Presynaptic inhibitory terminals are functionally abnormal in a rat model of posttraumatic epilepsy.

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ABSTRACT

Partially isolated “undercut” neocortex with intact pial circulation is a well-established model of post-traumatic epileptogenesis. Results of previous experiments showed a decreased frequency of miniature inhibitory postsynaptic currents (mIPSCs) in layer V pyramidal (Pyr) neurons of undercuts (Li and Prince 2002). We further examined possible functional abnormalities in GABAergic inhibition in rat epileptogenic neocortical slices in vitro by recording whole cell monosynaptic IPSCs in layer V Pyr cells and fast-spiking (FS) GABAergic interneurons using a paired pulse paradigm. Compared to controls, IPSCs in Pyr neurons of injured slices showed 1) increased threshold and decreased peak amplitude at threshold; 2) decreased input/output slopes; 3) increased failure rates; and 4) a shift from paired pulse depression towards paired pulse facilitation (increased paired pulse ratio or PPR). Increasing [Ca++]o from 2 to 4mM partially reversed these abnormalities in Pyr cells of the epileptogenic tissue. IPSCs onto FS cells also had an increased PPR and failures. Blockade of GABA_B receptors did not affect the paired results. These findings suggest that there are functional alterations in GABAergic presynaptic terminals onto both Pyr and FS cells in this model of posttraumatic epileptogenesis.

Key words: undercut, neocortex, GABAergic interneuron, paired pulse, inhibition.
INTRODUCTION

Partially isolated “undercut” (UC) or “injured” neocortex is a well-established in vivo model of posttraumatic epileptogenesis in cats and monkeys (Echlin and Battista 1963; Echlin and McDonald 1954; Grafstein and Sastry 1957; Sharpless 1969; reviewed in Halpern 1972). Spontaneous (s) and evoked (e) epileptiform activity persists in rodent neocortical slices cut through the UC neocortex and maintained in vitro (Hoffman et al. 1994; Prince and Tseng 1993; Salin et al. 1995; reviewed in Graber and Prince 2006). Alterations in glutamatergic excitatory synaptic activity/connectivity are present in this model and likely contribute to the development of hyperexcitability (Jin et al. 2006; Li and Prince 2002; Li et al. 2005; Salin et al. 1995).

Results of anatomical and electrophysiological experiments in UC cortex indicate that decreases in GABAergic inhibitory activity/connectivity may also contribute to epileptogenesis. Biocytin-filled axons of fast-spiking (FS) GABAergic interneurons in the chronically epileptogenic cortex are structurally abnormal with decreased bouton size and marked reduction in axonal lengths (Prince et al. 2009). Such alterations in presynaptic inhibitory terminals might be associated with functional abnormalities such as increased failure rate, decreased amplitude of inhibitory postsynaptic currents (IPSCs) and decreased probability of GABA release (Pr) that would make inhibitory transmission less effective (Harris and Sultan 1995; Pierce and Lewin 1994). There is also a decrease in the frequency of miniature inhibitory post synaptic currents (mIPSCs) in layer V pyramidal (Pyr) neurons of in vitro slices from UC cortex (Li and Prince 2002; Prince et al. 2009) that may be due to a decrease in inhibitory synapses on layer V Pyr cells (J Wenzel, PS Schwartzkroin and DA Prince, unpublished results) and/or a decreased Pr from GABAergic terminals. Probability of GABA release is an important variable affecting the strength of cortical network activity that has previously been examined in a model of temporal lobe epilepsy (Kobayashi and Buckmaster, 2003), and requires further investigation in chronic posttraumatic epileptogenesis. To explore this issue, we recorded electrically-evoked, pharmacologically-isolated (monosynaptic) whole cell inhibitory currents from layer V Pyr neurons and from FS interneurons in in vitro slices from partially isolated epileptogenic rat sensorimotor cortex. Pairs of stimuli were used to measure the paired pulse ratio (PPR) of response amplitudes, which is considered an index of release probability (Kravchenko et al. 2006; Murthy et al. 1997). Results indicate that the paired pulse depression present in control layer V Pyr neurons shifts towards facilitation in the epileptogenic tissue, indicating a decrease in Pr in GABAergic terminals. This conclusion is supported by an increase in the failure rate for evoked IPSCs. Similar changes in PPR and failure rate of IPSCs evoked on FS cells were found in slices from the UC cortex. These findings provide new information about the cellular mechanisms responsible for the down-regulation of inhibition in this model of posttraumatic epileptogenesis and suggest that GABAergic inhibitory terminals targeting FS and Pyr cells are functionally abnormal. Portions of these results were published in an abstract (Faria and Prince 2008).
METHODS

