# Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury

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**[ study below press releases]**

This study showed that mercury can cause neurodegeneration in the brain central ring ganglia of the snail Lymnaea stagnalis. The resultant defective microtubule assembly and the aggregation of neurofibrils observed can also be found in the brains of Alzheimer's patients. However, the species difference between snail cells and human cells does not necessarily provide a direct link between chronic exposure to mercury vapour and Alzheimer's.

In dentistry the most commonly used material for fillings today is amalgam. Low concentrations of mercury vapour are constantly released from these amalgam fillings, accounting for 70% of mercury ions found in human urine. There have been several clinical studies over recent years which have reported altered neurobehaviour in dental personnel and this may well be due to chronic exposure to low level mercury vapour.

Growth cones are found at the tip of developing and regenerating neurons and play an important role in the development and maintenance of the neuron. The scaffolding of the growth cone is mainly made up of proteins called microtubules. Microtubules are composed of molecules called tubulin which in pairs, join together in a process called polymerisation to form a long-chained structure which is ultimately a microtubule.

Using time-lapse photography with microscopy, the authors observed the microtubule structure at the growth cone in the brain neurons from the snail. The concentrations of mercury used were of the same order of magnitude as those reported in human and animal brains after chronic exposure to mercury vapour. Within a few minutes of exposure to mercury, the growth cone lost its motility and even exhibited robust collapse and retraction. The bare fibres of the neuron eventually formed aggregates. Over a 2-year period in over 40 different cultures, it was found that an average of 77% nerve growth cones were affected by exposure to mercury ions. When neurons were exposed to the heavy metals aluminium, lead, cadmium and manganese, there was no observed degeneration of the growth cones. The collapsed growth cones were also stained for actin/tubulin immunoflorescence. Mercury treated growth cones exhibited a high disintegration of the microtubule structure compared with controls indicating that it was most probably this part of the growth cone that is affected by the mercury ions resulting in growth cone collapse.

To look at the extent of this effect of mercury on the growth cones, the authors then measured the total neural outgrowth over a 48-hour period in both control and mercury treated neurons. Less than 5% of neurons that were treated with mercury showed some sort of outgrowth in

comparison with just over 93% of control neurons which displayed robust outgrowth.

The chronic exposure to mercury may be a potential factor in neurodegeneration in humans that could ultimately be observed as altered behaviour.

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UNIVERSITY OF CALGARY GAZETTE / APRIL 4, 2001

# Researchers present evidence of mercuryís effect on brain neurons

A University of Calgary Faculty of Medicine research team has found that exposure to mercury causes degeneration of brain neurons in animals.

The scientific findings are being published in a cover story in the April edition of the British journal NeuroReport. The researchersí academic paper is supported by a time-lapse video recorded from a microscope camera showing how neurons degenerate when they are exposed to mercury.

"Our study illustrates how mercury ions alter the cell membrane structure of developing neurons," says Fritz Lorscheider, physiology and biophysics. "This discovery provides visual evidence of our previous findings that mercury produces a molecular lesion in the brain."

The research paper, coauthored by Lorscheider and U of C professor Naweed Syed as well as medical student Christopher Leong, looks at brain neurons from snails. The researchers added mercury ions to cell cultures of developing neurons and observed the neurons undergoing rapid degeneration.

Nerve processes in snails and other animals, specifically the microtubules in neurons, are similar to those of humans.

The team has identified how this degeneration takes place: ï mercury ions attach to a neuron, causing its microtubules to disassemble or break down and, ultimately, leave that neuron stripped of its protective membrane

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**Research indicates how exposure to less than micromolar amounts of mercury for as little as 10 minutes may disrupt neuronal growth.**

