Purkinje neuron dendrite structure: Regulation by the MAP and PI-3 kinase pathways

Presented to the faculty of Lycoming College in partial fulfillment of the requirements for the Departmental Honors in Biology

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Abstract

The structure of a neuron determines much of its function within the central nervous system. The signaling events that regulate neuron morphology and function have only recently been discovered. The Purkinje cells of the cerebellum are ideal subjects for this type of study because they have been well characterized and have highly branched dendritic trees that develop in a stereotyped series of steps. Previous studies have found that interactions between granule cells and Purkinje cells play a key role in Purkinje cell development. Specifically, brian-derived neurotrophic factor (BDNF) contributes to an increase in spine density, and interfering with TrkB signaling creates longer-necked dendritic spines. BDNF binding to TrkB receptors triggers several intracellular signaling pathways. The current study analyzes the effects of the Mitogen Activated Protein (MAP) kinase pathway and the phosphatidylinositol 3-kinase (PI3-K) pathway on the dendrite morphology of cultured Purkinje cells. The MAP kinase inhibitor PD98059 decreased dendrite outgrowth but not cell body size, while the PI-3 kinase inhibitor LY294002 decreased dendrite outgrowth without significantly decreasing cell body area or dendritic length. These results highlight a role for the MAP and PI-3 kinase pathways in regulating Purkinje cell dendrite morphology.

Introduction

The morphology of neurons, including their dendrites and spines, is an important determinant of normal cell function. Although the critical influence of structure on function is well accepted, the detailed mechanisms behind the development of specific neuronal structures are not. An exceptional model in which to study the development of

dendrite and spine morphology is found in the Purkinje cells of the cerebellum. Within the cerebellum, they are the only cells to express calbindin-D_{28K} protein, facilitating their identification. These cells pass through several well-characterized developmental stages that produce a highly branched, easily recognizable dendritic tree (Figure 1; Baptista et al, 1994).

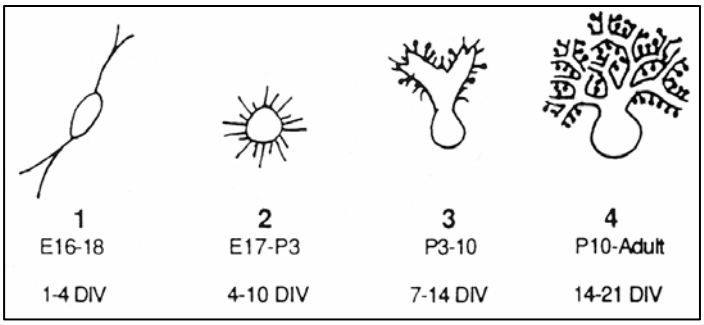


Figure 1. Stages of Purkinje cell development. Neurites are extended to assess the surrounding environment, retracted, and replaced by developing dendrites which become fully mature at 14-21 DIV. E=embryonic day, P=postnatal day, DIV=days in vitro (Morrison, Personal Communication).

In the early stages of development, neurites, processes that cannot be distinguished as either axons or dendrites, are extended to assess whether the Purkinje cell is in an optimal environment in which to grow. If the environment is optimal, the neurites are retracted and dendrites and an axon begin to develop until they reach maturity at about 14-21 days in vitro (DIV) (Figure 1). Throughout this process other cerebellar cells make synaptic contacts with the Purkinje cells and help to coordinate the development of the Purkinje cell dendrites, therefore also influencing proper Purkinje cell function. When Purkinje cells do not make enough contacts with their synaptic partners they do not produce mature dendrites and/or survive poorly, resulting in cerebellar disorders, known as ataxias (Morrison, Personal Communication).

Of the many synaptic partners of a Purkinje cell, which include climbing fibers, mossy fibers, deep nuclei, and the parallel fibers of granule cells, it is the interaction between granule cells and Purkinje cells that influence the survival and development of the Purkinje cell most significantly. Baptista et al (1994) reported that when Purkinje cells were cultured without granule cells, they survived poorly and development was abnormal. The same study also reported that the more granule cells in contact with a Purkinje cell, the better the Purkinje cell develops; this trend was seen between plating densities from $1x10^5$ cells/cm^2 to $11x10^5$ cells/cm^2. Further studies were conducted to discover which molecular signals control Purkinje cell survival and differentiation. Brain-derived neurotrophic factor (BDNF) was a candidate because both granule and Purkinje cells have TrkB receptors on their surface; Purkinje cells contain BDNF protein, but only granule cells contain BDNF RNA, implying that granule cells produce BDNF protein and then transfer it to Purkinje cells (Morrison and Mason, 1998). BDNF treatment increased cell survival when Purkinje cells were cultured alone, but decreased survival when Purkinje cells were cocultured with granule cells (Morrison and Mason, 1998). The same study also showed that the Purkinje cells that survived treatment with BDNF in the cocultures with granule cells had a higher spine density than the controls.

Further study of BDNF and its receptor, TrkB, showed that spine morphology is regulated independently of dendrite development (Shimada et al, 1998). BDNF increased spine density, but did not change overall morphology of either the dendrites or the spines. Blockage of TrkB signaling via application of TrkB-IgG consistently increased the length of the spine necks by approximately 109%, suggesting that normal levels of TrkB signaling keep spine neck lengths short (Shimada et al, 1998).

