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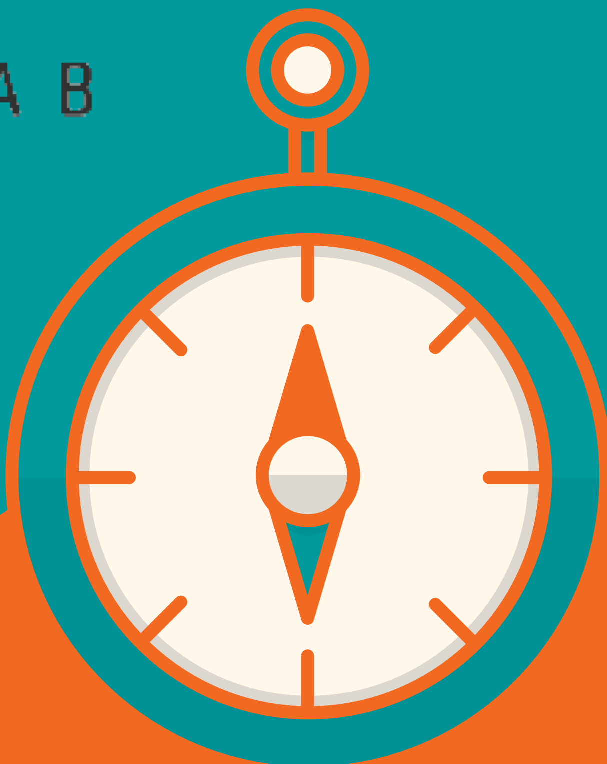

NAVIGATE Abstracts

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CERVOLET ASSOCIATION ÉTUON

Combining digital holographic microscopy and deep-learning methods to extract structural connectomes from living neurons in cultures

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Digital holographic microscopy (DHM) is a powerful non-invasive imaging technique that enables the study of living cells by providing quantitative phase images (QPI). However, this imaging technique alone lacks a comprehensive analysis pipeline for recognizing neurons and their connections in cultures. To overcome this limitation and construct detailed maps describing neural connections, known as structural connectomes, the integration of additional computational tools becomes indispensable. Consequently, we developed a computational pipeline that leverages state-of-the-art deep-learning methods to obtain a structural representation of living neural networks cultured from either rat cells or human induced pluripotent stem cells (hiPSC). Our deep-learning models are based on the U-Net architecture, which is a convolutionnal neural network developed for biomedical image segmentation. To train our models, we conducted manual segmentation of two sets of 8 images obtained using a 5X microscope objective. One set was used for cell body segmentation, and the other set was used for neurite segmentation. We created training and validation sets, on which we then performed different data augmentation methods to have more diverse data sets. We got one model for cell bodies and one for neurites, with the outputs being respectively a probability map of cell bodies and a probability map of neuronal connections. To evaluate the accuracy of our models, we utilized receiver operating characteristics (ROC) curves and calculated the area under the curve (AUC) for each prediction, with an AUC equal to one indicating an ideal model. Remarkably, we achieved an AUC of 0.99 for the rat cell body model, indicative of its high performance for cell body segmentation. Similarly, the neurite model reached an AUC of 0.88, demonstrating its very good ability to segment neurites. Furthermore, we acquired 8 DHM images of cultures of neurons derived from hiPSC. We used manual segmentation and data augmentation to prepare the training set for the U-Net model, focusing on both cell body and neurite annotation. We currently have a human cell body model with an AUC of 0.99, whereas our human neurite model reached only 0.66. The sensitivity and specificity of the neurite model are expected to increase as we add more images to the training set. The structural connectomes are then extracted by binarizing the probability maps and using a pathfinding algorithm to find the shortest paths between cells. By adopting this approach, the neuronal network can be represented as a structural connectome on which we will apply various graph-theoretical measures to identify significant differences between cell lines at different stages of development in vitro. Additionally, we plan to investigate potential differences between control lines and patients with brain disorders, providing valuable insights into the changes in the connectomes associated with these conditions.

Development of an image processing algorithm to improve the quantification of dopaminergic axons in the motor cortex of rats

Ann-Sarah Trudeau, Christian Ethier

INTRODUCTION: After obtaining brain images labeled by immunohistochemistry, the challenge is to find an image processing method to improve the isolation and quantification of dopaminergic axons while avoiding interferences from background noise and other artifacts.

METHODS: There are image processing softwares (such as ImageJ) with thresholding and contrast modification tools. Since we have hundreds of sample images to process in the same way, we need to find a way to automate the process to save time. Therefore, it is necessary to implement a code that performs the task on multiple images to avoid manual work. Python offers a specialized image processing library called OpenCV. It encompasses several functions that we use to enhance quantification at the end of the image processing.

First, we use the function that separates the channels (red, green, and blue) of an image, only the color containing the fluorescence of interest. Next, we convert this image to black and white, which is essential for quantification in ImageJ. Finally, the most significant challenge is to reduce background noises that interfere with the axons and hinder quantification. For this purpose, the denoising function utilizing the Non-Local Means Denoising algorithm proves to be beneficial.

The thresholding step allows us to isolate the pixels representing the axons in the image, quantify these pixels, and determine the density of dopaminergic axons in the brain's area of interest. We select the brain region we want to quantify, and the software counts each pixel within this selected area. This enables us to calculate the percentage of axons in the total selected area.

RESULTS: Thanks to the denoising function of OpenCV in Python, thresholding becomes more accurate, and the isolation of axons is improved compared to when background noise is not reduced. A significant time gain is observed since there is no longer a need to open, process, and save the image manually, as these tasks are now automated.

CONCLUSION: To declare Python algorithm as the best solution, a comparison between the thresholding in ImageJ and the Python algorithm needs to be carried out. Manual quantification is still required in ImageJ, but the software manipulations can be recorded and applied to each opened image, streamlining the process.

Étude comportementale et analyse d'activité neuronale chez le poisson-zèbre au stade larvaire

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Le portrait actuel des neurosciences diffère substantiellement de celui des dernières décennies. Cela est dû en bonne partie au recours au numérique, désormais crucial dans l'étude de la quantité importante de données générées par les instruments d'acquisition de toutes sortes à la disposition des chercheurs. En ce qui nous concerne, le groupe de recherche multidisciplinaire du laboratoire de Paul De Koninck étudie surtout le poisson-zèbre, modèle animal émergent.

