Astrocytes restore connectivity and synchronization in dysfunctional cerebellar networks

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Evidence suggests that astrocytes play key roles in structural and functional organization of neuronal circuits. To understand how astrocytes influence the physiopathology of cerebellar circuits, we cultured cells from cerebella of mice that lack the *ATM* gene. Mutations in *ATM* are causative of the human cerebellar degenerative disease ataxia-telangiectasia. Cerebellar cultures grown from *Atm*^{−/−} mice had disrupted network synchronization, atrophied astrocytic arborizations, reduced autophagy levels, and higher numbers of synapses per neuron than wild-type cultures. Chimeric circuitries composed of wild-type astrocytes and *Atm*^{−/−} neurons were indistinguishable from wild-type cultures. Adult cerebellar characterizations confirmed disrupted astrocyte morphology, increased GABAergic synaptic markers, and reduced autophagy in *Atm*^{−/−} compared with wild-type mice. These results indicate that astrocytes can impact neuronal circuits at levels ranging from synaptic expression to global dynamics.

astrocyte | neural circuit | synchronization | disease | ATM

Brain degenerative diseases (BDDs) disrupt brain function by impairing the ability of specific neuronal circuitries to locally process (segregate) and distantly communicate (integrate) information across brain-spanning networks (1, 2). At the neuronal circuit level, BDDs induce the loss of different cell types and cellular functionality (2, 3) and alter circuit topology and dynamics. Currently, it is unclear how particular diseases explicitly alter specific brain circuits and neuro-glial populations.

In the last two decades, it has become appreciated that glial cells play a critical role in BDDs (4). The symptoms of BDDs arise from pathological changes to neuro-glia interactions (5), leading to neuronal cell death, disrupted neuro-glia communication, and impaired cell function (3), all of which affect global dynamics of brain circuitry. Astrocytes, a particular glial cell type, play key roles in regulating the pathophysiology of neuronal functions (6). Specifically, perisynaptic sheaths of astrocytes cover the majority of synapses in the central nervous system and are essential for synaptogenesis, maturation, and maintenance of synapses (7). This structural-functional unit, also called the “tripartite synapse,” enables astrocytes to influence synaptic communication via gliotransmission (5).

In this work, we tested the hypothesis that neuronal circuit dynamics were impacted as a consequence of disrupted neuron-astrocyte physiology in a mouse model of the BDD that results from a deficiency in Ataxia Telangiectasia Mutated (ATM) protein. The gene encoding ATM is mutated in the human genetic disease ataxia-telangiectasia (A-T) (8, 9). One of the most devastating symptoms of A-T is the cerebellar ataxia, with significant loss of Purkinje and granule neurons in the cerebellum, that leads progressively to general motor dysfunction (10).

We used primary cerebellar cultures grown from postnatal wild-type (WT) and *Atm*^{−/−} mice to study how *Atm* deficiency influences the structure and dynamics of cerebellar neuronal-astrocyte circuits. We hypothesized that *Atm* deficiency impairs the neuronal-astrocytic interactions underlying spontaneous

neuronal synchronizations (NSs), a hallmark activity pattern of the developing nervous system that could be observed in many different circuits in various species and in vitro preparations including primary neuronal cultures (11–13).

We report that the absence of *Atm* in neurons and astrocytes severely alters astrocyte morphology and the number of pre-synaptic and postsynaptic puncta, disrupting NSs within cerebellar networks. Higher numbers of synaptic puncta in *Atm*^{−/−} networks relative to numbers in WT cultures were associated with lower levels of autophagy. These reported structural and functional anomalies were all rescued in chimeric neuronal networks composed of *Atm*^{−/−} neurons and WT astrocytes. In contrast, cultures of WT neurons with *Atm*^{−/−} astrocytes led to significant neuronal cell death. Characterizations of adult *Atm*^{−/−} cerebella similarly showed disrupted astrocyte morphology, up-regulated GABAergic markers, and dysregulated mTOR-mediated signaling and autophagy. These results, obtained in an in vitro model system and confirmed in adult mice, demonstrate a possible role played by astrocytes in the physiopathological, structural, and functional organization of the cerebellar circuits.