More recent studies have found similar results using other cell types. Danzer et al (2002) showed that by increasing expression of BDNF via plasmids in dentate granule cells of the hippocampus, both dendrites and axons showed an increase in branching. Increased expression of BDNF was found to induce the formation of basal dendrites and increase the number of short dendritic processes found on the apical side of the cell. By using Sholl analysis, a test to determine how branchy dendrites or axons are, this study also found that within 50um of the cell soma, primary axonal branching was increased three- to fourfold. When transfected cells were treated with the Trk inhibitor K252a, there was no difference in branching compared to control cultures. A study using cortical slices of pyramidal neurons also found that BDNF induced dendritic branching (Horch and Katz, 2002). The culture system utilized in this study allowed for the determination that the increase in dendritic branching is directly correlated with the distance from the source of the BDNF; a significant increase in dendritic branching was only found to appear when the receiving cell was within 4.5um of the source of BDNF.

Although the role of BDNF/TrkB signaling has been well documented in relation to neuron development, the exact mechanisms through which this signaling system works in Purkinje cells is poorly understood. This study aims to increase the understanding of the mechanisms behind Purkinje cell spine development by looking downstream of the TrkB receptor, to the Mitogen Activated Protein (MAP) kinase pathway and the phosphatidylinositol 3-kinase (PI3-K) pathway. In other types of central nervous system (CNS) cells, the activation of the MAP kinase pathway or the PI3-K pathway leads to the activation of transcriptional events that are involved in cell differentiation and survival (Figure 2; Chao, 2003). In the current study, mixed cultures

of cerebellar cells were treated with PD98059 or LY294002, pharmacological agents that inhibit the MAP kinase and PI3-K pathways, respectively. Inhibition of the MAP kinase pathway with PD98059 decreased dendrite outgrowth without affecting cell body size. Inhibition of the PI3-K pathway via LY294002 decreased dendritic branching without affecting cell body size or dendritic length.

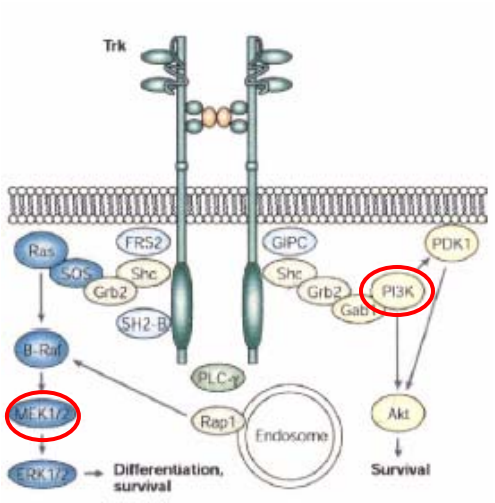


Figure 2. MAP and PI-3 kinase pathways. BDNF binds to the TrkB receptor which activates signaling cascades. The MAP (here, MEK½) and PI-3 kinase pathways are activated and lead to cell differentiation and survival (Chao, 2003).

Methods

Cell culture & immunohistochemistry. C57BL6/J mouse pups from a timed-pregnancy breeding colony at Lycoming College were used. All animal handling was in accordance with IACUC and GCULA guidelines. Brains were removed by decapitation of newborn pups. The cerebella were dissected and the meninges removed. The whole cerebellum was transferred to ice-cold calcium- and magnesium-free (CMF) phosphate buffered saline (PBS) until all brains were dissected (Hatten et al, 1998). Cerebellar cells were dissociated using trypsin, CMF-PBS, and trituration and then plated in 6mm diameter Nunc LabTek poly-D-lysine coated wells at 1.17×10^{6} cells/cm² in horse serum-containing medium. 100mL of horse serum-containing medium was prepared from

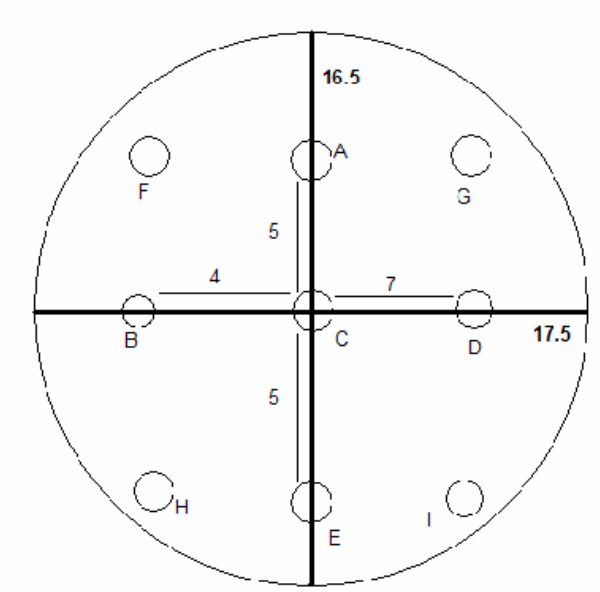
84mL BME, 200uL penicillin-streptomycin, 10mL horse serum, 160uL L-glutamine, 4.8mL 10% glucose, 71uL 10% NaCl, and 769uL dH_2O, calibrated to pH=7.4 and then filter-sterilized, bringing the final pH to 7.6. The next morning the medium was changed to serum-free medium to reduce the effects of growth factors present in the serum-containing medium. Serum-free medium was prepared with 93mL BME, 1g BSA, 1mL Sigma ITS, 200uL penicillin-streptomycin, 70uL L-glutamine, 4.8mL 10% glucose, 79uL 10% NaCl, and 851uL dH_2O, calibrated to pH=7.4 and then filter-sterilized, bringing the final pH to 7.6. The serum-free medium was then exchanged every three to four days thereafter (Hatten et al, 1998).

