Short-Term Facilitation and Depression in the Cerebellum

Some Observations on Wild Type and Mutant Rodents Deficient in the Extracellular Matrix Molecule Tenascin C

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ABSTRACT: Short-term plasticity was studied on synapses to Purkinje cells (PC): paired-pulse facilitation in parallel fibers (PF) and paired-pulse depression in climbing fibers (CF). Both phenomena relate to synaptic strength. These forms of short-term plasticity were tested on cerebellar slices in rat by early postnatal synchronous stimulation of olivary neurons (i.e., CFs) with harmaline and by inhibition of a metabotropic glutamate receptor (mGluR) as well as in mice that were deficient in the extracellular matrix glycoprotein tenascin-C. Harmaline stimulation delayed the developmental competition between CF inputs and maintained multiple innervation. Paired-pulse depression of the CF-PC synapse after harmaline treatment was more expressed. However, paired-pulse facilitation in PF-PC synapses remained unchanged. Electrophysiological responses of postsynaptic mGluR1 in CF-PC synapses could be obtained only with AMPA receptors blocked and glutamate uptake impaired. The mGluR1-specific antagonist CPCCOEt suppressed the CF-mGluR EPSC in some PCs and potentiated it in other PCs. CF paired-pulse depression was not changed with CPCCOEt, thus excluding a presynaptic effect. The postsynaptic effect was underlined by CPCCOEt-induced rise in amplitude of EPSC and by a prolongation of its decay time. Tenascins are extracellular matrix glycoproteins that may restrict the regenerative capacity of the nervous tissue. Testing short-term presynaptic plasticity in tenascin-C–deficient mice showed that CF paired-pulse depression was less expressed while PF paired-pulse facilitation was augmented except in a group of cells where there was even depression. The results underline differences in forms of short-term plasticity with regard to susceptibility to diverse modulatory factors.

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INTRODUCTION

Synapses usually exhibit a change in their efficacy with repeated use that can last for seconds to minutes.[1,2] This behavior is termed short-term plasticity—as opposed to the phenomenon of long-term plasticity,[3] which can be experimentally maintained for hours, days, or even weeks. Another difference is that short-term plasticities are primarily of presynaptic origin[4,5] while long-term plasticity phenomena generally depend on postsynaptic mechanisms. An important role in short-term plasticity phenomena is played by residual Ca^{2+}, which persists in the terminal after presynaptic activity.[1,5] While long-term plasticity is assumed to be at the basis of learning and memory,[6,7] short-term plasticity, at its milliseconds-to-seconds time scale, allows synapses to modulate neurotransmitter release continually in response to presynaptic activity.[8] Widespread forms of the latter plasticity are paired-pulse facilitation and paired-pulse depression, both obtained by stimulating the presynaptic cell with a paired-pulse protocol. It is generally accepted that paired-pulse facilitation is a characteristic of synapses with a low initial probability of release and is manifested by an increased release in response to repeated presynaptic action potential generation.[9,10] In contrast, paired-pulse depression is predominant at synapses with a high initial probability of release that becomes depressed in response to sequential presynaptic action potentials.[11,12] In addition, the recovery from depression is a Ca^{2+}-dependent phenomenon.[13,14] Study of these short-term plasticity phenomena and their changes can thus give information on presynaptic release mechanisms, synaptic strength, and/or the role of Ca^{2+}. Namely, more expressed paired-pulse facilitation as well as a decrease in paired-pulse depression should indicate a drop in probability of release of respective synapses.