Results

Absence of *Atm* Impairs in Vitro Cerebellar Neuronal Network Synchronizations. All *Atm*^{−/−} or WT astrocyte and neuronal cultures/networks referred to in the text were derived from single animals. A total of 80% of the cells in the primary cerebellar

Significance

Within the long-standing debate of whether glial cells contribute to neuronal circuits, we provide evidence of the impact of astrocytes on the physiopathology and the structural-functional organization of cerebellar neuronal circuits derived from *Atm*-deficient mice. In vitro and adult mouse characterizations show how disrupted astrocyte morphology is associated with an increased presence of synaptic markers in *Atm*^{−/−} compared with wild-type circuits, also related to reduce autophagy. Our study is corroborated by an in vitro demonstration of how replenishment of neuronal circuits with healthy astrocytes can restore *Atm*^{−/−} neuronal circuits' dynamics.

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cultures were granule neurons (NeuN-positive; $n = 19$) (14, 15). The majority of the remaining cells were astrocytes (GFAP- and Vimentin-positive), with a small portion of only Vimentin-positive cells (oligodendrocytes and glia precursors) (refs. 16–18 and *SI Appendix*, Fig. S1 *A* and *B*).

The spontaneous activities of cultured cerebellar networks derived from *Atm*^{−/−} and WT mice were monitored by using calcium-based imaging (Fig. 1). The calcium signals that originated from the neuronal activity were characterized by stereotypical sharp calcium transients (on the order of a few dozens of milliseconds, abolished by tetrodotoxin; $n = 3$) and were generated and recorded from granule cell bodies (isolated or organized in multicellular clusters, as shown in *SI Appendix*, Fig. S1 *C–H*). Extended wider segmentation of calcium images including astrocytic processes (*SI Appendix*, Figs. S1*H* and S2) confirmed the presence of fast calcium signals only in neuronal cells. Stereotypical slow calcium signals of astrocytes were rarely observed (and not included in the network analysis; *Methods*). The neuronal calcium signals were mediated by glutamatergic synaptic connectivity, since they were blocked by an AMPA receptor antagonist (2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide; $n = 7$) (*SI Appendix*, Fig. S1*E*, *Right*). Spontaneous NSs (*Methods*), a general common feature of primary cultures independent of the originating circuit (11–13), were defined as neuronal coactivations of at least two neurons and were observed in both *Atm*^{−/−} and WT cultures at similar frequencies of ~ 0.07 Hz (Fig. 1*E*). Global synchronizations (GSs) that recruited nearly the entire neuronal population were significantly more frequent in the WT compared with the *Atm*^{−/−} cultures [$P < 0.0001$; Mann–Whitney *U* (MWU) test; Fig. 1*E*]. Conversely, sparse synchronizations (SSs), recruiting $< 3\%$ of the active neurons, were significantly more frequent in *Atm*^{−/−}

compared with WT cultures; in WT cultures, SSs were very infrequent (Fig. 1*E*; $P < 0.0001$, MWU test).

When analyzing the GS build-up, we observed that early activated cells repeatedly preceding the GS in WT and *Atm*^{−/−} networks represented $9 \pm 1\%$ ($n = 28$) and $13 \pm 3\%$ ($n = 22$) of the active neuronal population, respectively, with no significant difference between the two groups (*SI Appendix*, Fig. S3; $P > 0.05$, two-sample Kolmogorov–Smirnov test). Cells recruited in the SS ($n = 2,615$ from 22 *Atm*^{−/−} networks) were spatially closely located, and this localization was significant compared with randomly computationally generated SSs (*SI Appendix*, Fig. S4).

No cell showed higher significant participation in SS since recruitment frequencies were similar in the *Atm*^{−/−} networks and in randomly computationally generated sequences of SSs that preserved the recruited cellular populations (Kolmogorov–Smirnov test, $P > 0.05$; *Methods*).

