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OBSERVATION ARTICLE

The curious case of the spinning neuronal mass [version 1; peer review: 1 not approved]

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Abstract

Rotating cells have been reported in past. Rotation of cells and cell clusters has been associated with migration and development. This observation reports for the first time a rotating cluster of cells isolated from the hippocampi of neonatal mouse pups. The speed of rotation in these clusters is immensely high. Further analysis of such rotating neurons can shed valuable clues on the origin of such cells, their electro-mechanical properties and their role in the development of the brain.

Keywords

cell rotation , neurons , hippocampus , brain development

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1. Dennis Steindler, University of Florida, Gainesville, USA	
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Introduction

Rotation in cells and in cell aggregates is an interesting biophysical phenomenon. Though the exact reasons behind such rotations are still largely unknown, it has been established that such rotations are important for the process of development. Cellular rotations have been previously observed in cilia-driven rotation of *Helisoma trivolis* embryos¹ and in cortical rotation of *Xenopus* eggs². Tanner *et al.* (2011) identified coherent angular motion (CAMo) in breast epithelial cells which maintain it cohesively while assembling into the acini. Such a rotation was lost in malignant cancerous cells. The authors conclude that such rotational movement is essential for constructing the overall geometry of the tissue³. Collective rotations have also been observed in endothelial cells^{4,5} and canine kidney epithelial cells^{6,7} on micropatterns. Most of these rotating cells follow the yin-yang pattern of movement⁸.

However isolated cells cultured in regular 2D cell culture systems have not shown full body rotation⁹.

Neurospheres are generated by three dimensional growth of neural stem cells and progenitors when primary neurons/neuronal precursors isolated from hippocampus are allowed to grow on a low attachment vessel under proper growth conditions in a non-differentiating environment. When these primary neurons/neuronal precursors are allowed to attach and grow in a differentiating environment they differentiate into mature neurons. The procedure of generating hippocampal neurospheres as well as primary neuron cultures requires single cell suspension of neurons derived from hippocampus and their immediate plating. This piece of work shows for the first time that neurons isolated from the hippocampi of four day old neonatal pups when kept in suspension for two-three hours in media instead of immediate plating, coalesce to form neuronal mass and exhibit angular momentum resulting in spinning of the mass.

Methods

Mice usage

Mice used for this experiment had been procured from the central Animal House facility (CCMB). They had been housed in polypropylene cages with shredded corn-cob bedding under proper care with 12 hour light and dark cycle. Four day old pups were generated by mating of male and female wild-type CD-1 (Charles River) mice. All the experiments adhered to the ethical guidelines of animal usage and were approved by the Institutional Animal Ethics Committee, CCMB (Reg. No. CPCSEA 20/1999).

Isolation and plating of cells

Hippocampi were isolated from brains of three male neonatal pups of four days age. The complete protocol followed has been described elsewhere¹⁰. They were pooled and fine dissected in DMEM (Himedia) without serum and then exposed to trypsin-EDTA (Sigma) for 15min at 37°C. Trypsin inhibitor was added to stop the reaction. Cells were collected after a short spin (700 rpm for 5min at room temperature in Kubota 2800 tabletop centrifuge) and re-suspended in NeuroCult Basal (StemCell Technologies) media (>1million/ml). The cells were passed through a 40micron column filter (Millipore) for ensuring single cell suspension. A fraction of this was used

for generation of neurospheres in NeuroCult Basal Media supplemented with Mouse Proliferative Supplement (StemCell Technologies), antibiotics (Pen-Strep, Sigma), 0.02% BSA (Sigma), 0.2% Heparin (Sigma), 20ng/ul EGF (Invitrogen) and 10ng/ul β FGF (Invitrogen). The remaining was put in a 50ml falcon and incubated at 37°C for 2–3 hours. They were then plated in 4 well tissue culture dishes and in T25 flasks. In two wells of the 4 well plate, Neurobasal + B27 (Life Technologies) were added to Neurocult Basal Media in 1:1 ratio.

Biological duplicates of this experiment were carried out.

Capturing video

In the absence of video capturing capability of Image Acquisition software Image-Pro Express, video recording was done through the eye-piece using a handheld video camera (Sony Xperia Acro S) in real time.

Observation

The observations were recorded in three video files.

