Limnology and Freshwater Biology 2022 (3): 1273-1276 DOI:10.31951/2658-3518-2022-A-3-1273

SI: «Mercury in Biosphere: Environmental and Geochemical Aspects»

Short communication

ISSN 2658-3518

LIMNOLOGY FRESHWATER BIOLOGY

www.limnolfwbiol.com

Vector field techniques for the detection of neuronal dynamics in the presence of mercury

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ABSTRACT. The role of mercury in the development of various neuropathologies is well known. The biogeochemical cycles of mercury indicate the possibility of its introduction into various organisms (including humans) through the corresponding trophic chains. However, as it turns out from the literature analysis, until recent years, there have been almost no works on the dynamic microscopic study (either *in situ* or *in vivo*) of the processes of connectome involution in aquatic organisms (including fishes, marine mammals, etc.) under the action of mercury. In this regard, we attempted to study the dynamics of morphogenesis and breakdown of the emerging connectome under the mercury exposure. A dynamic study of frames with the dynamics of the axonal path search was carried out in a series with an address-time code, on which it is possible to track the vector fields of displacements and reaction-diffusion processes in the cell culture immediately after the introduction of a drop of mercury or a mercury-containing liquid using capillary pipettes. This paper describes the observed effects and the possible mechanisms underlying them.

Keywords: mercury, ecotoxicology, axonal guiding, axonal pathfinding, motion estimation, vector fields

1. Introduction

The role of mercury in the formation of various neuropathologies is well known (O'Donoghue et al., 2020), including neurodegenerative diseases (Cariccio et al., 2019); it has been proven, in particular (Lee et al., 2018; Bjørklund et al., 2019; Siblerud et al., 2019; Azar et al., 2021), that mercury can cause the development of Alzheimer's disease. It can also lead to the development of brain tumors (Bjørklund et al., 2020) and the development of autism in children (Kern et al., 2020; Kaur et al., 2021), which is caused by disturbances in the machinery of neuro(morpho)genesis and impaired efficiency of connectome development (Abbot and Nigussie, 2021). Such effects may be due to exposure to mercury at the early stages of development, as well as at the most vulnerable stages of age-related physiology (Dórea et al., 2020). Effects of this type are also observed in marine mammals (López-Berenguer et al., 2020) inevitably exposed to mercury due to its presence in seawater (Brown et al., 2018; Wang et al., 2021; Jinadasa et al., 2021).

However, until recent years, there have been practically no works on the effects of mercury on the development of the neural structure of the brain of marine mammals during natural or model exposure to mercury, and, moreover, no works with dynamic observation of development with such exposure of their surviving brain slices under time-lapse microscopy and multi-angle 3D imaging techniques (confocal microscopy, SPIM, microtomography, holography, and holographic microscopy), which would answer the question of Hg-inhibitor diffusion in space and time. How does axonal guiding / axonal pathfinding change with introducing amounts of mercury characteristic of natural exposure into the nervous tissue? However, full-scale studies of this on marine mammals have not been conducted.

The situation is even worse with the study of the reactivity of the nervous system in fish to introducing mercury into the environment. Analyzing recent literature (because the author has not studied the nervous tissues of fish since 2015 (Gradov, 2015), due to the lack of equipment and infrastructural capabilities to maintain them), we came to a paradoxical, in fact, conclusion. Despite the increase in works postulating the toxic effect of mercury demonstrated on fish (up to its toxicokinetics and biotransformation at various stages of this effect) and works postulating mercury contamination under various hydrochemical conditions (Zheng et al., 2019; Jinadasa and Fowler, 2019; Wang and Wang, 2019; Mendes et al., 2019; Lahrich and El

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Received: June 10, 2022; ***Accepted:*** July 12, 2022;
Available online: July 31, 2022

Mhammedi, 2019; Rahmanikhah et al., 2020; Tamele and Vázquez Loureiro, 2020; de Paula Gutierrez and Agudelo, 2020; Askary Sary, 2020; Mahmudiono et al., 2020; Canham et al., 2021), the number of works describing and interpreting this effect at the cellular level, providing cytophysiological, immunohistochemical and morphometric data, allowing quantifying the effects with topographic reference, is extremely small (Pereira et al., 2019).

2. Materials and methods

Our approach implemented in this work was based on methods that provide for the dynamic study of frames with the dynamics of axonal pathfinding with a timecode, based on which it is possible to track vector displacement fields and reaction-diffusion processes in the culture immediately after introducing a drop of mercury or a liquid containing mercury using a capillary patch pipette. In this paper, we describe the method for the first time and test it on data, according to generally accepted ideas, proving (at a didactically understandable level) the presence of effects of exposure to mercury on neurogenesis. We initiated work on more complex neural structures, but their results, due to the multiplicity of interacting elements of the neural structure of the brain, are more difficult to describe and interpret and require reference to unpublished data. Therefore, we decided to start publication by a simpler version of the experiment known since the end of the past century.