Pharmacologic Inhibitors. Cultures were treated with pharmacological inhibitors PD98059 or LY294002, which were diluted in dimethyl sulfoxide (DMSO; Calbiochem/EMD Biosciences Inc). Cultures were treated with 0, 10, 20, or 100uM concentrations of PD98059 or 0, 1, or 10uM concentrations of LY294002. After incubation for 14 days, the cultures were fixed with paraformaldehyde and immunostained using anti-Calbindin-D_{28k} (Swant, Bellinzona, Switzerland, 1:2000 dilution) as the primary antibody and goat anti-rabbit peroxidase (Jackson Immunoresearch, 1:3000 dilution; Baptista et al, 1994; Hatten et al, 1998) as the secondary antibody. Slides were dehydrated by placing them in solutions of increasingly higher concentrations of ethanol and coverslipped using Permount.

Image collection and analysis. Images were collected using a Nikon TE2000U inverted microscope and differential interference-contrast (DIC) optics, which confers a 3-dimensional appearance to the cells, with the 60X objective lens. Nine cells were selected from each of two replicate wells according to the method previously described

in which 9 predetermined visual fields were chosen within each well in order to minimize bias (Figure 3; Shimada et al, 1998). Images were analyzed using the MetaVue Imaging System version 6.3 (Molecular Devices Corporation) with custom-designed algorithms compiled by the authors of this article with the assistance of a previous paper that used a similar imaging system (MetaMorph) to analyze comparative data (Klimaschewski et al, 2002).

A. B.



24	9	10	11	12
23	8	1	2	13
22	7	Origen	3	14
21	6	5	4	15
20	19	18	17	16

Figure 3. Method of Cell Selection. A) Nine cells were selected from visual fields A-I. Wells contained 17.5 visual fields horizontally and 16.5 vertically (thick lines). Thin lines indicate the number of visual fields between lettered visual fields. B) If no usable cell was found within the predetermined visual field (origin), the surrounding visual fields were scanned using a pre-arranged method until a usable cell was found.

All images were calibrated prior to measuring the cell parameters so that 1 pixel equaled 0.106 um (Metavue). The dendritic length was measured with the 'multi-line'

tool on the toolbar. With this tool, the dendrite was traced beginning at the center of the longest dendrite's width, where it left the cell body, and following the contour of the center of the dendrite until terminating at the tip. If there was more than one possible longest dendrite, all the possible longest dendrites were measured to determine which was longest. The data were then entered into a Microsoft Excel spreadsheet.

Cell body area was determined by using the 'auto-trace' tool on the toolbar and clicking the mouse in the center of the cell body. This automatically traced around the cell soma based on pixel intensity. The trace was then manually adjusted by clicking and dragging the outline of the cell body to account for areas of the soma that were not included in the original trace because their pixel intensity was too low. The region inside the trace was painted white, then thresholded so only the cell body area was measured, and the data were logged in an Excel spreadsheet (Appendix, 21).

Total cell area was determined in much the same way as cell body area. The entire cell, including cell body and dendrites, but not the axon, was traced using the 'trace region' tool on the toolbar (Appendix, 22). The image was thresholded and the measurement was logged into an Excel spreadsheet. Dendritic tree area was calculated by subtracting the cell body area from the total cell area. For more details, see Morphometric Analysis Routine and Logging Data protocols in the Appendix. Results of each treatment were compared with those for untreated control cells using 2-tailed Student's *t* tests. Significance was based on comparison to untreated controls using a $p \leq 0.05$.

Results

Cerebellar cells from newborn mice were cultured for 14 days in vitro and Purkinje cells were identified by immunostaining with anti-Calbindin-D_{28k}. Figure 4 shows a typical Purkinje cell, with a highly branched dendrite and numerous spines.

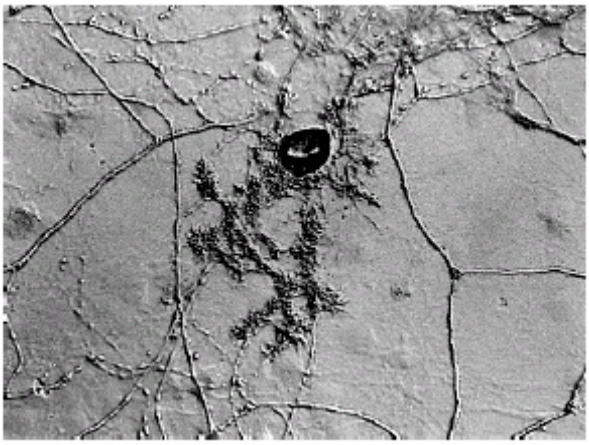
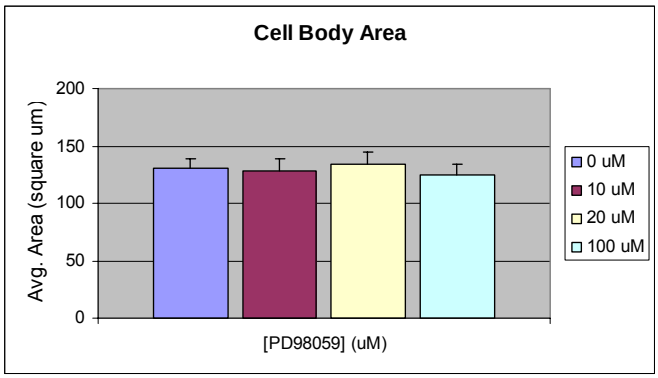