Inhalation of mercury vapor (Hg°) inhibits binding of GTP to rat brain tubulin, thereby inhibiting tubulin polymerization into microtubules. A similar molecular lesion has also been observed in 80% of brains from patients with Alzheimer disease (AD) compared to age-matched controls. However the precise site and mode of action of Hg ions remain illusive. Therefore, the present study examined whether Hg ions could affect membrane dynamics of neurite growth cone morphology and behavior. Since tubulin is a highly conserved cytoskeletal protein in both vertebrates and invertebrates, we hypothesized that growth cones from animal species could be highly susceptible to Hg ions. To test this possibility, the identified, large Pedal A (PeA) neurons from the central ring ganglia of the snail *Lymnaea stagnalis* were cultured for 48 h in 2ml brain conditioned medium (CM). Following neurite outgrowth, metal chloride solution (2 µl) of Hg, Al, Pb, Cd, or Mn ($10^{-7}$ M) was pressure applied directly onto individual growth cones. Timelapse images with inverted microscopy were acquired prior to, during, and after the metal ion exposure. We demonstrate that Hg ions markedly disrupted membrane structure and linear growth rates of imaged neurites in 77% of all nerve growth cones. When growth cones were stained with antibodies specific for both tubulin and actin, it was the tubulin/ microtubule structure that disintegrated following Hg exposure. Moreover, some denuded neurites were also observed to form neurofbrillary aggregates. In contrast, growth cone exposure to other metal ions did not effect growth cone morphology, nor was their motility rate compromised. To determine the growth suppressive effects of Hg ions on neuronal sprouting, cells were cultured either in the presence or absence of Hg ions. We found that in the presence of Hg ions, neuronal somata failed to sprout, whereas other metalic ions did not effect growth patterns of cultured PeA cells. We conclude that this visual evidence and previous biochemical data strongly implicate Hg as a potential etiological factor in neurodegeneration. *NeuroReport 12*:733-737 © 2001 Lippincott Williams & Wilkins.

Key words: Mercury; Microtubules; Neurite growth cone; Neurodegeneration; Neurofbrillary aggregates; Tubulin

## INTRODUCTION

Growth cones located at the tip of developing mid regenerating neurites are responsible for neurite extension, axonal pathfinding mid target cell selection in the nervous system. Actin and tubular that comprise the bulk of growth cone cytoskeleton are highly sensitive to various environmental cues that are present in the extracellular milieu of growth cones. A growth permissive environment facilitates growth cone assembly whereas various growth inhibitory molecules dissemble microtubular structure, induce growth cone collapse mid neurite retraction [1]. Microtubules, a principal protean of the cytoskeleton, are composed of polymerized tubular dimer subunits. Brain neurons require intact microtubules for axoplasmic

transport, membrane structure, mid normal neurite outgrowth; the cytoskeletal architecture being dependent upon microtubular stability [2,3]. Methylmercury (MeHg) is a potent neurotoxicant, mid its effects on microtubule integrity during CNS neuronal development are well documented [4].

Attention has also focused on potential CNS toxicity resulting from chronic exposure to another predominant toxic mercury species, that of mercury vapor (Hg°); the principal source being dental amalgam tooth fillings [5]. Approximately 70 % of all Hg ions in human urine originate solely from amalgam [6]. Recently, we have reported that inhalation exposure of rats to Hg° causes disruption of brain microtubule metabolism by inhibiting the polymerization of tubular molecules. Such polymerization is dependent upon the ability of GTP nucleotide to band to (3tubulai, banding that is markedly reduced by the presence of Hg ions. A similar in viva molecular lesion was observed in brains of 80 % of Alzheimer disease (AD) patients, but was not seen in brains from age-matched control patients [7].

Since the amino acid sequence of tubular from all animals brains (vertebrates and Invertebrates) is highly conserved, with > 97 % sequence homology across animal species [8], the present investigation employs a well-established snail neuronal culture model [9] to study microtubule metabolism in the presence of Hg. The development of time-lapse imaging techniques for intact isolated neurons, using cell culture systems, has allowed the direct observation of axonal microtubule structure mid protean synthesis at the neurite growth cone [10,11]. Therefore, the primary objective of the present study was to determine whether the marked Inhibition in microtubule metabolism following Hg° exposure, tie measured at the molecular level [7], could actually be directly observed by imaging the membrane dynamics of neurite growth cone activity in the presence or absence of Hg ions or other toxic heavy metals.

