1. Sir Henry Wellcome Postdoctoral Fellowship application

Reference number	UNS112977
Applicant name	Dr Adna Dumitrescu
Title of application	Seeing the forest and the trees: understanding the contribution of both axonal and glial activity-dependent plasticity in neural circuit function
Total amount requested	£300,000
Duration of funding	48 months

2. Application summary

Application title This is the title of your proposed project.
Seeing the forest and the trees: understanding the contribution of both axonal and glial activity-dependent plasticity in neural circuit function

Proposed start date This date must be at least six months after the full application deadline.	01/01/2021

Name of administering organisation If your application is successful, this is the organisation that will be responsible for administering the award.
University of Edinburgh

Lead applicant's address at administering organisation If your application is successful, we will use this address in your award letter.	
Department/Division	Centre for Clinical Brain Sciences
Organisation	University of Edinburgh
Street	49 Little France Crescent
City/Town	Edinburgh
Postcode/Zipcode	EH16 4SB
Country	United Kingdom

Research area Select the most relevant area, based on the key aims of the research. This allocates your application to the relevant Grants team. We may reallocate your application to another area if we consider it appropriate.
Cellular and Molecular Neuroscience

3. Lead applicant

Lead applicant details	
Full Name	Dr Adna Dumitrescu
Department	Cente for Discovery Brain Sciences
Division	Czopka Lab
Organisation	University of Edinburgh
Address Line 1	Chancellor's Building, Edinburgh BioQuarter, 49 Little France Crescent
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ORCID iD	
ORCID iD	https://orcid.org/0000-0002-7354-145

Career history (current/most recent first)

From	To	Position	Organisation
04/2020	01/2022	Postdoctoral Fellow	University of Edinburgh
03/2017	03/2020	Postdoctoral Fellow	Institut du Cerveau et de la Moelle Épinière (ICM)
04/2016	09/2016	Postdoctoral Fellow	King's College London

Education/training

From	To	Qualification	Subject	Organisation
09/2012	03/2016	Doctor of Philosophy (PhD;DPhil)	Neuroscience	King's College London
09/2011	07/2012	Master of Science (MSc)	MRes Neuroscience	King's College London
09/2010	07/2011	Master of Science (MSc)	Developmental Neurobiology	King's College London
09/2007	07/2010	Bachelor of Science (BSc)	Cognitive Science	University of Westminster

Source(s) of personal salary support
State all your sources of salary funding (for example, through your organisation's block grant from a higher education funding body), and the percentage of your salary they contribute. Answer 'not applicable' if you are not currently employed.

ERC Grant awarded to Dr Tim Czopka

Current/last appropriate salary details

If you are currently unemployed give salary details from your most recent employment.

Salary grade
UE07/ 34

Basic salary (per annum)
38017

Currency
GBP

Date of last increment	04/05/2020

Proposed starting salary for Fellow (£)
39593

Clinical status Are you a healthcare professional?	No

Career breaks Have you taken any career breaks or periods of part-time work, for example parental, long-term sick leave, carer responsibilities?	Yes

Provide details
Oct 2016 - Feb 2017 Full-time paid internship with the European Commission in Brussels in a Joint Research Centre team involved with promoting and researching evidence based policy making.

Do you wish to undertake this award part-time? If you wish to undertake this award part-time, either from the start or part way through the grant, your host organisation must employ you on a part-time basis during that time.	No

Career contributions What are your most important research-related contributions? These may be from any stage of your research career. State what each contribution was, when it came about, why you think it is important and what impact it has had. Examples include publications, patents and impacts on policy.
During my training as a neuroscientist, I actively contributed to enhancing our knowledge of how the brain works and also built tools that improve neuroscience research. One of my key research contributions, which I made during my Ph.D. project in the Grubb Lab at King's College London, was to identify a new form of neural plasticity. I was able to show that the axon initial segment can change its structure quickly (within hours) in response to changes in network activity, and in doing so, regulates the overall excitability of a neuron. This finding, published in the journal Cell Reports in 2015, is essential because it reveals that, in addition to well-established synaptic plasticity mechanisms, neurons can alternatively also use rapid axonal adaptations to respond to changes in baseline activity quickly. This research has since been replicated in other systems and opened up a new avenue of axonal plasticity investigation. Following a productive Ph.D., I received a Marie-Curie Individual Fellowship for my first postdoctoral project in the Wyart Lab at the ICM in Paris.

During my time here, I generated an optogenetics research toolbox, which was recently published in Elife. Before this article, the scientific community was missing crucial information about the efficacy of opsins in vivo. Taking advantage of Dr. Claire Wyart's world-renowned expertise in optogenetics and my in vivo electrophysiology skills, I was able to address this issue head-on by performing the first systematic opsin response calibration in vivo. The publication which stemmed from this project was enthusiastically received by reviewers, as well as colleagues in the field, and is poised to become an important reference point for the wider zebrafish neuroscience community. While I consider the publications mentioned above to be the most impactful of my career to date, I have also been interacting with colleagues and actively contributed to shaping research by presenting my work at over 11 conferences during the past seven years. I plan to continue my work on axonal plasticity using the tools and neural circuits, which I became an expert on during both my Ph.D. and my first post-doc.

Personal statement
How will this Fellowship further your research and career aspirations?

My long-term goal is to lead a lab researching the effects of exercise on neural plasticity. I have been tailoring my academic training to be able to address this question using the best available systems. For this reason, my PhD was focused on neural plasticity, while in my first postdoc, I studied the locomotor system with in vivo tools. With this Fellowship, I would be able to combine these two research areas, use all skills I have developed so far, and perform the necessary research that will make my future larger-scale study of exercise-induced plasticity possible.

Due to its broad scope and complex combination of research techniques, the project I designed cannot be hosted by a single lab, as a traditional postdoctoral position. The Henry Wellcome Postdoctoral Fellowship will give me the freedom and flexibility to work on a challenging project and collaborate with experts in glial in vivo physiology (Czopka Lab) and axonal computational modelling (Brette Lab), while being mentored by a world-leading expert in myelination plasticity (Professor David Lyons). These collaborators are essential for the success of this proposal, and will continue to support me as I transition to an independent career, and beyond.

In the future, I plan to apply similar techniques to other neuronal subdomains and locomotor cells, and also further develop the basic exercise manipulations used here. I strategically chose to have a computational modelling component at the end of the project, because it will help me generate hypotheses that will directly shape the 1st phase of research in my future independent lab.

Your Fellowship will provide me with the financial support and autonomy to lead a research project which will overall advance our knowledge of neural plasticity in a significant manner, and in doing so, secure my path to an independent research position.

Research outputs
List up to 20 of your most significant research outputs; at least five of these must be from the last five years. For 10 of these outputs, provide a statement describing their significance and your contribution (up to 50 words maximum per output).

Research outputs may include (but are not limited to):

- Peer-reviewed publications and preprints
- Datasets, software and research materials
- Inventions, patents and commercial activity

For original research publications, indicate those arising from Wellcome funded grants in **bold**, and provide the PubMed Central ID (PMCID) reference for each of these. You can find more information on this in the guidance to this question.

Give the citation in full, including the title of paper and all authors (unless more than 10, in which case you may use 'et al', ensuring that your position as author remains clear). Citations to preprints must state "Preprint", the repository name and the articles persistent identifier (e.g. DOI).
1. **Dumitrescu AS**, Fidelin K, Wyart C (2020). Towards a comprehensive model of circuits underlying locomotion: What did we learn from zebrafish? In Whelan PJ, Sharples SA (1) Model Systems and Tools to Study Locomotor Function. Elsevier Contribution: main co-author together with FK. I made major contributions specifically describing how motor neurons and excitatory interneuron networks have been studied in zebrafish. Significance: This book chapter will highlight the key findings that were made possible by using the zebrafish as an animal model for the study of the locomotor network. In particular, we highlight the ease with which integrative studies across multiple brain areas are uniquely possible in this small vertebrate model. **2**. Antinucci P*, **Dumitrescu A***, Deleuze C, Morley HJ, Leung K, Hagley T, et al. A calibrated optogenetic toolbox of stable zebrafish opsin lines. Elife. 2020;9. (*first co-authors) Contribution: In vivo electrophysiology experiments design, data collection and analysis, paper writing. Significance: Previous publications generally used intracellular recordings in reduced in vitro or ex vivo to calibrate opsin efficiency. This is the first publication in which opsins are calibrated comprehensively and directly in vivo. The electrophysiology dataset I provided is particularly useful to design more foolproof optogenetic stimulation experiments **3**. Evans MD, Tufo C, **Dumitrescu AS**, Grubb MS. Myosin II activity is required for structural plasticity at the axon initial segment. Eur J Neurosci. 2017;46: 1751–1757. Contribution: experiment design and data collection Significance: The axon initial segment is a key regulator of axonal excitability and can display activity-dependant structural plasticity adaptations. This paper provides the first line of evidence that Myosin II is a key axonal cytoskeletal factor which mediates axon initial segment plasticity. **4. Dumitrescu AS**, Evans MD, Grubb MS. Evaluating Tools for Live Imaging of Structural Plasticity at the Axon Initial Segment. Front Cell Neurosci. 2016;10: 1–17. Contribution: experiment design, data collection and analysis, paper writing. Significance: This paper constitutes the first major advance towards live AIS plasticity imaging, by carefully characterising all tools that are available for this purpose. **5.** Evans MD*, **Dumitrescu AS***, Kruijssen DLH, Taylor SE, Grubb MS. Rapid Modulation of Axon Initial Segment Length Influences Repetitive Spike Firing. Cell Rep. 2015;13: 1–13.(*first co-authors) **Contribution:** In vitro electrophysiology experiments design, data collection and analysis, paper writing. **Significance:** This paper describes a new type of activity-dependent plasticity at the axon initial segment. While this subcellular structure was previously shown to change on a scale of days, this is the first report of rapid plasticity, happening on the hours time-scale. **6**. Evans MD, Sammons RP, Lebron S, **Dumitrescu AS**, Watkins TBK, Uebele VN, Renger JJ, Grubb MS. Calcineurin Signaling Mediates Activity-Dependent Relocation of the Axon Initial Segment. Journal of Neuroscience. 2013;33: 6950–6963. Contribution: data collection Significance: This paper constitutes the first comprehensive analysis of the signalling pathways which mediate axon initial segment structural plasticity, and demonstrate that the calcium-sensitive phosphatase calcineurin is a key enabler of AIS plasticity.