Figure 4. Purkinje cell in cerebellar culture for 14 days. Cultures were fixed with paraformaldehyde, stained with anti- Calbindin-D_{28k}/peroxidase, and imaged at 600X magnification with DIC optics.

Cultures were treated with the MAP kinase inhibitor PD98059 or the PI3-kinase inhibitor LY294002 at different doses. MetaVue software was used to trace around the cell body and to measure its area on micrometer-calibrated images (0.106 um/pixel at a total magnification of 600X). Untreated control cultures had average cell body areas of 141.02 um^2 ± 8.32 um^2. None of the concentrations of PD98059 or LY294002 tested had a statistically significant effect on the cell body area (Figure 5).

A.



B.

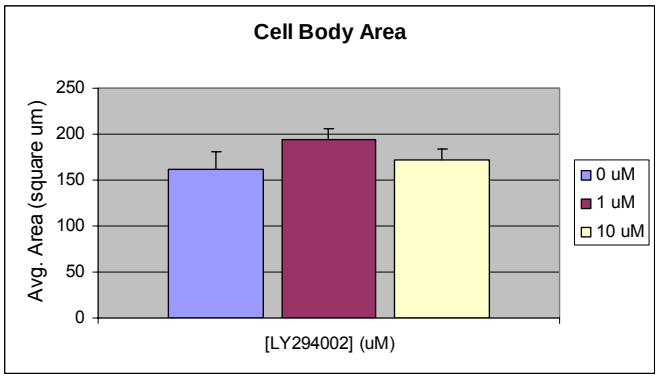


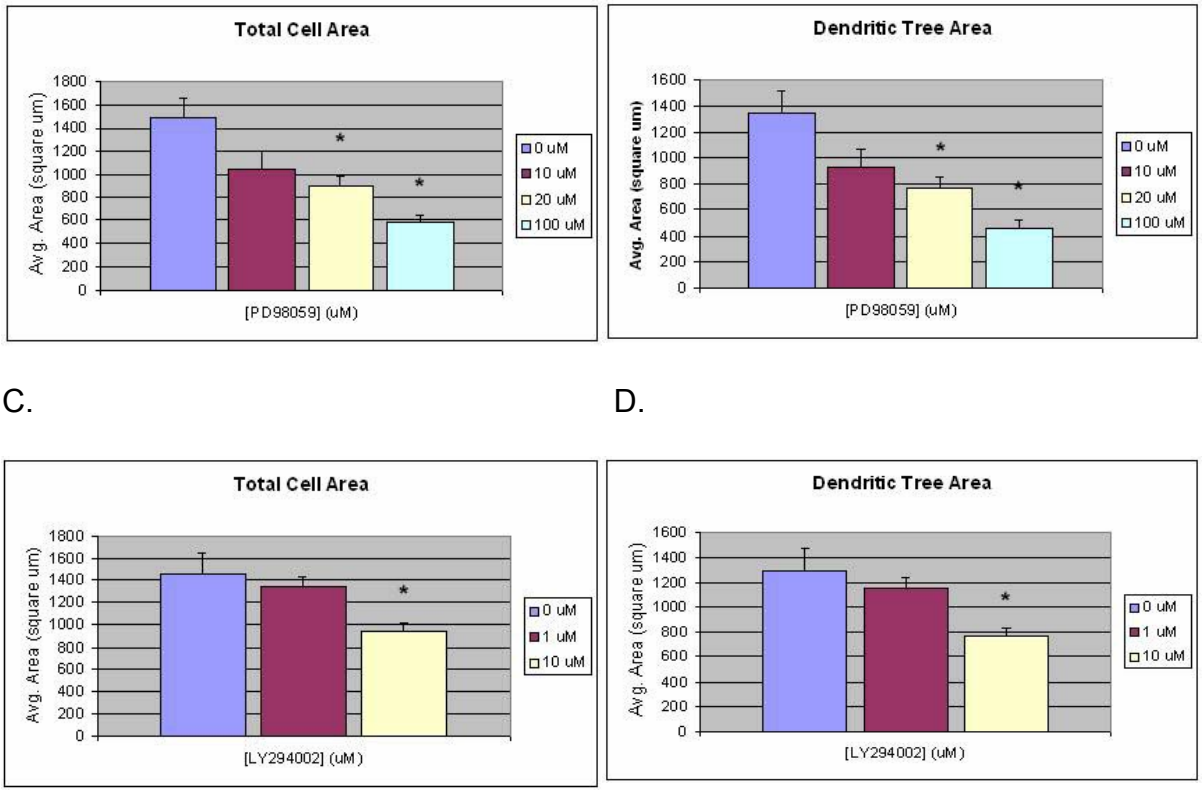
Figure 5. Purkinje cell body area was not altered by treatment with several different doses of the MAP kinase inhibitor PD98059 (A) or the PI3-kinase inhibitor LY294002 (B). The cell body area in um is shown as mean +/- SE. N = 9 cells from each of 2 replicate culture wells for each treatment. PD98059 cultures were from slide MEM71PD14D and LY294002 cultures were from slide SZLY14D. Both slide cultures were grown for 14 DIV.