All experiments were carried out according to protocols approved by the Stanford Institutional Animal Care and Use Committee. Sprague-Dawley rats aged P35-50 (P0 = date of birth) were used for in vitro recordings. UC rats had neocortical lesions placed at ages P21-25. They were deeply anesthetized with ketamine (80 mg/kg ip) and xylazine (Rompun 8 mg/kg ip), and a ~3 x 5-mm bone window centered on the coronal suture removed, leaving the dura intact and exposing a portion of the frontoparietal cortex unilaterally. Partial isolations of sensorimotor cortex were made as previously described (Graber and Prince 2006; Hoffman et al. 1994). A 30-gauge needle, bent at approximately a right angle 2.5–3 mm from the tip, was inserted parasagittally ~1–2 mm from the interhemispheric sulcus, advanced under direct vision tangentially through the dura and just beneath the pial vessels, and lowered to a depth of 2 mm. The needle then was rotated through 120–135° to produce a contiguous white matter lesion, elevated to a position just under the pia to make a second transcortical cut, and removed. An additional transcortical lesion was placed ~2 mm lateral and parallel to the initial parasagittal cut in a similar manner. The skull opening was then covered with sterile plastic wrap (Saran Wrap), and the skin sutured. Animals were given carprofen 5 mg/kg sc postoperatively. Lesioned animals recovered from surgery uneventfully and were reanesthetized for slice experiments 2-3 wks later. Techniques for preparing and maintaining brain slices in vitro were as previously described (Li and Prince 2002). Neocortical slices (~350 µm) were cut with a vibratome in cold (4 ± 1°C) "slicing" artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 1 CaCl2, 2 MgSO4, 26 NaHCO3, and 10 glucose; pH 7.4 when saturated with 95% O2-5% CO2. After 1 hr of incubation in regular ACSF (32 ± 1°C), containing (in mM): 126 NaCl, 5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgSO4, 26 NaHCO3, and 10 glucose, single slices were transferred to a recording chamber where they were minimally submerged and perfused at the rate of 2.5 -3 ml/min with regular ACSF (32 ± 1°C). Patch electrodes were pulled from borosilicate glass tubing (1.5 mm OD) and had an impedance of 2–3 MΩ when filled with intracellular solution containing (in mM): 70 K-gluconate, 70 KCl, 2 NaCl, 10 HEPES, 4 EGTA, 4 Mg ATP and 0.3 GTP. The osmolarity of the pipette solution was adjusted to 275–285 mosM with KOH. Whole cell voltage-clamp recordings were made from visually identified layer V Pyr neurons and FS interneurons using infrared video microscopy and differential interference contrast optics (Zeiss Axioskop2) and a Multiclamp 700A amplifier (Axon Instruments). The estimated chloride equilibrium potential (ECl) was ~16 mV based on the Nernst equation. Access resistance was measured in voltage-clamp mode from responses to 2 mV hyperpolarizing voltage pulses using software provided by Dr. J. Huguenard. Pyramidal neurons were identified as cells with large somata and a single emerging apical dendrite extending toward the pial surface. Firing behavior in current clamp, and absence of a large apical dendrite, was used to identify FS cells (Bacci et al. 2002; Xiang et al. 1998). In addition, in some slices, intracellular labeling with biocytin (0.1%) was used to confirm cell type. Only data from neurons in which access resistance was <15 MΩ, were used for analysis. Pharmacologically isolated (monosynaptic) IPSCs were reliably evoked with monopolar tungsten stimulating electrodes, placed 80 ± 10 µm from the recorded soma in the same lamina. 50µM 2-amino-5-phosphonovaleric acid (D-AP5) and 6,7-Dinitroquinoxaline-2,3-dione (DNQX) (Ascent Scientific) were diluted to a
final concentration in ACSF and continuously applied via bath perfusion. The final 
concentration of DMSO in the ACSF, 0.0005%, was not expected to have effects on 
GABA-induced currents (Nakahiro et al. 1992). The time to wash in drug was typically < 
5min, as judged by alterations in peak amplitude of the ePSCs. To obtain the threshold 
(T) for evoking IPSCs, the stimulus duration was initially set at 100 µs and stimulus 
intensity increased until a stable IPSC with a failure rate ≤50% was evoked. The pulse 
duration was then increased to 150 µs (1.5 x threshold; “1.5T” below) and responses to 
pairs of pulses or trains of 8 stimuli with interstimulus intervals (ISIs) of 20, 50 and 75 
ms, delivered at a repetition rate of 0.04 Hz, were recorded in various experiments. Single 
stimuli (0.1Hz) were used to obtain input-output curves of responses at various stimulus 
intensities. To decrease variability, responses evoked by at least 10 pairs or trains of 
stimuli in each condition were averaged. The PPR was calculated as the peak amplitude 
of the second evoked (e)IPSC (R2) / peak amplitude of the first eIPSC (R1). Spontaneous 
IPSCs were commonly observed during recordings but did not interfere with the 
assessment of evoked events. Responses in which sIPSCs were superimposed on evoked 
EPSCs were not included in the following results. Failures were not included in 
calculations of mean amplitudes. The failure rate was quantified and analyzed separately. 
Paired pulse ratios of <1 and >1 indicated paired pulse depression (PPD) and paired pulse 
facilitation (PPF), respectively. Statistical significance was determined with a two-tailed 
Student's t-test or with paired t-test (P < 0.05). Data are expressed as means ± SE and “n” 
corresponds to the number of neurons. One neuron was recorded per slice and no more 
than 2 slices were used per rat.
RESULTS

Whole cell recordings acceptable for analysis were obtained from 56 layer V Pyr neurons and 19 FS interneurons in control slices of sensorimotor cortex and 62 Pyr neurons and 16 FS cells in UC slices. The properties of evoked monosynaptic IPSCs (Methods) were examined at a holding potential ($V_{h}$) of -60mV for both cell types. In some slices, spontaneous and evoked events were initially recorded from layer V Pyr neurons in standard ACSF to verify that epileptiform synaptic activity was present. Bursts of both evoked (Fig. 1A) and spontaneous PSCs (Fig. 1B), likely consisting of mixed EPSCs and IPSCs under these recording conditions ($E_{Cl}$ -16 mV, $V_{h}$, -60 mV), were present in UC cortex. Although epileptiform burst responses were more pronounced following pairs of stimuli, they were also regularly evoked by single pulses (not shown). Glutamate receptor antagonists DNQX (20µM) and DAP-5 (50µM) were included in the bath in subsequent experiments to isolate eIPSCs. The threshold for evoking IPSCs was increased and IPSC peak amplitude significantly decreased in the glutamate blockers (data not shown; Salin and Prince 1996). eIPSCs were completely blocked by bath application of 10µM gabazine indicating that they were due predominantly to activation of GABA$_A$ receptors.

The threshold for evoking monosynaptic IPSCs is increased in layer V pyramidal cells of slices from undercut cortex

Monosynaptic eIPSCs recorded in cortical Pyr neurons vary in amplitude with the position of the stimulating electrode. Stimuli in all experiments were delivered in layer V, just below the soma of the recorded cell (methods) where the input/output slope of peak eIPSC amplitudes is steep (Salin and Prince 1996). This allowed us to minimize variability and compare eIPSCs with similar properties from control and UC slices. The stimulus intensity required to evoke IPSCs at threshold with pulse width set at 100µs (Methods) was higher in UCs (0.24 ± 0.02mA, n = 27) than in control group (0.11 ± 0.01mA, n = 34, P < 0.000001; Fig. 2A). At threshold and 1.5T, eIPSCs from control had peak amplitudes that were significantly larger than those of undercuts neurons (Fig. 2B; Table 1) and within the range previously reported for monosynaptic IPSCs in deep lying Pyr cells (Salin and Prince 1996). Input/output slopes of eIPSC amplitudes were plotted by increasing stimulus width in 50 µs steps from 100 µs (at threshold) up to 300 µs (3x threshold). In agreement with previous results in control rats (Salin and Prince 1996), IPSCs evoked by stimuli delivered in layer V just below the cell soma tended to have steeper input/output slopes of than those elicited by stimuli at other sites. The linear regression ($R^2$) for the slope of the IPSC input/output relationship was calculated for each neuron. Mean slopes were steeper in control (34.6 ± 4.9 pA/µs) than in UC Pyr cells (13.7 ± 2.8 pA/µs; $R^2$ control = 0.77 ± 0.03, n = 28; $R^2$ UC = 0.61 ± 0.07, n = 22; P < 0.05; Fig. 2B). The kinetics of eIPSCs in Pyr neurons from undercuts were not different from control when 1T stimuli were used, however, at 1.5T, eIPSCs in UCs had slower 10 – 90% rise times (Table 1). Representative responses for 1.5T stimuli are shown in the insert of Fig. 2B. Mean rise time increased in control (1.9 ± 0.3 ms at 1T and 2.5 ± 0.3 ms at 1.5T, n = 25, paired t-test; P < 0.05) and UC (2.7 ± 0.4ms at 1T and 4.6 ± 0.6ms at 1.5T, n = 19, paired t-test; P < 0.01) with increasing stimulus intensity.
Decreased probability of release for evoked IPSCs in Pyr neurons of slices from undercuts