## MATERIALS AND METHODS

***Animals:*** An established stock of the fresh water snail *Lymnaea stagnalis* derived from that of the Department of Biology at the Free University of Amsterdam was used. Animals were maintained in an aerated, filtered pond water aquarium at room temperature in the University of Calgary Animal Resources Centre and were fed lettuce tie described by Ridgeway et al. [12]. In all experiments, central rang ganglia were used for neuronal cell isolation mid to make brain conditioned media (CM). Snails with a shell length of 2530mm (3-4 months old) were used in all experiments.

Cell culture: Animals were de-shelled mid anesthetized for 10min in normal *Lymnaea* saline ((hi mM): 51.3 NaCl, 1.7 KCl, 4.0 $CaCl_2$ and 1.5 $MgCl_2$; buffered in HEPES to pH 7.9) containing 10 % Listerine. All primary cell culture procedures from tine point forward were carried out in a laminar flow hood to prevent Infection of culture samples from air-borne microorganisms. Anesthetized snails were pained down in a dissection dish containing antibiotic saline (ABS) (autoclaved normal *Lymnaea* saline; gentamycin 150 dug/ml) mid their CNS removed tie described previously [9,12]. The isolated central rang ganglia were washed in plastic culture dishes (Falcon; Becton Dickinson, Meylan Cedex, Prance; 35 x 10mm)

containing ABS to ensure an aseptic culture [9]. Three consecutive 10-15min washes were completed, each in a culture dish containing 3ml ABS. Brains were then transferred into a culture dish with 3ml defined media (DM; 50% L-15 medium with added inorganic salts (in mM): 40 NaCl, 1.7 KCl, 4.1 $CaCl_2$, 1.5 $MgCl_2$, 10 N-2 hydroethyl-piperzine-n'-2-ethanesulfonic acid, pH 7.9; and 20 pM gentamycin; (Gibco BRL, Gaithersburg, MD; special order) containing bug trypsin (2 mg/ml to yield a 0.2 % volume solution; Type T-4665; Sigma, St. Louis, MO), mid left at room temperature (18-20°C) for 23min. Following tine enzyme treatment, the central rang ganglia were placed Into a 0.2% volume trypsin Inhibitor (Type 1-S; Sigma)/DM solution mid left for 15min. The brains were then transferred to a dissection dish containing high osmolarity DM (750 VI of 1 M glucose added to 20 ml DM to yield a 180-190m0em solution) and pinned down dorsal surface up. Pine forceps were used to remove the outer and timer connective tissue sheathes surrounding each ganglion. A Sigmacote (Sigma)-treated glass capillary pipette was attached to polyethylene tubing and sterilized with 70% ethanol for 5min. Following tine sterilization, a micro-syringe (Gilmont, Model GS1100) was connected to the tube and the pipette/tubing/syringe system rinsed thoroughly with ABS prior to being filled with high osmolarity DM. A micromanipulator was used to maneuver the pipette tip overtop a Pedal A (PeA) neuron cell body and gentle suction pressure was applied through the micro-syringe to isolate the neuron from its ganglion. This PeA neuron was then gently flushed Into a poly-L-lysine coated glass coverslip/culture dish [12,13] containing brain conditioned media (CM, described below). Three to five neurons were plated ~5-10 soma diameters apart per dish mid were left undisturbed overnight to allow for cell attachment mid neurite outgrowth.

To prepare CM, 12 isolated central ring ganglia, washed seven times in ABS, were incubated in Sigmacote-treated glass culture dishes containing 6ml DM for 3 days tie described by Wong *et al.* [13]. These ganglia were then removed from the culture dish mid the CM (first time) was discarded. The ganglia were incubated for an additional 4 days in fresh DM and removed. This medium (second time) was filtered (0.22 µm pore; Nalgene) mid placed in a poly-L-lysine-coated plastic culture dish. The ganglia were added (2/ml filtered media) and the dish incubated for one additional day. These ganglia were then discarded mid the culture dishes with tine CM were used immediately.

