Decrease in Filopodia Sprouting after Exposure to Methyl Mercury

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Neurobiology Short Report

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Introduction:

Neurons grow by extending out growth cones supported by microtubules and actin that act to elongate the axon of the growing neurite. The microtubules form the length of the axon and polymerize out until they reach the actin cortex which they cannot penetrate (Morris lecture 09/23/2011). The proteins needed for continuing growth are all trafficked down the microtubules into the growth cone. The actin cortex at the end of the growth cone forms lamellipodia in its gel form and spikes protruding from the cone called filopodia in its bundled form (Morris lecture 09/23/2011). The actin cytoskeleton in these two forms controls the motility and directionality of the growth cone and therefore controls the rate of axon elongation as well as direction of elongation (Tanaka and Kirschner, 1991). Each filopodium serves as an antenna to help the cell decide which direction to grow by using receptors to sense ligunds in the environment that the cell should grow towards. These receptors trigger an event that depolymerizes the actin in the cortex which allows the microtubules to get through and carry all of the proteins needed for growth to the area with the most ligunds (Morris lecture 09/23/2011). If the microtubules cannot transport the materials needed for growth to the actin cortex including the filopodia those filopodia cannot continue to elongate the axon and search for ligunds.

Mercury is a well known neurotoxin that can cross the blood brain barrier and damage nerve cells. In adults exposure can cause parethesia, visual and hearing loss, and ataxia. In the fetal brain, which is more susceptible to the effects of mercury, exposure can lead to deficits in motor function, memory, and language development (Benoit lecture 09/21/2011). The EPA’s current reference dose for mercury is 0.1µg/kg body weight/day. This dosage level is intended to protect the nervous system of developing fetuses (http://www.epa.gov/mercury/exposure.htm).
A study by K. Miura et al. 2000 documented that mercury extensively decreased microtubule mass and depressed neurite growth rate by 50%. Leong et al found that exposure to mercury inhibited motility in growth cones (2001). The results of both of these studies suggest that exposure to mercury disrupts cytoskeletons of neuronal growth cones, causing depolymerization of microtubules which in turn stops filopodia functioning and growth. In the current study I propose that after exposure to one form of mercury, mercuric chloride, filopodia sprouting will no longer occur in a neuronal growth cone in chick embryos (*Gallus gallus*,) as measured by images taken using video-enhanced phased microscopy.

**Materials**

See materials from Primary Culture of Chick Embryonic Peripheral Neurons Version 09/28/2011b

Methyl mercury solution of 19mLs 100nMHgCl$_2$ in HBSS

Tubulin antibody nerve growth factor

VALAP

Clean slides

Cover slips

HBSS

Sympathetic nerve cells from 10 day old *Gallus gallus* kept at 37 degrees Celsius

Forceps

Clean plastic and glass pipettes

Nikon Eclipse E220 microscope

Sony DWF-X700 with a 1.0C mount camera

BTV software version 6.0bl

IMac computer (Cancer) with Mac OS X 10.5.8 software
Method

Dissection of chick embryonic cells

The dissection of the embryos was done using 10 day old *Gallus gallus* embryos. The dissection followed the procedure outlined in "Morris (2011a)".

Observation of cells using Video-enhanced Phase Microscopy

The observation of the cells was done following the procedure outline in "Morris (2011c)" “Video-Enhanced Phase Microscopy” section. For the observation a Nikon Eclipse E220 microscope was used in combination with the BTV software version 6.0bl on the IMac computer listed in the materials section.

Control data collection

Using cells that had been cultured for 17 hours a flow chamber was created using the protocol from "Morris (2011c)" “Video-Enhanced Phase Microscopy” steps 1- 18. The slide was then mounted onto a Nikon Eclipse E220 microscope, a heater was placed next to the microscope to keep the cells at 37 degrees Celsius, and a picture was taken at 40x magnification. After 3 minutes had elapsed another image was taken at the same magnification. A third image was taken 3 minutes after the second picture. After these 3 images were taken 1.0 mL of buffer was flowed through the chamber to simulate the procedure that would be followed when the mercury was flowed through the chamber and ensure that the flowing of any material is not what caused the change in the cells. The entire flow procedure took approximately 30 minutes. After the buffer flow was complete an image was taken. Just as before the flow once 3 minutes had passed a second picture was taken and after another 3 minutes a third image after buffer flow was taken.

Experimental data collection

Using cells that were cultured for 19 hours the flow chamber was created using the procedure from "Morris (2011c)" “Video-Enhanced Phase Microscopy” steps 1- 18. The slide was then mounted onto a Nikon Eclipse E220 microscope, a heater was placed next to the microscope to keep the cells at 37 degrees Celsius, and an image was taken at a 40x magnification. After 3 minutes had passed a second image was taken. 3 more minutes elapsed and a third image was taken. 1.0mL HgCl₂ was passed through the flow chamber then after a 20 minute exposure 1.0mL was flowed through the chamber. In total this flow procedure took about 25 minutes. Immediately after the flow was concluded another image was taken. After 3 minutes a second image was taken and after another 3 minutes the third image after flow was taken.