Total cell area was determined by tracing around the outline of each Purkinje cell, including cell body and dendrites. Treatment with 20 and 100 uM PD98059 or 10 uM LY294002 significantly decreased the total cell area in each of the two cultures used (MEM71PD14D and SZLY14D). Lower concentrations of PD98059 or LY294002 did not significantly alter total cell area (Figure 6A and 6C).

Dendritic tree area was determined by subtracting the cell body area from the total cell area. Treatment with 20 and 100 uM PD98059 significantly decreased the dendritic tree area, from an average of 1348 um^2 +/- 169 um^2 in the untreated controls to 762 um^2 +/- 86 um2 (p=0.004) with 20 uM PD98059 and 457 um^2 +/- 57 um^2

(p=1.7x10-5) with 100 uM PD98059 (Figure 6B). Treatment with 10 uM LY294002 also significantly decreased dendritic tree area from 1292 um^2 +/- 178 um^2 to 768 um^2 +/- 64 um^2 (p=0.002; Figure 6D).

A. B.



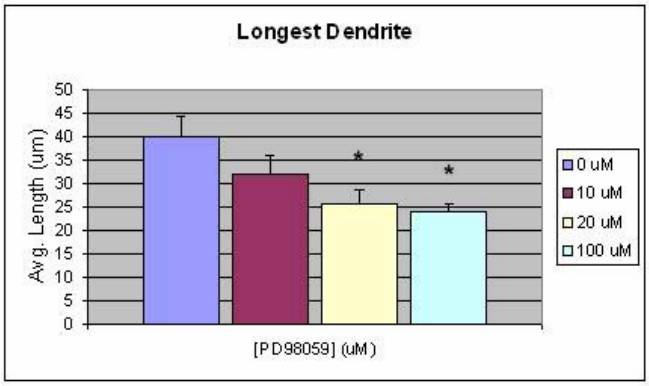
C. D.

Figure 6. Total cell area and dendritic tree area are decreased by the MAP kinase inhibitor PD98059 and the PI-3 kinase inhibitor LY294002. Cultures were grown for 14 DIV and N = 9 cells from each of 2 replicate wells for each treatment. Total cell area and dendritic tree area in um^2 is shown as mean +/- SE. Asterisks represent significance ($p \leq 0.05$)

The length of the longest dendrite on each cell was determined using the MetaVue polyline tool, which allows the measurement to follow the bends in each

dendrite. Treatment with 20 and 100 uM PD98059 significantly decreased the length of the dendrites, while none of the concentrations of LY294002 significantly affected dendritic length (Figure 7).

A.



B.

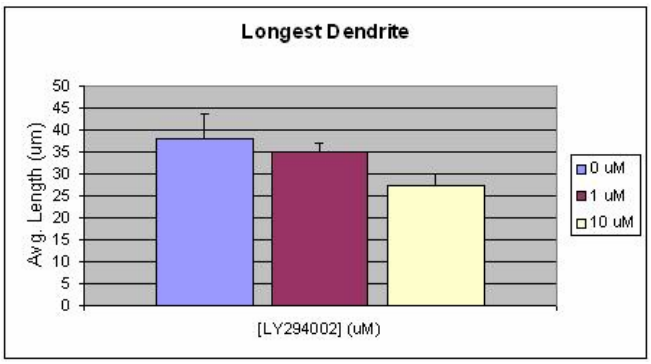


Figure 7. The length of the longest dendrite is significantly decreased by the MAP kinase inhibitor PD98059 (A) but is not significantly affected by the PI3-kinase inhibitor LY294002 (B). Traces followed the bends in the dendrite to reflect actual length. N = 9 cells from each of 2 replicate wells for each treatment. Asterisks represent significance ($p \leq 0.05$).

Discussion

Alterations in Purkinje neuron morphology have serious implications for signaling in the cerebellum, and for the control of balance and locomotion. Purkinje cells are the main output neuron for the cerebellum. They collect information about body position and movement from over 200,000 contacts with granule neurons, integrate it, and send the resulting signals and orders for adjustment back to the body via their axonal contacts

with the deep cerebellar nuclei. To make this huge computational task possible, the Purkinje cells develop highly branched dendrites, with spines that serve as sites of synaptic contact with the granule neurons. When this dendrite is damaged, or when it degenerates as in patients with cerebellar ataxia, its computational capacity is affected, with disastrous results for the patients: they can no longer walk, read, or move in a coordinated way. This study is part of a larger effort to understand how normal Purkinje cells attain their unique branched structure, and what can be done to stem their degeneration in ataxia patients.

