Final publishable summary report

Executive summary

The brain is one of the most complex organs in the human body. Fundamental knowledge about how the brain develops during embryogenesis is still fragmented, as techniques that allow the study of organ development *in vivo* without interfering with the organism were largely unavailable. The adaptation of sophisticated imaging techniques to rapidly developing embryos from non-mammalian species that naturally occurs outside the body have greatly contributed to closing this gap in our knowledge in recent years. These methods have now made it possible to observe biological phenomen at early stages of brain formation that previously required the surgical intrusion or destruction of the embryos and their parent. One such biological phenomenon is neuronal migration.

The brain is a highly complex organ made up from a large number of different cell types that are precisely arranged into regions and layers in order to exert their functions. The spatial arrangement of neurons is created by neuronal migration, which occurs in the early stages of brain formation. Neurons that arise from a cell-creating site (the germinal zone) typically leave this region in a highly time-controlled developmental window along precisely defined routes.

As migration organizes the different cell types and brings the correct partners in contact, it is thought that neuronal migration is fundamental to forming a functional brain. While the routes and the main guidance signal that influence the cells have been identified in recent years, we still lack a thorough understanding of how the progress along a given route is controlled in neurons. In our project, we aimed at elucidating whether and how neurotransmitter-mediated activity could act as control mechanism in neuronal migration, using the *in vivo* migration of tegmental hindbrain nuclei neurons (THNs) in live zebrafish embryos as a model system.

Using pharmacological and optogenetic methods, we found that different neurotransmitters regulate the progress of THNs either positively or negatively. We have summarized these results in a model that describes the effects as regions of influence acting in different parts of the cerebellum that the THNs could use to navigate the tissue. Next, we have investigated the molecular basis of the conversion of such (external) signals into active forward movement. In this, we have concentrated on the local distribution of Cadherin-2, which had previously been identified as an important regulator for THN migration. Lastly, we have probed the role of microtubules in neuronal migration as it occurs in its natural surroundings, as current models had proposed a prominent role for this part of the cytoskeleton in force generation in migrating neurons. While we can demonstrate that microtubules are essential to THN migration, contrary to expectations, our results suggest that a regulatory role for microtubules could be more important than force generation, which could be related to its function in the intracellular transport and subcellular distribution of Cadherin-2.

This project has yielded valuable insights into the fundamental regulation of neuronal cell migration. As neuronal migration is considered to be essential to the formation of a functional brain, it provides the starting point for future studies into the working of the brain and biomedical applications.

Project context and objectives

The brain exerts its complex tasks by the coordinated cooperation of different cell types which are arranged in distinct regions and layers. Individual neurons are connected in an intricate network of axons and dendrites to ensure correct communication. Synaptic transmission of information in the mature network is mediated by neurotransmitters, e.g. glutamate or glycine.

The structure of the brain forms during early embryogenesis by active neuronal migration. Neuronal precursors arise at distinct sites, from which the differentiating neurons emigrate along specific routes to reach their destinations. It is thought that this migration is not only necessary to bring cells into their correct regions, but also for the neurons to meet their communication partners in the forming network. Neurons that have reached their destination terminally differentiate in this position and stabilize their connections in the neuronal network.

Previous studies from rodents had hinted that neurotransmitter might act in some way during neuronal migration, which occurs much earlier than thought, as synapses are not present at this developmental stage. A detailed investigation of this was, however, precluded by the limited accessibility of embryos at this early developmental stage without the destruction of the organism and its parent in mammalian models. In recent years, concurring with the rise of non-invasive, in vivo-imaging-based techniques, non-mammalian models have become more popular to study questions in developmental techniques. One such model is the zebrafish, as its embryos are translucent and develop rapidly outside the body.

While the involvement of neurotransmitters in neuronal migration had not been established for zebrafish, a previous study (Rieger et al. PLOS Biology, 2009) had identified the subcellular localization of Cadherin-2 to the front of a migrating cell as an important regulator of motility in tegmental hindbrain nuclei neurons. Cadherin-2 is a member of the Cadherin protein family of cell-cell adhesion molecules. Besides mediating cell-cell contacts, Cadherin-2 also possesses signalling functions via its interactor beta-Catenin, and is also able to directly influence the cytoskeleton. In particular, a direct link to microtubules has been proposed via its direct interactor IQGAP1.