Absence of *Atm* Reduces Morphological Complexity of Astrocytes.

Previous studies showed that astrocyte coverage of blood vessels is dramatically reduced in the retinas of *Atm*^{−/−} mice (10). We performed immunostaining to determine whether astrocyte complexity was also altered in *Atm*^{−/−} cerebellar cultures. Staining of primary astrocytes with GFAP revealed a less complex cell arborization (Fig. 2*A* and *B*) and fewer processes originating from the cell bodies in *Atm*^{−/−} cultures than in WT cultures ($P < 0.0001$, MWU test). In addition, the mean total length of astrocyte processes in the entire astrocytic network (marked by GFAP) normalized to the number of the nonneuronal cells (i.e., DAPI–NeuN-negative cells) was significantly shorter in *Atm*^{−/−} compared with WT networks (Fig. 2*C*; $P < 0.0001$, *t* test).

WT Astrocytes Restore WT NSs in *Atm*^{−/−} Cerebellar Networks. Next, we tested the hypothesis that impaired neuro–astrocyte interactions induced by *Atm* deficiency disrupted spontaneous NSs. We generated cerebellar chimeric cultures from two mice with half the cerebellum of each animal plated as a control and half used to create the chimeric network composed of neurons from one animal and astrocytes from another animal with a different genotype (*SI Appendix*, Fig. S5). The cellular type and origin in the chimeric cultures will be specified according to the following nomenclature: N_{WT} , A_{WT} , $N_{Atm^{-/-}}$, and $A_{Atm^{-/-}}$, where *N* and *A* refer to neurons and astrocytes, respectively, and subscripts *WT* and *Atm*^{−/−} refer to the genotype of the donor animal. To validate the procedure, we prepared chimeric cultures from two different animals with the same genotype. The chimeras showed no statistical differences in terms of synchronization compared with single-animal cultures ($P > 0.05$, MWU tests for GS, SS, and NS).

Immunostaining of neurons (NeuN) and astrocytes (GFAP) in [$N_{WT} A_{Atm^{-/-}}$] and [$N_{Atm^{-/-}} A_{WT}$] chimeras compared with single-animal WT and *Atm*^{−/−} cultures showed that the morphology of the astrocytes were not influenced by the presence of neurons from a different mouse and/or genotype [Fig. 3*A* and *B*; $P < 0.0001$, Kruskal–Wallis (K–W) with Dunn’s multiple comparison (Dunn’s) test; *SI Appendix*, Fig. S6]. Notably, only a few neurons in [$N_{WT} A_{Atm^{-/-}}$] chimeric cultures survived (Fig. 3*A*).

We next analyzed neuronal dynamics in the WT, *Atm*^{−/−}, and chimeric cultures. The frequencies of NS events were similar in WT, *Atm*^{−/−}, and [$N_{Atm^{-/-}} A_{WT}$] chimeric cultures (Fig. 3*C*; K–W with Dunn’s test). In contrast, synchronizations were absent in the 3 of 10 [$N_{WT} A_{Atm^{-/-}}$] chimeric cultures that survived 17 d in vitro (DIV). [$N_{Atm^{-/-}} A_{WT}$] chimeric cultures were statistically similar to WT cultures in terms of GSs and SSs, compared with *Atm*^{−/−} networks (Fig. 3*D* and *E*; $P < 0.001$, K–W with Dunn’s test).