To assess release probability, pairs or trains of stimuli were applied at ISIs of 20, 50 and 75 ms to slices of control and UC rats and evoked IPSCs recorded from layer V Pyr neurons. The PPR (R2 / R1) was calculated after excluding response failures. In control cells, in contrast to UCs, the mean PPR for all ISIs was always <1 as shown in Fig. 3A and Table 2. Group data showed that there was no significant difference in the PPR between the control and UC groups at an ISI of 20 ms. However, while PPR remained <1 at ISIs of 50 and 75 ms in controls, in UC rats it was significantly larger at these ISIs (Table 2). Thus there was a significant shift away from paired pulse depression towards facilitation for IPSCs in Pyr neurons recorded in injured cortex. The peak response amplitude was also measured for each of the 8 IPSCs evoked by stimulus trains applied at an ISI of 50 ms. As shown in Fig. 3B, exponential fits for normalized curves of response amplitudes revealed differences in the mean slope for Pyr cells in UC vs control slices (control R²: 0.51 ± 0.04, n = 20; UC R²: 0.35 ± 0.06, n = 17; P < 0.05). As expected from the paired pulse results, the peak amplitude of the first response in the train was smaller in injured (peak amplitude R1 = 96.5 ± 12.8 pA, n = 31) than in control cells (151.1 ± 15.9 pA, n = 34 control; P < 0.05; inserted traces, Fig. 3B). To exclude the possibility that the intensity of stimulation affected the PPR, in 7 layer V Pyr neurons from UCs, stimulus intensity was increased, so that the peak amplitude of R1 was significantly larger than that obtained at 1.5 T. Although the peak amplitude of eIPSCs in injured Pyr neurons increased from 114.7 ± 23.5 pA to 218.7 ± 28.8 pA when stimuli were increased from 1.5 T to 2.5–3 x threshold (n = 7; P < 0.005), PPR in the same cells was not significantly altered (1.06 ± 0.05 at 1.5 T, and 1.04 ± 0.1 at 2.5 – 3 x threshold; P > 0.05). Results thus indicated that the stimulus intensity, and therefore the amplitude of responses, did not affect the PPR.

In previous experiments on unitary IPSCs in Pyr cells of naïve rat neocortical slices (Xiang et al. 2002), we found that the amplitude of R2 appeared to be independent of previous release for both FS-Pyr and low threshold spiking interneuron-Pyr synapses. To determine whether this was also the case for monosynaptic IPSCs in UC cortex, we analyzed the relationship between peak amplitudes of R1 and R2. An example of an R1-R2 plot is shown in Fig. 4A, and mean group values for linear regression in control (R² = 0.15 ± 0.03, n = 33) and UCs (R² = 0.21 ± 0.04, n = 26; NS) in Fig. 4B. Results indicate that the amplitude of R2 is not influenced by prior release in these experiments. The above abnormalities in PPR suggest a decrease in the probability of release of GABA from inhibitory presynaptic terminals onto layer V Pyr cells in the undercut cortex (Harrison et al. 1988; Discussion below). To further support this interpretation, we examined the failure rate for eIPSCs.

Response failures are more frequent for eIPSCs in injured Pyr neurons

Threshold intensity, at which ≤50% of stimuli failed to evoke an IPSC, was established for each cell in control and UC groups. Response failures to the first or second stimulus of pairs were then assessed at ISIs of 50 and 75 ms and stimulus intensities of 1.5 T. In control slices, IPSCs were evoked by each stimulus on almost every trial (Fig. 5A, upper...
trace). However, failures of either R1 or R2 often occurred in the UC group (Fig. 5A, middle and lower traces). At an ISI of 50 ms, there were no failures of R1 and 0.3 ± 0.3% for R2 in control cells, in contrast to 5.16 ± 1.2% and 5.5 ± 1.3% failures for R1 and R2, respectively, in the UC group (Fig. 5B and Table 2; R1: P < 0.00005; R2: P < 0.0005). At the 75 ms ISI (not shown), there were 3.9 ± 1.8% failures of R1 and 5.4 ± 1.8% failures of R2 in the undercut slices vs. none and 0.5 ± 0.5% for R1 and R2 in controls (P < 0.05 and P < 0.005, respectively). In an additional 2 neurons from 2 undercut rats there were 100% of failures of R2 at ISIs of 20 and 40 ms, even though R1 was evoked by most stimuli (data not included in Fig. 5).

*Increases in [Ca$^{++}$]$_o$ decrease failure rate for evoked IPSCs in UC cells and PPR in control and UC cells*

