Supporting Information

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SI Materials and Methods

Animals. We used early postnatal CD-1 mice (both sexes). All animal procedures were approved by the Internal Animal Care and Use Committee of the Cleveland Clinic Foundation and the University of Rouen.

Measurement of the Translocation of Granule Cells in the Early Postnatal Mouse Cerebella Using BrdU. P8 mice were i.p. injected with BrdU (50 μg/g bw) (1). At 2 h, 1 d, and 2 d after BrdU injection, all animals were transcardially perfused with 4% paraformaldehyde. Brains were postfixed in 4% paraformaldehyde for 24 h, stored in a 30% sucrose solution, and sectioned sagittally into 30-μm-thick slices. In each section, cells that had incorporated BrdU into their DNA were detected by an anti-BrdU monoclonal antibody (BrdU labeling and Detection Kit I; Boehringer Mannheim) and fluorescein-conjugated secondary antibody (1). Fluorescent signals were detected and processed using a confocal microscope.

Observation of the Migration of Granule Cells Using Acute Cerebellar Slices. Cerebella of P10 mice were sectioned transversely or sagittally into 150-μm-thick slices on a vibrating blade microtome (VT1000S; Leica Instruments) (2). To label granule cells, cerebellar slices were incubated for 4 min in 2 μM CellTracker Green CMFDA (5-chloromethylfluorescein diacetate; Invitrogen), which was added to the culture medium (2). The culture medium consisted of DMEM/F12 (Invitrogen) with N2 supplement, 90 U/mL penicillin, and 90 μg/mL streptomycin. The slices were subsequently washed with the culture medium, and put in a CO_2 incubator. Two hours after labeling, slices were transferred into the chamber of a microincubator attached to the stage of a confocal microscope (SP5; Leica). The speed of granule cell movement is closely related to the temperature of the medium; lowering the medium temperature slows cell movement (3). Therefore, the chamber temperature was kept at 37.0 ± 0.5 °C using a temperature controller (TC-202; Medical System Corp.), and the slices were provided with constant gas flow (95% O_2, 5% CO_2). To prevent movement of the slice preparations during observation, a nylon net glued to a small silver wire ring was placed over the preparations. A confocal microscope was used to visualize migrating granule cells labeled with CellTracker Green CMFDA in the slices. The use of this microscope permitted high-resolution imaging of migrating granule cells up to 100-μm deep within the tissue slices. The tissue was illuminated with a 488-nm wavelength light from an argon laser through an epifluorescence inverted microscope equipped with a 40× oil-immersion objective (N.A. 1.25; Leica), and fluorescence emission was detected at 530 ± 15 nm. To resolve the movement of granule cells clearly, we typically collected image data at an additional electronical zoom factor of 1.5–2.0. Images of CellTracker Green CMFDA-labeled granule cells in a single focal plane or up to 20 different focal planes along the *z* axis were collected with laser scans every 5–30 min for up to 4 h. At the beginning and end of each recording session for each preparation, frame images were recorded with 40× magnification (electronical zoom factor of 1), or 20× magnification (electronical zoom factor of 1) to determine the orientation of the slice preparations, the borders of cortical layers (the EGL, ML, PCL, and IGL), and the position of granule cells by optical sectioning of several different focal planes along the *z* axis.

Observation of Migration of Isolated Granule Cells Migration Using Cerebellar Microexplant Cultures. Cerebella of P0–P3 mice were placed in ice-chilled HBSS, and freed from meninges and choroid plexus (4). Cerebellar slices were then made with a surgical blade, from which white matter and deep cerebellar nuclei were removed. Rectangular pieces (50–100 μm) were dissected out from the remaining tissue, which mainly consisted of cerebellar gray matter, under a dissecting microscope. Small pieces of cerebellum were placed on 35-mm glass-bottom microwell dishes (MatTec Co.) coated with poly-L-lysine (100 μg/mL)/laminin (20 μg/mL), with 50 μL of the culture medium. We used poly-L-lysine and laminin as substrata, because these materials provide a scaffold for migrating granule cells and promote their movement. We were aware that the speed of granule cell movement depends on the concentrations of laminin coated on coverslips. Higher (50–100 μg/mL) or lower (1–5 μg/mL) concentrations of laminin significantly reduced the speed of granule cell movement (4). Therefore, we used a concentration of 20 μg/mL of laminin, which allows granule cells to migrate at the fastest speed. Each dish was put in a CO_2 incubator (37 °C, 95% air, 5% CO_2). Two hours after plating, 1 mL of the culture medium was added to each dish. The incubation medium consisted of Neurobasal medium (Invitrogen) with N2 supplement, 90 U/mL penicillin and 90 μg/mL streptomycin. In these cultures, more than 95% of the migrating neurons were granule cells, which were easily distinguished from other neurons by the small size of their cell bodies. Although granule cells were prepared from the EGL and IGL of all lobules of the cerebellum, the vast majority of granule cells were derived from the EGL, because at the age of P0–P3, the IGL contains only very small numbers of postmigratory granule cells (4). Therefore, the majority of granule cells were at the same developmental stage. One day after plating, dishes were transferred into the chamber of a microincubator (Medical System Corp.) attached to the stage of a confocal microscope (Leica). Chamber temperature was kept at 37.0 ± 0.5 °C, and the cells were provided with a constant gas flow (95% air, 5% CO_2). The transmitted images of migrating granule cells at 488 nm were collected every 60 s for up to 4 h.