Application of heavy metal solutions: Only neurone with well-developed neurites were used for experimentation to ensure a well established microtubule cytoskeletal structure. PeA cells were allowed to extend neurites for 2448h. after plating in CM before exposure to a heavy metal solution. Heavy metal chloride salts of mercury, aluminum, lead, cadmium, mid manganese were obtained from J.T. Baker (Phillipsburg, NJ; room temperature solubilities in water respectively (g/100ml): 6.9, 69.9, 0.99, 140, 151) to make the experimental solutions used. Stock solutions were made in 5.0ml Falcon sterile centrifuge tubes with autoclaved normal *Lymnaea* saline at room temperature (18-20°C) to obtain a concentration of 1 x 103 M. This stock solution was then serially diluted, also in normal *Lymnaea* saline, to obtain a final working experimental solution concentration of 1 x 10-7 M. Mercury chloride stock and experimental solutions were made fresh every few days due to a moderate lose of ions adsorbed on the container surfaces. The 1 x 10-7 M heavy metal solutions were loaded Into

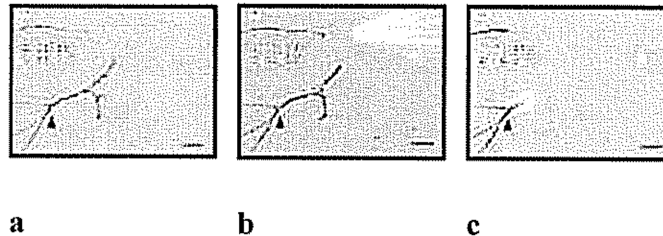
wide-bore, firepolished glass microinjection pipettes mid delivered via pressure ejection into the CM in a region adjacent to growth cones at 2-5 pen using an Eppendorf microinjector (Model 5242). Rattier than using a pulse ejection, the holding pressure of the microinjector was set at 2 pen to deliver a constant stream of experimental solution for 20min. The volume of metal solution delivered to the culture dish (containing 2ml CM solution) was estimated tie 2 VI. A peristaltic pump (Gilson, Model Minipuls -2) was used to provide a constant flow (400 µl/min) of sterile normal *Lymnaea* saline through the cell culture dish during heavy metal exposure. Neurone were observed as controls for 40min prior to heavy metal treatment and for an additional Albumin after the cessation of mercury ejection into the culture.

***Imaging***: Neurone were viewed with a Zeiss Axiovert Model 135) inverted microscope using a x40 objective. A time lapse video recording of the neurite growth cones during heavy metal exposure was captured using a CCD camera (Hitachi Denshi, Japan, Model KP-M1U) connected to a time-lapse frequency VCR (Panasonic model no. AG 6720A) set at 1 frame/e using Sony VHS SP tape. Linear growth rates for neurite growth cones were estimated using a stage micrometer scale.

A section of the video tape was converted to Betacam SP tape mid a digitized edition was developed by the Advanced Media for Learning unit at the University of Calgary's Learning Commons. Tape editing was performed with a Media 100 XS System, version 4 (Media 100, Marlboro, MA) and compressed for web delivery with Media Cleaner Pro, version 4 (Terran Interactive, Los Gatos, CA). The supporting animation was created with Softimage, version 3.8 sp 2 (Avid Technology Inc., Tewksbury, MA). This digital tape is replayed at a normal VHS speed of 30 frames/e mid can be accessed for web viewing at http://movies.commons.ucalgary.ca/mercury [14].