How many peer-reviewed publications have you authored/co-authored? Include systematic reviews and meta analyses but exclude abstracts and literature reviews.	5

Current and recent research funding (including Wellcome grants)
List all research funding you have held in the last five years and any key funding before then.

List the most recent first. State the name of the funder, name(s) of grantholder(s), title of the project, total amount awarded (and how much of this you received), your role in the project, and the start and end dates. State the percentage of your time spent on the research; if the grant is active state the number of hours per week that you spend on the research.

1. Research funding
Funder: Marie-Sklodowska-Curie European Individual Fellowship
Grant Holder: Dr. Adna Dumitrescu
Project Title: Mapping the functional connectome for speed control in spinal motor circuits
Total amount awarded: 173,080€
Role: Lead Applicant for a 2-year postdoctoral project in the Wyart Lab at the ICM in Paris, France.
Start - End dates: Feb 2018 - Jan 2020

2. Research funding
Funder: Boehringer Ingelheim Fonds Travel Grant
Grant Holder: Dr. Adna Dumitrescu
Project Title: An investigation into spinal V2a intra-connectivity during swim speed modulation
Total amount awarded: 3000€
Role: Lead applicant for a grant supporting a short collaborative research visit in the McLean Lab at Northwestern University in the USA.
Start - End dates: Oct 2017 - Jan 2020

3. Research funding
Funder: Physiological Society Travel Grant
Grant Holder: Dr. Adna Dumitrescu
Project Title: Larval mutant ninja zebrafish: an axon initial segment in vivo imaging project
Total amount awarded: £450
Role: Lead applicant for a travel grant to present at the SfN 2015 meeting in Chicago, USA.
Start - End dates: Nov 2015

4. Research funding
Funder: King's College London School of Biomedical & Health Sciences Travel Bursary
Grant Holder: Dr. Adna Dumitrescu
Project Title: Live imaging of the axon initial segment
Total amount awarded: £500
Role: Lead applicant for a travel grant to present at the SfN 2014 meeting in Washington DC, USA.
Start - End dates: Nov 2014

5. Research funding
Funder: King's College London Graduate School Travel Bursary
Grant Holder: Dr. Adna Dumitrescu
Project Title: Rapid activity-dependent plasticity at the axon initial segment
Total amount awarded: £300
Role: Lead applicant for a travel grant to present at the FENS 2014 meeting in Copenhagen, Denmark.
Start - End dates: June 2015

6. Ph.D. funding
Funder: Medical Research Council
Grant Holder: Dr. Adna Dumitrescu
Project Title: 4-year fully PhD
Total amount awarded: £70,000
Role: In a highly competitive scheme, I was awarded a fully-funded scholarship for a 4-year Ph.D.

at the MRC Centre for Developmental Neurobiology
Start - End dates: September 2011 - March 2016

7. Education funding
Funder: Dinu Patriciu Foundation, Romania & King's College London
Grant Holder: Adna Dumitrescu
Project Title: MSc Neuroscience, King's College London
Total amount awarded: £3000 & £9000 respectively
Role: I received a 15,000$ scholarship for my MSc in Neuroscience from the Dinu Patriciu Foundation, as part of a scheme recognising the top 100 Romanian postgraduate students. In addition, I was offered a £3000 fee bursary for the MSc in Neuroscience due to my excellent BSc degree results.
Start - End dates: September 2010 - July 2011

Recommendation by applicant's present sponsor/supervisor
Upload a letter of support from your current sponsor/supervisor (500 words maximum).

6. Collaborators

Are any collaborations essential for this proposal? This could be through sharing facilities, providing access to resources (essential reagents, samples, data) or sharing subject-specific knowledge and guidance.	Yes

List any key collaborators (name and organisation) and provide a very brief outline of their role in the proposed research.
Dr Misha Ahrens will provide assistance with voltage imaging experiments using a genetically encoded indicator recently developed in his lab at Janelia Farm. Professor Maarten Kole has published extensively on projects in which voltage sensitive dyes were used to study of axonal function. He has agreed to help us optimise imaging and analysis parameters of voltage imaging experiments.

I confirm that the collaborators named above have agreed to be involved, as described, in the proposed research and are willing for their details to be included as part of this application.
Confirmed

7. Related applications

Is this or a similar application for funding currently under consideration elsewhere?	No

Is this a resubmission of an application submitted to Wellcome within the last 24 months? Contact us before resubmitting an application.	No

8. Research summary

Research summary Provide a summary of your proposed research, including key goals, for an expert audience
Neurons adapt to changes in network activity with structural and functional modulations at the level of their dendrites, somas, and axons. While plastic adaptations of the somato-dendritic compartment have been intensely studied, considerably less is known about axonal contributions to network plasticity. Recent work indicated that different axonal subdomains are responsive to changes in activity – the axon initial segment, its myelination, and presynaptic boutons. However, the coordination of these processes and their implications for axon and network function remain unclear. Here, I propose an integrated approach of structural, functional, and computational analysis to reveal how axonal and glial plasticity mechanisms regulate axon function. Taking advantage of the zebrafish locomotor network and newly developed reagents, I will first study how entire neurons dynamically form and refine their axonal subdomains. Next, I will test how axonal subdomains structurally adapt to elevated network activity and how this in turn affects cell function. Using my experimental data, I will build a computational model to further test how subcellular axon components can tune cell function. Together, this work will reveal principles of how axonal and glial adaptive mechanisms are coordinated to control network function in vivo, for the first time.

Lay summary
Provide a summary of your proposed research for a non-specialist audience. You don't need to oversimplify your research, but try to explain it as clearly as possible. Write in the first person ("I" and "we") and structure your summary in this order: background to the research problem; your approach; expected impact of your work.

A key question in neuroscience is how experience changes brain function throughout an individual's lifetime. This feature, known as neural plasticity, involves anatomical/structural alterations, and is what makes learning and recovery from injury possible. We are now at a research crosspoint where we know which neuron subcompartments can be plastic. However, it remains unclear how structural changes are coordinated and how they affect cell function. To address this question, I will use newly developed genetic tools to image multiple neuronal domains simultaneously during plasticity adaptations in a larval zebrafish model system. These data will then be integrated in computational models of neural circuits, where we can check how each individual neuron subcompartment adaptation contributes to cell function. This will be the first time when neural plasticity is studied in such a comprehensive manner directly in an intact animal, and will lead to a better understanding of brain function.

9. Details of research project

Detail:
(a) Aims and research questions;
(b) Work which has led up to the project;
(c) Approach and how you will address challenges;
(d) Key stages in your research plans, indicating location and timelines.
Do not exceed **1,400** words.

Seeing the forest and the trees: understanding the contribution of both axonal and glial activity dependent plasticity in neural circuit function

(a) Aims and research questions

If you learned a second language, or became good at kicking a ball - this was possible because brains can dynamically adjust over time as they experience changes in network activity. To date, plasticity mechanisms have been studied extensively at the level of synapses[1–3]. In contrast, axonal plasticity has been investigated much less, despite being a key component in propagating information between neurons[4,5]. In principle, all critical domains of axons are responsive to neural activity via structural adaptations: the axon initial segment [6,7], myelination by glial cells[8,9], as well as presynaptic boutons[10,11]. **However, how axonal and glial plasticity are coordinated to regulate cell and network function remains a critical open question**. A major reason for this gap in our knowledge is that it is inherently difficult to directly study the many structural and functional adaptations of axons that occur over extended periods of time in the intact CNS.

Here, I developed a project that allows the investigation of structural changes along entire axons over time together with probing their functional output in vivo, using a relatively simple motor circuit in larval zebrafish. I plan to (i) investigate the dynamics of distinct axonal domains and their impact on function as circuits mature, (ii) manipulate network activity to look at structural and functional plasticity responses across multiple axonal subdomains, (iii) build a computational model to better understand how axonal and glial plasticity mechanisms regulate circuit function.

(b) Background

Action potential generation and propagation depends on the organisation of axonal subdomains. I, together with others, have previously shown that spike initiation depends on the size and position of the axon initial segment (AIS), which can change in response to neural activity[12–17]. As action potentials propagate along axons, it was long assumed that myelin simply maximised conduction speed. However, myelination can be present in highly specific patterns[18], and axons are adorned with nodes of varying sizes and in functionally organised motifs [19] to precisely time the arrival of an action potential at presynaptic terminii [20]. Furthermore, recent work including that of my host institution has shown that myelination itself is an activity-dependent process with the capacity to remodel[21–24]. Ultimately, presynaptic boutons connecting to downstream neurons are dynamic structures that can change in response to altered neural activity[11,25–27]. Overall, each axonal subdomain can, in principle, display activity-dependent plasticity.

However, it remains unclear if activity-regulated modulation of distinct axonal domains:

i. are all employed by individual neurons,
ii. occur during overlapping or distinct timescales,

iii. act in concert or independently,
iv. affect neuronal output.

I will address these fundamental questions through the following aims (Fig 3).