Pour supporter l'étude comportementale chez le poisson-zèbre, des outils numériques de suivi de position d'un individu en nage libre ou encore de suivi de mouvement oculaire ont été codés. À l'aide de tels outils, nous avons caractérisé le mouvement des yeux d'un poisson immobilisé lorsque celui-ci était visuellement exposé à un congénère en nage libre. Ce type d'expérience s'inscrit dans la même lignée que d'autres déjà en cours qui étudient la sociabilisation.

Dans une perspective de rendre plus accessibles les travaux de recherche auprès du reste du groupe, plusieurs solutions pour visualiser et animer des données ont été développées. Une interface graphique visant à peaufiner le choix de paramètres d'un algorithme de suivi de position a notamment été construite, de même que plusieurs animations pour mettre en lumière certains résultats obtenus tant lors d'études comportementales que lors d'études d'activité neuronale.

Comme de fait, nous nous sommes également intéressés à la réponse neuronale de poissons-zèbres au stade larvaire lorsqu'exposés à certains stimuli visuels. Afin de voir la distribution spatiale des neurones selon leur réponse préférentielle à un stimulus ou à un autre, les séries temporelles d'imagerie calcique obtenues par microscopie à 2-photons ont fait l'objet d'analyse de corrélation avec des régresseurs correspondant aux stimuli. Ensuite, dans le cadre d'un projet de développement d'environnement de réalité virtuelle pour mener des études comportementales approfondies chez le poisson-zèbre, un algorithme simple permettant de prédire la trajectoire de nage a été mis sur pied. L'algorithme simple sera comparé à un réseau de neurones récurrent (RNN) entraîné sur des données réelles. Le reste du stage est d'ailleurs consacré à l'entraînement de ce type de système dynamique complexe. L'objectif est alors de reproduire au moyen d'un RNN des données réelles d'activité neuronale telles que mesurées dans le tectum optique du poisson-zèbre. La connectivité de ce premier modèle sera ensuite étudiée. Si le temps le permet, un second modèle plus complexe sera également entraîné, cette fois-ci en tenant compte d'un mécanisme de plasticité synaptique hebbienne. Cet incrément en réalisme permettra notamment d'étudier l'habituation à court terme des réponses neuronales à des stimuli répétés.

Ally Champoux, Cédric Goffard, Marie-Ève Paquet

Pour ce stage d'été, mon projet est issu d'une collaboration entre la Dre. Marie-Ève Paquet de l'Institut Cervo et la Dre. Tomoko Ohyma de l'Université de McGill. Le laboratoire du Dre. Ohyma a développé un modèle comportemental de nociception chez la larve de drosophile et a démontré l'importance de quatre souches bactériennes du microbiote de ce modèle dans la perception de la nociception. Les quatre souches bactériennes en question sont Lactobacillus brevis, Lactobacillus plantarum, Acetobacter tropicalis et Acetobacter pomorum.

L'intérêt du projet est d'insérer une protéine fluorescente dans les quatre souches afin de les utiliser pour coloniser le poisson-zèbre et étudier l'impact de ces souches sur ce modèle. De plus, la production de protéines fluorescentes par ces bactéries permettra de mener des études chez la larve de drosophile.Le but du stage était donc d'élaborer et d'insérer une construction plasmidique contenant une protéine fluorescente dans ces bactéries par électroporation. Les conditions d'électroporation ont été testées avec succès pour L. plantarum, mais ont nécessité une optimisation pour L. brevis. Par la suite, plusieurs constructions déjà présentes à la Plateforme d'Outil Moléculaire ont été testées, sans succès, probablement en raison de l'origine de réplication ou du promoteur utilisé (pBAD, Rset).

Une construction contenant la GFP (Green Fluorescent Protein), adaptée aux Lactobacillus, a ensuite été commandée chez Addgene. L'électroporation n'a pas été concluante pour une raison inconnue. Une construction plasmidique avec une protéine fluorescente liée au promoteur (IDH-GFP) a été insérée dans le plasmide fonctionnel (pNZ123) et intégrée par chimioporation dans E. coli pour le multiplier. Par la suite, cette construction a été électroporée avec succès dans L. plantarum et L. brevis. Cependant, une diminution de la fluorescence a été notée après 48 heures d'incubation dans la première souche (L. plantarum). Il est possible que la bactérie dégrade la protéine fluorescente. Une prochaine étape consistera à tenter une construction avec une protéine plus stable comme la protéine mCherry.

En parallèle, le milieu de culture des Acetobacter a été optimisé pour avoir une croissance en moins de 48 heures. Les deux milieux présentant les meilleurs résultats sont l'Acetobacter acid medium et le Sabouraud medium.

En conclusion, deux des quatre souches bactériennes, soit les Lactobacillus, ont intégré un plasmide contenant la GFP. Le milieu de culture des deux souches d'Acetobacter a été optimisé, constituant ainsi la première étape vers la production d'Acetobacter fluorescentes.

Conception d'AAV mutant pour une transduction cellulaire individuelle dépendante de CLaP

Charles-Antoine Dubois, Anne-Marie Lapointe, Rochelin Dalangin, Marie-Ève Paquet

L'usage du virus adéno-associé (AAV) pour la thérapie génique est une avenue de la médecine qui possède un potentiel immense et qui est déjà utilisée pour le traitement de certaines maladies. Seulement, le contrôle sur l'action du vecteur pourrait être optimisé. Ce virus d'environ 25 nm de diamètre contient un simple brin d'ADN de 4,8 kb entouré d'une capside formée de trois corps protéiques dans un ratio de 1 : 1 : 10, soit VP1, VP2 et VP3. C'est en modifiant la composition de ces protéines de la capside que l'on peut diriger le tropisme cellulaire du virus. Cependant, il est impossible de limiter les infections à des cellules individuelles seulement en modifiant les protéines de la capside.