Previous work established a role for the BDNF/TrkB ligand/receptor pair in regulating Purkinje cell development (Shimada et al. 1998; Morrison and Mason 1998). BDNF/TrkB signaling also helps to regulate cortical neuron structure and some aspects of sympathetic nervous system development (reviewed in McAllister 2001 and Glebova and Ginty 2005). These studies left open the question of which intracellular signaling pathways downstream of the TrkB receptor were involved in dendrite development. The current study begins to provide an answer: Blocking the MAP and PI-3 kinase pathways with PD98059 and LY294002, respectively, decreases dendritic outgrowth in cultured Purkinje cells. The mechanism by which inhibition of these pathways decreases dendritic area is not the same for both pathways. While MAP kinase inhibition decreases dendritic outgrowth by either shortening the dendrites and/or by reducing dendritic branching, inhibition of the PI-3 kinase pathway specifically decreases dendritic branching without affecting dendritic length (Figure 6B and 6D; Figure 7A and 7B).

Although LY294002 decreased dendrite outgrowth (Figure 6), it did not alter cell body size (Figure 5) or dendritic length (Figure 7). The latter result may at first glance seem to conflict with the finding that dendrite outgrowth overall is decreased. However, the conflict is resolved by the observation that the branch order (amount of branching) of the LY294002-treated dendrites is decreased relative to controls. The longest dendrite is equally long in both conditions, but there are fewer branches along the way from the cell body to the tip of the dendrite when PI-3 kinase is inhibited. Future work will develop algorithms using MetaVue to quantify this effect on branch order, either through direct counts of branch order/branch points, or through traditional Sholl analysis.

Understanding the control of dendrite and spine development has implications beyond the cerebellum. Abnormalities in neuronal dendrites or spines have been observed in human diseases including mental retardation, Down's syndrome, Tay-Sachs disease, gangliosidoses, Menke's disease, in addition to cerebellar ataxias (Purpura 1979; Hamilton et al. 1996; Kaufmann and Moser 2000; Hadj-Sahraoui et al 2001). Neuronal dendrites may also "die back" with normal aging (Scheibel et al 1975; Chen and Hillman 1999). Studies such as this one, which identify pharmacologic interventions that can alter dendrite and spine form and function, may also yield insights into fighting aging and many forms of degenerative diseases.

Acknowledgements

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Notes for students continuing this work:

- Learning to dissect the cerebellum fast enough to make usable cultures takes a lot of time and patience.
- MetaVue analysis gets faster as more cells are analyzed and the program becomes familiar.
- More slides are needed for the data sets to reach a publishable total of 3 independent experiments with 2 wells per experiment and 9 visual fields per well giving a total of 18 visual fields per slide for each treatment concentration.

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Morphometric Analysis Routine (For MetaVue)

Image Modifications:

1) Open image.

2) Calibrate distances.
Measure → Calibrate Distances → Apply tab → **60X Lens → Apply → Close**
* Zoom feature does not change calibration or pixel count.

3) Duplicate flattened image.
Edit → Duplicate →Image

4) Minimize the duplicate image.

Longest Dendrite:

5) **Region toolbar → Multi-line tool**
Zoom in if necessary

6) Draw a poly-line from the center of the longest dendrite's width, where it leaves the cell body and follow the path of the dendrite to the tip.

7) Label Logged Data.
Check **'Log label 1'** → Type in or highlight from dropdown menu **'Longest Dendrite'**
****KEEP 'Label Logged Data' DIALOGUE BOX OPEN****

8) Log Data (See Logging Data protocol).

9) Close data file and re-open.

Cell Body Measurement:

10) Delete poly-line.
Make poly-line the active region and hit the 'Delete' key.

11) Auto trace cell body.

Region toolbar → Auto trace → Uncheck Use Threshold → Length = 100, Angle = 5, Delta = 50, Hole size = 3, Edge detection = Dark to Bright, Edge Smoothing = Intelligent.

Click middle of cell body → Close Auto trace dialogue box.

Adjust position of trace.

Double click trace line, so that dots appear on the trace line. Click and drag dots to include/exclude more or less area.

12) Paint region.
Display → Graphics → Paint region → Paint mode = Inside region area → Paint Color = Gray value → Gray value = 255 → Paint → Close

13) Threshold (See Figure A).
Measure → Threshold image → Threshold tab → State = Inclusive → Move top bar ('Low') all the way right (255) so region of interest (ROI) is orange → **Close**