Here we review the information gained in our studies of short-term plasticity phenomena on acute cerebellar slices. In the cerebellar cortex two different excitatory inputs project to separate domains of the Purkinje cell (PC) dendrites. The distal one, rich in spines, is innervated by hundreds of thousands of parallel fibers (PFs), the axons of granule cells, while the proximal one has only a few clusters of spines innervated by a single climbing fiber (CF) the terminal arbor of neurons located in the inferior olivary nucleus. The PC mono-innervation by the CF is the result of a regressive phenomenon from a state of polyinnervation that in the rat starts around postnatal day 5 (P5) and is completed at P15.[15] In cerebellum as well as in other neural tissues during development and regeneration, synapses are under morphogenetic control of extracellular matrix molecules (collagens, proteoglycans, and glycoproteins).[16] In these synapses glutamatergic transmission involves ionotropic and metabotropic glutamate receptors (mGluRs). In the cerebellum the two forms of short-term plasticity, paired-pulse facilitation and paired-pulse depression, can be studied on synapses to Purkinje cells from PFs and from CFs, respectively. These forms of short-term plasticity were tested (1) after early postnatal synchronous stimulation of olivary neurons with harmaline, (2) upon inhibition of the metabotropic glutamate receptor 1 (mGluR1), and (3) on transgenic mice deficient in the extracellular matrix glycoprotein tenascin-C (TN-C). We show how short-term plasticity can be used to

test the role of presynaptic mechanisms and to elucidate how they depend on different functional and constitutive modulatory factors.

RESULTS AND DISCUSSION

Modulation of CF-PC Synaptic Redundancy by Harmaline

Neural activity plays a critical role in establishing the highly refined and specific pattern of connections that leads to the final architecture of the brain.[17–20] We wanted to check whether CF–PC synapse regression from multiple to mono-innervation could be affected by induction of a synchronous activation of the inferior olive neurons in the rat. As seen in visual and neuromuscular systems, synchronous activity favors polyneuronal innervation while asynchronous one leads to competition, synapse elimination, and refinement of connections.[21,22] Since direct chronic stimulation is not feasible in postnatal pups, we induced a change in the firing pattern by administering harmaline. The effect of this drug is to induce a synchronous activation of the inferior olive neurons thus increasing the CF firing frequency from a basic value of about 1 Hz up to 8 or 10 Hz.[23–25]

Stimulation of CFs was performed by applying current pulses through a soda lime glass pipette filled with extracellular solution and placed in the white matter or several cell diameters away from the patched PC in the molecular layer. The position of the stimulating pipette was only changed if no evoked excitatory postsynaptic current (EPSC) was observed. Cells that did not reveal CF-EPSCs were discarded or used for the study of PF responses. In order to block inhibitory GABA-ergic input recordings were performed in the presence of bicuculline. CF-PC EPSCs were identified by their characteristic all-or-none response and the presence of paired-pulse depression (FIG. 1A). After finding the EPSC threshold, the intensity of the stimuli was gradually augmented up to maximal values. In cases of polyinnervation, increasing the stimulus strength beyond the threshold for an EPSC another current amplitude component could be recruited (FIG. 1B). Cases of multiple CF innervation (about 30%) were observed only in harmaline-treated animals ($N = 35/112$; P15–36) and none in the controls ($N = 54$; P15–P37). In harmaline-treated rats only two-step polyinnervation was observed.[26]

The current-voltage relationship for CF-EPSCs was linear as expected[27] and similar in both harmaline- and saline-treated rats.[26] In order to check further for possible changes at the presynaptic level we next studied the paired-pulse depression as a function of interpulse interval.[4] For all interpulse intervals studied CF-PC synapses from the harmaline-treated group, showed a significantly higher paired-pulse depression as compared to the control. This was found in both mono- and multi-innervated PCs (FIG. 1C). Fitting a double exponential curve to the data for the control and apparently mono-innervated harmaline-treated group gave recovery time constants[13] of 117 msec and 1.9 sec vs. 241 msec and 3.6 sec, respectively,[26] thus confirming a higher release probability in the treated group.