Pour assurer une transduction contrôlée au niveau cellulaire, nous avons élaboré une méthode qui entend confier la spécificité de transduction à la technologie de cell labelling via photobleaching (CLaP). Cela consiste à projeter un faisceau laser sur une cellule individuelle pour générer des radicaux libres sur la membrane, collant la biotine mise au préalable dans le milieu à la membrane cellulaire. Puisque la biotine est capable de former un complexe avec la protéine streptavidine, on peut attacher la streptavidine au virus à l'aide d'un sucre non naturel et de la click chemistry pour ensuite que l'endocytose se produise exclusivement grâce à la formation du complexe entre la biotine collée à la membrane cellulaire et la streptavidine attachée au virus. Il est important de noter que cette stratégie ne permet pas d'empêcher le virus d'appliquer son tropisme naturel sur les autres cellules qui n'ont pas été ciblées par le laser. C'est pourquoi une grande partie du stage a été consacrée à modifier les protéines de la capside de l'AAV pour obtenir un virus incapable d'infecter une cellule quelconque sans le CLaP. La méthode employée pour le design de la capside a été bio-informatique, ce qui comprend en premier l'identification des résidus qui interagissent le plus fortement avec les glycanes et le récepteur principal AAVR pour endocyter. Ensuite, une mutagenèse in silico de tous les résidus d'intérêt a été effectuée à l'aide du logiciel MutateX qui a déterminé quelles mutations perturbent le plus l'interaction entre AAVR et le virus pour une position donnée. Le sérotype d'AAV sur lequel se base le nouveau mutant non infectieux est l'AAV9, qui a été choisi pour sa capacité à traverser la barrière hémato-encéphalique.

Avec l'aide du logiciel, nous avons pu déterminer 9 mutations d'intérêt qui bloqueraient la capacité de transduction naturelle du virus. Ces mutations ont été testées en culture cellulaire pour observer l'expression de CRE-eGFP.

Nous prédisons que cette nouvelle méthode permettrait à la médecine d'utiliser la thérapie génique avec davantage de précision et de confiance à l'avenir.

Elaboration of a mathematical model to reveal the hidden mechanisms underlying chloride transports in neurons

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INTRODUCTION: Ionic transmembrane exchanges at the cell are critical for the maintain of neuronal homeostasis. Dysregulation of the intracellular concentration of ions such as Cl^- may lead to impaired cell function, often observed in brains affected by neurological disorders or diseases. Experimentally measuring precisely and simultaneously these exchanges is an arduous task, leaving mathematical models as a possible alternative for the study of these interactions. The objective of this project is to understand how a neuron regulates its ionic and volumetric homeostasis in response to various challenges, as well as the impact of multiple biophysical parameters on the cell's response to these challenges. The parameters included in the model are the passive conductance of K^+, Na^+ and Cl^- channels, the conductance of NKCC1 and KCC2 cotransporters, the peak current of the Na^+/K^+ pump and the permeability of water.

METHODS: A set of ordinary differential equations (ODEs) based on the Goldman-Hodgkin-Katz (GHK) formalism relating the intracellular and extracellular concentrations of K^+, Na^+ and Cl^-, as well as the cell's volume and its membrane potential, was solved numerically using an initial set of biophysical parameters. To verify the validity of the model, three potassic shocks of growing concentrations (from 3.5 to 8.5 mM, from 3.5 to 13.5 mM, and from 3.5 to 23.5 mM) were induced sequentially to groups of cells from a neuron culture at different development stages (5-7-DIV and 12-15-DIV). The intracellular chloride concentration ($[Cl^-]_i$) was measured in single neurons using MQAE. The simulated curve of $[Cl^-]_i$ obtained by the model was compared to the experimental data by an optimization algorithm based on the least squares method. By fitting the data for the consecutive potassic shocks with the simulation, after having sorted cells that followed similar changes of $[Cl^-]_i$ into different groups, a set of values of biophysical parameters was returned for each group. The resulting vector was then used to generate a better fit.

RESULTS: The program was successful in resolving the set of ODEs at each timepoint for multiple shocks. When provided with a noisy simulated curve, the fitting algorithm was able to return the biophysical parameters that generated it with high accuracy, confirming its validity. Furthermore, by using the average $[Cl^-]_i$ data obtained experimentally during the consecutive potassic shocks, the model managed to return a satisfactory fit with a set of possible biophysical parameters.

CONCLUSION: The present model appears able to approximately reproduce experimental intracellular chloride data following multiple external perturbations y finding the biophysical parameters that produce the best fit. Further tests are required to verify the accuracy of the simulation when considering the data from a single cell, and to determine the quantitative impact of each biophysical parameter.

An axicon is a conic lens widely known for its non-diffracting and stable Bessel beam generation. This work explores the GRIN-axicon optical configuration to generate a Bessel beam and how it can be used to replicate and replace a given axicon for extended depth of field microscopy applications. A GRIN-axicon consists on the combination of a hyperbolic secant index profile GRIN and a lens, and was suggested to be a cheap and modular alternative to a traditional axicon. During the course of the internship, equations were derived to model a GRIN-axicon using a set of parameters and then used to power a Python graphical interface. This interface was programmed to make the designing of the optical component more intuitive and user-friendly, providing in depth information about the required optical setup and real-time simulation features such as a dynamic view of light propagation in a GRIN-axicon optical system. With this configuration tool, a GRIN-axicon was designed to mimic an axicon of 2.5° and tested to validate their optical concordance. The axicon of a microscope was then replaced by the GRIN-axicon to verify if the behaviour of the system would stay unchanged. It was found that the intensity profile along the optical axis was significantly similar for both lens' and that the axicon in an optical system could easily be swapped by the GRIN-axicon without any change in performance. It can thus be concluded that the GRIN-axicon configuration offers a cheap, modular and reliable alternative for an axicon. Further testing on the scaling of a GRIN-axicon could also turn out to be an advantage over the regular axicon due to a GRIN's malleability property.

Investigating Speech Perception in Noise (SPiN)

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INTRODUCTION: Difficulties understanding speech in noise (SPiN), are commonly experienced by middle-aged and older adults and can have significant negative impacts on self-esteem, socialization, and overall well-being. Despite the prevalence of SPiN difficulties, the causes remain uncertain, and current treatments often prove ineffective. Therefore, understanding the underlying factors contributing to SPiN disorders is crucial for developing interventions and preventive measures to improve communication outcomes and enhance the quality of life for individuals affected by this condition. The aim of our study is to gain a better understanding of the origin of SPiN difficulties in healthy middle-aged and older individuals as well as those with auditory and cognitive impairments. Our overarching hypothesis is that the decline in SPiN performance associated with aging is linked to the aging of the dorsal speech stream, with more severe manifestations expected in individuals with auditory and cognitive impairments.

