.7\% \) and \( 21.7 \pm 2.0\% \) \( (n=6) \) of that of the first fEPSP before and after lowering the stimulation strength, respectively \( (p > 0.05, \text{paired } t \text{ test}; \text{see also Figure 2c}) \).

2.4 | Bath application of CHA

In some experiments, the adenosine A1 receptor agonist \( \Delta^6 \)-cyclohexyladenosine (CHA) \( (4 \text{ nM}) \) was applied to alter release probability. Notably, the wash out of CHA did not result in full recovery but to a remaining steady depression of \( 16.8 \pm 3.3\% \) \( (n=4) \). Such a maintained depression following application of an A1 receptor agonist has also previously been described (Brust et al., 2007). Because this maintained depression may differ mechanistically from the more acute effect of adenosine in lowering release probability, the effect of CHA on the fEPSP responses during brief train activation was evaluated by comparing the fEPSP responses observed during the CHA application with those observed following wash out of CHA.

2.5 | Unmasking of depression from a concurrent facilitation

The analysis of the present data disclosed that while an increased number of conditioning stimulations resulted in little further increase in the magnitude of depression, the depression became associated with a decrease in paired-pulse (PP) plasticity (50-ms interval, PP\(_{50}\)). This result suggested that the process underlying the depression actually accumulates with repetitive activation but that its increase is masked by a concurrent process that increases \( p_{\text{ves}} \), such as augmentation (Garcia-Perez & Wesseling, 2008). To remove the effect of such a process, and thus to unmask the depression, one would have to know in what manner this facilitatory process alters the PP\(_{50}\) ratio as a function of its effect on the fEPSP amplitude. To our knowledge, there has been no systematic study on a central synapse in what manner augmentation affects the PP plasticity. However, as noted in a study of augmentation in corticogeniculate synapses (Graneth & Lindström, 2004), augmentation preferentially increases the first synaptic response of a PP stimulation causing only a minor increase of the second response. The relationship between the percentage decrease of the PP\(_{50}\) ratio and the percentage increase of the fEPSP amplitude resulting from an unchanged second response was thus calculated in order to be of potential use for unmasking the depression.
However, in the present study, we also, for other purposes, altered \( p_{\text{ves}} \) by adding CHA or varied the Ca:Mg ratio in the perfusion fluid, thus obtaining a relation between the percentage change of the PP50 ratio and the percentage change of the fEPSP amplitude. We also applied a high concentration (800 \( \mu \text{M} \)) of CHA to the perfusion solution to rapidly induce a PP50 versus fEPSP relationship. All these procedures indicated a 1:1 relationship between the percentage decrease of the fEPSP and the percentage increase of the PP50 ratio. Such a 1:1 relation was subsequently used to construct the relationship between a percentage decrease of PP50 ratio and the percentage increase of the fEPSP amplitude. Interestingly, this relationship was close to that obtained under the assumption that the second synaptic response was unaffected, indicating that only minor changes of the second synaptic response occur as reported for augmentation (Granseth & Lindström, 2004). To unmask the depression, we have thus used this 1:1 relationship.

Data are expressed as means ± SEM. Statistical significance for independent samples was evaluated using Student’s \( t \) test.

**FIGURE 2** Effects of a preceding single-pulse stimulation on the synaptic response given by a brief train. (a) A schematic drawing of the stimulus arrangement indicating a 20-Hz 10-impulse train given before (red) and 1 s after (blue) a single-pulse stimulation. Example fEPSP responses during a 20-Hz 10-impulse train (\( n = 15 \) sweeps) before (red) and 1 s after (blue) the single-pulse stimulation. fEPSP amplitude is plotted against stimulus position in the train from such experiments (\( n = 9 \)). The values were normalized with respect to the first fEPSP of the train that was applied before the single-pulse stimulation. The cumulative fEPSP amplitude during the 10-impulse train is plotted against stimulus position in the train. Linear regression was performed on the last 5 points of the curves (\( n = 9 \)), and the regression lines are shown as dashed lines with the equations indicated in the figure. (b) Same as in (a) but performed using SR-CA1 synapses (\( n = 7 \)).

(c) Comparing the synaptic responses to a 20-Hz 10-impulse train evoked at two different stimulation strengths, the responses to the control strength (red) and after having reduced the strength (blue). fEPSP amplitude is plotted against stimulus position in the train, from such experiments (\( n = 6 \)) before and after normalization of the first fEPSP in the trains to the same amplitude. The experiments were performed in the presence of 100-\( \mu \text{M} \) LY341495, 100-\( \mu \text{M} \) PTX, 50-\( \mu \text{M} \) D-AP5, and 1-\( \mu \text{M} \) CGP.
obtained from Sigma-Aldrich (Stockholm, Sweden). All the other chemicals were obtained from Abcam plc (Cambridge, UK) and tetrodotoxin (TTX) and UBP 302 from Tocris (DPCPX), and AM251 were from Abcam plc (Cambridge, UK). The tetrodotoxin (TTX) and UBP 302 from Tocris (DPCPX), and AM251 were from Abcam plc (Cambridge, UK). The other chemicals were obtained from Sigma-Aldrich (Stockholm, Sweden).

3 RESULTS

When previously nonstimulated (naïve) second postnatal week SR-CA1 synapses are subjected to prolonged activation (>100 stimuli) at 0.05 Hz, the fEPSP becomes depressed to about half its naïve level, the amount of depression being no larger when the same number of stimuli are given at 0.2 or 1 Hz (Strandberg et al., 2009). This depression is explained by a stimulation-dependent postsynaptic AMPA silencing at about half of the activated synapses (Xiao et al., 2004). It does not require NMDA receptor activation for its induction, and it reverses slowly (20–30 min to hours) following stimulus interruption (Abrahamsson et al., 2007; Strandberg & Gustafsson, 2011). When instead naïve SLM-CA1 synapses of the same age were subjected to such very low frequency stimulation (0.03–0.05 Hz), the amount of AMPA silencing was much less (~20%), but the depression was found to incrementally increase when the stimulation frequency was increased within the 0.05- to 1-Hz range (Ma et al., 2016). Following stimulus interruption, this frequency-dependent depression that was added on top of the AMPA silencing, reversed within tens of seconds, and it was tentatively attributed to a depletion of the readily releasable pool of vesicles. Because this added depression was present already within the very first activations, our analysis of it has been focused on the depression following single-pulse, or brief train, stimulations.

3.1 Paired-pulse plasticity at the second postnatal week SLM-CA1 and SR-CA1 synapses

Figure 1 shows the effect of a conditioning single-pulse stimulation on a test fEPSP using interstimulus intervals from 50 ms to 20 s, this paired-pulse (PP) stimulation repeated at 1/min. These experiments were performed in the presence of GABA<sub>A</sub>, GABA<sub>B</sub> and NMDA receptor antagonists (see text-Figure 1) to isolate the AMPA receptor component of the fEPSP. Figure 1a shows that for the SLM-CA1 synapses there is a prolonged biphasic depression of the test fEPSP, the late phase of the depression displaying a peak at a 1 s PP interval (19.4 ± 2.1%, n = 5). The depression is still significant at 5 s (p = 0.001) and at 10 s (p = 0.003) inter-stimulus intervals but is no longer observed at a 20-s interval. The PP depression at these neonatal SLM-CA1 synapses thus demonstrates an overall time course quite similar to that of the field potential for the more adult perforant path–granule cells synapses (White et al., 1979). In conformity with these authors, we will refer to the late phase of the depression as late depression, and its magnitude will be that measured 1 s after a conditioning stimulation, unless otherwise stated. For the SR-CA1 synapses (Figure 1b), there is a PP facilitation that is largely observed for inter-stimulus intervals <1 s, although a very small ~3%, but significant (p < 0.05) facilitation, was still observed at intervals up to 10 s. The late depression at the SLM-CA1 synapses was at its peak not associated with any apparent change in the fEPSP time course, or in the afferent volley, as exemplified by sample records in Figure 1c (see also Figure 9e).

Thus, in contrast to the SR-CA1 synapses, the transmission at the SLM-CA1 synapses is depressed for a considerable time period even after a single-pulse stimulation, a late depression with kinetics suitable to produce a frequency-dependent depression in the 0.05- to 1-Hz range.

