Impaired Locomotor Learning and Altered Cerebellar Synaptic Plasticity in pep-19/pcp4-Null Mice

Peng Wei, Jay A. Blundon, Yongqi Rong, Stanislav S. Zakharenko, and James I. Morgan*
Department of Developmental Neurobiology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, MS 323, Memphis, Tennessee 38105-3678

Received 14 February 2011/Returned for modification 18 April 2011/Accepted 2 May 2011

PEP-19/PCP4 maps within the Down syndrome critical region and encodes a small, predominantly neuronal, IQ motif protein. Pep-19 binds calmodulin and inhibits calmodulin-dependent signaling, which is critical for synaptic function, and therefore alterations in Pep-19 levels may affect synaptic plasticity and behavior. To investigate its possible role, we generated and characterized pep-19/pcp4-null mice. Synaptic plasticity at excitatory synapses of cerebellar Purkinje cells, which express the highest levels of Pep-19, was dramatically altered in pep-19/pcp4-null mice. Instead of long-term depression, pep-19/pcp4-null mice exhibited long-term potentiation at parallel fiber-Purkinje cell synapses. The mutant mice have a marked deficit in their ability to learn a locomotor task, as measured by improved performance upon repeated testing on an accelerating rotarod. Thus, our data indicate that pep-19/pcp4 is a critical determinant of synaptic plasticity in cerebellum and locomotor learning.

MATERIALS AND METHODS

Generation of pep-19/null mice. A recombineering approach (39) was used to generate the pep-19 conditional knockout construct. An 11.68-kb DNA fragment containing exon 3 of the pep-19 gene was isolated from a bacterial artificial chromosome (BAC) DNA (Sanger Wellcome Trust) and cloned into the PL253 vector. loxP sites were engineered to flank a 3.43-kb DNA region containing exon 3 that encodes the majority of the Pep-19 protein, including its IQ motif. An Fmi restriction site was inserted adjacent to the 5' end of the first loxP site, whereas an SA-ires-T-lacZ-neo cassette was inserted at the 3' end of the second loxP site. W9.5 embryonic stem (ES) cells derived from the 129S1/SvImJ strain (kindly provided by P. J. McKinney, Department of Genetics, St. Jude Children's Research Hospital) were electroporated with NotI-linearized targeting vector and selected with G418 and fialuridine (FIAU). DNA from ES cells was digested by MfeI and analyzed by Southern blotting using a 0.9-kb external probe. Chimeras derived from two different ES clones underwent germ line transmission and generated founder strains. To delete pep-19 in germ cells and generate pep-19+/− mice, male pep-19+/− mice were crossed with female mice carrying Sox2Cre (Jackson Labs, Bar Harbor, ME). pep-19−/− mice were further inbred for at least 7 generations to generate 129S1-C57BL/6 mixed-background pep-19−/− mice for this study. Genotyping was performed by Southern blotting and PCR. PCR primers were pep-19 knockout 5' primer 5′-ATT GCT TTG TGA GGC ATT GTA A-3' and pep-19 knockout 3' primer 5′-GCT TCT TCT TCT TGT GTC GTC-3′, as well as pep-19 wild-type (WT) 5' primer 5′-CCG AGA AGC CAC CAT GAT CTC G-3′ and pep-19 WT 3' primer 5′-GAG CCT GAT CTC TTA GCC CTC ACC A-3′. Mice were maintained at St. Jude Children's Research Hospital and had free access to food and water. Investigational procedures conformed to all applicable federal rules and guidelines and were approved by the Institutional Animal Care and Use Committee.

Immunoblotting. Cerebellar extracts were run on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and electrotransferred to polyv-
nylode dihydroxy membranes (Bio-Rad, Hercules, CA). Membranes were probed successively with a rabbit polyclonal anti-Pep-19 antiserum (63) and a mouse monoclonal antibody to β-actin (Sigma, St. Louis, MO) as a loading control. Bound antibody was detected by the ECL chemiluminescence system (Amer sham, Piscataway, NJ).