Thus, although the evoked CF-EPSC current-voltage relation did not show a clear difference from the control, the paired-pulse data indicate that the chronic harmaline-induced CF stimulation induced a presynaptic long-term plasticity change in their synapses onto PCs.[26] A similar paired-pulse depression change was demon-

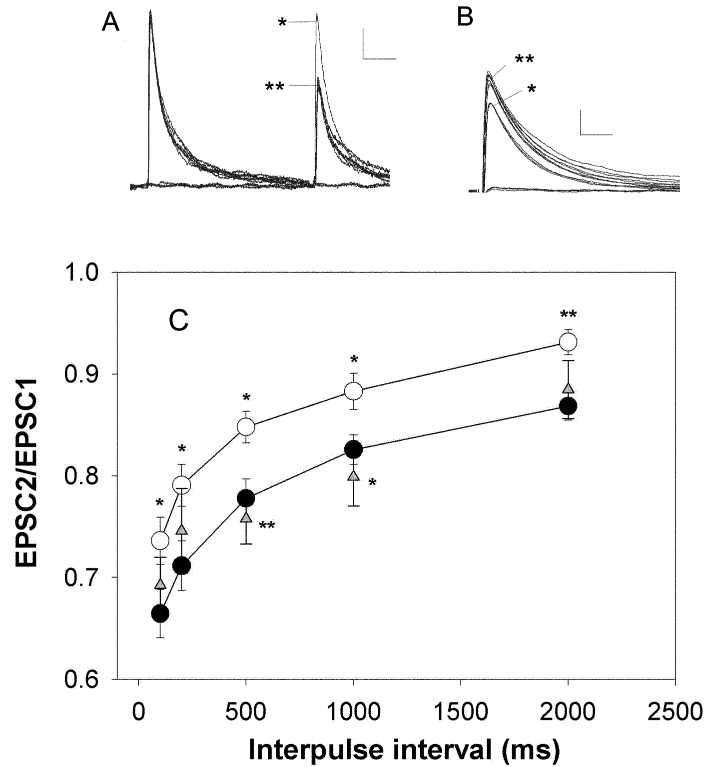

FIGURE 1. Paired-pulse depression of the CF-PC synapse in cerebellar slices from harmaline-treated rats. (**A**) Superimposed original recordings of CF-EPSCs evoked by a series of paired stimuli of increasing intensity. With threshold stimuli one of the responses may be lacking (failure). Thus, instead of being depressed (**) the second response can appear with normal amplitude (*). Calibration bars: 200 pA, 20 msec. (**B**) Original recordings showing the first amplitude step (*) to a series of graded stimuli and the second EPSC step (**) obtained with stimuli of higher intensity. Calibration bars: 100 pA, 10 msec. (**C**) Recovery from paired-pulse depression (expressed as the ratio of second over first EPSC amplitude) in slices from saline-treated (*open circles*) vs. harmaline-treated rats with single- or double-step EPSCs (*filled circles* and *gray triangles*, respectively). *Asterisks* indicate differences in pairs of data (control vs. single-step EPSCs, and double-step vs. control EPSCs), with $P < 0.05$ (*) or $P < 0.01$ (**). Here and in subsequent figures the data points present means ± S.E.M.[26]

strated for slow type CF-EPSCs observed in GLAST mutant mice[28] and in GluRδ2 mutants,[29] both mutants also having an impaired elimination of multiple CF innervation. A rise in the probability of transmitter release as indicated by a rise in paired-pulse depression should cause a rise in the amplitude of the evoked synaptic current. However, the current-voltage relation for the CF-EPSCs showed a similar magnitude of this response in harmaline-treated vs. control rats.[26] A detailed discussion of this apparent discrepancy can be seen in Andjus and collegues,[26] however, the most plausible explanation for the absence of a change in the current-voltage relation, in spite

of the change in paired-pulse plasticity, could lay in the fact that in these synapses due to multivesicular release the postsynaptic AMPA receptors are saturated.[30–32] This type of release, which saturates postsynaptic receptors, may thus cause an underestimate of paired-pulse depression. However, even in the case of saturation the observed enhancement of this short-term plasticity in harmaline-treated cells can only indicate a rise in the amount of released glutamate. In addition, the measurements of the kinetic parameters (rise time and decay) of the CF-EPSCs have not shown a change with harmaline treatment, thus further excluding a post-synaptic effect.[26]