3.2 Effect of the late depression on the fEPSP response evoked by a brief train stimulation

To analyse in what manner this late depression affects the synaptic transmission, we tested how it affected the synaptic response to a brief high-frequency activation (see Section 2). In these experiments, a single-pulse stimulation was paired with a 20-Hz 10-impulse train (at a 1-s interstimulus interval), this paired stimulation repeated at 1/min with an alternating order of presentation (see schematic drawing in Figure 2a). As can be observed in Figure 2a from the fEPSP responses from a sample experiment, and from the graph where the fEPSP amplitude is plotted as a function of its position in the train, the depression is largely observed only for the first fEPSP in the train (18.6 ± 2.3%, n = 9), leaving later release much less affected. Thus, for the cumulative fEPSP amplitude developing during the train, the “depressed” cumulative curve grows, after its initial phase, roughly in parallel with the control curve, the slopes of the regression lines in Figure 2a differing by only ~3%. In line with this slope difference, an average of the last three fEPSPs in the train showed that also the later release was depressed, but only to a very small
extent (2.3 ± 0.6%, n = 9, p < 0.05, paired t test). When the same experiment was performed on SR-CA1 synapses, the curves completely overlapped (Figure 2b), as might be expected from the PP data in Figure 1b. A similar overlap was also observed (after normalization to the same first fEPSP amplitude) when the train was evoked at two different stimulation intensities (Figure 2c; see also Section 2), indicating that the effect of the late depression on the fEPSP response shown in Figure 2a is not secondary to the reduction of the first fEPSP of the train.

Thus, the depression process is preferentially acting on the initial responses during the train, an action that supports the notion of a modulation of the presynaptic release characteristics rather than of the postsynaptic receptor properties.

### 3.3 The late depression is not explained by a reduced calcium influx and \( p_{\text{ves}} \) modulation

As indicated by the preferential action of the late depression on the first fEPSP of the train, the PP ratio estimated from the first two fEPSPs in the 20-Hz train (PP\(_{50}\) ratio) was substantially larger for the depressed fEPSP than for the control fEPSP, this PP\(_{50}\) ratio being 16.1 ± 2.1% (n = 9) greater than the control PP\(_{50}\) ratio (p < 0.001). This result would suggest that the late depression is explained by a decrease in \( p_{\text{ves}} \) possibly explained by a reduced calcium influx. In order to test this notion, we examined in what manner procedures known to alter \( p_{\text{ves}} \) affect the fEPSP response evoked by a brief train. In the first set of experiments, the Ca:Mg concentrations in the perfusion solution were switched from 2:6 to 4:4 mM and back (Figure 3a). This procedure resulted in a depression of the first fEPSP of 27.6% (± 2.4, n = 6) and an increase of the PP\(_{50}\) ratio with 31.2% (± 2.1, n = 6) (Figure 3a). The ratio between the percentage change in fEPSP amplitude and in PP\(_{50}\) ratio was thus ~1, in common with that caused by the late depression (18.6% vs. 16.1%). This result would then agree with the notion that the late depression can be explained by a decrease in \( p_{\text{ves}} \). However, the change in the Ca:Mg ratio also substantially affected the later release in the train in that this release became facilitated in the presence of the reduced Ca:Mg ratio (Figure 3a). In fact, the reduced Ca:Mg ratio resulted in an enhancement of the cumulative fEPSP amplitude reached at the end of the train (10.4 ± 2.1%, n = 6, P < 0.05, paired t test) (Figure 3a) rather than the decrease produced by the late depression (Figure 2a).

In a second set of experiments, the A1 receptor agonist CHA (4 nM) was applied to the perfusion solution to reduce \( p_{\text{ves}} \) (see Section 2). As shown in Figure 3b, CHA had essentially the same effect on the fEPSP response during the brief train as the reduced Ca:Mg ratio. Thus, the first fEPSP was reduced by 24.1 ± 1.1% (n = 4) and the PP\(_{50}\) ratio increased by 21.3 ± 2.3% (n = 4). Moreover, the later release was also facilitated, although in this case the increase in the cumulative fEPSP amplitude (6.8 ± 2.5%, n = 4) was of borderline significance (p = 0.05, paired t test).

Thus, the results from these two sets of experiments show that the effect of the late depression on the synaptic response evoked by a brief train stimulation, despite the observed change in the PP\(_{50}\) ratio, is not simulated by procedures that lower presynaptic calcium influx and thereby lower \( p_{\text{ves}} \).

### 3.4 Late depression is release independent and thus not explained by vesicle depletion

A depression of the fEPSP that is largely specific for the initial release in a train seems most easily explained by an activity-induced prolonged depletion of the vesicles within a small immediately releasable pool of vesicles. A very strong prediction would then be that the depression should be release dependent, that is, be relatively larger with respect to the test fEPSP when a larger fraction of the vesicle pool is released, and vice versa. The absence of such a depression at the SR-CA1 synapses might then be more apparent than real. This is because, as judged from the difference in short-term plasticity between SR-CA1 and SLM-CA1 synapses (Figure 1), the release probability at SR-CA1 synapses might be substantially lower (on average) than that at the SLM-CA1 synapses, this resulting in less depletion. PP plasticity was thus examined (as in Figure 1) for both SLM-CA1 and SR-CA1 synapses, using several different Ca:Mg ratios (from 7:1 to 1:7) in the perfusion solution. This variation in the Ca:Mg ratio substantially altered the PP\(_{50}\) ratio for the SLM-CA1 synapses, from 0.6 up to 1.4 (Figure 4a), without essentially altering the magnitude of the depression (as fraction of the test response) at PP intervals of 1 s, or more (Figure 4a,b). Moreover, for the SR-CA1 synapses, a similar variation in the Ca:Mg ratio failed to result in any depression at PP intervals of 1 s, or more, despite PP\(_{50}\) ratios within the range of the SLM-CA1 synapses (Figure 4c). The depression at these longer PP intervals thus seems to be a feature of the SLM-CA1 synapses distinguishing them from the SR-CA1 synapses independent of their different basal release states. Importantly, the observed release independence of this depression at the SLM-CA1 synapses is not compatible with vesicle depletion as an underlying mechanism.
The fEPSP depression developing with more prolonged low-frequency stimulation will also contain a component related to postsynaptic AMPA silencing (Ma et al., 2016). Nevertheless, we also examined the release dependence of such depression in experiments in which prolonged 0.1- and 1-Hz stimulation was given at two different Ca:Mg concentrations in the perfusion solution. These experiments showed that the strong reduction in release probability given by a change in the Ca:Mg concentration from 4:4 to 1:7 did not reduce the 0.1 and 1 Hz-induced frequency depressions (Figure 4d). In fact, the depression induced by 1-Hz stimulation in 1:7 Ca:Mg (67.7 ± 2.4%, n = 10) was even somewhat greater than that observed using 4:4 Ca:Mg (57.4 ± 1.7%, n = 10, p < 0.005). In agreement with the depression induced by single-pulse stimulation, the low-frequency induced depression appears also release independent.

Thus, from the above results, the late depression exhibits features of mechanisms based on a change in $p_{\text{ves}}$ (increase in the PP50 ratio) as well as on vesicle depletion (a reduced cumulative synaptic response) but cannot be explained by either of these two mechanisms. The
question then arises whether such results, for example, the associated change in the PP<sub>50</sub> ratio, indicative of a \( p_{ves} \) modification as an underlying mechanism, can be explained in a manner not involving \( p_{ves} \) changes.

### 3.5 Does the late depression have a fast onset?

Based on the chance observation of a PP experiment in which an absence of late depression was combined with a PP<sub>50</sub> facilitation (White et al., 1979), these authors surmised that the PP<sub>50</sub> depression, which was otherwise observed for the perforant path–granule cell synapses, was secondary to an interaction with the late depression. That is, the late depression might be sufficiently developed already within 50 ms after the presynaptic action potential to alter the PP<sub>50</sub> plasticity from a facilitation to a depression. As shown in Figure 5, where the PP<sub>50</sub> ratio and the late depression (expressed as the PP ratio at a 1 s PP interval) are plotted against postnatal day in a and b, respectively, these ratios varied quite considerably in magnitude, this variation to some extent dependent on animal age (see regression lines in a and b). When examined for the subpopulation of the above experiments in which both these ratios were obtained, there was a strong correlation between them (Figure 5c), such that a large late depression (low PP ratio at 1 s) was associated with a low PP<sub>50</sub> ratio, and a small late depression with almost no PP<sub>50</sub> depression. Because a large variation in the PP<sub>50</sub> ratio resulting from an altered \( p_{ves} \) did not affect the magnitude of the late depression (Figure 4a), the above correlation, if a causal one, can thus not be accounted for by a primary variation in \( p_{ves} \) that alters the PP<sub>50</sub> ratio and therefore the late depression. Instead, this correlation strongly supports the above notion (White et al., 1979) that the late depression could affect the PP<sub>50</sub> ratio.