Measurement of Changes in Intracellular Ca^{2+} Levels of Migrating Granule Cells. Small pieces of P0–P3 mouse cerebellum were placed on 35-mm glass-bottom microwell dishes coated with poly-L-lysine (100 μg/mL)/laminin (20 μg/mL). Each dish was put in a CO_2 incubator (37 °C, 95% air, 5% CO_2). The incubation medium consisted of Neurobasal medium (Invitrogen) with N2 supplement, 90 U/mL penicillin, and 90 μg/mL streptomycin. One day after plating, granule cells were incubated for 30 min with the cell-permeant acetoxymethyl ester form of 1 μM Oregon Green 488 BAPTA-1 (Invitrogen) diluted in the culture medium (5). The cells were subsequently washed 3× with the culture medium, and the dye was allowed to de-esterify for an additional 30–60 min in the CO_2 incubator. A confocal microscope was used to examine the changes in the intracellular Ca^{2+} levels. The granule cells loaded with Oregon Green 488 BAPTA-1 were illuminated with a 488-nm wavelength light, and fluorescence images for Ca^{2+} measurements (at 530 ± 15 nm) were collected every 1–10 s for up to 2 h. The changes in fluorescence intensity of each granule cell were normalized to its baseline fluorescent intensity.

Real-Time Observation of Granule Cell Migration in the Early Postnatal Mouse Cerebella Using a Confocal Microscope and DiI. At P10, deep anesthesia was induced by i.p. injection of urethane (1.0 μg/g bw). The skin and bone covering on the dorsal surface of cerebella were surgically removed under a dissecting microscope. A small volume of DiI solution (Invitrogen) was injected into the EGL of

the lobules V, VI, and VII of the cerebellum through glass electrodes with the use of a pressure injection system. Then, warmed gelatin was applied to the surface of the cerebellum and the incision site was sealed with a coverglass by dental glue. Two hours after DiI injection, the mice were transferred to the stage of a confocal microscope (SP5; Leica) and held dorsal-side down on the stage. Using a heating pad, the rectal temperature of animals was maintained at 37.0 °C during the entire period of observation. A confocal microscope was used to visualize the tangential migration of DiI-labeled granule cells in the EGL. Images of DiI-labeled granule cells in up to 10 different focal planes along the *z* axis were collected every 3 min for up to 4 h. Anesthesia wearing off caused problems for monitoring granule cell migration over time. To avoid this potential problem, throughout the observation session, animals were monitored every 30 min for changes in respiratory rate, return of toe-withdrawal reflexes, or any other body movement. Any of the indications that the anesthesia was wearing off was followed by supplemental injections of urethane (0.3 μg/g bw).

Detection of Apoptotic Cell Death of Granule Cells and Granule Cell Precursors. At P10, mice were transcardially perfused with 4% paraformaldehyde. Brains were postfixed in 4% paraformaldehyde for 24 h, stored in a 30% sucrose solution, and sectioned into 30-μm-thick slices. In each section, the apoptotic cell death of granule cells and granule cell precursors was determined by the TUNEL assay kit (Roche Diagnostics) according to the manufacturer's instructions (6). After staining with TUNEL, the sections were incubated with 400 μL of 0.33 mM TO-PRO3 (Invitrogen) for 5 min at room temperature for nuclear staining.

Statistical Analysis. Comparisons between groups were made using the Student *t* test throughout the study. $P < 0.05$ was considered statistically significant.

1. Komuro H, Yacubova E, Yacubova E, Rakic P (2001) Mode and tempo of tangential cell migration in the cerebellar external granular layer. *J Neurosci* 21:527–540.
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6. Kumada T, Lakshmana MK, Komuro H (2006) Reversal of neuronal migration in a mouse model of fetal alcohol syndrome by controlling second-messenger signalings. *J Neurosci* 26:742–756.