Astrocytic Genotype Impacts Synaptic and Autophagy Levels in Cerebellar Networks. Based on the known roles of astrocytes in the development, maturation, and dynamics of synapses (5, 7, 19, 20), we hypothesized that the impairment of neuronal dynamics

Fig. 1. NS impairment in *Atm*^{−/−} cerebellar networks. (*A* and *B*) Raster plots of neuronal activity for WT network (*A*) and *Atm*^{−/−} network (*B*). (*C*) Calcium signals from an *Atm*^{−/−} network displaying a SS (marked in green arrow) and followed by two GS events (marked in black arrows). The relative variation in fluorescence to the baseline level ($\Delta F/F$) is reported. (*D*) Calcium dye loading of the *Atm*^{−/−} network. Cells marked in green are recruited in the SS reported in *B*. (*E*) Synchronization frequencies in WT and *Atm*^{−/−} cultures. Significant differences are marked by asterisks. *** $P < 0.001$ (MWU test; $n = 43$ networks from 19 WT animals, $n = 43$ networks from 21 *Atm*^{−/−} animals).

Fig. 2. Astrocytic processes atrophy in *Atm*^{-/-} cerebellar cultures. (A) Images of a single astrocyte stained with GFAP (green) in WT (Upper) and *Atm*^{-/-} (Lower) culture. The yellow dashed squares highlight the processes originating from the cell body. (B, Left) Immunocytochemical staining of neurons (NeuN; red), astrocytes (GFAP; green), and nuclei (DAPI; blue) in WT (Upper) and *Atm*^{-/-} (Lower) cultures. (B, Right) Automatic extraction of processes contours (red lines) from GFAP staining (gray scale). (C) Quantification of processes length (normalized to the pixel size) per astrocyte. Significant difference is indicated by asterisks. ****P* < 0.001 (*t* test). *n* = 7 networks from four WT cultures, *n* = 7 networks from four *Atm*^{-/-} cultures.

in the cerebellar networks containing *Atm*^{-/-} astrocytes was due to disruption of neuronal connectivity. To test this hypothesis, we analyzed synaptic protein levels in *Atm*^{-/-} and WT cultures. We found that levels of both presynaptic [Synapsin1 (Syn1)] and postsynaptic (PSD-95) markers were significantly higher (68% and 58% increases, respectively) in the *Atm*^{-/-} cultures in comparison with WT cultures (Fig. 4 *B* and *C*; *P* < 0.01, MWU test). To evaluate the contribution of astrocytes to the neuronal circuit, we compared the levels of Syn1 and PSD-95 protein in *Atm*^{-/-}, [*N*_{*Atm*^{-/-}} A_{WT}] chimeric, and WT cultures: Syn1 (MWU test) and PSD-95 (MWU test) were expressed at similar levels (Fig. 4 *D* and *E*). Using immunocytochemistry, we further compared the synaptic termini in WT, *Atm*^{-/-}, and [*N*_{*Atm*^{-/-}} A_{WT}] chimeric cultures. In *Atm*^{-/-} cultures, we observed a higher average numbers of presynaptic puncta (Syn1) (Fig. 4 *F* and *G*; *P* < 0.001, K-W with Dunn's test) and postsynaptic puncta (PSD-95) (Fig. 4 *H* and *I*; *P* < 0.05, K-W with Dunn's test) per neuron in comparison with the WT and [*N*_{*Atm*^{-/-}} A_{WT}] chimeric cultures.

Given these results and the previous reports of disrupted number of synapses in rodent models of inherited developmental disorders (21, 22) and impaired pruning with dysregulated mTOR-autophagy signaling observed in autism (23), we investigated whether mTOR hyperactivation and reduction of autophagy occurs in *Atm*^{-/-} cerebellar cultures. We characterized the levels of the phosphorylated p70S6K protein, indicative of mTOR activation (24), and p62/SQSTM1 (abbreviated here as p62), a scaffold protein that binds LC3 and ubiquitinated substrates destined for degradation (24). The levels of p62 decrease when autophagy is induced (24). Both the p70S6K and the p62 protein were significantly increased in *Atm*^{-/-} cultures in comparison with WT cultures (Fig. 4 *K* and *L*; *P* < 0.05, *P* < 0.01, K-W with Dunn's test), indicating that mTOR activation and inhibition of autophagy occurs in *Atm*^{-/-} cultures. Although intermediate values of p70S6K were observed in the [*N*_{*Atm*^{-/-}} A_{WT}] chimeric cultures with no significant difference from either WT or *Atm*^{-/-} networks, we found similar levels of p62 in WT and [*N*_{*Atm*^{-/-}} A_{WT}] chimeric cultures that were significantly lower than levels in the *Atm*^{-/-} culture (Fig. 4*L*; *P* < 0.01, K-W with Dunn's test), implying that, to some extent, the autophagy process occurs even when mTOR is induced.