To evaluate the possible effect on the PP<sub>50</sub> ratio by a fast onset of the late depression we fitted an exponential function to the decay of the late depression (Figure 5d), and extrapolated this function to \( t = 50 \text{ ms} \). Assuming that the late depression is mechanistically independent of other processes determining the PP<sub>50</sub> ratio, an independence that is often assumed when dissecting out various components in short-term synaptic plasticity (Klyachko & Stevens, 2006), removal of the late depression observed in the 4:4 Ca:Mg solution (~19%) was found to increase the PP<sub>50</sub> ratio (Figure 1a) from 0.82 to 1.07, that is, resulting in a ~30% increase of the ratio. Taking a PP<sub>50</sub> ratio of 1.07 as the "true" PP<sub>50</sub> ratio in 4:4 Ca:Mg without the late depression component, we also calculated the PP<sub>50</sub> ratio expected from a late depression of 10% and 30%, respectively, and plotted the calculated...
values in Figure 5c (blue closed circles). As can be noted, these calculated values fall roughly within the confines of the experimental values, strengthening a causal relationship between the amount of late depression and the magnitude of the PP50 ratio.

As shown in Figure 5a,b, both the PP50 ratio and the late depression varied with age ($p < 0.0005$), the slope of the regression line being steeper for the PP50 ratio, although not significantly so ($p > 0.05$). Even so, a steeper slope for the PP50 ratio is in fact to be expected because the impact of the late depression on the PP50 ratio is not the PP1s values shown in Figure 5b, but the values for late depression at 50 ms, which are $\approx 20\%$ higher. The age dependence of the late depression at the time when it affects the PP50 ratio would thus have a slope of $1.2 \times 0.024$, that is, 0.029, which is rather close to the slope of 0.035 for the PP50 ratio.

Thus, the above data suggest that the PP50 ratio is not only correlated with the amount of late depression but also correlated in a manner such that most of the quantitative variation in the PP50 ratio can be accounted for by the quantitative variation in the late depression. The data are thus well compatible with the notion of a fast onset of the late depression, implying that the PP50 ratio is not only a function of $p_{ves}$ but also of how much it is affected by the late depression.
3.6 | A fast onset of the late depression can explain the associated changes in the PP<sub>50</sub> ratio

As calculated above, a total removal of late depression in the 4:4 Ca:Mg solution would result in a 30% increase (on the average) of the PP<sub>50</sub> ratio in the 4:4 Ca:Mg solution (from 0.82 to 1.07). Thus, if a single-pulse conditioning stimulation induces a considerable fraction of the late depression, leaving little to be evoked 1 s later by the first presynaptic spike in a test PP<sub>50</sub> stimulation, the test PP stimulation will be associated with a PP<sub>50</sub> ratio that may (on the average) be up to 30% larger than that associated with an unconditioned PP<sub>50</sub> stimulation. The ~16% change in the PP<sub>50</sub> ratio observed in association with the late depression (Figure 2a) does then not have to reflect a decrease in P<sub>ves</sub>, but rather a fast onset of the depression.

Such a fast onset of the late depression might also account for the substantial difference in PP plasticity at short PP intervals between SLM-CA1 and SR-CA1 synapses indicated in Figure 1. To examine this notion, the PP ratios at short intervals (50–500 ms) were therefore “corrected” for the effect of late depression at some different values of Ca:Mg concentration ratio (7:1, 4:4, and 1:7). The “uncorrected” and the “corrected” PP ratios for the SLM-CA1 synapses are shown together with the PP ratios from SR-CA1 synapses at the same Ca:Mg concentration ratios in Figure 5e. This “correction” shows that the late depression does not fully explain the difference in PP ratio between the SLM and the SR synapses.

Thus, a fast onset of the late depression can potentially explain the increase in the PP<sub>50</sub> ratio associated with the late depression. Such a fast onset would also affect the fEPSP response during a brief high-frequency train activation, in particular if the late depression accumulates with the successive presynaptic activations during the train. If so, can a fast onset of the late depression also explain the reduced cumulative fEPSP response during a brief train activation that was not explained by vesicle depletion? We will first examine to what extent the late depression can accumulate with an increased number of conditioning presynaptic activations as occurs during a high-frequency train.

3.7 | Does the late depression accumulate with repetitive stimulation?

During brief high-frequency activation, there is a profound depression (at the population level) of the fEPSP. The unmasking of the late depression evoked by brief train activation is shown in Figure 6. A 2-impulse (20 Hz) and a 10-impulse (20 Hz) stimulation were given as conditioning stimulation, respectively, on the fEPSP amplitude and the PP<sub>50</sub> ratio 1 s later. The percentage change in the PP<sub>50</sub> ratio (blue closed circles) and the magnitude of the fEPSP depression (closed red circles) are plotted against the number of stimuli in the conditioning train. Included in the graph are also the values obtained when a single-pulse stimulation is used as conditioning stimulation (Figure 2). The upper blue dotted line indicates the change in the PP<sub>50</sub> ratio expected if the impact of late depression on the PP<sub>50</sub> ratio is fully removed. The lower blue dotted line indicates the percentage change in the PP<sub>50</sub> ratio given by a conditioning single-pulse stimulation. The dotted red lines and red closed circles indicate the estimated range of the unmasked fEPSP depressions. The percentage increase of the PP<sub>50</sub> ratio and the percentage decrease of the fEPSP magnitude obtained soon after the wash-in of a high concentration (0.8 mM) of CHA are plotted against each other for both SLM-CA1 (red closed circle) and SR-CA1 (blue closed circle) synapses. The straight line drawn in the graph indicates a 1:1 relation between fEPSP vs. PP<sub>50</sub> changes. Experiments were performed in the presence of 100-μM LY341495, 100-μM PTX, 50-μM D-AP5, and 1-μM CGP. Included in the graph are also the fEPSP amplitude vs. PP<sub>50</sub> changes induced by a lowered Ca:Mg ratio (inverted green triangle) and by CHA (4 nM) (black triangle) from the experiments shown in Figure 3. (c) The 1:1 relation shown in (b) is used to construct the percentage increase of the fEPSP amplitude expected from a given percentage decrease in the PP<sub>50</sub> ratio. Included is also the change in PP<sub>50</sub> ratio expected on the basis that the second fEPSP is unaffected by changes in release probability.
SLM-CA1 synapses (Figure 2a) that is not seen for the SR-CA1 synapses (Figure 2b). To examine the above notion that the late depression (with a fast onset) may contribute to this depression that develops during the train activation by accumulating with repetitive presynaptic activation, we repeated the experiment shown in Figure 2a but used a 2-impulse 20-Hz train or a 10-impulse 20-Hz train as conditioning stimulation and a PP_{50} stimulation as test stimulation (see schematic drawing in Figure 6a). The graph in Figure 6a shows that increasing the number of conditioning stimuli from 1 to 2 and to 10 had little effect on the magnitude of the late depression (continuous red line and red closed circles). Thus, while the late depression that followed a 2-impulse conditioning stimulation was greater (22.7 ± 1.5%, n = 9) than that following a single-pulse conditioning stimulation described earlier (18.6 ± 2.3%, n = 9), this difference was small and not significant (p > 0.05). This was also true for the late depression that followed the 10-impulse conditioning train (21.8 ± 2.8%, n = 4). On the other hand, as shown in Figure 6a (continuous blue line and blue closed circles), the late depression became associated with lower PP_{50} ratios. Thus, following a conditioning 2-impulse train, the PP_{50} ratio was only 9.4% (± 1.2, n = 9) greater than the unconditioned PP_{50} ratio. This increase of the PP_{50} ratio was significantly smaller (p < 0.05) than that associated with the single-pulse conditioning stimulation (16.1 ± 2.1%, n = 9). This effect on the PP_{50} ratio was even greater following the 10-impulse conditioning train, the PP_{50} ratio being even smaller than the unconditioned PP_{50} ratio (−10 ± 2%, n = 4).

Thus, this effect of an increased number of conditioning presynaptic activations on the magnitude of the late depression and its associated change in the PP_{50} ratio suggests that the process underlying the late depression actually accumulates with repetitive activation but that its increase is masked by a concurrent facilitation based on an increase in p_{ves}, such as augmentation (Garcia-Perez & Wesseling, 2008). This masking is not necessarily also operating during the brief train activation to oppose whatever contribution the late depression could have on the depression that develops during the train. To assess that potential contribution, we will thus have to unmask the late depression from this concurrent facilitation.