(c & d) Experimental approach / implementation

I plan to use the larval zebrafish for my project, as this is the only vertebrate model where it is possible to study entire neurons and dynamic alteration to their axonal domains at the required subcellular resolution in vivo and in real time. I will focus on a relatively simple locomotor network comprising of hindbrain and spinal cord excitatory interneurons (including well-characterised Chx10 descending interneurons) that connect to motor neurons. This offers several advantages: (i) specific transgenic reporters can be used to follow all axonal subdomains over time (Fig 1B, D), (ii) neuron subtypes with specific locomotor functions can be identified unambiguously and have a well-described connectome [28–35] (Fig 1A), (iii) network activity can be modulated with simple behavioural tasks (Fig 2B), (iv) activity-dependent myelination in zebrafish has previously been observed in descending motor circuits [21].

Aim 1: How do individual axonal subdomains develop and regulate cell function?

I will express fluorescent reporter constructs developed by my host lab in single neurons to label AIS, nodes, myelin, and presynaptic boutons (Fig 1B, D). I will follow single axons by time-lapse microscopy from immature to mature stages and determine how different axonal subdomains of neurons belonging to fast and slow locomotor modules develop. I will probe the function of these neurons during key stages of their maturation using the following methods: (i) intracellular recordings and voltage imaging of Chx10 neurons to reveal changes in excitability and conduction (ii) paired intracellular recordings of Chx10 neurons and their motor neuron targets as well as synaptic GCaMP imaging to investigate synaptic function and plasticity.

Given studies of individual axonal domains in different in vitro and in vivo contexts [36–39], I predict a development timeline in which the AIS is stabilised first, followed by axonal myelination and node formation as functional connections mature. Presynaptic boutons may remain dynamic throughout. This integrated structural/functional analysis will provide a unique dataset of how the emergence and refinement of the AIS, myelination, nodes, and boutons relates to function in an intact animal.

Aim 2: How do individual axonal domains contribute to activity-dependent plasticity adaptations?

I will manipulate Chx10 network activity via two methods: optogenetic stimulation mimicking locomotor activity for broad activation (Fig 2A). Chx10 neuronal subtypes control the speed of swimming by driving motor neuron activity: therefore I will use behavioural interventions which promote swimming at different speeds to activate subsets of these neurons and their targets (Fig 2B). I will monitor how axonal subdomains of Chx10 neurons adapt using the approaches presented in Aim 1. I will analyse single axons over time to reveal which structural changes are subject to activity manipulations (AIS size and position, node size and placement, myelin

amount, bouton number) and how this affects their function and connectivity to downstream motor neurons.

These experiments will reveal which axonal plasticity adaptations occur in vivo, and how they correspond to cell function. If all axonal subdomains exhibit plasticity, I predict that structural changes will match their developmental order: presynaptic boutons responding within minutes to hours, AIS remodelling across hours to days, and myelin and node changes happening over days or longer. Functionally, excitatory neurons typically show compensatory adaptations to enhanced activity by decreasing cell excitability. However, this is not a given for the locomotor system because increased activity can lead to muscle growth, which in turn might require increased excitatory drive [40]. Therefore, how structural and functional plasticity of axonal domains shape and maintain function of this neural circuit in vivo remains to be determined.

The approaches described in aims 1 and 2 are ambitious but entirely feasible because: (i) my host lab has extensive experience in live-imaging myelinated axons in zebrafish [37,41], (ii) I have extensive experience (>7 years) performing electrophysiological recordings [14,42], including in zebrafish [42], (iii) I have expert collaborators (Dr Misha Ahrens (Janelia) and Prof Maarten Kole (Amsterdam) [43–45] who will support more challenging voltage imaging aims.

Aim 3: Build a computational model of axonal plasticity physiology

I will use the structural and functional data acquired in aims 1 and 2 to build a model of a neuron with a detailed multi-compartmental axonal configuration that includes an AIS, nodes, myelin and presynaptic boutons. This will allow me to precisely control the onset, form and duration of each type of axonal microdomain plasticity, and to virtually follow their individual or coordinated effects on cell function. In doing so, I will test how different axonal adaptations might affect cell output, generating refined hypotheses that can be tested empirically.

While my in vivo manipulations targeted generally only two cells at a time, in silico I will be able to check the effect of axonal plasticity by connecting a model neuron to a larger network. More broadly, this model can also be fitted with data to match other neuron types such as inhibitory cells, or mammalian neurons where axons cannot be tracked in vivo in this level of detail.

Previous models were generally restricted by the data available and contained single axonal substructures [44,46]. The data acquired here will allow me to build the first complete axonal model containing detailed subcellular elements. This will provide insights into mechanisms of axonal plasticity that are not possible with traditional methods, and will generate a new research tool that will be useful for the larger research community.

Overall, while so far, we had only piecemeal evidence of axonal microdomain function, my study will provide the first holistic view of axon function in vivo. In this way, this work will lead to a better understanding of nervous system plasticity.

Does your proposal involve human participants?	No

Additional information
Figures and additional information cannot exceed 2 A4 pages.

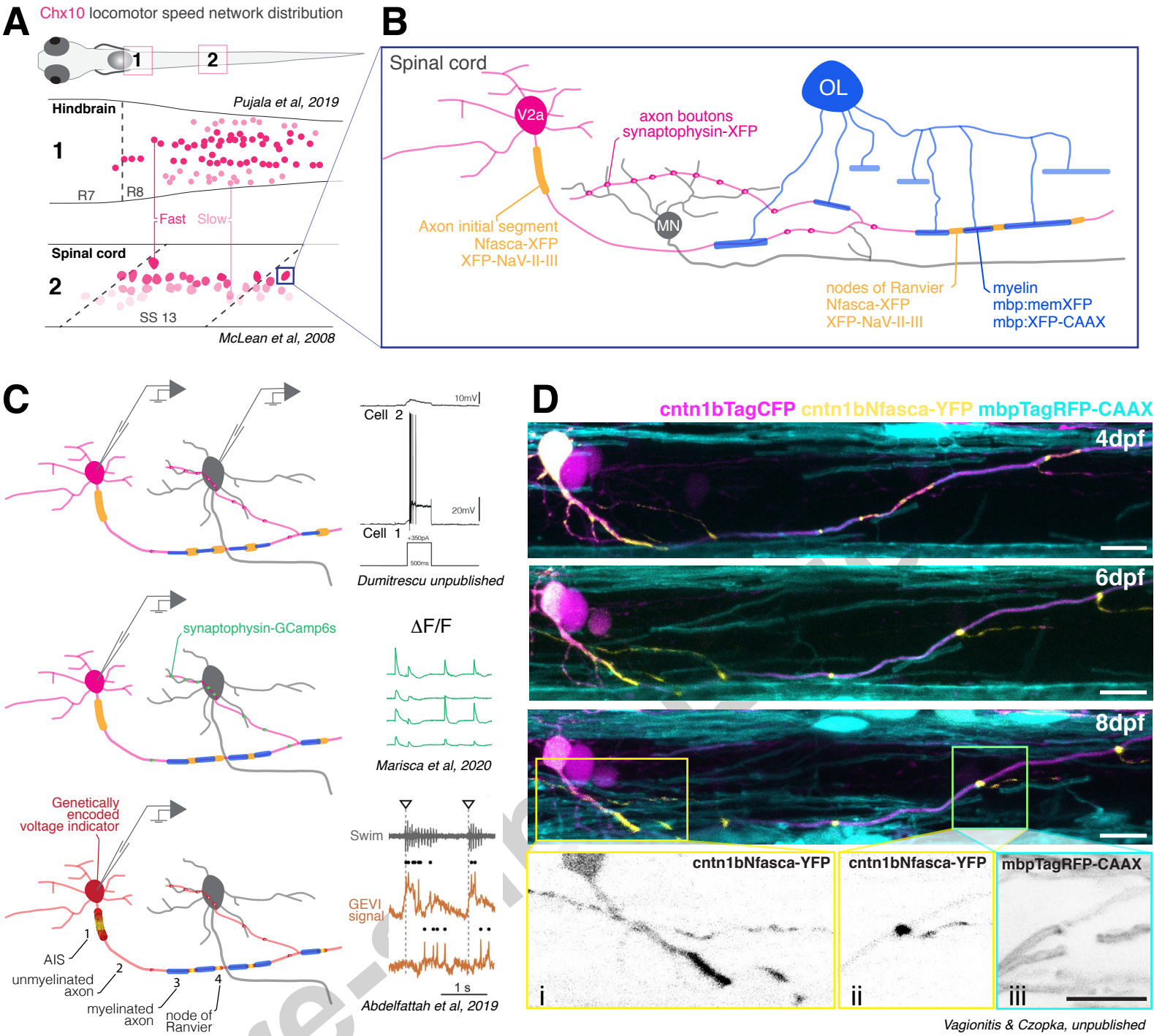


Figure 1: Methods to study myelinated axonal structure and function.
A: Illustration of the anatomical distribution of Chx10 positive neurons from the hindbrain and the spinal cord. The cells are pseudocoloured to highlight their contribution to the slow or fast swim locomotor network based on previously published work.
B: Schematic representation of the spinal cord network used for axonal plasticity investigations. Chx10 neurons (magenta) have ipsilateral projecting axons which contact motor neurons (grey). Fluorescent constructs (XFP) are available to label each individual axonal microdomain: axon initial segment and nodes (orange), myelination (blue) and presynaptic boutons (magenta).
C: Schematic representation of the planned approach to study functional effects of axonal plasticity: paired-patch recordings between V2a and motor neurons (top panel), calcium functional imaging of V2a presynaptic boutons (middle panel) and axonal voltage imagining (bottom panel). Example responses for each method are for illustration purpose only, and do not correspond to data from Chx10 neurons.
D: Example of a three-colour time-lapse imaging of a V2a neuron and its myelinated axon in a living zebrafish. The cell (cntn1bTagCFP), it's AIS and nodes (cntn1bNfasca-YFP) and axonal myelin (Tg:mbp-TagRFP-CAAX) have been imaged at 4, 6 and