To further examine the mechanisms for the increased PPR and response failure rate in Pyr neurons of UC slices, we assessed the effects of increasing [Ca$^{++}$] in the perfusate. Changes in [Ca$^{++}$]$_o$ should affect the probability of release at synaptic terminals (Li et al. 2005; Zucker 1993). Increases in Ca$^{++}$ entry during depolarization would lead to decreases in response failures, associated with increased Pr and decreased PPR, changes opposite to those found in UC slices perfused with control ACSF in the present experiments. We initially compared the PPR in each cell in control ACSF (2 mM [Ca$^{++}$]) and after perfusion with ACSF containing 4 mM [Ca$^{++}$] for 30 min. The increase in [Ca$^{++}$]$_o$ did not change PPR significantly in the control group (2mM Ca$^{++}$ PPR = 0.85 ± 0.03, n = 6; 4mM Ca$^{++}$ PPR = 0.74 ± 0.12, n = 5; P < 0.05). This lack of effect was likely due to slow equilibration between [Ca$^{++}$] in the perfusate and that in the tissue (Jones and Heinemann 1987). To circumvent this problem, slices from both control and UC groups, were pre-incubated for at least 1h in 4mM [Ca$^{++}$]$_o$ ACSF, and the PPR in Pyr neurons then measured in the same solution in the recording chamber. The PPR at ISI 50ms decreased from 0.76 ± 0.02 (n = 34) to 0.59 ± 0.06, (n = 12; P < 0.005) in control cells and from 1.04 ± 0.05 (n = 31) to 0.74 ± 0.04 (n = 15; P < 0.001) in UC cells (Fig. 6A). Similar results were obtained at an ISI of 75 ms (not shown). The PPR in Pyr neurons from slices of UC rats incubated in 4mM [Ca$^{++}$]$_o$ was not significantly different from that of control rats in standard 2mM [Ca$^{++}$] ACSF (P > 0.05). Representative pairs of responses with PPRs similar to group mean values are shown for a control and UC Pyr neuron in Fig. 6B,C. In UC cells, perfusion with 4mM vs. 2 mM [Ca$^{++}$] ACSF also markedly decreased the failure rate for R1 (from 5.2 ± 1.2% to 0.7 ± 0.7%, P < 0.05) and R2 (from 5.5 ± 1.3% to 0.7 ± 0.7%, P < 0.05; Fig. 6D). Values for failure rate in UC cells in high [Ca$^{++}$] were similar to those of control cells in 2 mM [Ca$^{++}$] ACSF.

*Activation of presynaptic GABA$_B$ receptors does not affect eIPSCs*

A number of presynaptic receptors that influence transmitter release are present on GABAergic presynaptic terminals (e.g., Freund and Katona, 2007; Harrison et al. 1988; Kruglikov and Rudy 2008; Rodriguez-Moreno et al. 1997). To increase response failures, either an increase in efficacy of a receptor negatively coupled to transmitter release or a decrease in a positively coupled receptor would have to occur in the injured tissue. Increased homosynaptic presynaptic inhibitory effects of GABA at GABA$_B$ receptors due
to increased release (Bennett et al. 1997), decreased reuptake (Deisz 1999) or receptor alterations (Merlo et al. 2007) could increase failures, but would presumably also decrease the amplitude of R2. We assessed the effects of the GABA_B receptor antagonist CGP54626 (10 µM) on PPR and IPSCs evoked by trains of 8 stimuli at 50ms ISIs in layer V Pyr neurons of control and UC slices. Plots of mean evoked peak IPSC amplitude during trains showed no significant change after GABA_B receptor blockade in either control or UC neurons (Fig. 7A; paired t-test, P > 0.05). In addition, mean PPR for evoked IPSCs in neurons of control and undercut slices was unaffected by perfusion with ACSF containing CGP54626 (Fig. 7B-D). Similar results were obtained at ISI 75ms (not shown).

Decreased probability of release and failures of eIPSCs in FS cells of undercuts

To determine whether the above abnormalities were target-selective (Reyes et al. 1998; Scanziani et al. 1998), i.e. involving inhibitory terminals onto layer V Pyr cells vs. inhibitory innervation of interneurons, we recorded monosynaptic eIPSCs in layer V FS interneurons and assessed mean peak amplitude, PPR and failure rate using the above protocols. FS interneurons were identified on the basis of their capacity to generate high frequency non-adapting trains of action potentials in response to depolarizing current pulses, the absence of a single emerging dendrite oriented towards the pial surface and, in some cases, their appearance as typical multipolar basket cells when filled with biocytin (Bacci et al. 2003; Xiang et al. 1998). In agreement with our results in injured Pyr neurons, FS interneurons from UCs showed multiple abnormalities of eIPSCs. The stimulus intensity needed to evoke IPSCs in FS interneurons at threshold with 100µs pulses was significantly increased in UC neurons (Fig. 8A; 0.21 ± 0.03 mA, n = 12 in UC vs 0.14 ± 0.01 mA in controls, n = 15; P<0.05). To assess release probability, pairs or trains of stimuli were applied at ISIs of 50 and 75 ms to slices of UCs while recording from FS cells, and the PPR calculated from averages of 15 sweeps. Representative responses in control (C1) and injured (C2) FS interneurons are shown in Fig. 8C. The PPR was increased significantly in FS cells of UCs at a 50 ms ISI (PPR_control = 0.83 ± 0.05, n = 15 and PPR_undercut = 0.99 ± 0.6, n = 14; P < 0.05; Fig. 8B, left graph), indicating a decrease in Pr. A similar shift at an ISI of 75ms did not reach significance (Fig. 8B, right graph; PPR_control : 0.95 ± 0.10, n = 9; PPR_UUC : 1.15 ± 0.12, n = 8; P > 0.05). Failures of R1 and R2 in control and injured FS neurons occurred with just threshold stimuli, however when stimulus width was increased to 1.5T, failures persisted in FS interneurons of UCs (Fig. 8C3) but not those of control (Fig. 8C1). There were no failures of R1 in control slices (n = 16) in contrast to 2.9 ± 1.5% (n = 13) in UCs (P < 0.05). These results show that there is a decreased Pr for eIPSCs on FS cells of UCs.
DISCUSSION

Recordings from injured layer V Pyr cells showed spontaneous and evoked bursts of synaptic currents (Fig. 1) not seen in control slices bathed in the same 5 mM K⁺ containing ACSF, confirming that slices from UCs were hyperexcitable (Hoffman et al. 1994; Prince and Tseng 1993; Salin et al. 1995). This allowed further analysis of alterations in GABAergic inhibition as a possible contributing pathogenetic factor.

Our results indicate that there are abnormalities in GABAergic inhibitory transmission onto both Pyr cells and FS interneurons in layer V of partially isolated, chronically epileptogenic neocortex, consisting of an increase in the paired pulse ratio for monosynaptic IPSCs, compatible with a decreased Pr; an increased failure rate; increased stimulus threshold for evoking IPSCs; and decreased eIPSC amplitude at threshold. The shift in these parameters in Pyr cells towards control values with increased [Ca++]₀ suggests possible abnormalities in presynaptic terminals that may contribute to the significant decrease in mIPSC frequency in layer V Pyr cells previously reported in chronically isolated neocortex (Li and Prince 2002).