### 3.8 Unmasking of the late depression

To unmask the late depression from the concurrent facilitation, we made use of the approximate 1:1 relation between the percentage decrease of the fEPSP amplitude and percentage increase of the PP_{50} ratio that results from changes in the Ca:Mg ratio and CHA application (Figure 6b; see also Section 2). This 1:1 relation was used to construct the relation between the percentage decrease of the PP_{50} ratio and the percentage increase of the fEPSP, that is, the fEPSP increase that will mask the depression (Figure 6c, closed red circles). Included in this graph is also the relation that would be observed if only the first fEPSP would be affected by the change in p_{ves}, leaving the second fEPSP unaffected (closed blue circles) (see Section 2).

Because we can have no knowledge regarding the actual change in the PP_{50} ratio that, in the absence of the concurrent facilitation, is associated with a 2-impulse or a 10-impulse conditioning train, the unmasking was done using two baseline conditions. That is, we used either the change in the PP_{50} ratio observed after a single-pulse conditioning stimulation (16.1%, dashed blue line in Figure 6a), giving a conservative estimate of the unmasking, or we used the change in the PP_{50} ratio that would be observed if the conditioning stimulation fully removes the effect of late depression on the PP_{50} ratio (~30%, dotted blue line in Figure 6a). Based on the percentage change of the PP_{50} ratio from these baseline values (16.1% or 30%) to the experimentally observed values of 9.4% and −10% after 2-impulse and 10-impulse train conditioning, respectively, and the relation shown in Figure 6c (closed red circles), the magnitude of the fEPSP facilitation that masks the depression can be computed. As shown in Figure 6a (dotted red lines and red closed circles), this calculation suggests that a 2-impulse train results in a depression of 28 to 38% (rather than the observed 22.7%). Similarly, following a 10-impulse tetanus, the depression would be between 44% and 50% rather than the 21.8% observed. Considering that the depression is likely close to maximally activated during the 10-impulse train, the increase in the PP_{50} ratio would in the absence of the facilitation be close to 30%, indicating that the higher value (50%) would be closer to the truth. These calculations would then suggest that the late depression increases considerably with repetitive activation from the ~18% observed after a single-pulse conditioning stimulation. Given first order kinetics, the ~50% depression (1 s after a 10-impulse 20-Hz train) would translate to a depression of ~60% at the end of the train, that is, to a depression about three times the ~20% that is observed 1 s after a single-pulse conditioning stimulation.

Thus, given that the process underlying the late depression has a fast onset and that the masking (augmentation) process has too little time to develop to any significant degree during the brief train activation, this amount of accumulation of the depression implies that it may contribute substantially to the depression that develops during a brief high-frequency train. If so, there
should be a close correlation between the amount of late depression, as observed 1 s after a conditioning single-pulse stimulation, and the depression during a brief high-frequency train.

3.9 The late depression can explain the preferential depression of the initial part of a high-frequency train

In common with the PP50 ratio and the late depression, the depression that develops during a 20-Hz 10-impulse train varies considerably within each postnatal day, and there is only a small, and statistically nonsignificant (p > 0.05), dependence on postnatal age (Figure 7a). On the other hand, this depression reached at the end of the train (fEPSPs8–10/fEPSP1) was found to be strongly correlated with the PP50 ratio (Figure 7b), as well as significantly correlated (p < 0.005) with the late depression following a single-pulse conditioning stimulation (Figure 7c, red closed circles). To substantiate this latter correlation, based on a limited set of values, we used the strong correlation between the PP50 ratio and the late depression (Figure 5c) to convert the strong correlation between the PP50 ratio and the depression at the end of the train (Figure 7b) to a relation between the late depression and the depression at the end of the train (Figure 7c, blue closed circles). The linear regression line through the values in Figure 7c suggests that there would still be a substantial depression at the end of the train (to a fEPSPs8–10/fEPSP1 value of ~0.65) even in the absence of the late depression. Nonetheless, the larger the magnitude of the late depression (i.e., the smaller the PP ratio [1 s]), the larger the depression at the end of the train. If we consider the effect of the late depression on this remaining part of the fEPSPs8–10/fEPSP1 value (~0.65), and setting that value to 1.0, the slope of the regression line in Figure 7c would increase from 1.9 to ~3. This means that the accumulation of the late depression during the train activation calculated above (three times the late depression following a single-pulse conditioning stimulation) would quantitatively suffice to account for the relation between the late depression and the depression reached at the end of the train (Figure 7c).

These results would suggest that the process underlying the late depression is strongly activated during the brief high-frequency train activation. A single-pulse conditioning stimulation applied 1 s before such a train (as in Figure 2a) would then add quite little to the depression that will be developed during the train stimulation itself. The single-pulse conditioning stimulation will thus largely affect only the initial part of the train, leaving the later part of it essentially unaffected, as also observed (Figure 2a). Thus, the seemingly specific effect of the late depression on the initial release does not have to reflect a specific depletion of the vesicles within an immediately releasable pool of vesicles but rather reflect a fast onset of the late depression.

Thus, a fast onset of the process underlying the late depression, and its accumulation with repetitive presynaptic activation, can then account for the fact that this depression process exhibits features of mechanisms based

![Figure 7](image-url)
on a change in $p_{\text{ves}}$ (an increase of the PP$_{50}$ ratio) as well as on vesicle depletion (a reduced cumulative synaptic response) but is not explained by any of these two mechanisms. Instead, as will be detailed in Section 4, it will be argued that a prolonged action potential-induced release site inactivation is a mechanism that best can explain our data.

3.10 Interaction between the late depression and the AMPA silencing during brief train activation

The analysis has up till now proceeded as if the AMPA silencing has had no influence on the observed depression. However, at these stimulation frequencies (0.1–20 Hz), there will also be depression caused by the AMPA silencing. Because very low frequencies (0.03–0.05 Hz) will induce only AMPA silencing (Ma et al., 2016), the contribution of the late depression to the total depression at any frequency >0.05 Hz could then possibly be uncovered by simply “subtracting” the AMPA silencing from the total depression. However, the onset kinetics of AMPA silencing is unknown. For example, when examined at SR-CA1 synapses, the depression induced by 1-Hz stimulation clearly lags that induced by 0.2-Hz stimulation when plotted as a function of stimulus number (Strandberg et al., 2009). Whether this lag depends on a slow onset of this process, or reflects the activation of e.g., a presynaptic facilitation masking the AMPA silencing, is unknown. The extent to which AMPA silencing contributes to the various depressions described above is thus unclear.

In order to explore this question of onset kinetics of the AMPA silencing and to possibly derive the activation characteristic of the late depression itself, forty 20-Hz 10-impulse trains were given at a rate of 1/min ($n = 5$). Twenty such trains were first given alone, followed by 20 such trains that each was preceded by a 1-Hz 20-impulse train (see schematic drawing in Figure 8a). To ascertain that the depression that develops during the 1-Hz activation is not a short-lasting depression (~1 s) distinct from that underlying the late depression, the 20-Hz 10-impulse train was positioned 5 s after the end of the 1-Hz 20-impulse train. Using this procedure, the AMPA silencing will show up as the decline of the first fEPSP at each of the successive stimulation trials (Figure 8b, closed red circles), that is, as the depression remaining 1 min after the stimulation. To observe the impact of the AMPA silencing on the fEPSP response evoked by the 20-Hz 10-impulse train, the response to the very first such train was compared with that averaged for the last five of the first 20 stimulation trials, at which time the AMPA silencing had levelled off. As shown in Figure 8c, these responses were essentially overlapping after normalization to the same peak amplitude of the first fEPSPs. When measured at the end of the train, there was no significant difference (paired $t$ test, $p = 0.40$) between the depression observed for the very first train (80.6 ± 1.7%, red closed circles) and the five last trains (79.5 ± 1.7%, blue closed circles), respectively. Similarly, when matching the fEPSP response to the very first 1-Hz 20-impulse train to that averaged for the last five such trains (Figure 8d), the depression observed for the very first train (32.0 ± 1.2%, red closed circles) did not significantly differ (paired $t$ test, $p = 0.22$) from the depression observed for the last five trains (30.5 ± 1.1%, blue closed circles). It should be noted that when stimulated at 0.05-Hz, the depression (AMPA silencing) after 20 stimuli is ~10% (Ma et al., 2016). It would thus seem that the AMPA silencing that follows each stimulation develops too slowly to have a significant impact on the fEPSP responses during the brief 1- and 20-Hz train stimulations.