Northern blotting. Total RNA from mouse cerebellum was extracted using Trizol reagent (Invitrogen, San Diego, CA). Total RNA (20 μg) was resolved by electrophoresis on a formaldehyde 1% agarose gel and transferred onto a nylon membrane by vacuum blotting. The probe was a 396-bp pep-19 cDNA fragment corresponding to nucleotides 213 to 609 of pep-19 (NCBI accession number NM_008791.2) and was labeled with digoxigenin according to the manufacturer's specifications (Roche, Indianapolis, IN). Hybridization was carried out in EasyHyb (Roche, Indianapolis, IN) using the manufacturer's protocol.

In situ hybridization. An RNA probe was prepared by in vitro transcription using digoxigenin (DIG)-UTP (Roche, Indianapolis, IN) and the pGEM-T plasmid vector (Promega, Madison, WI) carrying 396 bases of the mouse pep-19 cDNA fragment mentioned above. Wild-type and pep-19-null littermate mice were anesthetized at postnatal day 30 using 0.1 mg/ml chloral hydrate and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed after the perfusion and postfixed in the same solution for 4 h. After cryoprotection in 30% sucrose in phosphate-buffered saline, brains were frozen and sagittal sections cut at 12 μm. Cryosections were used for the hybridization. The hybridization and detection process was carried out by following the manufacturer's protocol (Roche), and sections were counterstained with nuclear fast red.

Histo logical methods. One-month-old mice were anesthetized and perfused transcardially with buffered 4% paraformaldehyde, and their brains removed and postfixed as described above. Paraffin sections (5 μm) were stained using 0.1% cresyl violet for standard histological analysis and granule cell counting. For immunohistochemistry, all sections were subjected to heat-mediated antigen retrieval using a 0.01 M sodium citrate buffer (pH 6.0) containing 0.05% Tween 20 (Sigma, St. Louis, MO) as described previously (62). A rabbit polyclonal antiserum to Pep-19 (1:4,000) or rabbit anti-calbindin D-28K (1:1,000; Chemicon, Sigma, St. Louis, MO) as described previously (62). A rabbit polyclonal antiserum to Pep-19 (1:4,000) or rabbit anti-calbindin D-28K (1:1,000; Chemicon, Phillippsburg, NJ) was used to immunostain cerebellar Purkinje cells as described previously (63). After immunostaining, the sections were counterstained with hematoxylin (Vector Labs, Burlingame, CA).

To estimate Purkinje cell numbers, matched 5-μm coronal paraffin sections spanning the cerebellum from 3 wild-type and 3 pep-19-null littermate mice (10 sections per mouse, total of 42 sections per genotype) were immunostained for calbindin D28K as described above. The total number of calbindin-positive Purkinje cells in each section was counted and data were analyzed using Student’s t test. To estimate granule cell numbers, nuclei were counted in two 100-μm2 squares from matched 5-μm cresyl violet-stained coronal paraffin sections spanning the cerebellum from 3 wild-type and 3 pep-19-null mice (10 sections per mouse, total of 30 sections per genotype). Data were analyzed using Student’s t test.

For β-galactosidase (β-Gal) staining, pregnant pep-19+/− dams were killed by CO2 overdose and embryos removed and immersion fixed in 2% paraformaldehyde in 0.1 M PIPES [piperazine-N,N'-bis(ethanesulfonic acid)] buffer, pH 6.9, whereas adult pep-19+/− mice were perfused transcardially and postfixed in the same fixative for 6 h. Subsequent processing of tissues, cryostat sectioning, and histochemical staining for β-galactosidase were as described by Oberlick et al. (43). Sections were counterstained with nuclear fast red to provide anatomical orientation.