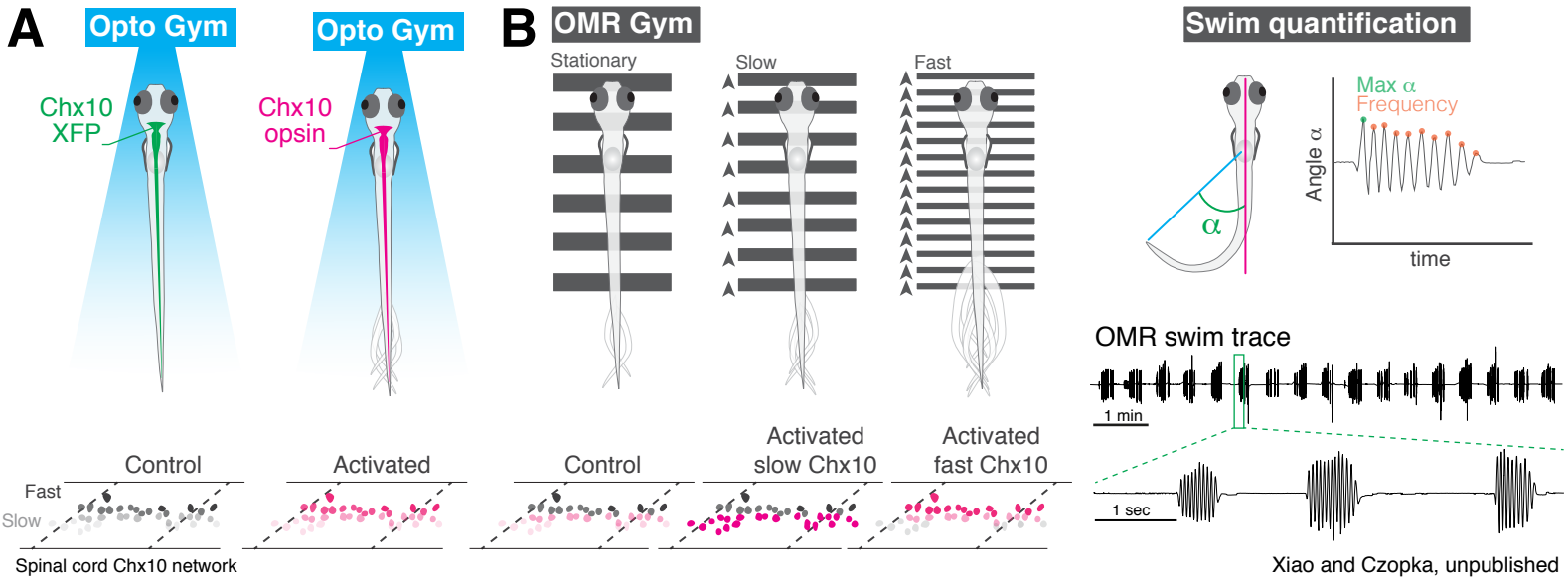
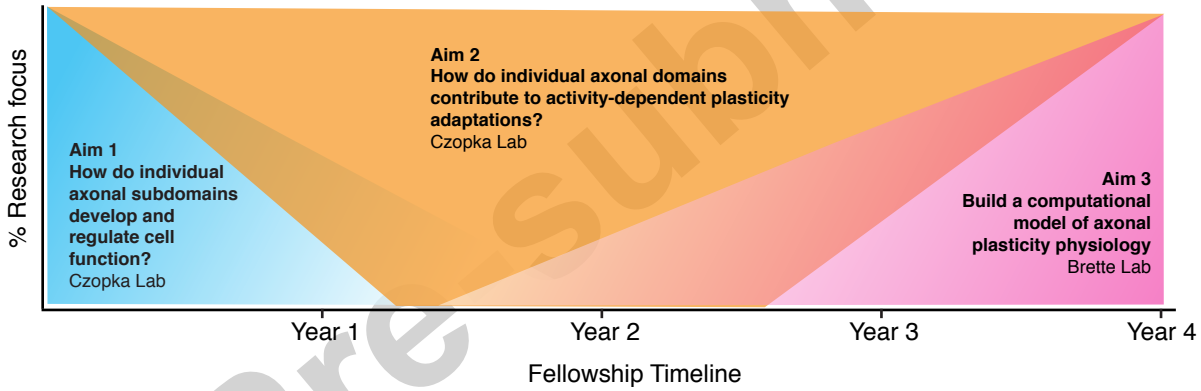


Figure 2: Network activity modulation methods
A: An optogenetic method of networks activation can be carried out by expressing an opsin under the Chx10 promoter. Control larvae expresing a fluorescent marker and opsin positive animals can then be stimulated with light stimuli which mimmick normal locomotor behaviour patterns. Bottom pannel displays expected cell map activation for the spinal cord area, where all Chx10 neurons will be equally activated and will lead to swim bouts of various intensities.
B: A schematic of a behaviour protocol that can be run to activate subsets of Chx10 neurons, tuned to different locomotor speeds. Using an optomotor protocol (OMR) we will display high contrast visual gratings which will be either stationary (control) or moving at slow or fast speeds. This visual stimulation is a reliable way to control swimming speed in zebrafish larvae. We expect control fish to display normal spontaneous swim bouts, which normally activate the ventral Chx10 cell population. The slow speed OMR will provide a net increased of network activation to the same ventral cell targets, while the fast OMR will stimulate proponderently more dorsal Chx10 populations. On the right there is a schematic of how swim bouts are usually analysed, and an example OMR trace from another set of experiments currently running in the Czopka Lab.

Figure 3:Timeline of research plan



References
Give the citation in full, including title of paper and all authors.

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myelinating potential of single oligodendrocytes in vivo. Development 2011;138:4443–50.
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Are there any papers listed in your 'References' section as being "in press" that you wish to submit to us?	No

10. Outputs management and sharing

Will the proposed research generate outputs of data, software, materials or intellectual property that hold significant value as a resource for the wider research community?	Yes

Select the approach you will use to maximise the impact of your significant research outputs to improve health and benefit the wider research community.
Make research outputs available for access and re-use

Provide an outputs management plan Our guidance on developing an outputs management plan sets out where such a plan is required and gives an overview of what you must consider.
My research project will result in a large in vivo axonal function and plasticity dataset and one computational model. These resources will be made available to the wider scientific community at the project publication stage. All raw imaging and electrophysiology files will be securely stored on University of Edinburgh data servers for an initial period of 10 years, which can be extended if needed. All processed and extracted data will be directly attached to the publications that will result from this project. Upon request, the Czopka lab will make available any unprocessed original datafiles. All analysis code will be published in full on Github under an MIT license. The multi-compartment axonal model will be stored on the ModelDB website, where it will be immediately available for other researchers to use.

11. Research locations

1

Name of sponsor/research sponsor You must invite the named research sponsor to participate in your application, under the 'Participants' section of this form.
Dr Tim Czopka

Organisation
University of Edinburgh

What work will you carry out here? Why did you choose this research environment?
At the end of my Marie Curie Postdoctoral Fellowship earlier in 2020, I had one clear aim for a second postdoctoral project. I wanted to apply the advanced in vivo research skills I developed during my first postdoc in the Wyart Lab, to my original interests in studying neural plasticity based on my PhD in the Grubb Lab. Dr Tim Czopka is the ideal research host for my current research plan as we have a mutually beneficial and complementary set of expertise and skill sets. As an experienced electrophysiologist

with an in depth knowledge of neuronal plasticity, I look forward to using the genetic tools that the Czopka lab developed, become versed in their exquisite time lapse imaging methods in vivo, and also expand my knowledge to include glial plasticity. The lab is equipped with all technical resources necessary for the planned in vivo structural and functional imaging, electrophysiology recordings, optogenetic stimulation and zebrafish locomotor behavioural protocols.

In addition to offering what I foresee to be a fruitful research collaboration, I was also drawn to Dr Tim Czopka as a postdoc advisor because he was acknowledged as a supportive mentor by his team. As evidence, the first Czopka lab graduate student is now continuing her research in the Schopik Lab, at NYU. Moreover, since establishing an independent research group, Dr Czopka has managed to acquire several high-profile international research grants, and has continued to be a prolific contributor to the field of oligodendrocyte function, as evidenced by his publication record.

More broadly, the University of Edinburgh is recognised internationally for the quality of its research output, specifically in the field of glial physiology and myelination. One of the people responsible for driving this work is my research mentor, Professor David Lyons, a world leading expert in myelination plasticity, and a topic that is central to my research plan.

The Czopka Lab and the University of Edinburgh will offer a stimulating academic work environment, and a professional support network that will put me in a competitive position as I approach my own move towards an independent research position.

Proposed time to be spent at research location (months)
30

Start date (if known)
1/01/2021

2

Name of sponsor/research sponsor You must invite the named research sponsor to participate in your application, under the 'Participants' section of this form.
Dr Romain Brette

Organisation
Institut de la Vision

What work will you carry out here? Why did you choose this research environment?
With increasingly complex neuroscience data sets, more researchers are using computational modeling methods to better grasp the full range of data interactions, or to run simulations when practical experimental techniques are lacking. During my first postdoc, I realised that the experiments I was planning could also benefit from a computational modelling approach. Dr Romain Brette is an axon modeling expert, whose prolific body of work I have been following since my PhD. I have approached him for a collaboration because of his specific expertise, and our overlap in scientific interests. While I previously used mostly imaging methods and electrophysiological recordings to study axon initial segment plasticity, Dr Brette has approached the same topic with computational modelling approaches. Under Dr Brette’s supervision, I will use some of the previous multicompartmental axonal models

developed in his lab to study the axon initial segment, and expand them to include other axonal subdomains such as nodes, myelin and boutons. Based on data availability, it is common for modelling projects to contain multiple estimated features. For this reason, I plan to actively consult Dr Brette during the data acquisition process, to be able to maximise the level of realistic detail that can be incorporated in this axonal multi compartment model.