***Immunostaining***: RITC, Bodipy mid FITC phalloidins (Molecular Probes Inc.) were used to label P-actin. Tublin was visualized with anti-(3tubulin, a mouse monoclonal antibody obtained from Boehringer-Mannheim. The secondary antibodies were obtained from Vector Labs Inc. Cultured cells were fixed for 30 man with 4 % paraformaldehye in PBS containing 3mM EGTA and 0.02% glutaraldehyde, then permeabilized in 0.5 % NP-40. The preparations were subsequently raised in PBS mid incubated for 1 h at room temperature with 25 units fluorescein phalloidin diluted with 20 VI PBS. The cells were raised with PBS mid incubated with (1:100) (3-tubulin diluted in PBS for 1 h. The cultures were then raised mid incubated with 1:20 dilutions of either FITC or rhodamine conjugated anti-mouse IgM for 1 h. Coverslips were mounted in PBS/glycerol (15-85%) containing 1%-n-propylgalate. Growth cones were viewed wider a Zeiss (Axioekop) fluorescent microscope mid photographed with a 35 mm camera.

**Fig. 1. Digital images of cultured nerve growth cones from identified *Lymnaea* neurons before (a), during (b) and after (c) mercury exposure.**



a b c

**The arrow indicates the same reference point in all three images. Bar=30XXXm. Neurons were cultured in the presence of brain conditioned medium and allowed to exhibit outgrowth. Following 24-48h neurite outgrowth, growth cone behavior was monitored for 40 min with time-lapse video imaging (a). Individual growth cones were subsequently subjected to Hg which was pressure applied locally under a fast perfusion system for 20 min (b). Hg exposure induced growth cone collapse within 10min (b). Neurite retraction continued under an additional 30 min of observation (c).**

## RESULTS

To test for both immediate and chronic effects of Hg ions on growth cone morphology and behavior, individually identified neurone from a homogeneous population of Pedal A cluster were isolated in vitro mid maintained in primary cell culture. All neurone cultured in the presence of CM exhibited robust outgrowth over night. Figure la-c shows sequential digital photographs, without image enhancement, of typical nerve growth cones from intact neurone cultured in 2ml media before, during mid after the addition of 2 μl of a $10^{-7}$ M Solution of $HgCl_2$. The tip of the microejection pipette is visible in Pig. 1b. Within a few minutes of Hg exposure, not only did the growth cone cease its motility but it also exhibited robust collapse mid retraction (Fig. lc). Consistent with tine image (Fig. lc) the denuded neurofibrils eventually formed neurofibrillary aggregates, an observation reflected in the enlarged bulbous bone structure that resulted from neurite retraction following growth cone collapse. This figure is from our June 1, 1999 experiment where tape frame times 15:41:31, 15:58:58, mid 16:15:41 were selected. The entire film sequence, illustrating the dynamics of neurite membrane disassembly mid retraction following Hg exposure, is available on the web [14]. The average linear growth rate for three of these growth cones was determined to be +28 μm/h before Hg exposure, compared to -102 μm/h during and -146 μm/h after Hg exposure. We have repeated tins experiment with similar results for -40 different neuron cultures wider the same conditions over a 2-year period. In these cultures, on average, ~77 % of all nerve growth cones were affected by Hg.

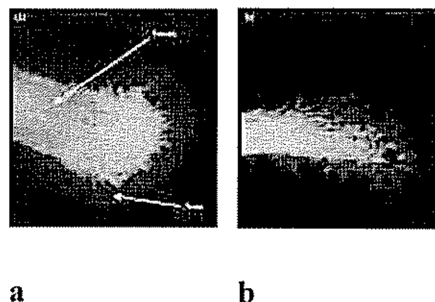
To test for the specificity of the effects of Hg ions on growth cone morphology, we next sought to determine whether other metalic ions such as Al, Th, Cd or Mn (10-7M chloride) would also alter neurite membrane integrity. Despite multiple exposure to the above ions, the growth cone morphology and behavior remained unperturbed suggesting that these ions do not affect growth cone cytoskeleton (n=3 different cultures for each metal, data not shown).