Behavioral analysis. Motor coordination, balance, and motor learning were evaluated with the accelerating-rotarod test. Wild-type, heterozygous, and pep-19−/− mice (gender- and age-matched littermates; n = 31/genotype) were tested on an accelerating rotarod (San Diego Instruments, San Diego, CA). The rotarod was programmed to accelerate from 0 to 40 rpm in 4 min and then hold constant speed for a further 1 min. The amount of time that elapsed before the mouse fell off was recorded. The maximum observation time was 5 min. Animals were given a session consisting of 5 trials per day with a 20-min intertrial interval. Data from 3 trials were averaged. Animals were repeatedly tested for two blocks of 5 consecutive days, separated by a 9-day rest period. The latency of the mice to fall from the rod was scored as an index of their motor coordination. Improvement in performance across training days indicates motor learning, and the time that elapsed until the animal fell was recorded, with the 1% off time being set at 60 s.

The daily activities of individual pep-19−/− and pep-19+/− mice were measured using exercise wheels from Lafayette Instruments (model 80820; Lafayette, IN). Gender-matched littermate mice (n = 10/genotype) were individually housed in running wheel cages, and wheel revolutions were automatically counted throughout the whole experimental period. Average daily revolutions were calculated over a 5-day period.

The treadmill stress test was performed on gender-matched pep-19+/− and pep-19+/− littermates aged 45 to 60 days (n = 18/genotype). Mice were exercised on a motorized treadmill (Exerc; Columbus Instruments) with a 5-degree incline. The regimen consisted of 5 days of familiarization running (10 min/m for 15 min) and 1 day of stress running. For the stress test, the treadmill was set at an initial speed of 10 m/min, and the speed was increased 1 m/min for 22 min. Mice were run until they could not maintain sufficient speed to remain off the shock grid, and the maximum speed they attained was recorded. The stress running stages began with a 5-min warm-up period at a speed of 10 m/min.

Electrophysiology and two-photon imaging. Cerebellar slices were prepared as follows: mouse brains were quickly removed and placed in cold (4°C) dissection artificial cerebrospinal fluid (ACSF) containing 125 mM choline-Cl, 2.5 mM KCl, 0.4 mM CaCl2, 6 mM MgCl2, 1.25 mM Na2HPO4·2H2O, 26 mM NaHCO3, and 20 mM glucose (300 to 310 mOsm) with 95% O2–5% CO2. Sagittal cerebellar slices (200 μm) were cut using a VT1000P vibratome (Leica Microsystems) and then transferred to an incubation chamber containing ASCF composed of 125 mM NaCl, 2.5 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 1.25 mM Na2HPO4·2H2O, 26 mM NaHCO3, 20 mM glucose (300 to 310 mOsm), with 95% O2–5% CO2. After a 1-h incubation at room temperature, the slices were transferred into a recording chamber and superfused (2 to 3 ml/min) with warm (30°C to 32°C) ACSF containing 30 μM D-28K.

Whole-cell recordings were obtained from cell bodies of Purkinje neurons using patch pipettes (open pipette resistance, 3 to 5 MΩ). The intracellular recording solution contained 140 mM K gluconate, 4 mM KCl, 10 mM HEPES, 5 mM Mg ATP, 0.1 EGTA, and 0.01 mM Alexa 594 (pH adjusted to 7.3 with KOH). All recordings were corrected for a liquid junction potential of the intracellular solution of 15 mV.

Whole-cell recordings were made using a Multiclamp 700B (Molecular Devices), digitized (10 kHz) (DigiData 1322A; Molecular Devices), and recorded using pCLAMP 9.0 software (Molecular Devices). A tungsten bipolar electrode (FHC Inc.) placed in the molecular layer was used to stimulate granule cell parallel fibers (see Fig. 5A). During voltage clamp recordings, the holding potential was set at −80 mV. In a subset of mice the stimulus input-current response output relationship was first determined by applying increasing stimulus currents (from 0 to 100 μA) in increments of 20 μA and measuring the 20 ms amplitudes of the excitatory postsynaptic currents (EPSCs). Stimulation intensity was then adjusted to evoke the first EPSC of approximately 150 pA to record a baseline for synaptic plasticity experiments. In all cells, baseline EPSCs were then evoked for 5 to 10 min with paired-pulse stimuli (60-ms interpulse interval) at a frequency of 0.067 Hz (57). The paired-pulse ratio (EPSC2/EPSC1) was used to monitor changes in synaptic function during LTD experiments.