The cytoskeleton is composed of several elements, of which actin and microtubules are the best studied. While cell migration in general is typically driven by actin, microtubules are considered essential to the migration of neurons. This notion is supported by in vitro studies, in which interference with microtubules produces a stop in migration, but also by medical data. Patients suffering from lissencephaly, polymicrogyria, microcephaly, but also psychological disorders, such as schizophrenia and autism, often have mutations in proteins that are directly linked to microtubules, and biopsy samples reveal unorganized regions and layers in the brain, sometimes with interspersed cell types foreign to the region. These findings point towards microtubules being essential to neuronal migration, and models on neuronal migration present microtubules as direct force generators.

In our study, we took advantage of the zebrafish model to investigate the role of neurotransmitter-mediated activity to the subcellular localization of Cadherin-2, the signal transduction from Cadherin-2's distribution into active migration and the contribution of microtubules to Cadherin-2's localization and forward motion.

Specifically, the questions underlying this project were:

(1) Can activity control neuronal cell migration?
(2) Can Cadherin-2 influence the microtubule array in migrating THNs?
(3) How do microtubules contribute to forward motion in migrating neurons?

By this research, we contribute to the fundamental understanding of how the brain forms, which could become the basis of medical interventions in future.

Main results/foregrounds

This is a summary of the most important insights gained from our study. For further details, please consult the midterm and period reports, or scientific publications.

(1) Investigation of the role of activity in neuronal cell migration

The first part of the scientific programme aimed to characterize the role of neurotransmitter-mediated activity in neuronal cell migration *in vivo* using zebrafish embryos as model organism.

To this end, we have obtained embryos from a zebrafish line in which a specific neuronal population, the tegmental hindbrain nuclei neurons (THNs), can be identified by the expression of green fluorescent protein. We recorded the migration of these neurons in vivo in 4 h videos and tracked the progress of the cells. As during this extended image acquisition the embryos continued to grow and the tissue expanded slightly, we set up a very successful collaboration with the Institute for Genetics at TU Braunschweig (head: Prof. R. Schnabel) to optimize a software and analysis algorithm to be able to quantify cell speeds reliably. This also allowed us to compare cell speeds across different embryos, making it possible to compare migration velocities under different conditions.

Next we applied a series of pharmacological treatments and identified acetylcholine, glutamate and glycine as neurotransmitters that influence THN migration. By regional analysis of the tracks we can also assign regions of influence for each neurotransmitter in the developing cerebellum. Using optogenetics and in vivo Calcium imaging, we can also directly manipulate the THNs and prove that depolarization increases and hyperpolarization decreases cell speeds. We summarize these findings in a new model that describes how regions of influence of the different neurotransmitters successively control the migration of THNs along their specific route through the cerebellum to reach their destination.

Our results strongly argue that neurotransmitter-mediated spatial regulation is a fundamental, evolutionarily conserved principle in neuronal migration which has been underestimated so far.

(2) Investigation of Cadherin-2's influence on microtubules

This task probed the molecular mechanism of how Cadherin-2 could regulate the cytoskeleton to control migration, as the cytoskeleton is the main driving force in cell migration. We hypothesized that IQGAP1 acts as bridge between Cadherin-2 in the plasmamembrane and the organization of microtubules which ultimately leads to forward movement of the nucleus and the cell.

In order to gain evidence for this notion, co-localization experiments involving the two proteins were performed in migrating THNs *in vivo*. These have revealed good co-localization between the factors, a prerequisite if IQGAP1 acts as mediator between Cadherin-2 and microtubules. Likewise, whole mount in situ hybridization experiments showed that IQGAP1 is expressed in the brain at the relevant developmental stage. Further evidence for a direct effect of IQGAP1 on microtubules will come from a series of mutations in IQGAP1 that are impaired either in the protein's binding to Cadherin-2, or to microtubules.

During the construction of these mutant versions, results from a group in the US suggested that instead of IQGAP1, its close homologue IQGAP3 could be the better candidate for regulating cell migration (Fang et al. Cytoskeleton 2015). We therefore included IQGAP3 in the colocalization and mutation analysis, after consultation with G. Bloom, University of Virginia, USA. The IQGAP1 part of this task was supported by material supplied by Anne Straube, University of Warwick, UK. At present, several mutants for both IQGAP1 and IQGAP3 have been constructed, but a detailed

analysis of each for colocalization with Cadherin-2 and the mutations' effect on THN migration is still under way. So far, the mutations affecting the subcellular targeting of the proteins appear to conform to expectations. In the earliest datasets available, some appear to negatively influence migration, but this remains to be confirmed by increased data volumes.
Once the data are fully evaluated, we will have a clear idea whether and how IQGAP1 and 3 are (differentially) involved in neuronal migration. We will be able to present a model of how the migration regulator Cadherin-2 could act to transduce the information into direct forward movement, and we will be able to attribute functions to specific parts of the IQGAP proteins. It will also contribute to the understanding of the function of IQGAP3, which remains understudied although it has been found upregulated in some cancers.