Astrocytic Atrophy and Impaired Autophagy Are Associated with Increased GABAergic Synaptic Levels in Adult *Atm*^{-/-} Mouse Cerebella. To understand whether the impact of astrocytic atrophy on the neuronal circuitry demonstrated in vitro results in similar consequences in adult cerebellum, cerebellar circuits in adult WT and

Atm^{-/-} mice were examined. First, similarly to the in vitro results, histological sections confirmed that there was a reduction in GFAP-stained area in both Bergmann glia (41% decrease, MWU test) and Velate astrocytes in the granule layer (47% decrease, MWU test) in the cerebella of adult (4 mo) *Atm*^{-/-} mice compared with WT mice (Fig. 5 *A–C* and *SI Appendix*, Fig. S7). Second, synaptic protein levels in adult cerebella were analyzed. In accordance with in vitro results, Syn1 levels were up-regulated in *Atm*^{-/-} relative to WT adult cerebella (25% increase, *t* test; Fig. 5*G*). Levels of PSD-95 (Fig. 5*I*) and Vglut, a glutamate transporter (Fig. 5*L*), were similar in both genotypes (*t* test and MWU test, respectively). Up-regulations of Gephyrin, an inhibitory postsynaptic protein (23% increase, *t* test; Fig. 5*H*), and Vgat, a vesicular inhibitory transporter (25% increase, *t* test; Fig. 5*K*), in *Atm*^{-/-} cerebella indicated that the increased synaptic levels in the adult cerebellum were confined to GABAergic synapses or pathways. Third, we examined the levels of the autophagy markers in the adult cerebellum. As we found in vitro, both p70S6K and p62 were significantly overexpressed in *Atm*^{-/-} compared with WT cerebella (Fig. 5 *M–O*; MWU test).

Discussion

To dissect the impact of WT and *Atm*^{-/-} astrocytes on the structure and dynamics of cerebellar networks, we first used WT, *Atm*^{-/-}, and chimeric cultures of cerebellar cells. Using the chimeric culture approach, we found that the absence of *Atm* in both astrocytes and neurons led to (*i*) reductions in lengths and

Fig. 3. WT astrocytes restore GSs in *Atm*^{-/-} cerebellar networks. (A) Immunocytochemical staining of the neurons (NeuN; red), astrocytes (GFAP; green), and nuclei (DAPI; blue). Half of the cerebellar cells from each mouse were plated as controls (Upper) and the other half as chimeric cultures (Lower). WT astrocytes are present in Left, whereas *Atm*^{-/-} astrocytes are present in Right, and in both cases they maintain their original morphology independently of the genotype of the cocultured neurons. (B) Number of astrocytic processes originating from the cell body in WT networks (*n* = 18; five cultures), [*N*_{*Atm*^{-/-}} A_{WT}] chimeric networks (*n* = 16; four cultures), *Atm*^{-/-} networks (*n* = 17; five cultures), and [*N*_{WT} A_{*Atm*^{-/-}}] chimeric networks (*n* = 8; three cultures) (*P* < 0.001). (C–E) Synchronization frequencies of NS (C; *P* > 0.05), GS (D; *P* < 0.001), and SS (E; *P* < 0.001) for the WT, *Atm*^{-/-}, and [*N*_{WT} A_{*Atm*^{-/-}}] chimeric networks. Note that the WT (*n* = 43; 19 animals) and the [*N*_{*Atm*^{-/-}} A_{WT}] chimeric (*n* = 11; six animals) networks are characterized by higher levels of GS and lower levels of SS events, whereas the *Atm*^{-/-} (*n* = 43; 21 animals) networks display low levels of GS and high levels of SS. Significant differences are marked by asterisks. ***P* < 0.01; ****P* < 0.001 (K-W test followed by Dunn's test).