LTD at parallel fiber-Purkinje cell (PF-PC) synapses was induced using a previously described protocol (34). This widely employed protocol is considered to simulate the events that occur during locomotor learning in vivo by concomitantly stimulating the climbing fiber and mossy fiber/parallel fiber pathways. In a typical cerebellum-dependent motor learning paradigm, such as eye blink conditioning, the conditioned stimulus (CS) such as a sound is paired with an unconditioned stimulus (US) such as a puff of air to the eye, and this pairing results in the establishment of a conditioned motor response (eye blink) to the sound stimulus alone. At the level of the cerebellum, these two stimuli converge at the Purkinje cell via different routes to generate motor learning. Information encoding the US arriving at the inferior olivary nucleus is relayed to Purkinje cells by climbing fiber axons that synapse onto the cell body and proximal dendrites of Purkinje cells. The CS information is relayed to Purkinje cells by parallel fiber-Purkinje neurons that in turn activate Purkinje cells via their parallel fiber axons, which synapse onto the distal dendrites on Purkinje cells. Although other neuronal types and circuits are also involved, at its core the close temporal pairing of firing from parallel and climbing fibers results in LTD at synapses to Purkinje cells.
whose output ultimately controls muscles in the eye. In cerebellar slices, the LTD induction paradigm simulates CS-US pairing by coordinated stimulation of parallel fibers with a stimulating electrode and injection of depolarizing current into the Purkinje cell body via a whole-cell pipette mimicking climbing fiber activity. Specifically, LTD was induced in current-clamp mode by a train of pairing stimulations delivered at 1 Hz for 5 min. Every pairing stimulation consisted of a depolarizing step (100 ms, 1,500 pA) delivered through the patch pipette followed with a 200-ms delay by a pair of stimulations (60-ms interpulse interval) of the parallel fibers using the same stimulation intensity as during the baseline recording (24). The depolarizing step elevated the membrane potential to \(-110 mV\) and was sufficient to generate a train of 3 to 6 action potentials in recorded neurons. Access resistance (Rs, typically 15 to 25 M\( \Omega \)) was monitored throughout experiments by measuring the amplitude of the peak response to a 50-ms 5-mV hyperpolarizing step. If Rs deviated by more than 20% of its original baseline value, the experiment was terminated and the data were discarded.

To aid in the selection of healthy Purkinje neurons for electrophysiology and to quantify cell morphology, a fluorescent dye, Alexa 594, was included in the recording pipette solution and visualized at 820 nm with two-photon laser-scanning microscopy (TPLSM) using an Ultima imaging system (Prairie Technologies), a Ti:sapphire Chameleon Ultra femtosecond-pulsed laser (Coherent), and 40x (0.8 numerical aperture) water-immersion infrared (IR) objective (Olympus). As the recording pipette was lowered into the cerebellar slice, healthy neurons excluded the red dye and appeared as dark shadows. At the end of most experiments, the detailed morphology of the dendritic tree of the recorded neuron was reconstructed using Alexa 594 fluorescence. Projections of wild-type and pep-19/+/pep-19 null Purkinje neurons that were oriented parallel to the slice surface were measured by closely outlining the entire dendritic tree and cell body and calculating dendritic areas using ImageJ software (NIH). Higher-magnification z-axis scans of individual dendrites were also collected, and ImageJ was used to analyze the density of dendritic spines.

All data showing error bars represent means ± standard errors of the means (SEM), unless otherwise noted. Mean comparisons were performed with Student’s unpaired t test, with significant differences between means determined by a probability level (P) of 0.05. All statistics were computed using Microsoft Excel, Sigma Plot, and Sigma Stat (Systat Software, Inc.).