(3) Investigation of microtubule-based force transmission

In this part of the project, the contribution of microtubules to active neuronal migration was investigated, as different models are described in the literature for how forces are generated to move neurons forward. These models differ by how the microtubule array is arranged during migration.
To address this question, a detailed description of the microtubule cytoskeleton during the different phases of migration of the THNs was completed, by building on available markers and creating a novel marker for microtubules. This in-depth description allowed us to clearly correlate subsequent reorganizations of the microtubules with forward motility as measured by nuclear position.
Next we investigated the requirement for microtubules for THN migration in a series of experiments involving pharmacological, optical and genetic methods that interfere with the formation of microtubules, impair the function of molecular motors or alter the surface of microtubules. In sum, treatments that interfered with dynamic microtubules or the function of Dynein led to a migration decrease in THNs.
At first glance, these results fully agree with the hypothesis that microtubules and their motor Dynein provide accessory forces in migrating neurons that are necessary to move the nucleus against pressure from the surrounding tissue. However, when looking at experiments altering microtubule dynamics in detail, questions arose over the strength of the effect on migration and observed changes to cell morphology that could not easily be explained by a simple force generation model.
As microtubules are known to be a strong element in intracellular transport and contribute to the subcellular distribution of proteins, we tested whether microtubules could also be involved in the regulation of migration. It is also known that microtubules can regulate cell adhesion in vitro. Therefore, we investigated whether microtubules are directly implicated in the intracellular transport of Cadherin-2, as it is an adhesion molecule with regulatory function in THN migration. Using similar methods as described for the investigation of the effect of microtubules on migration, we find that Cadherin-2 is trafficked in live zebrafish neurons by microtubules and their motor proteins. This adds significantly to our knowledge about the interaction of Cadherin-2 and microtubules, which now appears as a two-way system, in which Cadherin-2 regulates microtubules, but microtubules control the localization of Cadherin-2.
Taken together, the results demonstrate the microtubules support migration and likely coinciding developmental processes such as axon formation in our in vivo model system, but the molecular details are intricate and provide the starting point for future research. We expect to add significantly to this knowledge about the interactions between Cadherin-2 and the microtubule cytoskeleton in future in order to further understand the basis of regulating the fundamental developmental process that is neuronal migration.

To facilitate this work, the fellow has established contact with Oliver Thorn-Seshold from LMU Munich, Germany, whose support was essential in the light-controlled ablation of microtubules (Borowiak et al. Cell 2015). This part of the project also received significant contributions from Anne Straube, University of Warwick.

Potential impact, dissemination activities and exploitation of results

In addition to a new in vivo tracking method that enhances our technological spectrum to investigate cell migration in many contexts, this project has yielded valuable insights into the fundamental regulation of neuronal cell migration on the physiological, molecular and cell biological level. These aspects enhance our fundamental understanding of how the brain forms. Studying these questions in detail will provide us with an understanding how some severe neurological disorders arise. Severe neurological diseases such as lissencephalies or polymicrogyry often involve misregulation of cell biological control of neuronal migration. Elucidation of the underlying causative pathogenic mechanisms provides entry sites for therapeutic interventions and also potential constraints. Therefore, this work interconnects the fields of basic research and translational approaches, which is facilitated by extensive dissemination of our results via: **a)** presentation of our data to experts at scientific conferences, **b)** preparing manuscripts for publishing our obtained results, **c)** training of undergraduate and graduate students in cell biological concepts of neuronal migration and the reading of original scientific literature, **d)** organizing a scientific symposium with international speakers (Brainswick 2016), **e)** oral and hands-on presentations to the interested public, **f)** visiting schools and educating high school students, and **g)** our lab webpage.

For a complete list of dissemination activities to scientific and non-scientific audiences please refer to the tables A1 and A2.

Online information and contact details

Further information can be found at http://www.zoologie.tu-bs.de/index.php/en/cellular-molecular-neurobiology/research/neuronal-migration

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