There is extensive evidence in the literature that for CF-PC synapses the transition from multiple- to mono-innervation is under the influence of the PFs.[33–37]

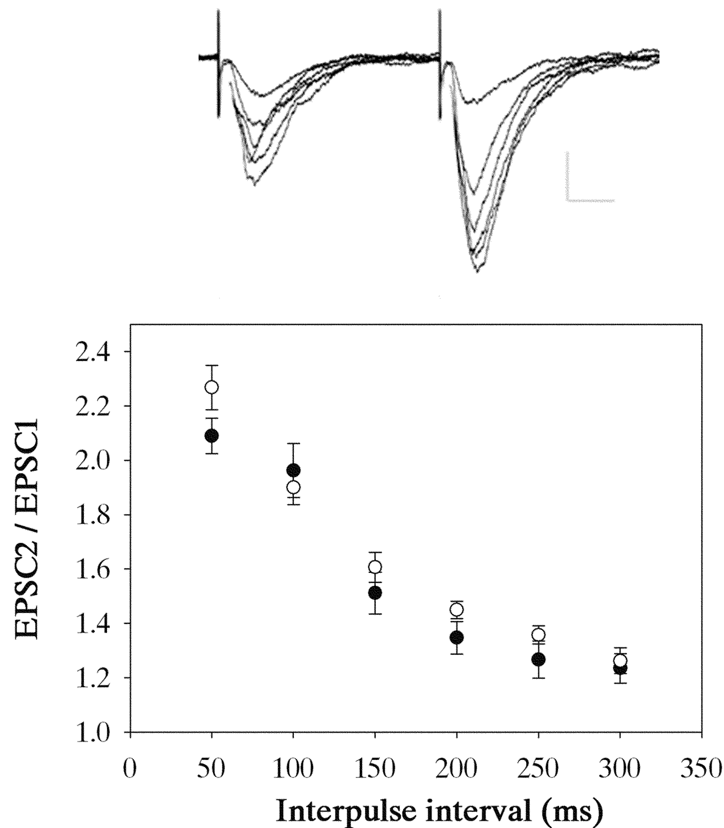

FIGURE 2. Paired-pulse facilitation of the PF-PC synapse in cerebellar slices from harmaline-treated rats. (*Above*) Several superimposed original recordings of PF-EPSCs obtained with paired stimuli of graded intensity. Calibration bars: 200 pA, 20 msec. (*Below*) dependence of paired-pulse facilitation (expressed as the ratio of second over first EPSC amplitude) on interpulse interval in slices from saline-treated (*open circles*, N=17) vs. harmaline-treated (*filled circles*, N = 14) rats.[26]

Moreover, a recent study showed a c-fos activation in a number of PF-forming granule cells during harmaline treatment.[38] Therefore, we tested whether in our harmaline-treated rats the PF-PC synapses displayed changed features. Measurements of PF EPSC (with tip of the stimulating electrode positioned in the molecular layer) at a holding voltage of –70 mV (FIG. 2, top), revealed no apparent changes in the function of amplitude on stimulus (not shown). In addition, testing the time course of the decay of paired-pulse facilitation showed that this short-term plasticity effect was very similar for all interpulse intervals applied in both control and harmaline-treated rats (FIG. 2, bottom). These electrophysiological results that did not reveal any long-term change were also confirmed by the morphological analysis of PF synaptic bouton density, which was also not altered after harmaline treatment.[26] Thus, it was concluded that it may be possible to modify the developmental plasticity of the CF-PC synapse by changing CF activity without affecting the basic morpho-functional characteristics of the PF input.