Fig. 5. In adult *Atm*^{-/-} cerebella, GFAP immunoreactivity is reduced in astrocytic processes; levels of Syn1, Gephyrin, and Vgat are increased; and autophagy is reduced. (A and B) Z-stack histological images of cerebella from 4-mo-old WT and *Atm*^{-/-} mice at different magnifications. Astrocytes are stained with GFAP (green), granule neurons with NeuN (magenta), and nuclei with DAPI (blue). The images show Bergmann glia processes (green) at the molecular layer of cerebellar folia. Note that there are fewer GFAP-stained processes in the *Atm*^{-/-} cerebella. (C) Morphology of a single representative astrocyte (*n* = 4 for both genotypes). These astrocytes had fewer numbers of processes in *Atm*^{-/-} mice than in WT mice. (D–F) Representative immunoblot (four independent mice) of Syn1 (D), Gephyrin (E), and PSD-95 (F) from 4-mo-old WT and *Atm*^{-/-} mice. Panel D and F are from the same blot; thus, measured using the same loading control. (G) Fold difference in Syn1 levels in *Atm*^{-/-} (*n* = 5) relative to WT (*n* = 5) cerebella (t test). (H) Fold difference in Gephyrin in *Atm*^{-/-} (*n* = 5) relative to WT (*n* = 5) cerebella (t test). (I) Fold difference in PSD-95 in *Atm*^{-/-} (*n* = 5) relative to WT (*n* = 5) cerebella (t test). (J) Representative immunoblot (four independent mice) of Vgat and Vglut from 4-mo-old WT and *Atm*^{-/-} cerebella. (K) Fold difference in Vgat levels in *Atm*^{-/-} (*n* = 5) relative to WT (*n* = 5) cerebella (t test). (L) Fold difference in Vglut in *Atm*^{-/-} (*n* = 5) relative to WT (*n* = 5) cerebella (MWU test). (M) Representative immunoblot (four independent mice) of p62 and p70S6K from 4-mo-old WT and *Atm*^{-/-} cerebella. (N) Fold difference in p62 levels in *Atm*^{-/-} (*n* = 5) relative to WT (*n* = 5) cerebella (t test). (O) Fold difference in p70S6K in *Atm*^{-/-} (*n* = 4) relative to WT (*n* = 4) cerebella (MWU test). Significant differences are marked by asterisks. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

are not present in vivo or in situ, coherent oscillations have been recorded in the granular layer in vivo driven by mossy fiber (33, 34) and synchronized by Golgi cells (35), inputs absent in the

in vitro model. The engineered in vitro chimeric networks, despite displaying artificial topologies, allowed a clear dissection of the role of astrocytes in cerebellar networks, which can be hardly obtained in in vivo experiments.

The cerebellar cultures' spontaneous synchronizations occurred at a frequency of 0.07 Hz, similar to frequencies reported for primary cultures from cortical, retinal, and spinal circuits (11–13, 36). Since the frequency of NSs in WT and *Atm*^{-/-} cultures is similar, but presents a lower number of GSs and an increased number of SSs in *Atm*^{-/-} cultures, we hypothesize that SSs could represent aborted GSs (i.e., network synchronization failures). Similar results have been observed in the neocortex of a mouse model of AD, showing a greater number of false up-state transitions and a smaller number of true up-state transitions in mutant compared with WT animals (37). The presence of spurious or aberrant burst (i.e., SSs) in *Atm*^{-/-} cerebellar cultures that do not lead to GSs (consistent with the fact that cells recruited in the SSs do not play a role in the GS build-up) can be explained by the overexpression of synaptic contacts. This overexpression is possibly a consequence of an impaired synaptic pruning mechanism (38) as discussed above. The apparent contradiction between a larger number of synapses in the *Atm*^{-/-} circuits and lower occurrence of network synchronizations could result from the homeostatic downscaling of synaptic weights between neurons (aborted effective connectivity hypothesis) or from the presence of nonfunctional connections (aborted functional connectivity hypothesis) (39). Both scenarios are consistent with the reduced autophagy in *Atm*^{-/-} cerebellar networks and the hypothesis of lack of synaptic pruning.