Studies of decreased cortical inhibition as a potential contributor to chronic epileptogenesis have focused predominantly on loss of GABAergic interneurons (DeFelipe 1999; Marco et al. 1996; Ribak et al. 1989), changes in postsynaptic GABA_A receptors (Brooks-Kayal et al. 2001; DeFazio and Hablitz 1999) and disturbances in postsynaptic transmembrane chloride gradients as contributing factors (Jin et al. 2005; Woo et al. 2002). However, anatomical abnormalities in axons and presynaptic terminals are present in some models of epileptogenesis (Hovorka et al. 1989; Salin et al. 1995; Prince et al. 2009) and in human epileptogenic cortex (Marin-Padilla 1997), suggesting that disorders in their function might be present. Alterations in axonal voltage-dependent conductances and presynaptic receptors on axonal terminals can contribute to long-lasting changes in synaptic activity that have been associated with hyperexcitability and/or epileptogenesis (Asprodini et al. 1992; Behr et al. 2002; Brew et al. 2007; Chen et al. 2007; Ganetsky and Wu 1983; Goussakov et al. 2000; Kral et al. 2003; Vervaeke et al. 2006). Bursts of antidromic action potentials arising from axonal terminals occur in models of both acute and chronic epilepsy and presumably play a role in epileptogenesis (Gutnick and Prince 1972; Noebels and Prince 1978; Pinault and Pumain 1985; Stashshef et al. 1993; reviewed in Pinault 1995).

The injury associated with the partial cortical isolation causes damage to callosal, thalamic and other subcortical and intracortical afferents. In addition, portions of the axonal arbors of layer V Pyr neurons, and likely those of interneurons as well, are severed, although both cell types usually survive in a structurally and functionally altered state (Li et al. 2005; Prince and Tseng 1993; Prince et al. 2009; Salin et al. 1995; Tseng and Prince 1996). Structural changes that might affect inhibitory transmission occur in FS interneurons (Prince et al. 2009), the largest subgroup of neocortical inhibitory cells in layer V (Uematsu et al. 2008). Their axonal arbors are significantly decreased in length, and their axons contain fewer large, and more small boutons, changes that would be expected to affect inhibitory synaptic transmission (Birô et al. 2006; Harris and Sultan 1995; Pierce and Lewin 1994).
**Evoked IPSCs in injured pyramidal neurons**

Pharmacologically isolated monosynaptic inhibitory currents evoked in control and UC layer V Pyr neurons had different properties. Increased stimulus intensity was required to evoke IPSCs at threshold in UCs vs controls (Fig. 2A), and input/output slopes were steeper for control cells (Fig. 2B) suggesting that injured GABAergic cells or their axons might be less excitable or were decreased in density in the vicinity of the stimulating electrode. The latter possibility is supported by previous results showing markedly decreased axonal lengths of biocytin-filled FS interneurons (see Fig. 3 of Prince et al. 2009). The kinetics of eIPSCs in Pyr cells of UCs were similar to control when threshold stimuli were used, but rise times became slower in both control and UC cells when stimuli were increased to 1.5T. Response amplitudes were decreased from control at both stimulus intensities. We were careful to position the stimulating electrode close to the soma to evoke more proximal, fast rising IPSCs in both control and UC slices (Kruglikov and Rudy 2008; Salin and Prince 1996), however more distally targeted non-FS interneuron or their axons (e.g. Xiang et al. 1998) were likely activated as the stimulus intensity was increased, and may have contributed to the slower rise time of IPSCs evoked in Pyr cells of control and UC by 1.5T stimuli. Decay time of eIPSCs at T and 1.5T was not significantly different in control vs. UC Pyr cells suggesting that significant alterations in GABAA postsynaptic receptors were not present.

A number of potential mechanisms might contribute to the decreased of eIPSCs in UC cells for both 1T and 1.5 T stimuli (Table 1), including reductions in quantal content (q), numbers of synapses/release sites (n) and the probability of release (Pr). Previous results showed a significant (~37%) reduction in frequency of mIPSCs, without a decrease in amplitude in this model (Li and Prince 2002). A reduction in numbers of GABAA receptors is therefore an unlikely explanation for the decreased eIPSC amplitude. The decreased Pr found in the present experiments would certainly contribute to the reduced mIPSC frequency, along with potential decreases in numbers of synapses or release sites. Data from biocytin-filled FS interneuronal axons in the UC model show a shift towards smaller boutons and decreases in those with detectable immunoreactivity (IR) for vesicular GABA transporter and proximity to postsynaptic gephyrin-IR, findings that suggest possible decreases in functional inhibitory synapses (Prince et al. 2009). Further, electronmicroscopic analysis has shown significant decreases in inhibitory synaptic density on somata of layer V Pyr cells of UCs (J Wenzel, PA Schwartzkroin and DA Prince, unpublished observations).

The paired pulse ratio (PPR) for monosynaptic IPSCs is increased in layer V pyramidal cells of partially isolated cortex

Although the PPR for eIPSCs in rat control layer V Pyr neurons in these experiments and in other reports (e.g. Xiang et al. 2002; Kruglikov and Rudy, 2008) usually shows paired pulse depression (i.e. amplitude of R2<R1), a significant increase in the PPR for eIPSCs was present in UC vs control layer V Pyr neurons, indicating a shift toward facilitation or reduced depression. Such changes are generally interpreted as indicating a decrease in presynaptic release probability (Lisman et al. 2007; but see Markram et al. 1998; Manita et al. 2007; Zucker and Regehr 2002). Postsynaptic alterations, such as changes in
GABA\textsubscript{A} receptor subunit composition with expression of receptors containing the \(\alpha_4\) subunit, do occur in both chronic epilepsy models (Schwarzer et al. 1997; Sperk et al. 1998) and human epilepsy (Brooks-Kayal et al. 1999), and could affect the kinetics and PPR of eIPSCs (Lagrange et al. 2007). However, such changes are associated with increased depression in responses to pairs or trains of stimuli, results contrary to those in the present experiments (eg, Fig 3A,B).

Increases in \([\text{Ca}^{++}]_o\) resulted in a significant decrease in the PPR and failure rate in UC slices toward normal values, as would be expected if Pr increased at presynaptic terminals (Fig. 5; Jiang et al. 2000). The relative percentage decreases in the PPR and in failures induced by increasing \([\text{Ca}^{++}]_o\) were not significantly different in UC vs. control cells (not shown).