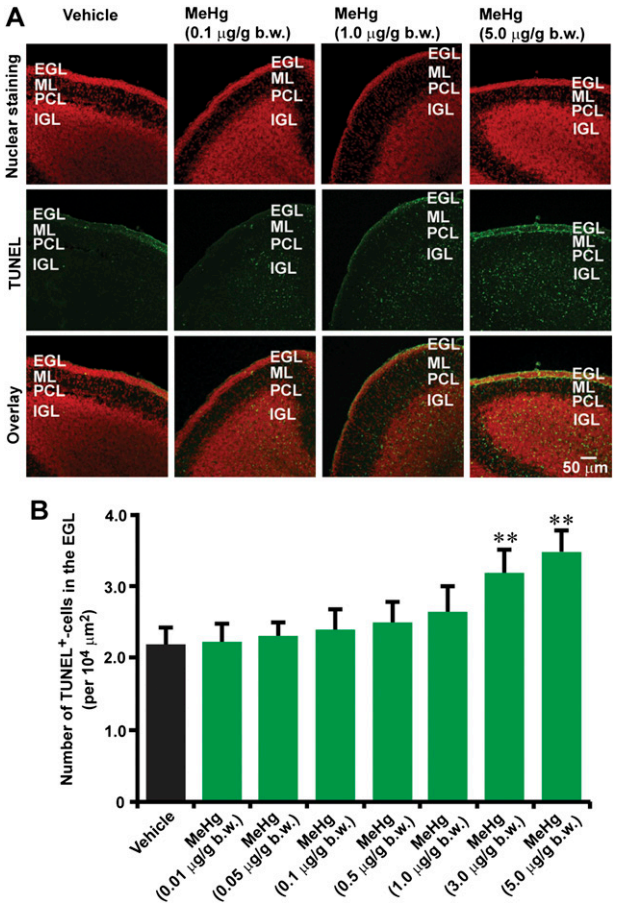

Fig. S1. Increase in the apoptotic cell death of granule cells and granule cell precursors in the EGL of P10 mouse cerebella by MeHg. (*A*) Photographs showing the apoptotic cell death of granule cells and granule cell precursors in P10 mouse cerebella by i.p. injections of MeHg (0.1, 1.0, and 5.0 μg/g bw) over 4 d (P6–P9). (*B*) Histogram showing the increase in the number of apoptotic cell death of granule cells and granule cell precursors in the EGL of P10 mouse cerebella by an i.p. injection of MeHg (0.01, 0.05, 0.1, 0.5, 1.0, 3.0, and 5.0 μg/g bw) for 4 d (P6–P9). Each column represents the average value obtained from at least 500 cells. Bars represent SD. $^{**}P < 0.01$ indicates statistical significance.

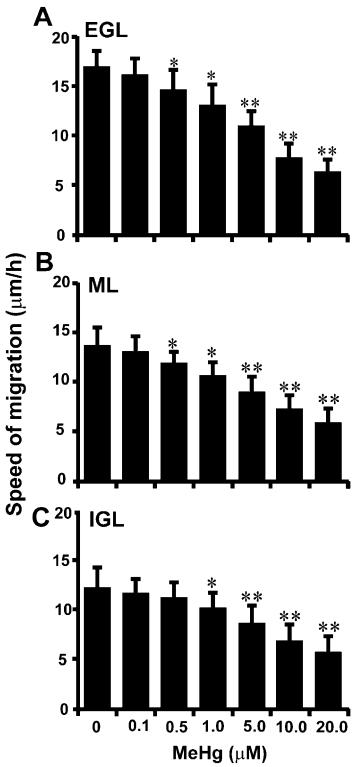

Fig. S2. Effects of seven different doses (0, 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 μM) of MeHg on the speed of granule cell migration in the EGL (*A*), ML (*B*), and IGL (*C*) of cerebellar slices obtained from a P10 mouse. Each column represents the average speed from at least 50 migrating cells. Bars represent SD. $*P < 0.05$ and $**P < 0.01$ indicate statistical significance.

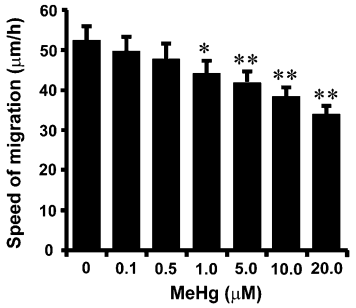

Fig. S3. Dose-dependent reduction of the migration speed of isolated granule cells in the microexplant cultures of P0–P3 mouse cerebella by MeHg (0, 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 μM). Each column represents the average speed obtained from at least 60 migrating cells. Bars represent SD. $*P < 0.05$ and $**P < 0.01$ indicate statistical significance.