We developed a research plan for the computational part of the project in which I will spend a total of 18 months under his supervision. After following an initial period of data collection during the first year and a half of my fellowship in the Czopka Lab, I plan to visit the Brette Lab regularly for periods of 1-2 months at a time, spread across the last 30 months of my fellowship. This would allow me to update my model in real time, with every new batch of data collection.

My computational modelling experience is restricted to attending the OIST summer school in 2019. However, based on this initial experience I feel confident that with Dr Brette's supervision and support I can develop the skills necessary to complete this part of the project.

Proposed time to be spent at research location (months)
18

Start date (if known)
01/07/2022

12. Public engagement

How could members of the public and non-academic communities, inform, use, or find value in your research?
During my time as an academic researcher I took part in several public engagement events, in both London and Paris, aimed at children as young as 5, teenagers and adults. For example, I was able to organise and lead a three-day science fair for school children. I have greatly enjoyed these events and they have helped me become a better communicator to lay audiences and fellow experts. Based on my previous experience, I can identify two ways in which my planned work at the University of Edinburgh can impact members of the public. First, I plan to continue my advocacy of responsible use of animals in research. I found that people are generally receptive when they are introduced to new animal models, such as zebrafish embryos. Second, my main research focus, neural plasticity, is inherently interesting and easy to grasp for non scientific audiences. Every person that learned a new language, or motor skill has been able to do so due to the brain's inherent plasticity. From my experience, making people aware of this concept is a good segue to more neuroscience research facts. One of the unexpected benefits of returning to an anglophone country for my second postdoctoral project, is that I now have more opportunities to take part in public engagement events as the University of Edinburgh has set up an extensive schedule of events aimed at the general public. I look forward to using my research work and contributing to public engagement during my fellowship.

Have you discussed your ideas and plan with your institutional Public Engagement Officer (if you have one?)	Yes

13. Location of activity

Will the funded activity take place at more than one location? List any locations outside of your administering organisation where you will be conducting research or redirecting funds. This includes, but is not limited to, anywhere in receipt of indirect funding, fieldwork sites, and time spent working in another organisation/laboratory. This does not include conference attendance.	Yes

For each location, select the country and, where applicable, state the organisation. You must include the administering organisation.
Enter the approximate percentage of the total funds that will be spent in each location. Enter zero for locations where activity will take place but no significant funds will be spent. If you are requesting salary costs, attribute them to the employing organisation.

Country	Organisation	Percentage of funds
United Kingdom	University of Edinburgh	100
France	Institut de la Vision	0

14. Research involving human participants, human biological material and identifiable data

Does your project involve human participants, human biological material, or identifiable/potentially identifiable data?	No

15. Proposals involving animals

Select any of the following that apply to your proposed work:
(Proposal involves the use of animals, Proposal involves the use of animal tissue, Neither of the above)

Proposal involves the use of animals

Select any of the following species you will use:
(Primate, Cat, Dog, Equidae, Pig, Genetically Altered Animals, Other animals)

Genetically Altered Animals
Other animals

Select 'Add...' to enter the animal species and total numbers required (this may differ from the number to be purchased, maintained).

Animal species	Strain (if appropriate)	Total number required to carry out proposed work
Fish - Zebrafish		469-3374

Provide a justification of the proposed sample size alongside details of the planned statistical

analyses. Describe experimental design, including any plans to reduce bias such as blinding or randomisation if appropriate. You must include power calculations if appropriate. (1,000 words max.)

The study proposed here relies on in vivo imaging of axonal microdomains coupled with assays to probe their function as a result of network activity manipulations. We plan to randomly assign sibling larvae to experimental conditions as control versus activity manipulated groups. All collected data will be blinded to the experimental group at the analysis stage.

For all statistical analyses, we will first test the data distribution profile with a normality test (Shapiro-Wilk) after which the appropriate parametric or non-parametric tests will be carried out to check for experimental effects. The criterion for statistical significance will be set a $p < 0.05$ and appropriate multiple testing corrections will be employed as needed. Our unit of assessment throughout the study will be the number of zebrafish larvae between 5-15dpf. Based on previous experiments, we expect to record data from one cell per animal.

Table 1 contains details of our planned statistical analysis and a priori sample size calculations for each experiment with $\alpha = 0.05$, Power β s= 0.8 and standardised Cohen's f values for a small, medium or large effect (equivalent to Cohen's d = 0.5, 1 or 1.5, as per NC3Rs guidelines for animal studies[1]). Since no previous data is available we could not use more specific effect sizes, and had to rely instead on standardised effects. For simplicity, we assumed the use of normally distributed datasets, however, we will use appropriate non-parametric tests when needed.

Aim 1 experiments:
Depending on the effect size of our manipulations (small, medium or large), sample size calculations show that our Aim 1 axonal development structural and functional imaging studies will need a minimum of 4 and a maximum of 32 zebrafish per experimental group (see Table 1, Aim 1 experiments for more details). For the planned in vivo electrophysiological recordings, we estimate a minimum of 5 and a maximum of 32 animals for each experimental group to be necessary (Table 1, see Aim 2 experiments). To minimise animal use for in vivo recordings, which is a more severe procedure, we plan to run experiments with a target sample based on a conservative small effect size, but use a sequential analysis design [2] with an intermediate analysis point at 60% of data collection (max n = 20 animals per group). Significance threshold α will be adjusted with the O'Brien–Fleming method [3], and data collection will be stopped at the intermediate point if the analysis results in a group difference with an $\alpha <$ adjusted target.

Aim 2 experiments:
To look at activity dependent axonal plasticity, In the first instance, we plan to run structural and functional imaging experiments which have a mild animal welfare impact using two different two activity manipulation protocols (Opto vs OMR Gym). For these types of experiments we calculated that a sample size of max n = 27 (small effect size) and a minimum of n =4 (large effects). To reduce animal number used for procedures with increased severity, such as intracellular recordings, we will first choose the manipulation which results in the most robust effect (Opto vs OMR Gym) and second, we will employ the same sequential statistical analysis design described in Aim 1. With these constraints, we estimate to need a minimum of n = 17 and a maximum of n = 27 animals to achieve sufficient power to uncover even small effects.

Overall, depending on the effect sizes of our manipulations we estimate that we will use a maximum of n = 3374 (small effect) and a minimum of n = 469 (large effects) zebrafish larvae at stages between 5-15dpf. These sample sizes will allow us to uncover even small effects in appropriately powered experiments.

References:
[1] https://eda.nc3rs.org.uk/experimental-design-group
[2] Neumann K, Grittner U, Piper SK, Rex A, Florez-Vargas O, Karystianis G, et al. Increasing efficiency of preclinical research by group sequential designs. PLoS Biol 2017;15:e2001307.
[3] O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics 1979;35:549–56.

Table 1: Statistical analysis specification and sample size calculations

Project AIM:	Measurement Type	Factor 1			Factor 2			Analysis Type	Group number	α	Power β	Effect size (Cohen's f)	Sample Size Total	Sample Size / Group
		Name	Type	Levels	Name	Type	Levels							
1.1	Structural timelapse imaging of AIS, myelin, and nodes	Neuron age (early, intermediate, mature)	repeated measures	3	Neuron type (fast or slow)	between subjects	2	3x2 Mixed ANOVA	6	0.05	0.8	0.25	158	27
												0.5	42	7
												0.75	22	4
1.2	Structural & functional timelapse imaging of boutons	Neuron age (early, intermediate, mature)	repeated measures	3	Neuron type (fast or slow)	between subjects	2	3x2 Mixed ANOVA	6	0.05	0.8	0.25	158	27
												0.5	42	7
												0.75	22	4
1.3	Functional voltage imaging (endpoint)	Neuron age (early vs mature)	between subjects	2	Neuron type (fast or slow)	between subjects	2	3x2 Mixed ANOVA	4	0.05	0.8	0.25	128	32
												0.5	34	9
												0.75	17	5
1.4	Single Intracellular recordings	Neuron age (early vs mature)	between subjects	2	Neuron type (fast or slow)	between subjects	2	2x2 ANOVA	4	0.05	0.8	0.25	128	32
												0.5	34	9
												0.75	17	5
1.5	Paired Intracellular recordings	Neuron age (early vs mature)	between subjects	2	Neuron type (fast or slow)	between subjects	2	2x2 ANOVA	4	0.05	0.8	0.25	128	32
												0.5	34	9
												0.75	17	5
2.1	Structural timelapse imaging of AIS, myelin, and nodes	Imaging time (pre, during, after stimulation)	repeated measures	3	Opto Gym (control vs stimulated)	between subjects	2	3x2 Mixed ANOVA	6	0.05	0.8	0.25	158	27
												0.5	42	7
												0.75	22	4
2.2	Structural timelapse imaging of boutons	Imaging time (pre, during, after stimulation)	repeated measures	3	Opto Gym (control vs stimulated)	between subjects	2	3x2 Mixed ANOVA	6	0.05	0.8	0.25	158	27
												0.5	42	7
												0.75	22	4
2.3	Structural timelapse imaging of AIS, myelin, and nodes	Imaging time (pre, during, after stimulation)	repeated measures	3	OMR Gym (control / slow / fast swim)	between subjects	3	3x3 Mixed ANOVA	9	0.05	0.8	0.25	196	22
												0.5	54	6
												0.75	28	4
2.4	Structural & functional timelapse imaging of boutons	Imaging time (pre, during, after stimulation)	repeated measures	3	OMR Gym (control / slow / fast swim)	between subjects	3	3x3 Mixed ANOVA	9	0.05	0.8	0.25	196	22
												0.5	54	6
												0.75	28	4
2.5*	Functional voltage imaging (endpoint)	Neuron type (fast or slow)	between subjects	2	OMR Gym (control / slow / fast swim)	between subjects	3	3x2 Mixed ANOVA	6	0.05	0.8	0.25	158	27
												0.5	42	7
												0.75	22	4
2.6*	Single Intracellular recordings	Neuron type (fast or slow)	between subjects	2	OMR Gym (control / slow / fast swim)	between subjects	3	3x2 Mixed ANOVA	6	0.05	0.8	0.25	158	27
												0.5	42	7
												0.75	22	4
2.7*	Paired Intracellular recordings	Neuron type (fast or slow)	between subjects	2	OMR Gym (control / slow / fast swim)	between subjects	3	3x2 Mixed ANOVA	6	0.05	0.8	0.25	158	27
												0.5	42	7
												0.75	22	4