Comparison between the fEPSP responses to the 20-Hz 10-impulse train before and after the addition of the 20-impulse 1-Hz trains (Figure 8e) shows that the first fEPSP became depressed by 15.5 ± 1.5% ($n = 5$) 5 s after the 1-Hz train. This depression can be compared with a late depression of ~10% 5 s after a single-pulse conditioning stimulation (Figure 1a). This 50% increase in depression given by the 1-Hz 20-impulse train, as compared with that given by a single-pulse stimulation, well matches the increase in depression from 22.0 ± 0.5% to 30.5 ± 1.1% ($n = 5$) that occurs from the second fEPSP to the last fEPSPs during the 1-Hz 20-impulse train (Figure 8d). The depression developing during this 1-Hz train is thus well accounted for by the late depression.

The depression induced by the 1-Hz train was also found to be largely specific for the early part of the 20-Hz 10-impulse train (Figure 8e). In fact, in this case, the average of the last three fEPSPs in the “depressed” train did not significantly differ from that of the control train (22.9 ± 2.8% vs. 22.6 ± 2.4%, $n = 5$, $p = 0.6$, paired $t$ test). Moreover, the depression produced by the 1-Hz 20-impulse train was not associated with an increased PP$_{50}$ ratio but with a decreased such ratio (~6.1 ± 1.9%, $n = 5$, $p < 0.05$). Thus, as is the case with the late depression following the 2-impulse and 10-impulse 20-Hz trains, the depression that develops during the prolonged 1-Hz train is likely partly masked by the development of a concurrent process that enhances $p_{\text{ves}}$. Using the same calculation for unmasking as used above, and assuming that the change in PP$_{50}$ during the depression decays in parallel with the depression itself, this unmasking suggests that the depression at the end of the 1-Hz 20-impulse train is ~42% to 48%, rather than the 30.5%
observed. It would thus appear that a facilitation process also impacts the fEPSP response during the 1-Hz activation.

Thus, the above results show that the AMPA silencing has had virtually no impact on the depressions induced by the brief train activations used in the present study. They also show that the process underlying the late depression does accumulate also at lower frequencies such as 1 Hz but that much of this accumulation is masked by an opposing facilitation. On the other hand, the above experiments also indicate that the AMPA silencing is likely to considerably contribute to the depression induced by more prolonged presynaptic activation.
3.11 Contribution of the late depression and the AMPA silencing to the depression induced by a prolonged 1-Hz stimulation

Using the above data presented in Figure 8b–d, the contributions of the late depression (with associated facilitation) and of the AMPA silencing to the depression that develops during a prolonged 1-Hz stimulation of a naïve synaptic input may be evaluated. In this calculation, the late depression (with associated facilitation) is assumed to reach a steady-state value at the end of the 1-Hz 20-impulse train, and its contribution to the further depression will stay at this value (Figure 8f). The AMPA silencing, on the other hand, will be represented by the depression accumulating 1 min after each 20-Hz 10-impulse train when given alone (Figure 8b). Because we are interested in the AMPA silencing produced by the 1-Hz 20-impulse train, the amount of AMPA silencing obtained by the 10-impulse trains is multiplied by two (Figure 8g). This is because the amount of AMPA silencing is a function of the number of stimuli, not their frequency (Strandberg et al., 2009; Xiao et al., 2004). Because the late depression and the AMPA silencing are independent processes, as indicated by their lack of interaction (Figure 8c,d), these depressions were multiplied with each other, resulting in the combined depression shown in Figure 8h. This combined depression is plotted in Figure 8i together with the actual depression produced by such a prolonged 1-Hz train, indicating a good agreement between the constructed and the experimental curves.

Thus, this agreement between the constructed and the experimental curves supports the above notion (Figure 8f) that the additional depression developing after more than 20 s of 1-Hz stimulation is explained by AMPA silencing while that of the initial 20 s is totally dominated by the late depression.

3.12 The late depression is homosynaptic

As previously shown (Ma et al., 2016), stimulation of a separate set of afferents in SLM does not induce any late depression in the test input, indicating that this depression is homosynaptic. However, to exclude possible local heterosynaptic effects, or heterosynaptic effects arising using prolonged low-frequency stimulation, we examined the effect of various receptor antagonists on the late depression following a single-pulse conditioning stimulation as well as on the depression induced by prolonged low-frequency stimulation. It may then first be noted that the above experiments were performed in the presence of GABA_A, GABA_B, and NMDA receptor antagonists, indicating that activation of these receptors is not necessary for the depression. As also described previously (Ma et al., 2016), the 1-Hz-induced depression was not affected by the broad spectrum mGlu receptor antagonist LY341495. In the neonatal rat, glutamate can depress transmission acting via presynaptic kainate receptors (KARs) (Lauri et al., 2006). However, after application of the KAR antagonist UBP 302 (10 μM), the late depression was 20.5 ± 2.5% (n = 5), which did not significantly differ from the 15.6 ± 3.8% (n = 5) obtained prior to the application of this drug (p > 0.05, paired t test). Likewise, in the presence of this drug, the 1-Hz stimulation resulted, after 250 stimuli, in a depression of 47.4 ± 2.6% (n = 5), which did not differ from that observed before application of this drug (43.0 ± 2.1% [n = 5], P > 0.05, paired t test).

The application of the adenosine A1 receptor antagonist DPCPX (200 nM) resulted in a decreased PP_{50} value, indicating the removal of a tonic adenosine-mediated depression of release. Nonetheless, the late depression was similar in the presence of DPCPX (21.1 ± 2.1%, n = 7) as before its application (19.5 ± 2.1% [n = 5] (p > 0.05, paired t test). Likewise, in experiments only made in the presence of DPCPX, the 1-Hz stimulation resulted after 250 stimuli in a depression of 46.9 ± 1.6% (n = 6), a depression that agrees well with that observed before any drug application in other experiment described here. Because endocannabinoid receptors are present in SLM (Xu et al., 2010), we also examined the effect of the endocannabinoid receptor antagonist AM251 (2 μM) on the depression induced by 250 stimuli at 1 Hz. Neither the initial depression from the first to the second fEPSP (17.2 ± 2.1%, n = 6) nor the depression reached after 250 stimuli (48.8 ± 1.5%, n = 6) differed from that obtained prior to the application of this drug (17.2 ± 1.6%, n = 6; 47.8 ± 1.1%, n = 6, respectively) (p > 0.05 in both cases).

Thus, these results thus support the notion that the late depression is homosynaptic and intrinsic to the activated synapse itself.

3.13 Late depression of the NMDA receptor-mediated fEPSP

As noted earlier, the preferential action of the late depression on the initial responses during a brief train activation is much more compatible with a modulation of the presynaptic release characteristics rather than of the postsynaptic receptor properties. The late depression should then not only be observed for the AMPA component of
the fEPSP but also for the NMDA component. To obtain an isolated NMDA fEPSP, a 2:0.1 mM Ca:Mg solution was used, and AMPA, GABA_A, and GABA_B antagonists (see Figure 9) were present in the solution. A low concentration of tetrodotoxin (2 nM) was also added to reduce excitability. Paired stimuli were given at 1/min using 20, 10, 5, and 1 s interstimulus intervals. As shown in Figure 9a, also the NMDA fEPSP exhibits a late depression. Compared with that of the AMPA fEPSP that peaks at about 20%, the late depression of the NMDA fEPSP is significantly larger, peaking at 33.4 ± 1.9% (n = 10). Nevertheless, when normalized to the same peak amplitude of depression as that of the AMPA fEPSP (dashed line in Figure 9a), the depression of the NMDA fEPSP decays with about the same time course as the depression of the AMPA fEPSP, in line with a presynaptic locus of the late depression.