**RESULTS**

**Generation of pep-19 -null mice.** To generate a conditional pep-19-null allele (Fig. 1A), we flanked exon 3 with a 5’ loxP site and 3’ with a LoxP-SA-IRES-T-lacZ-neo cassette by using standard techniques in ES cells (Fig. 1A; see Materials and Methods for more details). Male pep-19\(^{+/+}\) mice were crossed with female mice carrying Sox2Cre (Jackson Labs), which is
ous gross anatomical anomalies (data not shown). Viable and fertile, have normal life spans, and exhibit no obvious expression of \textit{pep-19} gene in the targeted allele, \textit{H9252} (Fig. 3A to D) and calbindin D28K (Fig. 3F and G) immunohistochemistry using a Pep-19 antibody shows marked expression of \textit{pep-19} in wild-type Purkinje cells (C) that is absent in \textit{pep-19}-null cerebella (D). The inset is an enlarged view of the boxed region in panel C. Nuclear LacZ staining (blue) in Purkinje cells of a \textit{pep-19} heterozygous mouse (E) confirms correct recombination of the \textit{pep-19} allele and establishes its utility in mapping expression of \textit{pep-19} in vivo. (F) Higher magnification of the box in panel E confirms nuclear localization. The sections are counterstained with nuclear fast red. Bars, 500 \(\mu\)m (A to E) and 15 \(\mu\)m (F).

Characterization of \textit{pep-19}-null mice. \textit{pep-19}-null mice are active in the female germ line (27), and heterozygous offspring were then intercrossed to obtain germ line \textit{pep-19}-null animals (Fig. 1A). Correct targeting was confirmed by Southern blotting (Fig. 1B). Northern (Fig. 1C) and Western (Fig. 1D) blotting showed that cerebella from homozygous \textit{pep-19}-null mice lacked \textit{pep-19} mRNA and protein, whereas heterozygous mice had intermediate levels of both compared to wild-type littermates. \textit{In situ} hybridization (Fig. 2A and B) and immunohistochemistry for \textit{pep-19} (Fig. 2C and D) confirmed the presence of \textit{pep-19} mRNA and protein in Purkinje cells, respectively, and its absence in \textit{pep-19}-null animals. As Cre-mediated recombination activates the nuclear \textit{lacZ} reporter gene in the targeted allele, \(\beta\)-galactosidase histochemistry can be used to map expression of \textit{pep-19}, and this revealed prominent expression in Purkinje cells (Fig. 2E and F).

Impaired locomotor learning in \textit{pep-19}-null mice. Repeated testing on the accelerating rotarod is used to assay motor learning in mice (19, 23, 36, 37, 41). Improvement in performance across training days, as measured by increasing latency to fall from the rotarod, indicates motor learning. We employed a protocol in which a 9-day rest period is interposed between two 5-day periods of training. This permits the measurement of both acquisition of locomotor learning and its retention (6). In naïve animals, there was no statistically significant difference among wild-type, heterozygous, and null littermates (Fig. 3E and H, respectively). Moreover, Purkinje cells of \textit{pep-19}-null mice did not show significant changes in dendritic spine density or dendritic arbor measured as area occupied by a dendritic tree (Fig. 3I to K).

There were no differences in the numbers of granule cells or Purkinje cells between wild-type and null littermates (Fig. 3E and H, respectively). Moreover, Purkinje cells of \textit{pep-19}-null mice did not show significant changes in dendritic spine density or dendritic arbor measured as area occupied by a dendritic tree (Fig. 3I to K).
performances at all times tested, pep-19-null mice had a markedly reduced ability to improve performance compared to the mice of other genotypes (Fig. 4A) (P < 0.01; Bonferroni’s post hoc test; n = 31/genotype). Upon initial testing following 9 days off trial, there remained a highly significant difference (P < 0.01, ANOVA) in performance between pep-19-null mice and both wild-type and heterozygote littermates (Fig. 4A). Nevertheless, all groups performed better in the first retest session than they did in their initial (naive) performance in the first training session (P < 0.01 for all groups, paired t test), indicating retention of locomotor learning.