METHODS: The study comprises three visits, and recruitment began last spring. Two groups of participants will be recruited: a control group consisting of 40 adults aged 35 to 60 years. This group will be used to establish age-related differences in SPiN. The second group of 120 adults aged 65 to 85 years will be divided into three categories: one-third with normal cognition, one-third with mild cognitive impairment, and one-third with subjective cognitive deficit. The first visit will involve a neuropsychological evaluation to establish a cognitive profile. The second visit will include assessments of peripheral and central hearing, as well as speech evaluations and EEG recordings (at rest and during a SPiN task). Finally, the third visit will consist of a multimodal MRI session.

RESULTS: In this poster, we present preliminary results obtained from the second visit. These results focus to the assessment of peripheral and central hearing, as well as SPiN evaluation, and will be summarized using descriptive statistical analyses and graphs.

CONCLUSION: This comprehensive study will enable us to conduct a robust analysis of SPiN performance, considering a broader range of cognitive and auditory factors. By providing new insights into the mechanisms underlying SPiN difficulties, our research holds the potential to open up new therapeutic perspectives for individuals affected by this condition, particularly for those who find limited benefit from hearing aids alone. The findings from this research could be instrumental in the development of rehabilitation techniques that leverage brain plasticity, such as transcranial magnetic stimulation (TMS).

Dynamiques moléculaires de l'adducine dans l'activité neuronale

Héloïse L'Homme, Rachel Morin-Pelchat, Paul De Koninck

Le squelette périodique associé à la membrane (MPS) des neurones est composé de filaments d'actine organisés en anneaux et régulièrement espacés (180-190nm) par des tétramères de spectrine. L'adducine, une autre composante du MPS, contribue la stabilité du cytosquelette en recrutant la spectrine aux filaments d'actine et en limitant l'élongation de ces derniers. Dans l'activité, les anneaux d'actine se réorganisent pour former des fibres qui s'allongent le long de la dendrite. Le rôle de l'adducine dans ces dynamiques d'actine n'est pas bien connu. Sachant que la phosphorylation in vitro de la sérine 724 de l'adducine diminue son affinité pour l'actine, nous avons émis l'hypothèse que cette modification post-traductionnelle pourrait jouer un rôle dans la régulation des dynamiques d'actine. L'objectif de mon projet est donc d'évaluer si la phosphorylation de l'adducine est liée à l'activité neuronale, puis d'investiguer le comportement de l'adducine phosphorylée dans des cellules dont l'activité a été induite ou inhibée.

Pour se faire, nous avons traité un premier groupe de neurones primaires de l'hippocampe du rat avec une solution de blocage, riche en magnésium, qui inhibe l'activité neuronale. Un autre groupe a été stimulé avec une solution de KCl qui provoque une dépolarisation membranaire des cellules ainsi qu'une entrée importante d'ions calcium qui activent diverses voies métaboliques impliquées dans l'activité et la plasticité synaptique. Les cellules ont ensuite été fixées, puis ont subi une immunocoloration avec des anticorps spécifiques à l'adducine ou à l'adducine spécifiquement phosphorylée à sa sérine 724. Toutes les cellules ont également reçu un marquage de la protéine dendritique Map2. Ensuite, nous avons capturé des images à l'aide d'un microscope à super résolution par illumination structurée (SIM) de Zeiss (Elyra).

Les résultats préliminaires observés démontrent que le signal de la phosphoadducine(S724) est significativement plus faible dans les cellules dont l'activité a été bloquée, comparativement à celles stimulées au KCl. De plus, on observe un signal d'adducine périodique avec un intervalle régulier de 188nm dans nos images, caractéristique des anneaux d'actine qui sont connus pour colocaliser avec l'adducine. Lorsqu'on regarde le signal spécifique à la phosphoadducine, on retrouve davantage de ces motifs périodiques dans la condition KCl que dans la condition de blocage de l'activité. D'ailleurs, la phosphoadducine se localise principalement en périphérie des dendrites en condition KCl. Dans le cadre d'une autre expérience, nous avons également pu observer le signal périodique de l'adducine-GFP dans des cellules vivantes à l'aide de notre microscope SIM.

Nos observations suggèrent que la phosphorylation de l'adducine augmente lors d'une stimulation de type KCl. Il pourrait donc avoir une relation entre l'activité neuronale et la phosphorylation de l'adducine. De plus, nos résultats laissent croire que, contrairement à l'actine, l'adducine maintiendrait sa position périodique, caractéristique du MPS, dans l'activité. Finalement, la phosphoadducine semble être localisée principalement en périphérie des dendrites, et donc potentiellement au sein ou très près du MPS. Dans le futur, nous aimerions observer les dynamiques activité-dépendantes de l'adducine par rapport à l'actine dans une même cellule vivante avec un microscope de type SIM.

Characterization of AAV2 transduction profile in neuronal cell cultures

Jacob Perreault, Annie Barbeau, Marie-Ève Paquet and Antoine G. Godin

INTRODUCTION: Adeno-associated viruses (AAV) have proven highly useful for neuroscience research and promising for clinical applications such as gene therapy. AAVs are small (20-25 nm) icosahedral (20 faces) non-coated viruses. There are 11 types of AAVs identified to date. When AAVs encounter cells, they can attach to primary glycan receptors and secondary protein receptors on the cell membrane. The cell membrane then folds around the AAV by endocytosis, and an endosome transports the AAV to the cell nucleus. When AAV enters the nucleus through nuclear pores, single-stranded AAV DNA is released. The DNA will then be integrated into the cell DNA and will go through the replication process to infect the cell.

PROBLEMATICS: The capacity of an AAV to transduce different cell types and brain regions are of crucial importance. In neuroscience research, a plethora of sample types are used depending on the research question. Rodent samples or cells obtained from primary cultures are routinely used. The development and availability of induced pluripotent stem cells (iPS) allowed the development of human cellular models that are now also commonly used. The genetics tools needed for each specific context must be developed and tested in consequence. For this reason, being able to characterize the transduction profile of the serotype used is paramount, but systematically testing in each sample of interest would also be highly desirable.

This project aims at implementing a systematic method to assess the transduction properties of the AAV2, most used serotype, produced in neuronal cell culture. The protocol will ultimately be transposed in more complex cell samples.