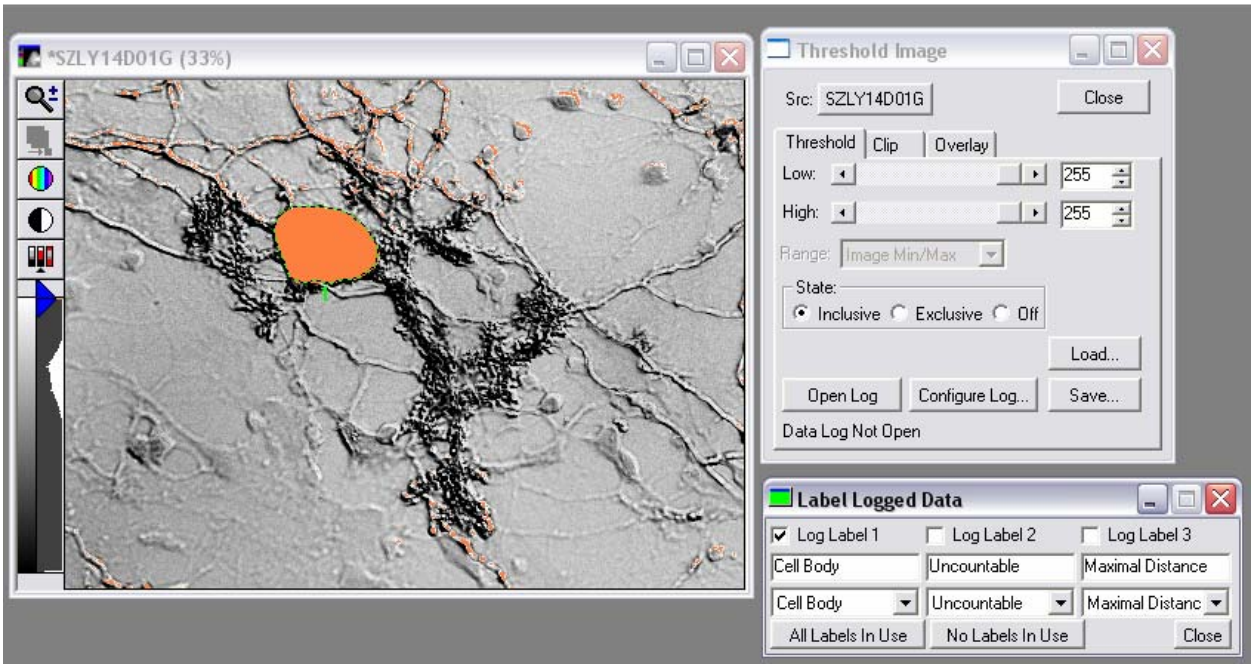

Figure A

14) Label Logged Data.
Log → Label logged data → Check **'Log label 1'** → Type in or highlight from dropdown menu **'Cell Body'**
*Label must appear in the top box.

15) Log Data. (See Logging Data protocol)

16) Close Image → **No**

Total Cell Area:

1) Maximize copy of flattened image.

2) Trace entire cell, including dendrites (See Figure B).
Region Toolbar → Trace Region Tool

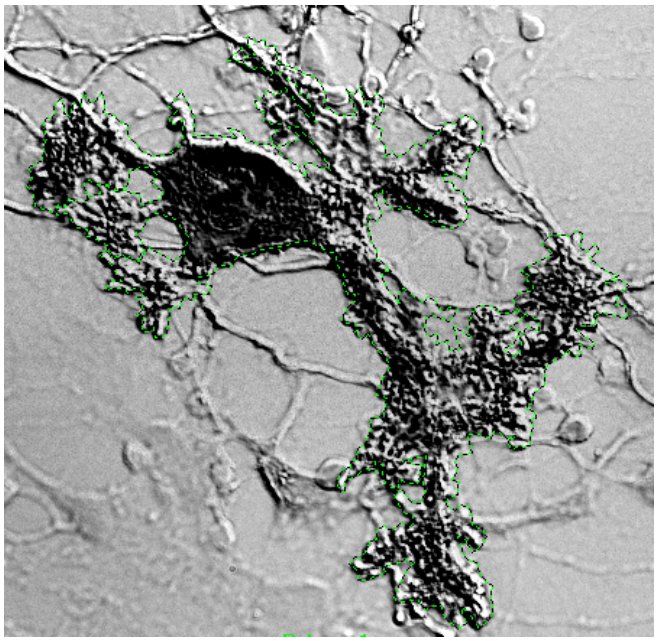

Figure B

3) Threshold.

Measure → Threshold image → Threshold tab → State = Inclusive → Move bottom bar (high) to left so region of interest (roi) is orange → **Close**

*********Total cell area – total dendritic area = area of soma***

4) Label Logged Data.

Check **'Log label 1'** → Type in or highlight from dropdown menu **'Total Cell Area'**

5) Log Data. (See Logging Data protocol)

6) Delete trace of cell.

Make outline of cell the active region and hit the 'Delete' key.

7) Close data file and re-open.

Number of Primary Dendrites:

8) Reset Measurements

Measure → Manually Count Objects → *RESET MEASUREMENTS* → Yes

Choose class: 1 primary dendrite OR 2 uncountable

9) Count primary dendrites OR a cell uncountable.

COUNT

Primary dendrites:

Click in the center of the dendrite's diameter as close as possible to the cell body.

OR

Cell uncountable:

Click anywhere in the cell one time.

Definitions:

Primary dendrite – the part of the dendrite where it leaves the cell body, before any branching occurs.

Axon hillock – a short, smooth protrusion from the cell body in which an axon is clearly radiating; has no branching or spines. ******DO NOT COUNT AXON HILLOCKS AS PRIMARY DENDRITES******

Cell uncountable – a 'fried egg' cell in which a person cannot readily determine the number of distinct primary dendrites.

→ Configure Manual Count Data...

Uncheck everything

→ OK

→ **Configure Object Counts Data...**
Check: **Image Name**
Primary dendrite
Uncountable
→ **OK**

10) Uncheck all log labels and close 'Label Logged Data' dialogue box.