It should be noted that these experiments were complicated by the fact that the 1/min PP stimulation by itself resulted in a large NMDA fEPSP depression (58.3 ± 2.8%, n = 14, after 60 paired stimuli) (Figure 9b) that interfered with the measurements of the late depression. Thus, when computed for each stimulus pair and plotted against stimulus number (Figure 9c), the late depression changed from 42.3 ± 2.3% for the first stimulus pair to 30 ± 2.1% for the last stimulus pairs (p < 0.005). Inspection of the NMDA fEPSPs at the beginning and at the end of the stimulation, respectively, revealed that the 1/min-induced depression was associated with a substantial shortening of the decay phase of the NMDA fEPSP (Figure 9d, upper records). Such a shortening, albeit less, was also observed when comparing the first and second fEPSPs of the first few stimulus pairs (Figure 9d, middle records) but was not evident when comparing the first and second fEPSPs for the last stimulus pairs (Figure 9d, lower records). The late depression of the NMDA fEPSP is thus contaminated by another depression process, and its value obtained at the

**FIGURE 9** Late depression of the NMDA fEPSP. (a) The depression of the NMDA fEPSP induced by a preceding single-pulse stimulation is plotted against interstimulus interval using a log time plot (n = 10). For the construction of this graph, each experiment was started with the longest interstimulus interval and ending with the shortest, and five sweeps were given for each interval. Example fEPSPs (n = 5 sweeps) taken at intervals indicated in the graph. Included in the graph is also the corresponding depression of the AMPA fEPSP, taken from values shown in Figure 4 (except those obtained in 1:7 Ca:Mg). For comparison, the NMDA fEPSP depression was normalized to that of the AMPA fEPSP (with respect to the values at a 1-s interstimulus interval) and replotted in the graph with its regression line (dashed line). (b) Depression of the NMDA fEPSP when subjecting the naïve (previously unstimulated) synapses by the 1/min repetition of the PP stimulation (1-s interstimulus interval) (see example fEPSPs). The amplitudes of the first and second fEPSPs of the pair are plotted as a function of sweep number. (c) The late depression of the NMDA fEPSP in experiments such as that shown in (b) is plotted against sweep number, indicating a larger depression of the NMDA fEPSP at the onset of the 1/min stimulation than later. (d) Example fEPSPs taken at time points indicated in (b) (a and c, n = 5 sweeps; b and d, n = 20 sweeps). (e) Example fEPSPs (n = 60 sweeps) are shown expanded and superimposed to illustrate the afferent volley. The experiments were made in the presence of 2-mM Ca^{2+}, 0.1-mM Mg^{2+}, 100-μM PTX, 1-μM CGP, 15-μM CNQX, and 2-nM TTX (n = 14 exps.)
end of the stimulation should represent a truer late depression of the NMDA fEPSP. Thus, for the experiments shown in Figure 9a, we prestimulated with 30 single stimulations (at 1/min) prior to applying the PP stimulation to reduce (but perhaps not completely) the impact of the 1/min-induced depression on the magnitude and time course of the late depression of the NMDA fEPSP.

Because the experiments examining the late depression of the AMPA fEPSPs were done in the presence of an NMDAR blockade while those examining late depression of the NMDA fEPSP naturally were performed without such a blockade, we also examined to what extent such a blockade could have impacted the depression of the AMPA fEPSP. In these experiments, the late depression was somewhat greater than usual, but it did not significantly change following the application of 50-μM D-AP5 (23.8 ± 1.1% vs. 25.2 ± 1.2%, n = 7) (p > 0.05, paired t test).

Thus, although there is some mismatch in the magnitudes of late depression of NMDA and of AMPA receptor mediated fEPSPs, the overall agreement in time course of the late depression of these fEPSPs, and the existence of factors that may explain the mismatch, strongly supports that the late depression is indeed presynaptically located.

3.14 Late depression is not associated with a change in the afferent volley

Because of temporal overlap between the afferent volley and the synaptic current, estimation of afferent volley amplitude and time course can be more favourable when recording NMDA than AMPA fEPSPs due to the slower onset kinetics of the former. Thus, for the experiments shown in Figure 9, the responses to the first and second stimulations for each experiment were averaged, and the peak amplitude of the afferent volley in response to the second stimulation was expressed as a fraction of that in response to the first stimulation. As also indicated by the records in Figure 9e where these volleys from one such experiment are shown superimposed, there was no difference in amplitude between these afferent volleys (second/first volley peak amplitude 0.99 ± 0.005, n = 14), or in their time course.

4 DISCUSSION

The present study on second postnatal week rats shows that a single-pulse stimulation of synapses onto the distal dendritic tree of CA1 pyramidal cells (SLM-CA1 synapses) results in a biphasic depression of release, the late phase of which requires ~20 s to fully decay. While this depression can only be observed in isolation at times later than 1 s after the stimulation, our data suggest that the process underlying this depression has a fast onset (<50 ms) and thus could contribute to synaptic plasticity also at short times after the single-pulse stimulation. In that sense the term “late depression” used in Section 3 may be a misnomer. Nevertheless, to be consistent within this article, we will continue using this term. This late depression was found to be specific for the SLM-CA1 synapses because it could not be induced in the more proximal SR-CA1 synapses originating from the CA3 region, despite a wide experimental variation in basal release conditions. While some data point to vesicle depletion, or to a Pves reduction, as an underlying process, other data clearly contradicted such explanations. What will be proposed below is that this late depression is explained by a stimulation-induced release-independent release site inactivation that results in depression in the low-frequency (0.1–1 Hz) range but that may also operate at higher frequencies to contribute to the profound depression observed during such stimulation of these synapses.

While the peak magnitude of the late depression following a single-pulse stimulation was not large, the fEPSP (on average) being reduced to ~80% of its control value, our data suggests that the depression accumulates with repetitive presynaptic activation. Thus, according to these data, as much as about half of the activated synapse population would be inactivated for several seconds following only half a second of 20-Hz presynaptic activity, implying a quantitatively significant role of this depression process.

Even though the present study was made on second postnatal week synapses, and the late depression became significantly smaller during the second postnatal week, this depression is likely not in itself a developmentally restricted phenomenon. The characteristics of the depression presently described agree well with those described in older animals for the entorhinal projection to the dentate granule cells (Teyler & Alger, 1976; White et al., 1979), making it likely that we are dealing with mechanically the same form of depression. When examined longitudinally for these dentate gyrus synapses the frequency-dependent depression observed in second postnatal week animals was not found to differ from that observed in 1- to 2-month-old animals (Abrahamsson et al., 2005), indicating little age dependence at least for these synapses. However, while in the present study the late depression was associated with an increased PP50 ratio, no such change was found in that study (Abrahamsson et al., 2005). It should then be noted that changes in the PP50 ratio in those experiments...
(Abrahamsson et al., 2005) were evaluated using PP stimulation at 0.2 Hz, a stimulation that, according to the present data, will likely mask the change in $P_{50}$ ratio by a concurrent facilitation process. Moreover, the increase in the $P_{50}$ ratio observed in the present study was also observed earlier (White et al., 1979), supporting the above interpretation of the absence of changes in the $P_{50}$ ratio in the Abrahamsson et al. study. A developmental reduction of the late depression at the SLM-CA1 synapses may rather be specific for these synapses and could suggest that the entorhinal projections to the CA1 region may develop along a different trajectory than those to the dentate gyrus, possibly because of different functional roles of these entorhinal projections in the more mature animal.

4.1 | The late depression is explained by a presynaptic modification

Short-term synaptic plasticity is generally thought to reflect changes in the release properties of the presynaptic terminal. For the SLM-CA1 synapses, we observed a late depression not only for the AMPA fEPSP but also for the NMDA fEPSP, both these depressions decaying within about 20 s with much the same time course. This similarity would seem to constitute definite evidence for a presynaptic locus of expression. However, the depression of the NMDA fEPSP was larger in magnitude than that of the AMPA fEPSP. A simple solution would be that the depression is modulated by activation of presynaptic NMDA receptors, these receptors being blocked in our experiments except in those in which the depression of the NMDA fEPSP was examined. However, application of an NMDA receptor antagonist did not affect the late depression of the AMPA fEPSP. Another factor that could contribute to such a discrepancy was the fact that PP stimulation of NMDA fEPSPs, even at a very low rate (1/min), resulted in an additional form of depression of the NMDA fEPSP. This depression was associated with a change in NMDA fEPSP time course that was similar to that of a depression observed in SR-CA1 synapses (Gambrill et al., 2011) attributed to a postsynaptic down-regulation of GluN2B-containing NMDA receptors. While steps were taken to saturate this depression prior to the quantitative evaluation of the late depression of the NMDA fEPSP, some remnants of such a depression could have contributed to the larger late depression of the NMDA fEPSP. Moreover, when using fEPSPs to monitor the synaptic response, the inherent voltage dependent nature of NMDA receptor channel opening might also contribute to some discrepancy in the magnitudes of depression of AMPA and NMDA fEPSPs. Nonetheless, irrespective of the fact that we may have left part of this discrepancy unexplained, it seems difficult to envisage a postsynaptic modification that preferentially affects only the initial part of the synaptic response during a brief train activation.

A presynaptic depression does not necessarily have to be located in the presynaptic terminal but could alternatively be explained by axonal changes. However, our data suggest that changes in axonal activation or conduction do not explain or contribute to the depression. Thus, the axonal volley, which was altered in a 1:1 relation with the fEPSP when the fEPSP was decreased by reduction in stimulation strength, was unaltered both in magnitude and time course when evoked at the peak of the late depression.