Rotation of neuronal masses derived from hippocampi of four day-old mouse pups

3 Data Files

<http://dx.doi.org/10.6084/m9.figshare.1292866>

Different sizes of rotating and spinning cluster of neurons can be visualized at different magnifications in video 1. Interestingly, not only large masses, but a cluster of four cells also exhibited such rotation/spin (Video 1e,f). The culture, since it was derived from four day old pups, resulted in a high amount of debris. Spinning and rotation of neurons can be visualized in spite of the resistance provided by the debris around it (video 2a).

These movements observed were not because of Brownian motion as a) there was no movement of the media and the plates and b) the videos were taken after 30min-1 hour of plating. Few of these masses continued exhibiting rotation for more than 48 hours (video 3) till they settled and attached. The angle of rotation/spin was not constant and the mass also exhibited change in direction/angle (video 1b, c; video 3). Also not all such neuronal masses exhibited such movements and a majority of them were attached to the plates (video 1d). Such a rotational movement persisted even when the media was changed to NeuroCult Basal with Neurobasal supplemented with B27.

Discussion

The observation is the first report of rotation observed in clusters of mammalian neural cells. Cells isolated from hippocampi in the same experiment were also used for generation of neurospheres. No such rotational movement was seen there. The reason for such rotation/spin is unclear and there can be numerous possibilities.

The most likely hypothesis is that two or more migrating neuronal cells couple to each other, resulting in the generation of torque, causing angular motion. Cell migration occurs in developing organs

and tissues, wound healing and cancer,^{11–13}. Collective migration associated with cell-cell interaction has been seen in neural crest cells¹⁴ as well as in neurons of the rostral migratory stream^{15,16}. Collective migration of cells can lead to collective rotation, as is the case of rotation of epithelial cells in the extracellular matrix¹⁷. Pernille Rorth argues that this might be because most of the cells in the cluster follow a ‘leader’ cell and, as with sheet migration, rotate in their bid to follow the leader cell¹⁸. There is also a possibility that usage of planar polarized signaling molecules such as the non-canonical Wnt-Fz pathway, could account for rotation of the epithelium^{18–20}.

Noting that even a four cell clusters exhibited such rotation (video 1e,f), it can also be argued that a single cell might be the unit responsible for such a phenotype. But considering the amount of energy required for moving a larger mass, as displayed in video 2 or 3, this is highly improbable. Hence, such a movement more likely results from the interaction of multiple cells. Since these cultures were generated from fourth day neonatal pups, there are many migratory neurons present in the hippocampus. The spin thus may be caused by the force exerted by these migratory neurons.

However it is also plausible that such a rotation arises as a result of change in charge difference between the membrane surface and the interior of the neurons. A continuous dynamic change in the charge could result in the generation of angular momentum in a cluster of cells. However a detailed analysis of electrophysiological parameters and calcium imaging of these mass of neurons needs to

be done to understand the exact reasons behind such an interesting phenotype.

The other most interesting fact about the rotating neurons was the speed at which they were rotating and that the rotation persisted for more than 48 hours. Whether such rotation occurs in neurons derived from other brain regions also needs to be assessed.

Further research needs to be done to find actual reason of such rotatory movement and the origin and fate of these cells.

Data availability

Figshare: Rotation of neuronal masses derived from hippocampi of four day-old mouse pups. doi: [10.6084/m9.figshare.1292866](https://doi.org/10.6084/m9.figshare.1292866)²¹

Competing interests

No competing interests were disclosed.

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I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Version 1

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The author describes spinning cells or spheres in neurosphere cultures from the mouse hippocampus. Although an interesting phenomenon, presenting a time lapse film of these structures, without any phenotyping, cell and molecular analysis of them does not allow any understanding of the nature of these structures. Furthermore, these have been described before in neurosphere cultures from the subventricular zone (see Laywell *et al*, PNAS, 2000; Figure 1), originating from ciliated ependymal cells that can form spheres with other ciliated cells or astrocytes. The hippocampal periventricular ciliated ependymal cells and B cell astrocytes are probably the origin of these spinning structures described here, but only characterization of them would allow this confirmation. Finally, referring to the potential physical mechanisms underlying rotation really only needs to consider the study of Sawamoto *et al.*, Science, 2014.

Competing Interests: No competing interests were disclosed.

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