* The type of activity manipulation used for these experiments will be chosen based on the experimental conditions where maximal effects are observed in 2.1-2.4 . For simplification, we used OMR stimulation for the current calculations

Why are the species to be used the most appropriate?
My research project will use the zebrafish as an in vivo animal model because it offers several specific research advantages. The Danio rerio genome has been fully sequenced and numerous transgenic lines and DNA fusion constructs are available to label specific cell populations with fluorescent markers or activity indicators. This is particularly helpful because zebrafish larvae are innately transparent, making them the only vertebrate species in which it is possible to perform time-lapse imaging of large scale neural circuits in an intact animal at single-cell resolution. Structural and functional Imaging studies can be further complemented by detailed kinematic analyses of zebrafish behaviour, or intracellular recordings from identified neurons, during fictive swimming. The mouse is an alternative vertebrate model in which some of the methods described above are also successfully employed, with several notable differences. By virtue of their increased nervous system size and anatomy, in vivo mouse studies rely on more severe protocols such as cranial or spinal windows, and in addition since axons span very large areas, it is not possible to trace their entire length in vivo. On the other hand, invertebrate unprotected models such as Drosophila or C elegans are not appropriate for our research question since they do not have myelinating glia. Therefore, by focusing our research in the zebrafish, we are not only gaining access to an easily amenable cell population, but also a neural circuit which cannot be studied in vivo, at a similar level of detail, in other vertebrate or invertebrate models.

Does your proposal include procedures to be carried out on animals in the UK which require a Home Office licence? Your organisation must ensure that research involving the use of animals complies at all times with UK laws and regulations.	Yes

Is there a current Home Office Personal Project Licence (PPL) that authorizes the proposed procedures to be carried out in the UK?	Yes

Provide the name of the licence holder and the PPL number.
Professor David Lyons PPL: PP5258250

If your proposal involves the use of animals, what would be the severity of the procedures? You can find guidance on assessing the severity of a procedure on the Home Office website.
Mild

Does your proposal involve the use of animals or animal tissue outside the UK?	No

Why is animal use necessary: are there any procedures of less severity that could be used?
In vivo experiments are necessary to look at the structural remodelling and function of myelinated axons in response to network activity manipulations. While our study requires the use of animal models, the experimental design has been tailored throughout by carefully considering animal welfare principles of replacement, reduction and refinement. By choosing the zebrafish larva as an animal model, we are using a vertebrate which conceivably has a lower capacity to feel pain due to their less developed nervous systems compared with adult zebrafish, or mice (**Replacement**). The majority of our experiments rely on

interventions of mild severity, such as non-invasive imaging approaches in vivo (**Refinement**). Overall, we have taken all necessary precautions to minimise animal number use, whilst carrying out adequately powered research studies (see section above). In addition we plan to generate pilot data and screen laboratory animals at unprotected stages under 5dpf, such that only the absolute minimum number of larvae are used past this stage (**Reduction**). Moreover, since zebrafish are prolific breeders (an adult couple can produce several hundred offspring in one mating session), we can run high-throughput experiments while maintaining a limited number of adult fish colonies (**Reduction**).

Where it was obvious that experimental constraints would not allow us to answer questions about axonal physiology in vivo, we chose to pursue a computational modelling approach instead. This has the benefits of **replacing** the spurious use of animals for current research experiments, and will also help narrow down the hypothesis testing space of future investigations.

16. Risks of research misuse

Confirm that you have considered whether your proposed research could generate outcomes that could be misused for harmful purposes.
Confirmed

Have you identified any tangible risks of this type?	No

17. Freedom to operate/conflicts of interest

Describe any freedom to operate or other intellectual property related issues that might affect your ability to carry out the proposed research and/or to use, share or commercialise the research outputs. Explain how you will address these.

In particular, consider the following:

- Will your research use technology, software, databases, materials or patented inventions that are owned or controlled by others and which you do **not** already have written permission to use?
- Will the ownership, use, commercialisation and/or sharing of research outputs with the wider research community, be subject to agreements with commercial, academic or other organisations? This includes arrangements with collaborators named in this application.

N/A

Describe any conflicts of interest which might affect your ability to carry out the proposed research and/or to share or commercialise the research outputs. Explain how you and your organisation will manage these and how you will comply with your organisation's requirements in relation to conflicts of interest.

In particular, consider the following: Does anyone involved in your project hold any consultancies, advisory roles, or equities in, or directorships of, companies or other organisations that might have an interest in the results of your proposed research?

Confirm in each case whether the conflict has been disclosed to your organisation.

N/A

18. Wellcome Trust supported facilities

Will the project be based in one of the following Wellcome Trust supported facilities: • the Wellcome Trust Sanger Institute • a Wellcome Trust Centre • an Africa and Asia Programme • the Francis Crick Institute?
No

19. Synchrotron radiation sources

Will you need access to a synchrotron source?	No

Summary	
Grant Type	Sir Henry Wellcome Postdoctoral Fellowship
Application ref.	221679/Z/20/Z
Lead applicant	Dr Adna Dumitrescu
Organisation	University of Edinburgh
Project title	Seeing the forest and the trees: understanding the contribution of both axonal and glial activity-dependent plasticity in neural circuit function
Reviewer	1
Reviewer organisation	Information Redacted

Peer Review: Early to Mid-Career Researchers

Candidate
What is your view of the candidate's suitability for this award?

Taking into account their career stage and any career breaks, please consider:
(i) the significance of their research outputs so far
(ii) whether the candidate has the potential to deliver the proposed research
(iii) how this fellowship will further develop their career.

(i) significance of candidate's research outputs so far: The candidate has demonstrated outstanding research output so far. Two publications from her PhD phase (one shared first author publication and one coauthor publication) have greatly impacted the field of axon initial segment (AIS) plasticity (Evans et al., 2017 and Evans*, Dumitrescu* et al., 2015), providing first evidence for rapid AIS plasticity in vitro. The candidate very successfully established her expertise in AIS live labeling techniques with a follow-up first author publication in 2016 and has continued to produce high quality output both in the form of manuscripts as well as at conferences.

(ii) whether the candidate has the potential to deliver the proposed research: I have no doubt that the candidate has the potential to not only delver the proposed research, but probably also extend on the concepts outlined in the present proposal.

(iii) how this fellowship will further develop their career: The candidate has demonstrated a very smart, tactical approach to building her career so far, with seeking out top leaders in their respective fields in order to advance her training (both in the lab as well as in writing), but never losing sight of her long-term goal in research - asking novel questions about axonal plasticity in order to build her own independent research expertise and ultimately, her own lab. The candidate is sincere in her goal to further the field of axonal plasticity by attracting the next generation of scientists once her own lab has been established. The candidate presents a clear strategy and structured approach to that end, and her own previous career clearly shows that she can build successful networks and collaborations that will support her goals.

Research proposal
What is your view of the research questions and approaches?

Please consider:
(i) whether the questions are original and important
(ii) the strengths and weaknesses of the approaches (including risks and feasibility)
(iii) whether the outputs will significantly impact the field
(iv) (if applicable) whether the clinical trial design is appropriate.

(i) whether the questions are original and important: The questions outlined in research aims 1-3 are original, important, and so far remain unaddressed in their complexity. As the candidate rightly points out, conventional

animal models (rodents) lack the ability to address several axonal domains at the same time due to technical difficulties. The candidate has built her recent career on zebrafish specifically; a smart move considering that her overall goal has always been to look at axonal plasticity at multiple points of interest in a single cell. Manipulating network activity in this model will certainly serve to gain a better understanding of the dynamics of axonal plasticity both from a developmental as well as learning/memory perspective in adult circuits. The field of axon plasticity will benefit from this work.

(ii) the strengths and weaknesses of the approaches (including risks and feasibility): The strengths of the approaches include the model itself (high throughout, ease of use, transparency, genetic tools available) and the fact that the candidate has already gained significant expertise working with this model. Furthermore, her seeking a lab that has documented expertise in a complementary fashion is ingenious and in my opinion, one of the most noteworthy strengths of this proposal. All technical approaches are feasible and while challenging, remain at lower risk. They are backed-up by the fact that the candidate and her sponsors for all three aims have documented their expertise in the respective areas. Another plus of this proposal is aim 3 – an aim that is based on theoretical models and will not further increase the number of animals required to complete the proposal. One potential weakness is the question whether the observations made during the course of this work will be transferrable to higher mammalian species, including humans. However, I could envision the candidate to seek further support after this grant proposal has been completed, and address the arguably more complex issues in more sophisticated neuronal networks at a later stage of her career.

(iii) whether the outputs will significantly impact the field: Please see my comment in section (i); Yes.

Research environment
What is your view of the research and training environment(s)?

Please consider whether:
(i) the supervisors/sponsors/mentors are appropriate for the candidate's career development
(ii) the research environment is appropriate to support delivery of the proposal

(i) the supervisors/sponsors/mentors are appropriate for the candidate's career development. Yes without a doubt, the supervisors, sponsors and mentors that are providing support are more than appropriate for the candidate's career development. They enthusiastically support the candidate as evidenced by the letters of recommendation. Furthermore, the additional outside collaborators are internationally recognized leaders in their respective fields and provide the candidate with exceptional research support and opportunity to grow her expertise, complete her proposed project and develop her career.