4.2 | The late depression is homosynaptic

A late depression, although briefer, with similar release-independent properties as shown here for the SLM-CA1 synapses, has been described in a neocortical synapse and tentatively attributed to the release of a modulator affecting the presynaptic terminal (Castro-Alamancos & Connors, 1997). As shown previously (Ma et al., 2016), activation of one set of SLM-CA1 synapses did not induce or affect the late depression in a separate set of synapses in the same region; that is, this depression seems homosynaptic. Any modulator would thus have to act in a very restricted manner. Moreover, as shown here, such a tentative modulator action cannot rely on GABA, glutamate, adenosine, or endocannabinoid release. Although we cannot fully exclude participation of some released modulator, we propose, based on the results above, that the depression is intrinsic to the synapse itself.

4.3 | The late depression is not explained by a $p_{ves}$ reduction

An intrinsic mechanism for the late depression could be an activity-induced reduction in $p_{ves}$. For instance, the release-independent depression at autapses onto cultured hippocampal pyramidal cells (Sullivan, 2007) was tentatively attributed to a calcium-activated inhibition of calcium entry, resulting in a reduced $p_{ves}$. In line with such a mechanism, the late depression was found to be associated with an increased $P_{50}$ ratio. However, a depression based on a $p_{ves}$ reduction should predict that only the initial part of the cumulative synaptic response during a brief high-frequency train should be affected. This is because the same amounts of vesicles will
eventually be released, albeit somewhat slower because of the lower $p_{\text{ves}}$. However, this expected convergence was not observed with respect to the late depression. Moreover, when experimentally altering $p_{\text{ves}}$ by decreasing the Ca:Mg ratio, or adding an adenosine A1 receptor antagonist, the effect on the cumulative synaptic response distinctly differed from that induced by the late depression. The observed increase in the $PP_{50}$ ratio associated with the late depression can also be accounted for in manners not related to a $p_{\text{ves}}$ reduction, such as a selective inactivation of high release probability synapses, or by a fast (within milliseconds) onset of the late depression (see below).

It can further be noted that the depression 1 s after a brief train activation (rather than after a single-pulse stimulation) was associated with an actual decrease in the $PP_{50}$ ratio, explained by the induction of a concurrent facilitation process, such as augmentation, partly masking the late depression. If the late depression, in common with augmentation (Garcia-Perez & Wesseling, 2008), should be explained by a $p_{\text{ves}}$ modification, these modifications being in opposite directions, the net result of a decreased $PP_{50}$ ratio following the train stimulation should lead to the expectation of a train-induced fEPSP facilitation. Instead, a considerable depression was observed.

### 4.4 The late depression is not explained by vesicle depletion

The effect of the late depression on the fEPSP response to brief train activation was largely restricted to the initial part of the train leaving the later part of the train much less affected. This result seems most easily explained by a lingering depletion of the immediately releasable pool of vesicles following the conditioning single-pulse stimulation. However, when release probability was altered by changes in the Ca:Mg ratio that caused large changes in PP plasticity at short PP intervals, the depression at long PP intervals ($\geq 1$ s) was not affected. Such independence of depression from changes in the release fraction of the vesicle pool has previously been observed at neocortical synapses (Castro-Alamancos & Connors, 1997), at autapses onto cultured hippocampal pyramidal cells (Sullivan, 2007), as well as at Aplysia cholinergic synapses (Doussau et al., 2010), and has there been taken to exclude depletion of the readily releasable vesicle pool as an explanation for these depressions. In addition, simulations on a release model based on experimental data from neonatal SR-CA1 synapses (Hanse & Gustafsson, 2001b, 2002) show that a reduction in release probability explained by vesicle depletion would not be associated with an increased $PP_{50}$ ratio. To account for the release independence of the late depression, a depletion of the vesicle pool would have to be combined with a facilitation ($p_{\text{ves}}$ increase) that, like the depletion, should increase when release probability becomes higher, these two processes counteracting each other. However, in that scenario, the late depression should be associated with a decreased $PP_{50}$ ratio (due to the higher $p_{\text{ves}}$) and not the increased $PP_{50}$ ratio that is observed.

### 4.5 Is the late depression explained by release site inactivation?

Studies of cholinergic neurons in the buccal ganglion of Aplysia have shown that an increase in stimulation rate from 0.025 to 1 Hz results in a synaptic depression that is independent of large changes in Ca:Mg ratio (Doussau et al., 2010). Stationary analysis of fluctuations in synaptic amplitude under various conditions of release probability suggested that a change in the number of release sites underlies this depression. What in turn may underlie such change in the number of release sites is unclear but some studies have indicated use-dependent effects on the release machinery causing release site inactivation (for review, see Fioravante & Regehr, 2011). If the neonatal SLM-CA1 synapse, like the SR-CA1 synapse (Hanse & Gustafsson, 2001c), only contains a single release site, such release site inactivation would be equivalent to a presynaptic silencing of that synapse. This explanation for the late depression will naturally explain the reduced cumulative fEPSP response, but can it also explain the change in the $PP_{50}$ ratio?

### 4.6 Heterogeneity in release properties among the SLM-CA1 synapses as an explanation for changes in $PP_{50}$

For the neonatal SR-CA1 synapses, the release probability in response to a single action potential (or the first action potential in a train stimulation) can vary from close to zero to close to one among the synapses, and the release during a high-frequency train can vary from profound depression to strong facilitation (Hanse & Gustafsson, 2001a, 2001d). The fEPSP response of SR-CA1 synapses to a 20-Hz train (Figure 2b) is thus not representative for any SR-CA1 synapse but is the sum of the response of synapses with widely different release dynamics. While no similar study of individual SLM-CA1 synapses has been performed, experiments on third postnatal week SLM-CA1 synapses (Speed & Dobrunz, 2009), using MK-801 to assess release probability, indicate that
such release heterogeneity also exists for the early postnatal SLM-CA1 synapse population. If so, a stimulation-dependent selective inactivation of SLM-CA1 synapses with the highest release probability to a single action potential would result in (i) a release-independent depression, (ii) an increased PP$_{50}$ ratio (because of the removal of high release probability synapses with a low PP$_{50}$ ratio), (iii) a depression that essentially only affects phasic release (because of the small later release of high release probability synapses), and (iv) a decrease in the cumulative fEPSP response. Such a selective inactivation of high release synapses would be efficient in reducing the response to low-frequency activation of the synapses, that is, in habituation, and would thus make functional sense. However, while there was a statistically significant reduction of the later release in association with the late depression, potentially corresponding to the later release otherwise produced by inactivated high release probability synapses, this reduction was very small and can be better accounted for in an alternative manner (see below). In addition, because no late depression was observed for the SR-CA1 synapses, such a selective inactivation of high release probability synapses cannot be a general property of such synapses but should be specific for those in SLM. Alternatively, the late depression may not only be selective with respect to which synapses it affects but also what release it affects. Thus, during a train activation the initial (phasic) release may be distinct from the later (tonic) release either due to the existence of two functional vesicle states (Neher & Brose, 2018), or which release locations within a release site (Gustafsson et al., 2019) that are used, indicating that phasic and tonic release can be separately controlled. The late depression may then be explained as a selective depression of phasic release. However, as mentioned above, the late depression could be associated with a small, but statistically significant, reduction also of the later release. Moreover, both the above explanations are based on a depression with a slow onset that does not interfere with fEPSP responses during a brief train stimulation, and would thus fail to account for the observed correlation between the depression 1 s after a single-pulse conditioning stimulation and the depression that develops during a brief high-frequency train.

4.7 Fast onset of the late depression as an explanation for a change in the PP$_{50}$ ratio and for a preferential depression of initial release during brief train activation

Observations suggesting a fast onset of the late depression at the perforant path–granule cell synapses have earlier been made (White et al., 1979). In the present study, we observed a strong correlation between the amount of late depression and the PP plasticity at a 50-ms interstimulus interval (PP$_{50}$). Because large changes in this latter PP plasticity brought about by large changes in the Ca:Mg ratio had no effect on the late depression, this correlation must be explained by the late depression affecting the PP$_{50}$ ratio, rather than the other way around. A release-site inactivation that affects synapses independently of their release probability and that is induced within millisecond after the arrival of the action potential can explain such a causal correlation. In Figure 10 is schematically indicated how such a release site inactivation following a single-pulse stimulation can account for our experimental results. As illustrated in this figure, such an inactivation would reduce the number of synapses contributing to the second fEPSP of a PP$_{50}$ stimulation (point a) and thus reduce the control PP$_{50}$ ratio, as well as result in depression at 1 s after the single-pulse stimulation (point d). The second fEPSP of the PP$_{50}$ stimulation that is elicited during the depression (at point e) will then be less affected (than in the control situation) by inactivation, because a considerable part of the inactivation has already been induced by the spike producing the late depression. Such an occlusion effect will then result in a PP$_{50}$ ratio that is higher than the control PP$_{50}$ ratio.