Sequences of frames taken before the introduction of mercury were considered an episode for control. As an episode to control the intrinsic dynamics of disturbances in vector fields during mercury diffusion (under conditions of advection and convection that inevitably arise due to the temperature difference between the introduced substance and the cultivation medium), frames were taken for the first one and a half seconds after introducing the toxicant into the medium, until the currents were established, which allowed us to observe dynamics and own behavior of neurons, without “convective artifacts”. As an episode to control the vector fields of the intrinsic behavior of neurons, frames taken after the establishment of a stationary state of the medium up to the extreme stage of involution (denudation) and the decay of trends in the formation of the connectome were used.

3. Results

The results and their descriptions are shown in the figures, which shall be published in the latest issue of the journal. Briefly, the content of the experiment states is as follows:

1. The structure of the vector fields of axonal guidance in the control (before the introduction of mercury into the liquid) demonstrates the presence of a high “search activity” of axons, spreading over the entire field of view, a kind of “field probing”;
2. after introducing a drop of Hg into the area free from cellular structures using a patch pipette, there are high-speed convective and advective flows in the medium, indicating the beginning of the distribution of mercury in the medium;
3. when mercury reaches the ends of neuronal structures, denudation begins accompanied by the contraction of the lateral processes and protrusions, while the machinery of axonal path finding, the formation of connections (structural units of the connectome) is blocked and ceases to be active (since the vector fields of the dynamics of the structures corresponding to it cease to be registered);
4. retrograde vector fields indicate further structural involution: there is a reduction in the growth cones of axons of all neurons located at an accessible distance capable of participating in axonal search / axonal guidance (relative to each other);
5. the terminal stage of the development of denudation processes represents a stationary state; it is fixed by the absence of representative vector fields of motion estimation (however, in the initial period of it, residual oscillations / fluctuations can be observed: reversible short-wave contractions, which, apparently, are due to the automatism of the elements of the cytoskeleton and filopodia).

4. Discussion

The production of aquatic environments that simulate known freshwater and oceanic or marine environments with an arbitrarily high complexity of composition reproduction does not present significant technical problems at the moment - up to models that include microbiological components, fluid models for specific geographical locations, specific exposure levels - imitating the photochemistry and photohydrochemistry of mercury and the presence of specific dissolved or precipitated forms, etc. (Regnell and Watras, 2018; Zhu et al., 2018; Jinadasa and Fowler, 2019; Kimáková et al., 2019; Yan et al., 2019; Luo et al., 2020; Branfireun et al., 2020; Helmrich et al., 2021; Gallorini and Loizeau, 2021). Moreover, in the presence of modern models that reconstruct trends (which is a consequence of the analysis of “big data” about natural ecosystems), not only reconstruction is available for known environmental conditions, but also for arbitrary conditions for which a plausible calculation of the state in computational models is possible. That is, in fact, there are no obstacles to modeling not only statics and adaptation, but also possible forms of the norm of reaction to the content of mercury in the evolutionary process or in bio(geo/hydro-)chemical pathology.

5. Conclusions

Drawing parallels between the bioavailability of mercury for consumers of different levels, including humans (Broadhurst et al., 1998; Okpala et al., 2018; Ong and MacKenzie, 2018; de Almeida Rodrigues et al., 2019; Cosio, 2020), it is possible to implement multilevel schemes of model systems, in which the conditions for the assimilation of mercury in ecological chains as a whole, and not only in individual organisms, will be reproduced. As a consequence, it is also possible to implement schemes of installations with a modified medium (analogues of stop flow or continuous flow, including their microfluidic implementations) to analyze the response of different neurons and for preparations of different types of aquatic organisms exposed to different (in terms of Hg content, at least) hydrochemical conditions. Applying to such "microchemostatic" systems vector-field methods for analyzing the results of microimaging obtained from inverted or lensless (which is suitable only for very large neurons) microscopes, it is possible to study the dependences of the search behavior of neurons in the process of axonal guidance depending on environmental conditions and the dynamics of its contamination. . In our opinion, such prospects can open a qualitatively new chapter in the history of mercury ecotoxicology, especially in terms of its neurophysiological and neuroembryological effects.

Acknowledgements

The author is very grateful to P.L. Aleksandrov (IBCh RAS) for automation of time-lapse recording at a given exposure, including camera modification for these purposes. The relevant data will be published in future papers. The author expresses his gratitude to E. Adamovich for the super-fast translation of the text of the article.

Conflict of interest

The author declares no conflict of interest related to this publication.

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