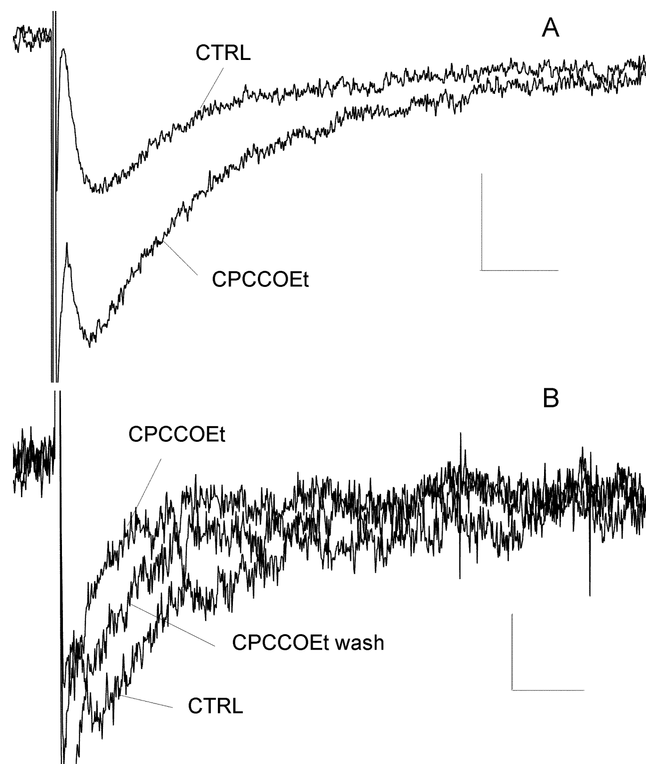

FIGURE 3. Dual effect of CPCCOEt on CF mGluR PSCs obtained with single pulse stimulation. (**A**) CPCCOEt-induced rise in amplitude. V_h= –70 mV. Calibration bars: 100 pA, 10 msec. (**B**) Suppression of PSC by CPCCOEt with subsequent partial wash. V_h= –50 mV. Calibration bars: 20 pA, 10 msec. Control recordings (*CTRL*) are obtained in TBOA (inhibitor of glutamate transport) and with AMPA, NMDA, $GABA_A$ receptors blocked.[51]

Dual Effect of Inhibition of CF-PC mGluR1

Different forms of synaptic plasticity expressed at both CFs and PFs require the presence of mGluRs. Such are the PF long-term depression (LTD),[39,40] the CF non-associative LTD,[41] the short-term presynaptic depression via retrograde signaling to cannabinoid receptors at CF terminals (CF cSTD),[42] and the activity-dependent pruning of the multiple CF inputs to each PC during development.[40,43] Activation of mGluRs is known to give rise to a slow postsynaptic current (mGluR PSC). This current was observed in PF-PC synapses and it was sensitive to antagonists of mGluR1 subtype.[44–47] Although earlier immunocytochemical studies revealed mGluRs also at the CF synapses,[48,49] only recently, a mGluR-mediated all-or-none postsynaptic current was also demonstrated at the CF-PC synapse.[50,51] In order to reveal this current in rat cerebellar slices, in addition to the inhibition of ionotropic AMPA, NMDA and $GABA_A$ receptors, glutamate transporters also had to be blocked (DL-*threo*-β-benzyloxyaspartic acid, TBOA) thus augmenting the concentration of endogenous transmitter in the CF-PC synaptic cleft. However, for the CF-PC synapse we have shown[51] that in some cells the mGluR1 antagonist, 7-(hydroxyimino)cyclopropa[β]chromen-1α-carboxylate ethyl ester (CPCCOEt), augmented the mGluR PSC amplitude more than twice (see example in FIG. 3A) but in others caused its suppression by 50% (see example in FIG. 3B). This dual behavior was apparent with single-pulse ($N = 6$ vs. 6; FIG. 3) as well as with train stimulation ($N = 8$ vs. 13; not shown).