METHODOLOGY: A cell nuclei segmentation algorithm[1] was implemented, and a protocol was developed to determine the number of infected cells and the cell types of infected cells. The method and algorithm are tested on primary neuronal culture from rats[2] to verify the accuracy of the method. DAPI was used to identify the cell nuclei (Fluoroshield Abcam ab104139). GFP identified infected cells that had mature fluorescent proteins. GFP was amplified using an anti-GFP primary antibody (chicken igG, Aves, catalog GFP-1010, diluted 1/1k) and an anti-chicken igG secondary antibody coupled to Alexa fluor 647 (goat igG, Invitrogen, A-21449, diluted 1/1k), identifying infected cells with or without mature fluorescent proteins. Finally, Neurons were revealed using an anti-Neun primary antibody (mouse igG, Merck-Millipore, catalog MAB377, diluted 1/1k) and an anti-mouse igG secondary antibody coupled with Alexa Fluor 568 (goat igG, Invitrogen, A-11004, diluted 1/1k).

Samples were imaged using a wide-field microscope (Nikon Eclipse Ti2-E wide-field microscope). A 4x objective (CFI Plan Apochromat Lambda 4X/NA 0.20/WD 20.0 mm/CG 0.17 mm/Dry) was used and pixels were binned 4x4. The peak excitation wavelengths were 398 nm, 470 nm, 547 nm, and 636 nm. The filters collected peak emission wavelengths of 432 nm, 514 nm, 595 nm, and 740 nm. To quantify, the coverslip is imaged in frames.

Images must be segmented and analyzed. The first step is to partially segment images using ImageJ [3]. We labelled regions of background noise, limits between superposed cells, and the position of a few cells. We

repeat this for a portion of our images (at least 20). Then we use the algorithm to train a model, modify thresholds and use the model to segment images.

CONCLUSION: The images that were taken are being used to optimize the methodology. The methodology will be useful to determine the transduction capacity of AAVs.

[1] Cicconet, Marcelo, NucleiSegmentationBot, 2017, https://www.mathworks.com/matlabcentral/fileexchange/64720-nucleisegmentationbot

[2] CERVO Brain Research Centre, Laval university, https://cervo.ulaval.ca/en/neuronal-cultures-platform-pcn-cervo

[3] Rasband, W. S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018

Validation de séquences d'« enhancer » pour cibler l'expression génique dans les neurones

Jade Corbeil, Anne Sébilo, Marie-Éve Paquet

L'Initiative Québécoise d'Adresses Neuronales (IQAN) est un projet collaboratif ayant pour but de développer des outils moléculaires spécifiques afin d'innover des traitements contre la douleur chronique. Le transfert de gène est une alternative thérapeutique aux drogues comme les opioïdes pour soulager la douleur chronique.

De courtes séquences d'ADN nommées communément « enhancers » ou amplificateurs déterminent la spécificité cellulaire de l'expression génique par un effet promoteur ou inhibiteur. Certaines de ces séquences régulent l'expression des gènes en lien avec la douleur dans les neurones sensitifs. Il faut donc vérifier que ces séquences peuvent contrôler ces gènes d'intérêts et donc être utilisées dans la fabrication d'outils moléculaires. Les vecteurs viraux de type « Adeno-Associated Virus » (AAVs) sont les outils de transfert génique utilisés dans le cadre de ce projet en raison de leur stabilité dans les neurones, de leur variété d'injection et de leur caractère peu ou pas immunogène.

L'hypothèse est qu'il serait possible de contrôler de façon spécifique l'expression des gènes d'intérêt en combinant une séquence amplificatrice avec un promoteur minimal. Les séquences amplificatrices qui seraient en lien avec le contrôle de l'expression de la douleur ont été identifiées par une autre équipe de recherche avec la méthode RNAseq dans les zones ouvertes de la chromatine des neurones de la moelle épinière. Ces séquences, une fois identifiées, sont amplifiées à partir d'ADN génomique de souris Wild Type C57/Bl6. Des techniques de clonage moléculaire, principalement l'assemblage Gibson, permetient de créer des plasmides recombinés qui serviront à la production d'AAVs. Le plasmide, en plus de contenir la séquence amplificatrice, contient un promoteur minimal, les ITRs (« inverted terminal repeat ») et le gène de la protéine GFP (« green fluorescent protein »). Une triple transfection dans des cellules HEK293T17 avec le plasmide purifié permet la production d'AAVs. Les AAVs sont ensuite récoltés, 'trés et testés. L'efficacité et la spécificité du type neuronal ciblé par la séquence amplificatrice sont validées in vitro dans des modèles de cultures neuronales, ce qui nécessite aussi despratiques de cultures cellulaires aseptiques.

Jusqu'à présent, la production de huit AAVs est terminée. Quatre d'entre eux ont été envoyé dans un autre laboratoire pour les tests in vivo chez les souris et les rats. Leur fonctionnement a préalablement été validé lors d'infections dans des plaques de cultures neuronales. Cinq autres constructions plasmidiques sont terminées et quatre sont en cours de clonage.

Le projet est toujours en cours. D'ici la fin du stage, les constructions plasmidiques seront transfectées dans des cellules HEK293T17 et les AAVs seront testés in vitro par infection de cultures neuronales. Ensuite, les AAVs seront testés in vivo par d'autres équipes de recherche chez les souris et les rats. Éventuellement, les tests pourront se faire chez les humains.

Production d'AAVs avec des protéines VP1 modifiées

Jaëlle Méroné, Marie-Ève Paquet

INTRODUCTION : Les virus adéno-associés (AAV) sont des vecteurs utilisés pour transférer des outils encodés génétiquement dans des cellules. L'efficacité de la transduction AAVs dépend de plusieurs facteurs, tels le sérotype et les modifications apportées à la capside. Il est possible de modifier les caractéristiques des protéines structurales de la capside d'un AAV en ajoutant des peptides fonctionnels ou d'autres molécules. Ainsi, la production d'un AAV contenant des étiquettes de 6 histidines sur sa capside est testée. Cette modification a pour but de produire des AAVs qui peuvent facilement être purifiés à l'aide de billes Ni-NTA. De plus, le domaine de liaison au récepteur de SARS est ajouté à la capside d'un AAV pour voir si cela a un effet positif sur sa capacité à entrer dans une cellule. Une autre modification, l'étiquette DBCO, est ajoutée à la capside d'un AAV pour qu'il soit ensuite possible de le marquer avec des fluorophores qui se lient au DBCO.