(ii) the research environment is appropriate to support delivery of the proposal. Indeed it is; the proposal outlines that all lab work will be done at the Univ of Edinburgh, an internationally recognized institution with world-class axon researchers, some of which are the direct sponsor and advisors of the candidate (T. Czopka, D. Lyons). The lab and technical infrastructure available to the candidate is world-class. In addition, the candidate indicates in her proposal that she will also travel to Paris to work on the theoretical aspects of her research proposal (specifically aim 3) together with her sponsor R. Brette, another well-known axon expert.

Proposals involving animals
Please consider whether the applicants have addressed the following questions:
(i) Is the use of animals and the chosen species justified in terms of the likely outcomes of the research?
(ii) Is there adequate justification for the numbers of animals to be used?
(iii) Is the experimental design appropriate, including any plans to reduce bias such as blinding or randomisation?
(iv) If the use of primates, cats, dogs, or equidae is proposed, could any other animal species be used instead?
(v) Is there potential for improvement in the research approach to replace the use of animals, reduce the number of animals used, and/or reduce animal suffering? Would changes in the research approach allow the researchers to derive significantly greater scientific benefit from their use of animals?
(vi) Would any of the procedures be considered unacceptable in your laboratory –if so, why?

(i) Is the use of animals and the chosen species justified in terms of the likely outcomes of the research? Yes

(ii) Is there adequate justification for the numbers of animals to be used? Yes

(iii) Is the experimental design appropriate, including any plans to reduce bias such as blinding or

randomisation? Yes

(iv) If the use of primates, cats, dogs, or equidae is proposed, could any other animal species be used instead? N/A

(v) Is there potential for improvement in the research approach to replace the use of animals, reduce the number of animals used, and/or reduce animal suffering? Would changes in the research approach allow the researchers to derive significantly greater scientific benefit from their use of animals? No

(vi) Would any of the procedures be considered unacceptable in your laboratory –if so, why? No, all proposed procedures would be acceptable in my lab.

Outputs management and sharing
Will the proposed research generate outputs - including datasets, software, materials or intellectual property - that will hold significant value as a resource for the wider research community? If yes, please comment on:
(i) whether the outputs management plan clearly describes when and how the outputs will be made available,
(ii) whether these arrangements for managing and sharing research outputs are appropriate and in line with best practise in the field.

(i) whether the outputs management plan clearly describes when and how the outputs will be made available. Yes it does.

(ii) whether these arrangements for managing and sharing research outputs are appropriate and in line with best practise in the field. Yes they are. The candidate clearly states that all data will be shared via different platforms, open to any interested party and that even raw data that has not been assessed is available via direct contact to the sponsor. This is actually exceed the current practice in the field at this time.

Resources
This question is not applicable for the Sir Henry Wellcome Postdoctoral Fellowship applications.
Are the requested resources appropriate (staffing, equipment, running costs etc)?

Overall assessment
Please provide a rating which reflects your opinion of the overall merit of the proposal. You should refer to the 'Help' button for details of each rating category.

Outstanding

Overall assessment

As outlined above, this proposal is compelling on several levels. (i) It covers novel ground, asking pertinent questions about complete axon plasticity that due to technical limitations in rodent tissue, have not been addressed comprehensively at this time and will benefit from the proposed zebrafish model. (ii) The candidate is a highly accomplished young investigator with great potential to continue her high quality research output and build a lasting career in neuroscience, ultimately leading to the establishment of her own lab. (iii) The candidate is demonstrating her ability to create a highly competitive network of world-class collaborators to address the proposed questions.

Summary	
Grant Type	Sir Henry Wellcome Postdoctoral Fellowship
Application ref.	221679/Z/20/Z
Lead applicant	Dr Adna Dumitrescu
Organisation	University of Edinburgh
Project title	Seeing the forest and the trees: understanding the contribution of both axonal and glial activity-dependent plasticity in neural circuit function
Reviewer	2
Reviewer organisation	Information Redacted

Peer Review: Early to Mid-Career Researchers

Candidate
What is your view of the candidate's suitability for this award?

Taking into account their career stage and any career breaks, please consider:
(i) the significance of their research outputs so far
(ii) whether the candidate has the potential to deliver the proposed research
(iii) how this fellowship will further develop their career.

This candidate's research outputs demonstrate signficant interest and contributions to the field of cellular, especially axonal, neuroplasiticity and motor neurophysiology through molecular and electrophysiology (doctoral) and optogenetics (postdoctoral). The candidate is working toward an independant academic research career studying the effect of exercise on neuronal plasticity. This fellowship would clearly advance the candidate toward that goal through acquisition of new skills in structural time lapse imaging (Czopka lab) and computational modelling (Brette lab).

Research proposal
What is your view of the research questions and approaches?

Please consider:
(i) whether the questions are original and important
(ii) the strengths and weaknesses of the approaches (including risks and feasibility)
(iii) whether the outputs will significantly impact the field
(iv) (if applicable) whether the clinical trial design is appropriate.

The candidate's overarching question is: "How are the axonal initial segment, myelination by glia, and presynaptic boutons coordinated to regulate function in single neurons and in networks in-vivo during development and plasticity?" This is an timely, important, and vast question.

The strengths of the research proposal are:

1/ the appropriateness and detailed motivation for the model (larval zebrafish locomotion) to address this question at both single-cell and network scales;

2/ the diversity of tools to be used (paired whole-cell recordings, calcium imaging, voltage imaging, structural imaging);

The primary weakness of the research proposal is that, whilst the candidate's enthusiasm and motivation for this question shine through clearly, the candidate does not describe how they plan to integrate data from all of these modalities to draw meaningful inferences. Two vague predictions are presented:

"I predict a development timelines in which the AIS is stabilised first, followed by axonal myelination and node formation as functional connections mature."

and

"I predict that structural changes will match their developmental order: presynaptic boutons responding within minutes to hours, AIS remodelling across hours to days, and myelin and node changes happending over days or longer."

How do these predictions, and the experiments described to test them, address the overarching question? What roles will the different proposed experimental techniques play in supported or refuting these predictions and their underlying hypotheses? What are the hypotheses? What data would support or refute these hypotheses? Are the effects to be observed only correlated with development and plasticity, or can causality be established in some cases? How?

In summary, the motivation for the work is clear. The candidate plans to observe how the AIS, boutons, and myelin change during development and motor-induced plasticity changes by a variety of modalities. These experiments will generate a mountain of data. Plans for processing and interpretating these data in the context of specific hypotheses are not presented in this proposal.

Research environment
What is your view of the research and training environment(s)?

Please consider whether:
(i) the supervisors/sponsors/mentors are appropriate for the candidate's career development
(ii) the research environment is appropriate to support delivery of the proposal

The research/training environments are well matched to the candidate's research and career aims.

Proposals involving animals
Please consider whether the applicants have addressed the following questions:
(i) Is the use of animals and the chosen species justified in terms of the likely outcomes of the research?
(ii) Is there adequate justification for the numbers of animals to be used?
(iii) Is the experimental design appropriate, including any plans to reduce bias such as blinding or randomisation?
(iv) If the use of primates, cats, dogs, or equidae is proposed, could any other animal species be used instead?
(v) Is there potential for improvement in the research approach to replace the use of animals, reduce the number of animals used, and/or reduce animal suffering? Would changes in the research approach allow the researchers to derive significantly greater scientific benefit from their use of animals?
(vi) Would any of the procedures be considered unacceptable in your laboratory –if so, why?

(i) Is the use of animals and the chosen species justified in terms of the likely outcomes of the research?

Yes

(ii) Is there adequate justification for the numbers of animals to be used?

Difficult to say given lack of information about the hypotheses to be tested.

(iii) Is the experimental design appropriate, including any plans to reduce bias such as blinding or randomisation?

Difficult to say given lack of information about the hypotheses to be tested.

(iv) If the use of primates, cats, dogs, or equidae is proposed, could any other animal species be used instead?

N/A

(v) Is there potential for improvement in the research approach to replace the use of animals, reduce the number

of animals used, and/or reduce animal suffering? Would changes in the research approach allow the researchers to derive significantly greater scientific benefit from their use of animals?

No

(vi) Would any of the procedures be considered unacceptable in your laboratory –if so, why?

No

Outputs management and sharing
Will the proposed research generate outputs - including datasets, software, materials or intellectual property - that will hold significant value as a resource for the wider research community? If yes, please comment on:
(i) whether the outputs management plan clearly describes when and how the outputs will be made available,
(ii) whether these arrangements for managing and sharing research outputs are appropriate and in line with best practise in the field.

The electrophysiological data and images would be stored at UoE and shared on demand after publication. This is appropriate and in line with best practice in the field.

Resources
This question is not applicable for the Sir Henry Wellcome Postdoctoral Fellowship applications.
Are the requested resources appropriate (staffing, equipment, running costs etc)?

N/A

Overall assessment
Please provide a rating which reflects your opinion of the overall merit of the proposal. You should refer to the 'Help' button for details of each rating category.

Competitive

Overall assessment

The candidate has a competitive track record, has clearly motivated the research question to be studied, and has the background needed to make significant contributions through this research. The candidate has also clearly described what training s/he plans to acquire and how this will advance his/her career goals. My one reservation is the lack of hypotheses and plans for interpreting the data acquired during the project. Maybe these are clear to the candidate but were not communicated well in the proposal.

Summary	
Grant Type	Sir Henry Wellcome Postdoctoral Fellowship
Application ref.	221679/Z/20/Z
Lead applicant	Dr Adna Dumitrescu
Organisation	University of Edinburgh
Project title	Seeing the forest and the trees: understanding the contribution of both axonal and glial activity-dependent plasticity in neural circuit function
Reviewer	3
Reviewer organisation	Information Redacted

Peer Review: Early to Mid-Career Researchers

Candidate
What is your view of the candidate's suitability for this award?