In order to reveal the mechanisms underlying the dual effect of CPCCOEt, we studied the effect of this antagonist on the CF EPSC and its short-term plasticity without AMPAR antagonists or inhibitors of glutamate transport (only $GABA_A$ receptors were blocked).[51] Paired-pulse depression of the CF EPSC was not changed with the CPCCOEt (FIG. 4A). In standard 2 mM external calcium, used in these experiments, AMPARs should be saturated[30–32] and the paired-pulse depression change could be masked. Therefore, paired-pulse plasticity was also checked in low (0.5 mM) external Ca^{2+} (which causes less depression and changes recovery kinetics[13]) and again no change in paired-pulse depression was observed (FIG. 4B). Nevertheless, even with AMPARs saturated (in 2 mM Ca^{2+}), CPCCOEt induced a rise in the CF EPSC amplitude by 50% (FIG. 4E) and a longer decay time (FIG. 4C), thus pointing to a postsynaptic effect. In lower, 0.5 mM Ca^{2+} the decay of CF EPSCs was faster and it was also significantly prolonged with CPCCOEt (FIG. 4D). However, unlike the case in 2 mM Ca^{2+}, in 0.5 mM Ca^{2+} the CF EPSC amplitude was not significantly affected (FIG. 4F), which may indicate an underlying Ca^{2+}-dependent mechanism. Thus, in some cells the inhibition of postsynaptic mGluR1 may cause the expected decrease of its current[50] but in others this effect may be overcome by a rise of PSCs presumably originating from other mGluR types.[51] The latter mGluR currents of unknown origin would otherwise be suppressed through mGluR1 action. The lack of change in paired-pulse depression on one hand with a change of EPSC decay time and amplitude change in 2 mM Ca^{2+} on the other hand indicates that the observed mGluR1 effects originate postsynaptically.

Tenascin-C and Glutamate Release

Tenascins are extracellular matrix glycoproteins that contribute to the formation of the nervous system, are characteristic of perineuronal nets, and may restrict or en-