Quantification of data

To determine whether filopodia sprouting decreased after exposure to mercury a single growth cone was selected in both the
control and experimental data sets. The emergence of new sprouts on the growth cone was measured by comparing consecutive images to see if new filopodia appeared. If there was sprouting the new filopodia were measured from the base of the growth cone to the tip of the filopodium using the segmented line tool in imageJ software. The length in pixels of each new filopodium for an image were averaged together to ascertain the average sprouting length for that time point. This was done for every image in the control and experimental data sets, except for the first image in each set since there could be no new sprouts as it was the first image.

**Results**

Two sets of data were collected, a control set and an experimental set. In the control set continuous filopodia sprouting was observed for the majority of the images, even those after the buffer flow exchange, although in the last two images there was less filopodia sprouting then in the earlier images. This is likely due to the fact that cells were no longer growing at same rate since they had not been in growth medium or incubated for some time. The neuronal growth cone in the experiment was exposed to HgCl₂ for 20 minutes. In the first image taken after the exposure, the image shown in figure 2A, there was a large amount of filopodia sprouting and the average length of filopodia increased. In the subsequent images however there was no filopodia sprouting and from the image shown in figure 2B it appears the filopodia already present might have started shrinking back towards the growth cone.

Figure 1A

[Image of a growth cone before buffer flow]

Figure 1A shows the first image taken in the control data set. The image shows a growth cone of an axon and it’s filopodia before buffer flow.
Figure 1B shows the last image of the control data set. Specifically the image shows the growth cone of an axon and its filopodia after an exchange of buffer flow.

Figure 2A shows an experimental image of the growth cone, including the filopodia, of an axon directly after it had been exposed to methyl mercury for 20 minutes.

Figure 2B
Figure 2B shows the last image in the experimental data set. It is of the same growth cone on the axon as the one in figure 2A. This image was taken 6 minutes after the image in figure 2A and it is clear that no new filopodia have sprouted.

![Figure 3: Average Sprouting Length over Time](image)

Figure 3 shows a graph of the average length in pixels of the filopodia sprouts of each image for both the control and experimental data set. For both data sets the graph starts at the second time point since there is no new sprouting in the first image. The average length of filopodia sprouting for both data sets was measured at 2 growth cones coming off of 1 axon of a single neurite.

Discussion

The data collected in this experiment supported the hypothesis that after mercuric chloride exposure growth cones would no longer sprout new filopodia. Based on the graph in figure 3 above it is clear that after exposure to mercury there is a severe
decrease in the average length of filopodia sprouts because there were no longer any sprouts, whereas the control group shows continued growth after the buffer exchange.

If this data trend continued over many experiments it could be concluded with certainty that the exposure to mercury caused filopodia to stop sprouting. Since mercury is responsible for depressing microtubule functioning (Miura et al 2000, Leong et al 2001) and microtubules supply the actin making up filopodia with proteins needed for growth (Morris lecture 09/23/2011) it seems a logical conclusion that mercury would have negative effects on the filopodia of a neuronal growth cone. Filopodia sprouting, or lack thereof, may reflect growth cone retraction and microtubule depolymerization. Future studies could expand on this work by further looking at the changes that occur in filopodia when it is known that microtubules are being polymerized.

Expanding on the ideas explored in this study is important because exposure to mercury in daily life is high. A lot of different varieties of fish regularly consumed by most cultures contain high levels of mercury. In the United States mercury was used in dental amalgams until fairly recently and the exposure of individuals with these fillings to mercury vapor has become a concern (Benoit lecture 09/21/2011). In pregnant women these types of exposure could easily lead to the fetal brain suffering damage from the negative effects of mercury demonstrated in this study which has been shown to cause developmental delays after birth (Benoit lecture 09/21/2011). If the exact mechanism of mercury’s damaging effects on growing neurites was know it is possible that they could be combated.

This experiment did have a few sources of error. When the control data was being gathered there were problems with the flow chamber that resulted in a large time lapse between images and the need for the microscope lens to be cleaned. These errors do in part account for the amount of average length increase seen in the control data set. In conducting future experiments similar to this one it would be prudent to monitor the time it takes to flow both the buffer and the mercury through the flow chamber as that time lapse may have an effect on the length of sprouting observed.

Works cited


Morris, R.L. (2011a) Neurobiology Bio324 Primary Culture Of Chick Embryonic Peripheral


Morris, R.L. (2011c) Neurobiology Bio324 Primary Culture Of Chick Embryonic Peripheral