Because Hg ions have previously been shown to effect tubular polymerization, we next asked

whether Hg-induced degeneration of growth cone structure involved actin/tubular architecture of newly assembled cytoskeletal elements. Specifically, neurons were cultured and allowed to extend neurites. Following neurite outgrowth, individual growth cones were exposed to Hg ions mid following collapse, these were fixed and processed for actin/tubular immunofluorescence. We found that as compared with their control, untreated counterparts (Fig. 2a), the Hg ion treated growth cones exhibited a high degree of disintegration of tubulin/microtubule structure (Pig. 2b). These data demonstrate that Hg-induced degeneration of growth cone structure probably involves microtubular disassembly.

Taken together, the above findings demonstrate that Hg ions exert growth suppressive effects on the growth cone of PeA neurons. To test the extent of these effects, PeA cells were cultured in the presence of Hg ions mid the extent of total neurite outgrowth (sprouting) was measured after 48h. Consistent with our hypothesis we found that neurons cultured in the presence of Hg ions failed to initiate neurons (4.6 ± 2.4 % sprouting), whereas control neurons extended robust outgrowth (93.4 ± 3.1 % sprouting). These data, shown in Table 1, thus demonstrate that the effects of Hg ions are not restricted to individual growth cones, rattier they prevent neurite initiation from the entire neuron.

**Fig. 2. Fluorescent images of cultured nerve growth cones double-stained with antibodies against actin (red-rhodamine) and tubulin (greenfluorescene) before (a) and after (b) mercury exposure.**



**a** **b**

## DISCUSSION

The results of the investigation described herein clearly demonstrate that exposure to Hg ions markedly disrupts the membrane structural integrity of neurites mid the growth cones of identified neurons. This phenomenon appears to be specific for Hg, since exposure to four other heavy metals had no observable effect on either growth cone morphology or individual neurites. These findings are consistent with earlier biochemical evidence demonstrating that microtubule metabolism is compromised in the presence of Hg ions because Hg inhibits GTP nucleotide binding to (3-tubulin, a requisite step for tubulin polymerization in the formation of microtubules [7]. We believe that the Hg-induced disassembly of the neurite membrane, as seen in the present study, is a physical manifestation of a disrupted microtubulin polymerization cycle.

The question arises as to whether tins Hg-induced retrograde degeneration of the neuron membrane is solely the result of disruption in microtubule metabolism. Previous evidence indicates that the autoradiographed 45 kDa band of rat brain cortex proteins showed no change in GTP nucleotide banding in the presence of Hg [7]. This protean band is primarily composed of actin, another *cytoskeletal* protein involved al growth cone motility and which is ATP nucleotide-specific, mid tile band also contains lesser amounts of glutamine synthetase mid creatine kinase. Dully *et al. [15]* have previously demonstrated that Hg had no effect on GTP balding to actin. This supports tile interpretation al tile present study that tile structural disassembly of tile neurite membrane, observed herein, is a direct effect of Hg on tubular rattler titan actin; an interpretation confirmed by tile immunostaining evidence presented in this report.

These results do not, however, rule out other neurite constituents as potential targets for Hg. For example, neuromodulin (also known as B-50 or GAP-43), present in tile cytoskeleton mid inner plasma membrane surface of tile growth cone, also helps stabilize tile *neurite cell* membrane mid is involved in neurite outgrowth [16,17]. ADP ribosylation, an essential process in brain metabolism of cytoskeletal and growth associated proteins, is markedly inhibited after both *in vitro* mid *in vivo* exposure *to* inorganic Hg [18].

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**Table 1. Sprouting assay of neurite outgrowth.**

| | No.Cells plated | No.Cells sprouted | % sprouted |
|---|---|---|---|
| CM dish | | | |
| I | 10 | 9 | 90 |
| 2 | 13 | 13 | 100 |
| 3 | 8 | 7 | 87 |
| 4 | 9 | 9 | 100 |
| S | 10 | 9 | 90 |
| | | | Average 93.4+3.1 |
| CM +Hg dish | | | |
| I | 14 | I | 7 |
| 2 | 12 | 0 | 0 |
| 3 | 9 | I | 11 |
| 4 | 21 | I | 5 |
| S | 10 | 0 | 0 |
| | | | Average 4.6+2.4% |

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The actual Hg concentration present in our neuronal cultures was indeed lower titan $10^{-7}$M because of a dilution effect in the culture media. The Hg concentrations to which these neurone were exposed were of tile same order of magnitude as Hg levels reported al human mid animal brains after chronic exposure to $Hg^0$ (reviewed al [5]).