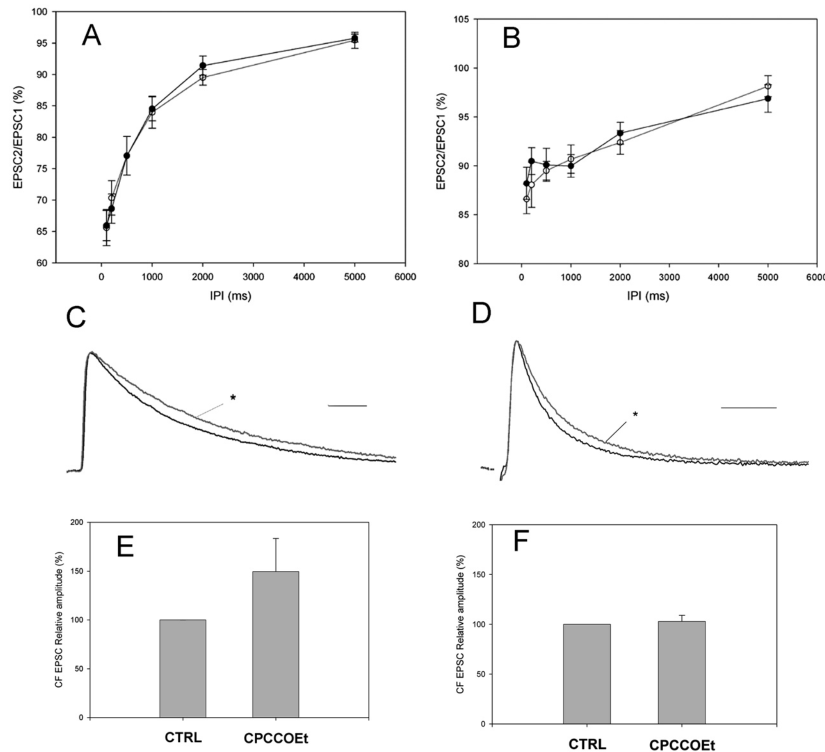

FIGURE 4. Paired-pulse depression recovery experiments in CPCCOEt. (**A**) Normal external medium (2.0 mM Ca^{2+}); control (*open circles*; *N*=8) vs. CPCCOEt (*filled circles*; *N*= 8). (**B**) Low external Ca^{2+} (0.5 mM); control (*open circles*; *N*=10) vs. CPCCOEt (*filled circles*; *N*= 10). *Ordinate*: amplitude ratio; *abscissa:* interpulse interval (IPI). (**C** and **D**) Examples of normalized average EPSCs in CPCCOEt (*asterisk*) vs. control (each trace is average of 3 recordings) in 2.0 and 0.5 mM Ca^{2+}, respectively. V_h = +30 mV. Calibration bar: 5 msec. (**E** and **F**) CF EPSC mean amplitude in CPCCOEt relative to control in 2.0 (*N*= 6) and 0.5 mM Ca^{2+} (*N*= 8), respectively.[51]

hance the regenerative capacity of the adult central nervous system.[16,52–54] TN-C represents such a glycoprotein that is highly conserved during evolution and prominently expressed in the course of neural development. TN-C has dual functional features: it is inhibitory for neurite outgrowth when presented as a sharp substrate border, but enhances neurite outgrowth as a uniform substrate in cell culture. After a chemical lesion in the inferior olivary nucleus TN-C mRNA was not detectable nor was it detectable prior to the lesion.[55] However, TN-C mRNA was transiently downregulated in Golgi cells of the cerebellar cortex. On the other hand, after chemical trauma or inducing stab wound lesions in the adult CNS, TN-C is upregulated in its expression, the functional consequences of which are still largely unknown. TN-C and the structurally related tenascin-R are capable of modulating electrophysiologi-