The shift in the PPR for eIPSCs towards facilitation and associated decreased Pr in Pyr neurons in slices from UCs differed from our previous results in unlesioned cortex (Xiang et al. 2002) and those of others (Beierlein et al. 2003; Kravchenko et al. 2006; Thomson et al. 1996) that showed paired pulse depression of IPSCs/Ps normally predominates at the inhibitory presynaptic terminals onto neocortical Pyr neurons (but see Fleidervish and Gutnick 1995; Thomson et al. 1996). Significant changes in presynaptic function have been reported in models of temporal lobe epilepsy, including a shift from PPD to PPF for IPSPs in dentate gyrus granule cells of rats with pilocarpine-induced temporal lobe epilepsy (Kobayashi and Buckmaster 2003), and decreased quantal release of GABA, associated with reduced synaptic vesicle density in both pilocarpine and kainate models of temporal lobe epilepsy (Hirsch et al. 1999). A similar shift from paired pulse depression to facilitation was reported in IPSCs evoked in layer IV fast-spiking cells in the irradiated model of cortical dysplasia (Zhou et al. 2009).

One potential mechanism for the shift toward facilitation in Pyr cells of UCs is decreased Ca\textsuperscript{++} buffering in presynaptic FS interneurons and a resultant increase in residual Ca\textsuperscript{++} in terminals (Caillard et al. 2000; Collin et al. 2005; Zucker and Regehr 2002). Fast-spiking inhibitory cells account for ~ 50\% of neocortical GABAergic neurons in layer V (Uematsu et al. 2008) and likely contribute significantly to the monosynaptic IPSCs in the present experiments, particularly since FS cells would tend to be differentially activated by perisomatic layer V stimuli (Kruglikov and Rudy 2008; Salin and Prince 1996). The calcium binding protein, parvalbumin (PV), is highly expressed in FS interneurons (Celio 1986), however PV-containing interneurons and their axon terminals in epileptogenic neocortex or hippocampus may lose or decrease their PV content (Andre et al. 2001; Rosen et al. 1998; Roper et al. 1999; Scotti et al. 1997; Wittner et al. 2001, 2005). Such changes would result in alterations in Ca\textsuperscript{++} buffering and could underlie the shifts toward paired pulse facilitation seen in these experiments (e.g. Vreugdenhil et al. 2003).

Alterations in Pr and PPR dependent on Ca\textsuperscript{++} are not likely due to a single process, as mechanisms for regulating \([\text{Ca}^{++}]_i\) undergo complex chronic changes in human temporal lobe epilepsy (Lie et al. 1999) and in models of status epilepticus and traumatic brain injury (DeLorenzo et al. 2006; Pal et al. 1999, 2000; Sun et al. 2008). Changes in the properties of Ca\textsuperscript{++} channels that might affect Pr can also be present (Becker et al. 2008; Su et al. 2002). The long-lasting increases in \([\text{Ca}^{++}]_i\) that occur in status epilepticus (Pal et al. 1999, 2000) and fluid percussion epilepsy models (Sun et al. 2008) may have presynaptic effects on GABAergic inhibition and, if sufficiently large, result in decreased
GABA release (De Koninck and Mody 1996; Maruyama et al. 1990). In our experiments, PPR in UCs was decreased as expected in the presence of increased \([\text{Ca}^{++}]_o\) (Jiang et al. 2000; Kravchenko et al. 2006; Li et al. 2005) and approached values that were similar to those recorded in control rats in standard ACSF (Fig. 6). Studies of changes in \([\text{Ca}^{++}]_i\) and calcium channel function in injured presynaptic terminals in the partial isolation model might contribute to a better understanding of abnormalities in efficacy of inhibitory synaptic transmission.

**Failures of synaptic transmission are increased in injured Pyramidal neurons**

The increased failure rate for eIPSCs in Pyr cells of the UC is another index of reduced synaptic efficacy (Fig. 5B). The failure rates for eIPSCs onto layer V Pyr cells in control cortex reported here were significantly lower than those for uIPSCs in earlier experiments (Xiang et al. 2002), perhaps due to the fact that extracellular stimulation at 1.5 T would recruit more presynaptic cells, and that ACSF in the prior experiments contained a lower \([\text{K}^+]_o\) (2.5 mM vs. 5 mM). Perfusion of injured slices with ACSF containing 4 mM \(\text{Ca}^{++}\) decreased the failure rate and PPR to levels similar to control in standard ACSF (Fig. 6), presumably by increasing \(\text{Ca}^{++}\) influx into terminals during the presynaptic action potential. This could indicate an abnormality in presynaptic \(\text{Ca}^{++}\) channels (Faria and Prince, 2009) or alterations at downstream sites controlling transmitter release (e.g. Catterall and Few 2008; Neher and Sakaba 2008; Sudhof 2004). A negative correlation between the amplitude of R1 and R2 was not present (Fig. 4), suggesting that failures were not due to vesicle pool depletion. Similar findings have been reported for unitary IPSCs recorded in rat hippocampal slices (Jiang et al. 2000). Another potential mechanism for synaptic unreliability, axonal branch point failure, occurs under some circumstances in invertebrates (Grossman et al. 1979; Smith, 1980), but has not been shown to date at least for excitatory axons in neocortex (Cox et al. 2000) or hippocampus (Allen and Stevens, 1994; Jiang et al. 2000; but see Debanne et al. 1997). In any case, increases in \([\text{Ca}^{++}]_o\) would be expected to decrease axonal membrane excitability (Frankenhaeuser and Hodgkin, 1957) and increase rather than decrease failures at branchpoints.

**Presynaptic modulation**

GABA release from terminals can be enhanced (Bacci et al. 2002; Rodriguez-Moreno et al. 1997; Ruiz et al. 2003) or decreased (Bowery et al. 1980; Burke and Hablitz 1994; Freund and Katona 2007; Harrison et al. 1988; Kruglikov and Rudy 2008) by activation of a variety of presynaptic receptors. To contribute to the increased failures and reduced amplitude of eIPSCs on Pyr cells of UC cortex (Table 1), a transmitter would have to decrease release of GABA or its effects at GABA_\text{A} receptors. GABA acting at GABA_\text{B} receptors might decrease release (Bowery et al. 1980; Harrison et al. 1988), however application of a GABA_\text{B} receptor antagonist did not change PPR or IPSC properties in our experiments. Also, the applied stimuli were likely not intense enough to release sufficient GABA to activate GABA_\text{B} receptors in control or UC Pyr neurons (Bennett et al. 1997). We cannot rule out activation of other presynaptic receptors that could reduce GABA release and increase failures such as cannabinoids (Freund and Katona 2007),
acetylcholine (Benardo 1991), or neuropeptide Y (Bacci et al. 2003), however release of such substances would require more intense stimulation than employed in the current experiments. Metabotropic glutamate receptor activation could also contribute to decreased inhibitory transmission in the present experiments (Bandrowski et al. 2003; but see Cahusac and Wan 2007; Salt and Eaton 1995).