Taking into account their career stage and any career breaks, please consider:
(i) the significance of their research outputs so far
(ii) whether the candidate has the potential to deliver the proposed research
(iii) how this fellowship will further develop their career.

The candidate has an excellent track record. She published several outstanding papers that attracted a lot of attention. She has all required experience to deliver this project.

The candidate seems to have a very good career development plans and the current fellowship aligns very well with these ideas. She will gain more experience in state-of-the-art imaging, electrophysiological and computational methods which will help her to establish herself as a group leader in few years.

Research proposal
What is your view of the research questions and approaches?

Please consider:
(i) whether the questions are original and important
(ii) the strengths and weaknesses of the approaches (including risks and feasibility)
(iii) whether the outputs will significantly impact the field
(iv) (if applicable) whether the clinical trial design is appropriate.

The research proposed by Dimitrescu will deal with an important question: the role of structural changes of the axonal domain in development and and learning. She rightfully argues that we know a great deal about dendritic plasticity and its role learning and memory and way less about how axonal plasticity affects development of neural circuits and learning. Some work from labs of Burrone and others have demonstrated that this is indeed a feasible question as axonal, for example, axonal initial segment changes its position during neuronal plasticity. To address this important problem, Dimitrescu proposes using zebrafish as a model system and using a large combination of imaging fluorescent reporters, electrophys and modelling to understand how individual axonal subdmains develop and how individual axonal domains contribute to the activity dependent plasticity. The questions are very good, well formulated and timely. However, in my view, she provided very little information regarding experimental paradigms to be used to address these questions. For example:

1. What fluorescent reporters will be used in Aim one? Is it GFP fused to some proteins to localise it to AIS and other axonal parts?

2. What voltage dyes will be used to image membrane potential?

3. How will individual neurons be labelled? Alx:GFP line label quite a dense population of descending neurons and it is difficult to follow individual neurons. Thus one will need to use some methods for single-cell labelling. Similarly, how will she identify connected neurons for paired recordings? (Aim 1)

4. Very little information is provided on the details of the computational model (Aim 3). Although I do agree that building good computational model of a neuron will enhance our understanding of axonal plasticity, it is difficult to judge from the Aim3 how the model will be useful.

Research environment
What is your view of the research and training environment(s)?

Please consider whether:
(i) the supervisors/sponsors/mentors are appropriate for the candidate's career development
(ii) the research environment is appropriate to support delivery of the proposal

Perfect choice of environment to study plasticity in zebrafish. Great lab, excellent collaborators possessing all required experience to perform the project.

Proposals involving animals
Please consider whether the applicants have addressed the following questions:
(i) Is the use of animals and the chosen species justified in terms of the likely outcomes of the research?
(ii) Is there adequate justification for the numbers of animals to be used?
(iii) Is the experimental design appropriate, including any plans to reduce bias such as blinding or randomisation?
(iv) If the use of primates, cats, dogs, or equidae is proposed, could any other animal species be used instead?
(v) Is there potential for improvement in the research approach to replace the use of animals, reduce the number of animals used, and/or reduce animal suffering? Would changes in the research approach allow the researchers to derive significantly greater scientific benefit from their use of animals?
(vi) Would any of the procedures be considered unacceptable in your laboratory –if so, why?

This is perfectly justified. There is no other way of study neuronal plasticity than using animal research. Zebrafish is an excellent model as it is transparent and allows for powerful genetic manipulations.

Outputs management and sharing
Will the proposed research generate outputs - including datasets, software, materials or intellectual property - that will hold significant value as a resource for the wider research community? If yes, please comment on:
(i) whether the outputs management plan clearly describes when and how the outputs will be made available,
(ii) whether these arrangements for managing and sharing research outputs are appropriate and in line with best practise in the field.

Good data sharing plan. All analysis and computational models will be provided immediately after publication. Raw data will be available upon request.

Resources
This question is not applicable for the Sir Henry Wellcome Postdoctoral Fellowship applications.
Are the requested resources appropriate (staffing, equipment, running costs etc)?

Overall assessment
Please provide a rating which reflects your opinion of the overall merit of the proposal. You should refer to the 'Help' button for details of each rating category.

Excellent

Overall assessment
As i mentioned above, this is a very good idea but it is a bit vague in terms of what experimental paradigms will be used. Clearly, some of the aims were not thought through.

Summary	
Grant Type	Sir Henry Wellcome Postdoctoral Fellowship
Application ref.	221679/Z/20/Z
Lead applicant	Dr Adna Dumitrescu
Organisation	University of Edinburgh
Project title	Seeing the forest and the trees: understanding the contribution of both axonal and glial activity-dependent plasticity in neural circuit function
Reviewer	4
Reviewer organisation	Information Redacted

Peer Review: Early to Mid-Career Researchers

Candidate
What is your view of the candidate's suitability for this award?

Taking into account their career stage and any career breaks, please consider:
(i) the significance of their research outputs so far
(ii) whether the candidate has the potential to deliver the proposed research
(iii) how this fellowship will further develop their career.

Dr. Dumitrescu is a strong candidate for this award.

(i) the significance of their research outputs so far.

The candidates research outputs are good for a person at this stage (fourth year of post-doc), although probably not outstanding. The two most substantial pieces of work to which they have made a strong contribution (judging by order of authors) are a recent paper in eLife (2020) and a second in Cell Reports (2015). The first is an interesting technical paper that will be useful to many labs using optogenetics in zebrafish but I can't see that it provides clear evidence of an ability to drive discovery science. But the second paper, showing the rapidity of activity-dependent plasticity at the axon initial segment, is indeed an important scientific contribution.

(ii) whether the candidate has the potential to deliver the proposed research.

Yes, think the candidate probably can deliver the research. Dr. Dumitrescu is technically skilled, judging from the research outputs, and has written a proposal that displays strong and mature scientific judgement (what is important? what can be achieved?).

(iii) how this fellowship will further develop their career.

This fellowship certainly provides an excellent opportunity for Dr. Dumitrescu to drive a project based on her own scientific ideas while collaborating with a number of laboratories with different strengths. The choice of collaborating labs provides excellent opportunities for integrating different techniques into the candidates research.

Research proposal
What is your view of the research questions and approaches?

Please consider:
(i) whether the questions are original and important

(ii) the strengths and weaknesses of the approaches (including risks and feasibility)
(iii) whether the outputs will significantly impact the field
(iv) (if applicable) whether the clinical trial design is appropriate.

(i) whether the questions are original and important.

The questions are certainly important and the approach to those questions is unusually "holistic". This proposal will investigatesthree processes that determine neuronal function: spike initiation at the axon initial segment, spike propagation affected by myelination and synaptic transmission. The aim is to obtain an integrated view of how these processes are adjusted, first during the development of motor circuits and then in the context of activity-dependent changes in the function of those circuits.

(ii) the strengths and weaknesses of the approaches (including risks and feasibility)

A great strength of the proposal is the use of zebrafish to study the dynamics of these processes in vivo and in the context of well-defined motor circuits. The experiments will be demanding but they are technically possible at the moment and the candidate has most of the necessary skills (or at least very strong technical ability that will allow these skills to be adapted to the project). I could not see any fundamental problems with the approach. Building models is not always useful in directing experiments, but you don't know until you have tried and it is good that the candidate is alive to the potential utility of modelling.

(iii) whether the outputs will significantly impact the field

I think the proposal is excellent and displays strong and mature scientific judgement in terms of assessing i) what is important to make significant advances in the field, balanced by ii) what can be achieved realistically with current techniques. Zebrafish are a key model for studying circuit function in vivo and I believe that the project has strong potential for making a significant impact.

Research environment
What is your view of the research and training environment(s)?

Please consider whether:
(i) the supervisors/sponsors/mentors are appropriate for the candidate's career development
(ii) the research environment is appropriate to support delivery of the proposal

Edinburgh provides an excellent research environment in general but also specifically for this proposal. Prof. Lyons and Dr. Czopka are ideal mentors/collaborators for this project.

It is notable that Dr. Dumitrescu has experience of a number of institutions that provide strong research environments (KCL, Paris) and I have no doubt that Edinburgh will also provide excellent training opportunities.

Proposals involving animals
Please consider whether the applicants have addressed the following questions:
(i) Is the use of animals and the chosen species justified in terms of the likely outcomes of the research?
(ii) Is there adequate justification for the numbers of animals to be used?
(iii) Is the experimental design appropriate, including any plans to reduce bias such as blinding or randomisation?
(iv) If the use of primates, cats, dogs, or equidae is proposed, could any other animal species be used instead?
(v) Is there potential for improvement in the research approach to replace the use of animals, reduce the number of animals used, and/or reduce animal suffering? Would changes in the research approach allow the researchers to derive significantly greater scientific benefit from their use of animals?
(vi) Would any of the procedures be considered unacceptable in your laboratory –if so, why?

All these questions have been addressed appropriately.

Outputs management and sharing
Will the proposed research generate outputs - including datasets, software, materials or intellectual property - that will hold significant value as a resource for the wider research community? If yes, please comment on:
(i) whether the outputs management plan clearly describes when and how the outputs will be made available,
(ii) whether these arrangements for managing and sharing research outputs are appropriate and in line with best practise in the field.

The outputs management plan is reasonable and in line with best practise in the field.

Resources
This question is not applicable for the Sir Henry Wellcome Postdoctoral Fellowship applications.
Are the requested resources appropriate (staffing, equipment, running costs etc)?

Overall assessment
Please provide a rating which reflects your opinion of the overall merit of the proposal. You should refer to the 'Help' button for details of each rating category.

Excellent

Overall assessment

The research proposal is very strong indeed. I would probably have rated the application as a whole "outstanding" if there was stronger evidence that Dr. Dumitrescu has the potential to drive research of this nature e.g though a significant piece of discovery science during post-doctoral work in Paris.