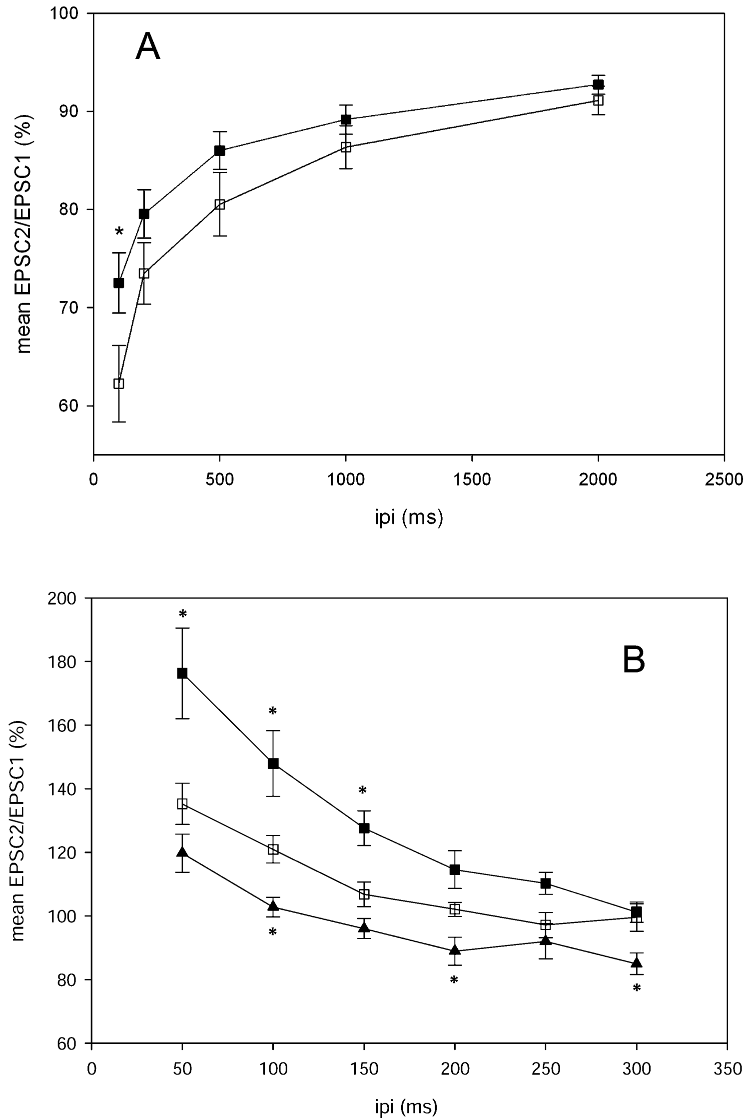

FIGURE 5. Paired-pulse plasticity in TN-C$^{-/-}$ mutants. (**A**) Dependence of paired-pulse depression of CF-EPSCs on interpulse interval in wild type (*open squares*; N= 13) vs. TN-C$^{-/-}$ (*closed squares*; N= 14). (**B**) Dependence of paired-pulse facilitation of PF-EPSCs on interpulse interval in wild type (*open squares*; N=7) vs. two groups of TN-C$^{-/-}$ —one showing more facilitation (*closed squares*; N= 13) the other showing depression for longer interpulse intervals (*closed triangles*; N= 4). Asterisks indicate significant difference (P<0.05) of single data points from corresponding wild-type values.

cal behavior of neurons, and *vice versa*—neural activity affects the expression of TN-C, for instance. In fact, TN-C is overexpressed upon hippocampal long-term potentiation.[56] Recently, it has been suggested that TN-C affects L-type Ca^{2+} channels and thus could control release and modulate synaptic plasticity.[57] In order to test the latter hypothesis we studied short-term plasticity on cerebellar slices from genetically modified TN-C–deficient (TN-$C^{-/-}$) mice.[57]

Measurements of paired-pulse depression on CF-PC synapses showed that in TN-$C^{-/-}$ mice the response to the second CF stimulus was less depressed (FIG. 5A). This difference was more pronounced for shorter interpulse intervals. In fact, only the response after 100 msec was significantly less depressed then in wild type. However, the two-way ANOVA test showed that the recovery curves differ significantly ($P<0.05$; FIG. 5A). In TN-$C^{-/-}$ mice measurements of paired-pulse facilitation in PF-PC synapses revealed a more complex behavior. Namely, some cells expressed a significantly higher facilitation, especially for shorter interpulse intervals (50–150 msec) while other cells, on the contrary, showed paired-pulse depression for longer interpulse intervals (150–300 msec). Two-way ANOVA for both curves showed significant difference ($P<0.05$ and $P< 0.01$, respectively) from the wild-type curve. This short-term plasticity behavior clearly demonstrates that the constitutive lack of TN-C affects glutamate release from CF as well as from PF terminals onto PCs. The prevailing effect is a decrease of probability of release at both synapses. However, in a group of cells PF-PC synapses seem to be strengthened by TN-C deficiency and in fact show paired-pulse depression instead of short-term facilitation. Whether this is the influence of the prevailing population of PF terminals in the sum of the responses of numerous synapses (about 150,000) onto each PC, and if this may be a consequence of different activity patterns[8] leading to functional duality of TN-C[16] remains to be elucidated.

CONCLUSION

Experiments with short-term plasticity may give information on the presynaptic origin of effects (experiments with harmaline and TN-C deficient mice as opposed to mGluR EPSCs). The presented studies on cerebellar slices lead to the information on synapse-specific long-term effects such as the potentiation of CF-PC synapses (while no such effect was observed for PF-PC synapses) after chronic stimulation with harmaline or to synapse-nonspecific effects as observed in the TN-C–deficient mutants. The latter result also points to a general mechanism of synapse weakening in TN-C–depleted animals, and justifies further studies, especially regarding the known effect of this extracellular matrix glycoprotein on L-type Ca^{2+} channel-mediated signaling and long-term plasticity phenomena.[57]

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