Our data support the hypothesis that *Atm*-deficient astrocytes induce a multiscale accumulation of structural and functional defects into cerebellar circuits. These defects have the potential to scale up the impact from molecular pathways to the synaptic level, to cell assemblies, and neuronal circuits.

Methods

Generation of WT and *Atm*-Deficient Mice. The construction of the *Atm*-deficient mice was described (40). All animal care protocols and all experimental protocols used for this study were conducted according to the animal research guidelines from the Institutional Animal Care and Use Committee, Tel Aviv University (ethical approval no. L-14-019).

Cerebellar Dissociated Cultures Enriched in Granule Neurons. Primary cultures of mouse cerebellar granule neurons were prepared from 8-d-old *Atm*^{-/-} and WT mice as described (14). Cultures were prepared from a single mouse (*SI Appendix, SI Methods*).

Generation of Chimeric Networks. Cerebellar cultures were prepared by using the cerebellar dissociated culture protocol (see above). For each pair of pups, a total of three 35-mm Petri dishes were prepared: a control Petri dish from each pup with neurons and astrocytes from the same animal and a single chimeric culture (neurons obtained from one pup and astrocytes from a second pup) (*SI Appendix, SI Methods*).

Neuronal Recording. Recordings were performed on cultures at 17 DIV. The loading procedure and recording setup was described (41) (*SI Appendix, SI Methods*).

Immunocytochemistry. At the end of calcium imaging experiments, cells on coverslips were washed with PBS and fixed with 4% paraformaldehyde for 10 min at room temperature. Details and antibodies list are provided in *SI Appendix, SI Methods*.

Immunohistochemistry. Fresh-frozen cerebella were obtained from 4-mo-old mice, sectioned sagittally in a cryostat to 25-μm sections, and mounted on slides. Details and antibodies list are provided in *SI Appendix, SI Methods*.

Western Blot Analysis. Western blots were done on extracts from both primary granular cultures and 4-mo-old WT and *Atm*^{-/-} mice cerebellar tissues as described (10) (*SI Appendix, SI Methods*).

Data Analysis of Network Dynamics Based on Calcium Imaging. The neuronal activity monitored through calcium imaging was analyzed by using custom code written in Matlab (MathWorks), which implements a few upgrades from what was described (41, 42).

Contour Detection of Neuronal Cell Bodies and Segmentation of Calcium Images. Segmentation of calcium images was aimed to identify neuronal cells. Cell soma boundaries were automatically identified by using the first 1,000 frames acquired at the beginning of the calcium imaging session (*SI Appendix, SI Methods*).

Automatic Detection of Astrocytic Arborization from GFAP Staining in Cultures. The total length of astrocytic arborizations was calculated by using the same automatic identification of boundaries described for neuronal cell body identification (*SI Appendix, SI Methods*).

Statistical Analyses. Following a Shapiro–Wilk normality test, data comparisons were carried out by using a two-tailed *t* test or two-tailed MWU test for

two groups and one-way analysis of variance followed by Tukey's multiple comparison test or K-W test followed by a Dunn's post hoc test for multiple group comparisons. All statistical analyses were performed by using GraphPad Prism (Version 6 for Windows). *P* values < 0.05 were considered statistically significant. Values are expressed as means ± SEM. Significant data are denoted with asterisks: **P* < 0.05; ***P* < 0.01; ****P* < 0.001. Error bars represent mean + SEM. Statistical details of experiments can be found in the figure legends

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