MÉTHODES : Pour ajouter les étiquettes d'histidines sur la capside d'un AAV, le gène de la protéine VP1 est amplifié par réaction de polymérisation en chaîne avec des amorces qui ont préalablement été conçues pour amplifier une région spécifique du gène de VP1 en ajoutant une séquence codant pour 6 histidines. La même technique est utilisée pour ajouter la séquence de l'étiquette DBCO à la séquence codant pour la protéine VP1. Pour ajouter le domaine de liaison au récepteur de SARS sur la capside d'un AAV, la séquence d'ADN du domaine de liaison au récepteur est envoyée à Twist Bioscience pour être synthétisée. Chaque fragment d'ADN est inséré dans un plasmide vecteur par assemblage Gibson. Les plasmides recombinants sont transformés dans des bactéries pour être clonés, puis l'ADN plasmidique est extrait et purifié. Les plasmides recombinants sont transfectés dans des cellules HEK293T17 pour produire des AAVs présentant les VP1 modifiés et qui contiennent le gène d'une protéine fluorescente. Les AAVs sont ensuite extraits des cellules, purifiés et quantifiés par digital droplet PCR.

RÉSULTATS : Les titres des AAVs produits sont bas et variables. La plus haute concentration est obtenue avec les AAVs qui ont des étiquettes d'histidines et qui sont purifiés avec des billes Ni-NTA. Leur titre est de 7,56x1012 copies du génome/millilitre. La méthode de purification par gradient mène à des concentrations plus faibles. Les cellules HEK293T17 infectés avec les AAVs qui ont l'étiquette d'histidines et le domaine de liaison au récepteur expriment la protéine fluorescente.

CONCLUSION : Les modifications apportées aux capsides des AAVs ont diminué l'efficacité de leur production. L'étiquette 6 histidines permet de purifier les AAVs avec une méthode alternative en utilisant des billes Ni-NTA. Il reste des tests à faire pour déterminer l'efficacité d'infection des AAVs qui ont le domaine de liaison au récepteur de SARS et pour valider le fonctionnement de l'étiquette DBCO.

Relation entre la cognition, l'audition et la perception de la parole et les difficultés de perception de la parole dans le bruit

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INTRODUCTION : Le vieillissement s'accompagne souvent de difficultés de perception de la parole dans le bruit. Ces difficultés, lesquelles apparaissent dès la fin de la trentaine, peuvent représenter un réel enjeu pour la communication. En effet, suivre des conversations, qui ont souvent lieu en présence de bruit, comme au restaurant, devient difficile, et la charge mentale associée à la communication augmente, monopolisant ainsi les ressources cognitives. Les difficultés communicationnelles ainsi créées peuvent impacter le bien-être des personnes, et entraîner une diminution de la participation sociale et l'isolement. Notre projet vise à comprendre la source de ces difficultés, en étudiant le lien entre la cognition, l'audition et la perception de la parole et les difficultés de perception de la parole dans le bruit, chez des personnes en bonne santé cognitive, et des personnes ayant un trouble cognitif subjectif ou léger.

MÉTHODE : L'objectif du projet est de recruter 160 personnes âgées de 35 ans et plus, réparties en 4 groupes. Le groupe 1 comprend 40 personnes âgées de 35 à 59 ans sans plainte ou trouble cognitif. Le groupe 2 comprend 40 personnes âgées de 60 ans et plus sans plainte ou trouble cognitif. Le groupe 3 comprend 40 personnes âgées de 60 ans et plus avec trouble cognitif subjectif (SCD+). Finalement, le groupe 4 comprend 40 personnes âgées de 60 ans et plus avec trouble cognitif léger de type amnésique (TCL-amnésique). Les critères d'inclusion sont : avoir une bonne santé neurologique, être compatible IRM, être droitier, et parler couramment le français québécois. Les critères d'inclusion incluent : la claustrophobie, des implants cochléaires ou des appareils implantés non compatibles IRM, un diagnostic psychologique, psychiatrique, neurologique ou neurodégénératif (excluant le TCL et le SCD), la prise de certains médicaments, et des troubles auditifs développementaux.

DÉROULEMENT : une entrevue téléphonique de dépistage est d'abord réalisée par un membre de l'équipe et l'éligibilité des participants est vérifiée. L'étude comporte 3 visites au centre de recherche CERVO. La première visite dure 3 h et consiste en une évaluation neuropsychologique complète (mémoire, attention, inhibition, langage, et planification). Les scores des participants pour cette visite sont ensuite comparés aux normes pour leur groupe d'âge, leur scolarisation et leur sexe, et une réunion de consensus est effectuée avec l'équipe du professeur Hudon afin de valider l'éligibilité. Si les participants sont éligibles après l'évaluation neuropsychologique et la réunion de consensus, ils peuvent alors passer à la deuxième visite. Celle-ci dure 3 h également et vise à évaluer la communication. La visite inclut des tests d'audition, de parole et de langage, et vise à évaluer tous les aspects de la communication affectant la perception de la parole dans le bruit (audition périphérique et centrale, discrimination et identification de syllabes,

écoute de phrases, accès lexical). Lors de cette visite, l'activité électrique du cerveau est mesurée au moyen de l'électroencéphalographe (EEG). Finalement, la dernière visite, d'une durée de 1 h 30, consiste en un examen d'imagerie par résonance magnétique (IRM). Différents types d'images anatomiques, vasculaires et fonctionnelles au repos et lors d'une tâche d'écoute dans le bruit est effectuée sont acquises. Les signaux physiologiques sont acquis durant les séquences fonctionnelles. Cette étude a été approuvée par le Comité d'éthique de la recherche sectoriel en neurosciences et santé mentale, IUSMQ (#2023-2718).

RÉSULTATS ET CONCLUSIONS : La collecte de données pour ce projet vient d'être amorcée et une trentaine de participants ont déjà été recrutés. Dans cette présentation, le projet sera décrit et expliqué, mais aucun résultat ne sera présenté.

Molecular Tools Platform

Julien McClish, Marie-Ève Paquet

The molecular tools platform consists of a group of experts in molecular biology who are divided into two interrelated parts. One of them offers services in the generation of various genetically encoded tools such as fluorescent protein markers and the other one is a group that develops new tools to vary our services. We specialize in gene transfer technologies based on viral vectors such as AAV. AAV offers great advantages over other viruses used as viral vectors such as they are recombinant, non-replicative, and have little inflammatory responses of the infected host. They can be used in vivo as well as in cultured cells. We have developed expertise in the following area: viral vectors, molecular biology constructions, and DNA-encoded tools for conditionally expressed markers, monitoring neural markers, deep tissue imaging, ion measurements, light activation/inhibition of neurons (optogenetics), or anatomical, cellular, and subcellular targeting.