To determine whether strength or general locomotor performance, rather than locomotor learning, was compromised in pep-19-null mice, additional tests were performed to measure these parameters. No significant differences were detected between the genotypes for spontaneous activity on a running wheel (Fig. 4B) (P = 0.56, 10 mice per genotype) or for stress running in an enforced treadmill test (Fig. 4C) (P = 0.14, 18 mice per genotype), nor were there any significant differences in grip/muscle strength, as assessed by a hanging wire test (Fig. 4D) (P = 0.46, 30 mice per genotype).

**Synaptic plasticity at parallel fiber-Purkinje cell synapses is altered in pep-19-null mice.** Long-term synaptic plasticity at the granule cell parallel fiber-Purkinje cell (PF-PC) synapse is generally thought to mediate cerebellar motor learning (5, 9, 14, 25, 29, 32). To investigate the role of Pep-19 in PF-PC synaptic plasticity, we carried out whole-cell recordings of Purkinje cells in freshly prepared cerebellar slices from adult wild-type and pep-19-null mice (Fig. 5). In these experiments, we also filled recorded neurons with the fluorescent dye Alexa 594 (40 μM) to image dendritic trees and dendritic spines of Purkinje cells using two-photon laser-scanning microscopy (Fig. 5A). We found that basal synaptic transmission at PF-PC synapses measured as an input-output relationship between intensity of PF stimulation and Purkinje cell excitatory postsynaptic current (EPSC) amplitude was not significantly different between wild-type (n = 7) and pep-19-null (n = 7) littermates (Fig. 5B). Normal basal synaptic transmission in mutant mice was also paralleled by normal dendritic structure and normal density of dendritic spines on dendrites of recorded Purkinje neurons (Fig. 3I to K). However, long-term synaptic plasticity at PF-PC synapses was dramatically altered in pep-19-null mice. Whereas the pairing induction protocol (see Materials and Methods) induced robust LTD of EPSC amplitude in wild-type mice (35.1% ± 10.9% of the baseline EPSC; P < 0.001, n = 6), it was ineffective at inducing LTD in pep-19-null mice. Instead,
the same induction protocol produced long-term potentiation (LTP) of EPSC amplitude (175.5% ± 23.6% of the EPSC baseline; \( P = 0.004, n = 14 \)) at PF-PC synapses in slices from mutant mice (Fig. 5C and D). At the same time, no significant changes in the paired-pulse ratio were observed after induction of synaptic plasticity in both pep-19-null and wild-type mice (data not shown), suggesting that both forms of synaptic plasticity are expressed postsynaptically. Together, these results suggest a critical role of Pep-19 in mechanisms of synaptic plasticity.

**DISCUSSION**

Loss of pep-19 in mice results in impaired locomotor learning and markedly altered synaptic plasticity in cerebellar Purkinje neurons. Notably, a stimulation protocol that normally induces LTD at PF-PC synapses instead induces LTP in pep-19-null animals. Despite the profound effect on synaptic plasticity, pep-19 deletion did not affect basal synaptic transmission or morphology of PF-PC synapses, indicating that Pep-19 does not affect overall cerebellar structure and baseline function but rather has a very specific role in mechanisms of synaptic plasticity at PF-PC synapses. The cellular phenotypes were paralleled by behavioral findings. pep-19-null mice showed no overt signs of cerebellar dysfunction such as ataxia and tremor. Furthermore, the knockout mice showed no significant differences from wild-type littersmates in strength, as assessed by the hanging wire test (Fig. 4D), or in general locomotor performance and endurance, as measured by spontaneous activity on a running wheel (Fig. 4B) or enforced running on a treadmill (Fig. 4C). Indeed, naïve pep-19-null mice and wild-type littermates were initially indistinguishable on the rotarod test. However, locomotor learning deficits became progressively apparent during subsequent training sessions (Fig. 4A). Following a 9-day rest period, mice of all three genotypes were initially indistinguishable on the rotarod test. However, trained wild-type and heterozygous mice perform much better than trained pep-19-null mice at the start of the second period of training. These results indicate that acquisition of locomotor learning is markedly impaired in pep-19-null mice, although retention of locomotor learning still occurs in the knockout mice.