11) Log data. (See Logging Data protocol)

12) Close image → **No**

Logging Data

For New Data File:

First Log:

Measure → Show Region Statistics → Configure Log → Uncheck **'Place log data on current line',** Check **'Log Column Titles' → OK → Close.**

Region Measurements → Configure tab →
Check: **Image name**
Distance (For Maximal Distance only)

Threshold area *(For Cell Body & Tot. Cell Area)*

Measure → Region Measurements → Open Log → Check **'Dynamic Data Exchange (DDE)',** Uncheck **'A text file' → OK**

Application: **Microsoft Excel**

Sheet Name: **XX Th Area** (For Cell Body & Tot. Cell Area measurements)

XX Distance (For Maximal Distance measurement only)

* XX = Your Initials

Starting Row: **1**

Starting Column: **1 → OK**

Log Data → Close

*****Minimize MetaVue and check to make sure the data logged in Excel and that it logged in the correct row and column.**

SAVE DATA!!! → Save file as 'XXdataLog

**XX = Your Initials

Second Log:

Log → Set Logging Row and Column

Log File: **Data Log**

Row Move Type: **Relative**

Row Offset: **1**

Column Move Type: **Relative**

Column Offset: **-3**

Check Set anchor column

Position reference for relative move: **Last log position**

OK → Close

Measure → Show Region Statistics → Configure Log → Check **'Place log data on current line'** (Log column titles is unchecked automatically) **→ OK → Close**

Measure → Region Measurements → Configure tab **→**

Check: **Image name**

Distance (For Maximal Distance only)

Threshold area (For Cell Body & Tot. Cell Area)

Log Data → Close

*****Minimize MetaVue and check to make sure the data logged in Excel and that it logged in the right row and column.**

SAVE DATA!!!

All Subsequent Logs:

Log → Set Logging Row and Column
Log File: **Data Log**
Row Move Type: **Relative**
Row Offset: **1**
Column Move Type: **Relative**
Column Offset: **-3**
Check **'Set anchor column'**
Position reference for relative move: **Last log position**
OK → Close

Measure → Show Region Statistics → Configure Log → Check **'Place log data on current line'** (Log column titles is unchecked automatically) → **OK → Close**

Measure → Region Measurements → Configure tab →
Check: **Image name**

Distance (For Maximal Distance only)
Threshold area (For Cell Body & Tot. Cell Area)
Log Data → Close

*****Minimize MetaVue and check to make sure the data logged in Excel and that it logged in the right row and column.**

SAVE DATA!!!

For Saved Data File:

First Log:

Open saved log file from My Computer.

***MAKE SURE YOU KNOW WHICH ROW IS THE NEXT BLANK ROW! If you log data on a row that already had data in it, the previous data will be over-rided and YOU WILL LOSE THAT DATA.

Measure → Show Region Statistics → Configure Log → Check **'Place log data on current line'** ('Log column titles' is unchecked automatically) → **OK → Close**

Measure → Region Measurements → Open Log → Check **'Dynamic Data Exchange (DDE)',** Uncheck **'A text file' → OK**

Application: **Microsoft Excel**
Sheet Name: **Your saved data file name**
Starting Row: **Your next empty row**
Starting Column: **1 → OK**

Measure → Region Measurements → Configure tab →
Check**: Image name**
Distance (For Maximal Distance only)
Threshold area (For Cell Body & Tot. Cell Area)
Log Data → Close

*****Minimize MetaVue and check to make sure the data logged in Excel and that it logged in the correct row and column.**

SAVE DATA!!!

All Subsequent Logs:

Measure → Show Region Statistics → Configure Log → Check **'Place log data on current line'** ('Log column titles' is unchecked automatically) → **OK → Close**

Log → Set Logging Row and Column
Log File: **Data Log**
Row Move Type: **Relative**
Row Offset: **1**
Column Move Type: **Relative**
Column Offset: **-3**
Check Set anchor column
Position reference for relative move: **Last log position**
OK → Close

Measure → Region Measurements → Configure tab →
Check**: Image name**
Distance (For Maximal Distance only)
Threshold area (For Cell Body & Tot. Cell Area)
Measurements tab → **Log Data → Close**

*****Minimize MetaVue and check to make sure the data logged in Excel and that it logged in the correct row and column.**

SAVE DATA!!!

For Number of 1° Dendrites:

For New Data Sheet:

Log → Open Data Log → Check **'Dynamic Data Exchange (DDE)',** Uncheck **'A text file' → OK**
Application: **Microsoft Excel**
Sheet Name: **XX Primary Dendrites**
* XX = Your Initials
Starting Row: **2**
Starting Column: **1 → OK**

'Manually Count Objects' dialogue box → **Log Counts → Close**

*** Minimize MetaVue and check to make sure the data logged in Excel and that it logged in the correct row and column.

Manually type column headings in each cell

Row 1, Column 1: **Image Name**

Row 1, Column 2: # **Primary Dendrites**

Row 1, Column 3: **Cells Uncountable**

SAVE DATA!!!

For Saved Data File:

Log → Open Data Log → Check **'Dynamic Data Exchange (DDE)',** Uncheck **'A text file'** → **OK**

Application: **Microsoft Excel**

Sheet Name: **XX Primary Dendrites**

* XX = Your Initials

Starting Row: **Your next empty row**

Starting Column: **1** → **OK**

'Manually Count Objects' dialogue box → **Log Counts** → **Close**

*** Minimize MetaVue and check to make sure the data logged in Excel and that it logged in the correct row and column.

SAVE DATA!!!