Significant changes in presynaptic terminals of Fast Spiking cells

Current results, taken together with those previously obtained from Pyr cells in partially isolated epileptogenic cortex (Li et al. 2005), indicate that axonal terminals of both excitatory and inhibitory neurons are functionally abnormal, but are differently affected by injury. Probability of release is increased in injured excitatory presynaptic terminals (Li et al. 2005) and decreased in inhibitory ones. These functional alterations are associated with anatomical abnormalities including sprouting with increased length, branch numbers and bouton density in Pyr cell axons (Salin et al. 1995) and decreased axonal lengths and putative inhibitory synapses from fast-spiking interneurons (Prince et al. 2009). In both cases, abnormalities in bouton size are present with a shift towards smaller boutons.

From the recordings of monosynaptic IPSCs from single cells in cortical layer V obtained in our experiments, it is not possible to determine whether specific subtypes of interneurons (eg Markram et al. 2004; Uematsu et al. 2008) are more or less affected by the injury, or whether all the inputs from GABAergic cells have the functional changes described. This question is relevant as specific sets of interneurons may be differentially affected by injury while other subtypes are not damaged (Cossart et al. 2001; DeFelipe, 1999; Morin et al. 1998). Also, short term dynamics of transmitter release vary in different subtypes of interneuron (Gupta et al. 2000; Xiang et al. 2002) so that the results obtained here represent the summated contributions of an aggregate of inhibitory inputs. As pointed out above, fast-spiking parvalbumin-containing cells make up more than half of interneurons in layer V (Uematsu et al. 2008) and the position of the stimulating electrode close to somata (Kruglikov and Rudy 2008; Salin and Prince 1996) makes it likely that these were the largest cohort of interneurons contributing to the eIPSCs. Recent experiments on synaptically-connected pairs of FS and excitatory cells in layer IV of UC and control cortex (Ma and Prince, 2009) have shown results compatible with those obtained from the current experiments. However, we cannot rule out effects on other subtypes of interneurons, e.g. somatostatin-containing cells that make up another large group of neocortical interneurons (Uematsu et al. 2008).

Results of experiments from paired recordings and examination of calcium channel function in terminals could provide more detailed information on the cellular mechanisms responsible for the reductions of inhibition in this model of posttraumatic epileptogenesis. The functional consequences of our findings are hard to predict. Although IPSCs evoked by single stimuli onto Pyr cells would be less effective in the UC, the shift toward facilitation results in maintenance of larger amplitude IPSCs during stimulus trains at higher frequencies (Fig. 3) such as might occur in vivo (Pare et al. 1998). This could result in increased synchronization of Pyr cell networks, enhanced rhythmic activity (Khazipov and Holmes, 2003) and increased susceptibility to epileptiform activity, as has been suggested to occur in PV−/− mice (Schwaller et al. 2004). The functional results of
decreased Pr for IPSCs onto FS interneurons (i.e. disinhibition) are also hard to translate into alterations in network excitability, as previous experiments in this model have shown that there are structural abnormalities in FS interneuronal terminals such as decreased axonal lengths and decreased bouton size (Prince et al. 2009) that might reduce the output of the disinhibited GABAergic cells.
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Table 1. Properties of IPSCs evoked in layer V Pyr cells of control and undercut. T: threshold (50% failures). *: p< 0.05; **: p< 0.005; ns: p>0.05. (n): number of cells tested. Values expressed as means ± SE (unpaired t-Test). (ns): Not significant.

Table 2. Paired pulse ratio (PPR) and failures of evoked monosynaptic IPSCs in layer V Pyr cells of control and UC. PPR values expressed as means ± SE. Failure rate expressed as percentage of the total number of responses. (n): Number of Pyr neurons tested. All values are expressed as means ± SE. *: p< 0.0005; **: p< 0.00005; ***: p< 0.00001, ns: p>0.0
FIGURE LEGENDS

FIG. 1. Epileptiform activity in injured layer V Pyr neurons.
A: Representative postsynaptic currents (PSCs) evoked by pairs of 1.5 T stimuli in a neuron of control (upper trace) and UC slice (lower trace). In UC cell, the second stimulus evokes a burst of PSCs. B: Spontaneous PSCs in neurons from control (upper trace) and UC slices (lower trace). Bursts of PSCs occur in UC but not in control cell. Segments in boxes shown at 3x gain and sweep speed. Vh: -60 mV; EC1: -16mV in A and B, so that both EPSCs and IPSCs likely contribute to the spontaneous and evoked PSCs.

FIG. 2. Threshold and mean slope of input output curves for eIPSCs in Pyr neurons of control and UC slices.
A: The stimulus intensity required to evoke IPSCs at threshold was higher in UCs than in control group. Pulse width: 100 µs. B: Mean slope in control (open triangles) is steeper than in UCs (filled triangles) R² control = 0.77 ± 0.04, n = 28; R² UC = 0.61 ± 0.07, n = 22; P < 0.05. Inset: Representative eIPSCs from UC (top) and control (bottom).

FIG. 3. Increased paired pulse ratio (PPR) in injured Pyr neurons of UC rats.
A: Plot of PPR vs. interstimulus interval for control and UC neurons. At ISIs of 50 and 75 ms, but not 20 ms, the PPR for eIPSCs in layer V Pyr neurons of UC slices was significantly different than control and shifted toward facilitation (values given in Table 2). Inset: Representative responses to paired stimuli (ISI = 50 ms) from control (upper) and UC neurons (lower), obtained at Vh = -60mV. Dashed lines mark peak amplitude of R1 here and in Fig. 7C. B: Normalized peak amplitudes for evoked IPSCs decrease progressively during trains of 8 stimuli. Mean slopes for normalized peak amplitudes are steeper in control cells (P < 0.05). Insert: Representative responses for a control (lower) and UC neuron (upper). C: Stimulus intensity affects peak amplitude but not PPR. Peak amplitude (left scale) increased when stimulus intensity was increased from 1.5 to 2.5 – 3 x threshold in UCs (n = 7; P < 0.005), without a change in PPR (right scale). Insert: Pairs of representative eIPSCs show increased peak amplitude for 2.5T vs. 1.5T stimuli.
Calibration: 100pA, 50ms.