Although more titan three-quarters of all Hg-exposed growth cones that we imaged showed evidence of neurite membrane disassembly, tile absence of any response by some growth cones may reflect maturational changes in microtubules. This interpretation is supported by rationale proposed by *Reuhl et al.* [4] al which they suggest that less differentiated developing neurone may be more *susceptible* to microtubule disruption al tile *presence of* MeHg.

It has been claimed that microtubule assembly is defective in AD brains. However, the relationship between tile paired helical filaments characteristic of neurofibrillary tangles al AD brains mid microtubule instability is unclear [19]. Given tile species differences between human mid snail neurone, tile aggregation of denuded neurofibrils observed in tile present study, following Hg exposure, may not be directly analogous to lesions seen al AD brains.

Recently, Escheverria *et al.* [20] have reported a variety of neurobehavioral effects in dental personnel resulting from chronic low-level exposure to Hg°. Their report is confirmed by tile results of several other clinical investigations conducted by tile same group. We suggest that tile cellular findings al tile present study, revealing that Hg disrupts tile integrity of tile neurite membrane al growth cones of intact neurone, may implicate Hg as a potential etiological factor in neurodegeneration that could ultimately be observed as altered neurobehavior.

**REFERENCES**

1. Spencer (:E, Lukowiak K and eyed NI. J Neurosci 20, 8077-8086 (2000).

2. Gelfand VI and Ber,had,ky AD. Annu Rev Cell Biol 7, 93-116 (1991).

3. Falconer MM, Vaillant A, Reuhl KR et al. NeuroToxicology 15, 109-122 (1994).

4. Reuhl KR, Lagunowich LA and Brown DL. NeuroToxicology 15, 133-146 (1994).

5. Lorscheider FL, Vimy MI and Summer, AO. FASEB 1 9, ;04-;us (199;).

6. Aposhian 11V, Bruce DC, Alter W et al. FASEB 16, 2472-2476 (1992).

7. Pendergrass JC, Haley BE, Vimy MI et al. NeuroToxicology 18, 319-324 (1997).

8. Little M, KraXXmmer G, Singhofer-Wowra M and Luduena RP. Ann NY Aced Sci 466, 8-12 (1986).

9. Syed NI , Bulloch AGM and Lukowiak K. Science 250, 282-289 (1990).

10. Feng Z-P, Klumperman J, Lukowiak K and eyed NI. J Neurosci 17, 7839-7849 (1997).

11. Van Minnen J, Bergman JJ, Van Kesteren ER et al. Neuroscience 80, 1-7 (1997).

12. Ridgway RL, eyed NI, Lukowiak K and Bulloch AGM. J Neurobiol 22, 377-390 (1991).

13. Wong RG, Marten EL and Kater SB. J Neurosci 1, 1008-1021 (1981).

14. Lorsheider FL, Leong CCW and eyed NI. http://movies.commons.ucalgary.ca/mercury (2000).

19. Dane EF, Pendergrass JC, Slevin JT and Haley BE. Toxicol Appl Pharmacol 122, 273-280 (1993).

16. Skene JHP. Alum Rev Neurosci 12, 127-196 (1989).

17. Liu Y, Fisher DA and Storm DR J Neurosci 14, 9807817 (1994).

18. Palkiewicz P, Zwiers 11 and Lorscheider FL. J Neurochem 62, 2049-2092 (1994).

19. lqbal K and Grundke-Iqbal 1. Aim NY Acad Sci 777, 132-138 (1996).

20. Echeverria D, Apoqnian IIV, Wood, JS et al. FASEB )12, 971-980 (INS).

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