Most of the project sent to the platform goes through four simple steps: 1- Definition of your experimental idea into specific objectives; 2- Designing a molecular construct that fits the viewpoint of the experiment; 3- Packaging it into a viral vector to express your gene of interest; and 4- Transduction of your gene of interest using a viral vector in vitro or in vivo.

Another part of the platform is there to develop new tools that are going to be implemented shortly. Some examples are new AAV serotypes which tend to improve the infection rate, or even optimization of fluorescent proteins to improve contrast in microscopy.

Function of neuron-derived neurotrophic factor (Ndnf+) expressing neurons in sensorimotor learning

Juliette Bourgeois, Joël Bou/n, Emmeraude Tanguay & Vincent-Breton Provencher

Neurons in the cortex are grouped into subtypes of excitatory neurons, and inhibitory neurons and these subtypes are distributed in functionally distinct layers. Within the superficial layer of the cortex - layer 1 - interneurons expressing the neuron-derived neurotrophic factor (NDNF INs) have been recently described. NDNF INs potentially integrate the signal from other cortical areas and from neuromodulatory inputs, and in turn inhibit different portion of the dendrite of excitatory neurons. This connectivity pattern is thought to be critical for plasticity processes underlying sensorimotor learning, however, direct evidence for this role is lacking. Here, we aimed at characterizing the role of NDNF INs by manipulating their activity in the motor cortex (MC) during a sensory detection task.

Using histology techniques, we verified the distribution of NDNF INs in the MC and verified the specifity of the NDNF-Cre transgenic mouse. Two NDNF-CRE mice were injected with a Cre-dependent AAV virus expressing GFP and were transcardially perfused. The brains from these mice were collected, sectioned, and immunostained for GFP. Confocal images of the MC were obtained and the density of NDNF+ INs were then analyzed using the ImageJ software. We found that while there was presence of NDNF+ cells in every layer, there was significantly more NDNF INs in the first layer of the MC. This confirms that NDNF+ is a marker for L1-residing interneurons.

Next, we performed pilot experiments to evaluate the role of NDNF INs in sensorimotor learning by using optogenetics to increase the activity of NDNF INs in the MC of three mice while they learned an auditory detection task. NDNF-CRE mice were injected with a Cre-dependent AAV virus expressing the excitatory opsin ChRmine2.0 in their MC and were implanted with a headplate. For light delivery, either an op/c fiber (2 mice) or a cranial window (1 mouse) was implanted above the MC. While head-fixed, the mice were trained to associate a sound with a lever press to obtain a water reward and an orange laser was used to photoactivate the NDNF neurons during each trial. With this pilot group of mice, we were able to trouble shoot the training protocol and optogenetics manipulation. Training of these mice is still ongoing, but results obtained in two mice indicate that learning could be slower when we stimulate NDNF neurons. In future experiments, we will use larger cohort of mice and include a control group expressing a fluorescent protein in place of the opsin to determine the effect of NDNF activation in sensorimotor learning.

Development and optimization of a mouse model of activity-based anorexia

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Anorexia nervosa (AN) is a highly debilitating eating disorder that impacts approximately 1 out of every 100 individuals. This mental illness is characterized by high mortality rates including by suicide. Loss of body weight in AN occurs through food restriction and/or increased physical activity. This pathology is characterized by increased anxiety, body image disturbance and alterations in the endocrine system. This illness affects predominantly adolescent and young-adult women. As the severity of AN intensifies, medical complications appear. Epidemiological studies indicate that ~50% of individuals diagnosed with AN will recover, while it will become chronic in ~20-30%, and 10% will ultimately lose their lives. The biological mechanisms underlying AN remain poorly understood and there is still no approved pharmacological treatment, highlighting the need to better understand this pathology.

In mice, activity-based anorexia (ABA) is a paradigm widely used to mimic human AN. Briefly, animals have free access to exercise wheels while being gradually food restricted which will lead to a voluntary decrease in food consumption and a marked increase in physical activity level, resulting in significant body weight loss. ABA induces anxiety- and depression-like behaviors. At the neurobiological level, massive body weight loss creates somatic symptoms via brain dysfunction, mainly for the dopaminergic and serotoninergic systems and in brain regions involved in motivation, memory, hunger, and emotion regulation. This project aims to implement the ABA mouse paradigm in the CERVO animal facility. I expect that the animals subjected to the ABA protocol will lose weight, display altered emotional behaviors and hyperactivity when compared to control groups.

C57BL/6 female mice were purchased from Charles River, single housed and habituated for 6 days to the running wheels. Animals were then divided into 4 groups: ABA (running wheels, food restriction), food restriction only (FR), physical activity only, and controls (Ctrl, no running wheel and ad libitum access to food). Food access was gradually reduced, from 8h to 2h a day for the ABA and FR groups. Physical activity in the wheels was monitored with an automated software throughout the whole paradigm. Finally, behaviors were assessed with a battery of behavioral tests including the elevated plus maze and open field for anxiety, and the splash, social interaction, and sucrose preference tests for depression.

Our results confirm greater weight loss in the ABA mice vs Ctrl group. Surprisingly, weight loss was comparable for the ABA and FR groups, suggesting that food restriction had more impact than free access to physical exercise. Analysis of the behavioral tests is ongoing to assess the establishment of an anxiety- and depressive-like phenotype in ABA mice. Once the model is validated, a second cohort of mice will be subjected to the same paradigm to collect brain and blood samples. This will allow to perform transcriptomic and morphological assessments in brain regions of interest and possibly identify circulating biomarkers that could help for AN diagnosis and treatment choice.