FIG. 4. Relationship between amplitudes of R1 and R2 in control and UC layer V Pyr cells.
A: Plot of peak amplitude of the first (R1) vs. the second response (R2) for 15 pairs of eIPSCs (ISI 50 ms) recorded in a representative control Pyr cell. Line: linear regression, R² = 0.15. B: Mean group values for slopes of R1 vs R2 amplitudes in control and UC. Control R² = 0.15 ± 0.03; UC R² = 0.21 ± 0.04; P > 0.05). Numbers of neurons shown in each column.

FIG. 5. Failure rate for eIPSCs in layer V Pyr neurons from UCs is increased vs. controls.
A: Representative responses to 1.5T stimuli showing failures of R1 or R2 that often occurred in the UC group (middle and bottom traces) but not in control (upper). B: At ISI = 50 ms, there were no failures of R1 and 0.3 ± 0.3 % failures of R2 in control cells. In contrast, failures occurred in 5.2 ± 1.2% and 5.5 ± 1.3% of R1 and R2 respectively in UCs.
FIG. 6. Effects of increased [Ca\(^{++}\)]\(_0\) on PPR and failure rate in Pyr neurons.

A: Increasing [Ca\(^{++}\)] in ACSF from 2 mM to 4 mM decreased PPR in control (white bars, left) and shifted values towards control in UCs (black bars; right). B-C: Representative eIPSC responses to paired stimuli (ISI 50 ms) in 4mM [Ca\(^{++}\)] in control (B) and UC cells (C). PPRs of both representative samples are similar to the mean group values in 4 mM [Ca\(^{++}\)] in graph of A. D: Incubation in 4mM [Ca\(^{++}\)] markedly decreased failures of R1 and R2 in Pyr cells from UC rats. The effect of high calcium on R1 and R2 was similar.

Calibration in B, C: 100 pA, 40 ms.

FIG. 7. GABA\(_B\) receptor blockade does not affect amplitude or PPR of eIPSCs in control or injured layer V Pyr neurons.

A: Slopes of mean peak amplitudes vs. pulse number in standard ACSF (open symbols) and after GABA\(_B\) receptor blockade (filled symbols). B: PPR (ISI = 50 ms) recorded before and after GABA\(_B\) receptor blockade was not affected in either control (n = 10) or UC (n = 9) group (control PPR = 0.77 ± 0.08; 10µM CGP54626 PPR = 0.79 ± 0.06; P > 0.05). C-D: Control (C) and UC group (D) variability in PPR during perfusion of standard ACSF and after bath perfusion of ACSF containing 10µM CGP54626. Lines connect PPR values for single neurons before and after GABAB receptor blockade.

FIG. 8. Inhibitory innervation of FS cells is altered in UCs.

A: Threshold for evoking IPSCs onto FS cells of UC is increased compared to control. Stimulus pulse duration was set at 100µs and intensity increased until ~50% of stimuli evoked an IPSC. B: PPR is increased in FS cells of UC at ISI = 50ms. A non-significant shift in PPR toward facilitation was present at ISI = 75ms.

C: Representative IPSCs evoked by paired stimuli show PPD in control neuron (C1) and decreased PPD in a cell from UC (C2) (ISI = 50ms, 1.5T, V\(_h\) = -60mV, E\(_{Cl}\) = -16mV). Failures persisted in UCs when stimulus width was increased to 1.5T (C3). Numbers in bars of A, B: total number of FS cells recorded. Dashed lines in C1,2 mark peak of R1 (C1) and of R2 (C2).
A
Control ePSC
UC ePSC

B
Control spont
UC spont
$V_e = -60\text{mV}$
$E_{Cl} = -16\text{mV}$

100pA
10ms
100pA
100ms

$3x$ $3x$
**Figure A**

Stimulus Intensity (mA) at threshold:

- Control: 34 mA
- UC: 27 mA

**Figure B**

Peak amplitude (pA) vs. Times threshold:

- UC: 300 pA
- Control: 100 pA

Scale: 100 pA, 20 ms
**A**

**B**

**C**
**A**

- Control
- UC
- UC

100pA
10ms

**B**

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
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<tr>
<td>Control</td>
<td>8</td>
<td>6</td>
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<tr>
<td>UC</td>
<td>4</td>
<td>2</td>
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</tbody>
</table>

Failures % (ISI = 50ms)

* Significant difference
A Stimulus Intensity (mA) for eIPSC at threshold

B PPR (R2/R1)

C ISIs (ms) 1.50 1.25 1.00 0.75 0.50 0.25 0.05 0

100pA 20ms

Control UC
<table>
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<th></th>
<th>Control</th>
<th>Undercut</th>
</tr>
</thead>
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<tr>
<td>Peak amp T (pA)</td>
<td>$77.7 \pm 12.2$ (28)</td>
<td>$42.7 \pm 7.7$ (22) *</td>
</tr>
<tr>
<td>Peak amp 1.5T (pA)</td>
<td>$125.5 \pm 16.1$ (28)</td>
<td>$64 \pm 9.8$ (22) **</td>
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<tr>
<td>Rise time T (ms)</td>
<td>$1.9 \pm 0.3$ (25)</td>
<td>$2.9 \pm 0.6$ (22) ns</td>
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<tr>
<td>Rise time 1.5T (ms)</td>
<td>$2.6 \pm 0.3$ (28)</td>
<td>$4.5 \pm 0.6$ (20) **</td>
</tr>
<tr>
<td>Decay Time T (ms)</td>
<td>$17.4 \pm 1.8$ (23)</td>
<td>$15.2 \pm 1.7$ (20) ns</td>
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<td>Decay Time 1.5T (ms)</td>
<td>$21.4 \pm 1.8$ (27)</td>
<td>$21.1 \pm 2.5$ (22) ns</td>
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<tr>
<td>Peak latency T (ms)</td>
<td>$4.6 \pm 0.3$ (27)</td>
<td>$4.7 \pm 0.4$ (22) ns</td>
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<td>Control</td>
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<tr>
<td><strong>PPR ISI = 20ms</strong></td>
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<td><strong>PPR ISI = 50ms</strong></td>
<td>$0.77 \pm 0.02$ (34)</td>
<td>$1.04 \pm 0.05$ (31) ***</td>
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<td><strong>PPR ISI = 75ms</strong></td>
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<td>$1.07 \pm 0.06$ (12) *</td>
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<td>Failures R1 (%)</td>
<td>0 (34)</td>
<td>$5.16 \pm 1.22$ (31) **</td>
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<tr>
<td>Failures R2 (%)</td>
<td>$0.29 \pm 0.29$ (34)</td>
<td>$5.48 \pm 1.3$ (31) *</td>
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