Deficiencies in, and compromised development of, motor skills and motor planning are among the most common findings in DS (17, 18, 55, 60). Furthermore, many cerebellum-dependent deficits are evident in mouse models of DS, including reduced granule cell number (3, 44), mimicking the cerebellar hypoplasia of DS (2) and variable locomotor deficits that, depending on the mouse model, can include gait anomalies and impaired performance on the rotarod test (3, 13, 20). We cannot directly compare our model with those of DS, as the latter involve increased expression of multiple genes including pep-19 (44), whereas ours exclusively involves pep-19 loss. Nevertheless, this study is the first to show that pep-19 per se is a critical determinant of synaptic plasticity in the cerebellum and locomotor learning. Thus, alterations of PEP-19 levels in DS could potentially contribute to the motor deficits in this disorder.

Distinct calcium/CaM-dependent signaling components are known to play critical but different roles in synaptic plasticity and locomotor learning in the cerebellum. Genetic elimination of either αCaMKII (26) or βCaMKII (59) results in deficits in locomotor performance and/or learning and, analogous to the situation seen for pep-19-null mice, PF-PC LTD is converted into LTP. Because Pep-19 is highly enriched in PC and not present in granular neurons, it is clear that this change in the direction of synaptic plasticity is due to postsynaptic mechanisms that most likely involve insertion or internalization of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid) receptor subunits (11, 38, 40, 46, 61). It has been proposed that the direction of synaptic plasticity at PF-PC synapses is governed by the balance between postsynaptic protein kinases and phosphatases, where kinases are responsible for LTD and phosphatases are responsible for LTP mechanisms (4, 12, 32, 49). Indeed, induction of PF-PC LTD is dependent upon a set of protein kinases that include αβCamKII (26, 59), whereas LTP at these synapses is dependent upon a series of protein phosphatases that include PP2B (4, 49). As Pep-19 lies upstream of these enzymes in the CaM signaling cascade, it can potentially influence both aspects of synaptic plasticity, with the net outcome being a reversal of LTD to LTP.

The precise mechanisms of this reversal of plasticity are not clear. The pep-19-null mouse would be predicted to have a gain of CaM-dependent function by increasing the fraction of calcium-bound CaM in the Purkinje cell. The increase in CaM activity might affect multiple pathways that are related to synaptic plasticity. However, the most parsimonious explanation is that the increase in CaM activity affects both CaMKIs and calcium/CaM-dependent protein phosphatases such as PP2B (49), with the balance favoring PP2B-dependent pathways over CamKII-dependent mechanisms, resulting in LTP as the net effect. Therefore, the pep-19-null mouse may be useful in dissecting the interrelationship between these two modes of synaptic plasticity.

**ACKNOWLEDGMENTS**

This work was supported in part by NCI Cancer Center Support Grant CA 21765, National Institutes of Health grants NS040361 and NS042828 to J.I.M., MH079079 to S.S.Z., and ALSAC (American Lebanese Syrian Associated Charities). We thank the Hartwell Center for Bioinformatics and Biotecltechnolog at St. Jude Children’s Research Hospital for all DNA sequencing and synthesis. None of us have any financial interests or conflicts of interest in the data presented in this paper.

**REFERENCES**

52. Sköld, K., et al. 2006. Decreased striatal levels of PEP-19 following MPTP lesion in the mouse. J. Proteome Res. 5:262–269.