A fast onset of release site inactivation can also explain the preferential depression of the initial part of the synaptic response during a brief train activation, this explanation again based on occlusion. Because the late depression was found to accumulate with repetitive activation, much of it will have been induced at the end of the 20-Hz 10-impulse train (point c, naïve train). A single-pulse stimulation preceding such a train by 1 s will depress the previously, nondepressed first fEPSP in the train (point d) but will have little impact on the later, already depressed, fEPSPs (point h, depressed train). In fact, the small (~2%) depression of the last few fEPSPs in the train may reflect such a small impact from the conditioning single-pulse stimulation. Thus, given a fast onset of the late depression, a release site inactivation can explain our experimental data, including the observed changes in the PP$_{50}$ ratio, the decrease in cumulative release during brief train activation, as well as a seemingly selective depression of initial release.

Thus, a release site inactivation with a fast onset and a slow decay, and that accumulates with repetitive spikes, can account for our data. However, the problem with this explanation is that we have no mechanistic basis for it. To our knowledge, no one has described how a presynaptic action potential can give rise to such a release site inactivation. It has been reported recently that every
The presently described depression process is not the only process that produces synaptic depression at the SLM-CA1 synapses. When starting with naïve (previously nonstimulated) synapses, the late depression process was found to contribute ~60% of the overall ~50% depression that is induced during 120 impulses at 1-Hz stimulation (Figure 8). This contribution of the late depression process to the 1 Hz-induced depression was found to be rapidly established in that about two thirds of presynaptic action potential, in addition to triggering release, also results in the distancing of vesicles from the membrane in the release zone, thus prohibiting further release (Kusick et al., 2020). However, this effect was found to be quite short-lasting (10–15 ms) or, if more prolonged (seconds), did not obey first-order kinetics. Nevertheless, it may be speculated that such a mechanism for release site inactivation could be adaptable in its kinetics in order to create synapses depressing strongly to both low and high-frequency activation.

### 4.8 The contribution of late depression to depression developing during train activation

The synaptic depression during a high-frequency train is commonly seen as resulting from the rapid emptying by the first few stimulations of an immediately releasable pool of vesicles, the release thereafter given by vesicles that become more or less slowly recruited and primed to the now vacant release locations. However, as noted above, a fast onset of the late depression would also contribute to the high-frequency-induced depression. In

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**FIGURE 10** Schematic illustration of the proposed interaction between the late depression and the PP$_{50}$ ratio. The first stimulation occurs at time = zero, and point (a) indicates the percentage of the synapses that are inactivated 50 ms after that first stimulation. The second stimulation occurring at this time will now activate fewer synapses, which will result in a PP$_{50}$ ratio lower than in the absence of the late depression. The second stimulation will also produce further inactivation, that is, move the number of inactivated synapses to point (b). Further 20-Hz stimulation will increase the number of inactivated synapses, reaching asymptotically point (c) after 10 stimulations. Following the single-pulse stimulation, the inactivation will decay, and after 1 s reach point (d). Similarly, the inactivation produced by the 20-Hz 2-impulse and 10-impulse stimulation, respectively, will decay to points (f) and (g). A stimulation given 1 s after a single-pulse stimulation (point (d)) will now activate a reduced number of synapses, which will show up as a late depression. It will also produce some additional inactivation, moving the number of inactivated synapses to point (e), a second stimulation that occurs at point (e), that is, 50 ms after the single-pulse stimulation at point (d), will now activate fewer synapses than the stimulation at point (d), which will again result in a PP$_{50}$ ratio lower than in the absence of the late depression. However, because fewer synapses became inactivated when moving from point (d) to point (e) than when moving from time zero to point (a), this lowering of the PP$_{50}$ ratio will now be smaller. Thus, the late depression will be associated with a larger PP$_{50}$ ratio than the PP$_{50}$ stimulation that occurred at time zero. Further 20-Hz stimulation will increase the number of inactivated synapses, reaching asymptotically point (h) after 10 stimulations. Because now point (h) is on the same level of inactivated synapses as point (c), the final level of depression induced by this train will be the same as that induced by a train evoked at time zero. Thus, only the initial part of the train evoked 1 s after a single-pulse stimulation will be depressed, whereas the later part of it is seemingly unaffected. It should be noted that the number of inactivated synapses at point (d) is the observed value of late depression after a single-pulse stimulation, whereas the number of inactivated synapses at points (f) and (g) are calculated by unmasking the effect of a concurrent facilitation. The number of inactivated synapses at points (a), (b), and (c) are extrapolated from the equation given in Figure 5.

The contribution of late depression to the 1 Hz-induced depression was found to be rapidly established in that about two thirds of it occurs already from the first to the second fEPSP, and the steady-state depression of ~30% is reached within 15–20 stimuli. This time course agrees well with that observed in the early studies of this form of depression at more adult perforant path–granule cell synapses (White et al., 1979). The increase in depression observed with more prolonged 1-Hz stimulation of the second week postnatal synapses is thus a consequence of postsynaptic AMPA silencing. It should, however, be noted that the steady-state value of ~30% is an underestimation of the late depression itself, because changes in the PP$_{50}$ ratio indicated an opposing facilitation that reduced the depression from an estimated value of ~45% to the observed value of ~30%. Thus, according to our interpretation of the data, a 1-Hz stimulation of these synapses would presynaptically silence almost half of the activated population of synapses within 15–20 s.
fact, we found the depression reached at the end of a brief high-frequency train to be well correlated with the magnitude of the late depression at 1 s after a single-pulse stimulation. We also found that the calculated increase in late depression with an increased number of conditioning action potentials could quantitatively account for this correlation. It should, however, first be noted that the above correlation showed that the second postnatal week SLM-CA1 synapses should still exhibit (on the population level) a substantial (~35%) depression during a 10-mpulse 20-Hz train even in the absence of the late depression. Because the SR-CA1 synapses did not (at the population level) display any such depression, the late depression cannot be the only factor differing between these two sets of synapses with respect to high-frequency-induced depression.

It should also be noted that if the correlation between the late depression and the depression developing during a high-frequency train should be a causal one, the facilitation process (augmentation) opposing the late depression must take time (>0.5 s) to develop to any significant degree. Otherwise, this facilitation process will largely cancel out the increase in depression that develops during the train activation, as indicated by the lack of increase in the late depression when the conditioning stimulation increased from single-pulse stimulation to a 10-impulse train. The rate of increase of various short-term synaptic enhancement processes is thought to reflect the decay rate of these processes (Fisher et al., 1997), indicating a somewhat slow onset of augmentation. However, to our knowledge, studies of augmentation has exclusively dealt with its decay, and not with its onset kinetics, leaving open the question whether it may have a delayed onset. At present, we will argue that the good correlation observed between the late depression and the depression developing during a high-frequency train is highly suggestive of a causal correlation, thus predicting slow onset kinetics for augmentation at the neonatal SLM-CA1 synapses. The very profound depression, with fEPSP responses at the end of the train being only 10%–30% of that of the first fEPSP in the train, should then, at least partly, be a consequence of a depression process that within half a second of 20-Hz stimulation should silence, on the average, as much as 60% of the activated population of second postnatal week SLM-CA1 synapses for several seconds.

The present study thus suggests that a large fraction of the depression in the 0.1- to 20-Hz range examined is explained neither by vesicle depletion nor by a reduction in $p_{\text{ves}}$. The depression is intrinsic to the presynaptic terminal and may be explained by a process directly affecting the release machinery, resulting in a stimulation-dependent release site inactivation that is rapidly induced and slowly (seconds) dissipated. It would thus appear that the SLM-CA1 synapses, forwarding sensory information from the entorhinal cortex/thalamic nuclei into the hippocampus, already in the neonatal rat possess a habituation-like neuronal process (Teyler & Alger, 1976), not exhibited by intrahippocampal synapses like the SR-CA1 synapses. Whether the similar form of depression, present in more adult animals for entorhinal cortex projections into the dentate gyrus, is also based on such a mechanism seems likely but remains to be confirmed.

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CONFLICT OF INTEREST
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS
R. M. and B. G. designed the study; R. M. performed the experiments and collected the data; R. M and B. G analysed the data and wrote the first draft of the manuscript; R. M., E. H., and B. G wrote the final version of the manuscript.

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DATA AVAILABILITY STATEMENT
The data supporting the findings of this study are available in the article and raw data of the study are available from the corresponding author on request.

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