Singers vs Instrumentalists: Ageing Effects on Speech Perception in Noise

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INTRODUCTION: Ageing is associated with a decline in our ability to focus on a signal whilst filtering out background noise decreases (Jin, Liu & Sladen, 2014). This can lead to decreased quality of life for the elderly (Parbery-Clark et al., 2011, cited in Coffey, Mogilever, & Zatorre, 2017). Given the importance of this function for quality of life, it is essential to better understand the mechanisms involved to reduce the effects of ageing. Studies have found several key areas involved in the process of speech perception in noise (SPiN), such as the superior temporal gyrus (STG), and prefrontal cortex (PFC) (Wong, Uppunda, Parrish, Dhar, 2008). Age is associated with a decrease in cortical thickness in these areas (Fjell et al., 2009). As well as functional changes such as speed of processing (Dennis & Cabeza, 2011; Bilodeau-Mecure, Lortie, Sato, Guitton, Tremblay, 2014). However, musical activity has been associated with improved SPiN functions and a reduction in the effects of ageing on these functions (Maillard, Joyal, Murray & Tremblay, 2023; Parbery-Clark, Skoe, & Kraus, 2009; Başkent & Gaudrain, 2016; Parbery-Clark, Strait, Anderson, Hittner & Kraus, 2011). This study aims to investigate whether the type of musical activity impacts the effects of ageing on SPiN functions. We hypothesise that there will be a significant difference in SPiN performance for musicians compared to non-musician participants. However, there will be a non-significant difference between instrumentalists and singers.

METHOD: 106 healthy participants (M age= 55.75, SD= 18.35, 50 F) underwent the fMRI. All participants were right-handed. Exclusion criteria included those with Contraindications to MRI, hearing, language, or psychological disorders, neurological and neuropsychological disorders, and Smokers. Participants were separated into three groups, 36 Control, 32 Singers, and 38 Instrumentalists. Characteristics such as the level of audition, age of onset, the ratio of practice, practise experience, years of practice, and intensity of practice were also calculated. MRI data was acquired using a 3 Tesla “Philips Achieva TX” and a 15-channel antenna (dStream HeadSpine coil). Participants were given a Speech in Noise Discrimination Task in the form of a classical AX discrimination task. The trial was manipulated in terms of SNR and talker identity: Different SNR levels (5db and 10 db) and different or the same speakers for both syllables. The level of background noise was normalised for each participant. Participants responded using an MRI compatible response box from CINQ (Current Design fORPS) and stimuli were presented through MRI compatible intra auricular headphones (NNL). FMRI data was analysed using the AFNI software (RW Cox & JS Hyde, 1997). For behavioural data, accuracy and reaction times (RT) were analysed using R studio.

RESULTS: Analysis is currently underway. Preliminary results will be discussed at Navigate

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Design and characterization of a fast scanning system for super-resolution microscopy

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Confocal microscopy was developed in the mid 50's to overcome the optical sectioning inherent to widefield microscopy by adding a pinhole. However, it is diffraction limited according to Abbe's law of 1873 and the resolution is limited to about 250 nm in the visible spectrum. Some biological structures are smaller than this limit, such as synaptic proteins, whose colocalization and nano organization would be interesting to measure. Therefore, super-resolution microscopy techniques have been developed in order to overcome the diffraction limit. In fact, some of these techniques, such as stimulated emission depletion (STED), were awarded the Nobel Prize in Chemistry in 2014. STED microscopy can reach resolution on the order of tens of nanometers even for live sample imaging. This is achieved by overlaying a donut-shaped laser beam, which depletes the fluorophores located under the donut, on top of the excitation laser beam. The goal of the internship is to implement an electro-optic deflector (EOD) into a STED microscope in order to scan faster or with arbitrary scanning patterns in a small field of view. An EOD is a device consisting of a crystal to which a voltage difference is applied. This phenomenon induces a refractive index gradient that deflects the incident laser beam. It can scan faster than other optical devices typically used to scan samples (e.g., mirror galvanometer) because it's not limited by inertia. Its scanning frequency can be as high as 250 kHz, while mirror galvanometers usually scan at a frequency of a few tens of hertz. Before implementing the EOD in the microscope optical path, it is important to characterize its behavior. The following features have been characterized: the polarization dependence, the power loss, the response time, and the deflection. First, it was confirmed that the transmission and efficiency of the EOD depend on the polarization orientation of the laser beam in relation to the device. Thus, the polarization of the laser beam and the polarization of the EOD must be parallel to maximize its transmission. Regarding the power loss, it has been measured at different wavelengths and voltage differences. The average is (85 ± 4) %, meaning that it is not wavelength or voltage dependent. As for the deflection as a function of the induced voltage difference, the linear function that was obtained matches the theoretical deflection previously calculated. In the long run, two EODs will be implemented on a STED microscope to allow fast scanning on both the x and y axis. The ultimate goal is to scan arbitrary patterns which would be assisted by artificial intelligence algorithms. The fast scanning is, however, limited to small regions of interest, so the larger overview of the sample would be scanned by two mirror galvanometers. The EOD scanning frequency should be faster than video microscopy and could image calcium events with a higher frame rate.

Whole-brain zebrafish calcium imaging with a HiLo microscope

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The understanding of animal behavior and changes in mental capabilities during growth starts with analyzing the connectome in the brain. Formed with thousands of neurons, we need to differentiate each of them and different imaging approaches were developed to do so. However, most of those approaches have accessibility issues, such as cost and complexity. In this study, I present the emergent technique of HiLo microscopy applied in the context of whole-brain zebrafish imaging. To verify its potential, I compare our results with two-photon microscopy, a technique already proven efficient in its ability to produce high-quality cellular activity data. Neuron segmentation as well as neuronal activity over time are compared and an efficient Python code for post-acquisition image processing is developed. It includes different image processing methods such as optically sectioning planes of interest with the HiLo algorithm, motion correction and image registration. Although the HiLo algorithm still needs tuning to segment single neurons, images taken with the HiLo technique show improvement in signal-to-noise ratio and the experimental setup permits easy whole-brain acquisition. With the different data acquired, I wish to characterize the neuronal activity of the zebrafish and try to show that the same type of data analysis made with two-photon microscopy can be done with HiLo microscopy.

Development of social behavior in zebrafish larvae

Mathilde Roland-Caverivière, Margaux Caperaa, Gabriel Bossé, Paul De Koninck

Many neurodevelopmental conditions such as ASD are associated with social deficits; however, the abnormal development of the neural networks involved in social behavior leading to those disorders is still poorly understood. With their rapid social development in the first few weeks of life, zebrafish larvae are a useful model for the study of these early social behaviors. A transgenic line of zebrafish, the GCaMP line, remains transparent after the embryonic stage and express a fluorescent Ca2+ sensor, making whole-brain imaging possible during the entire developmental period. According to previous studies [1, 2], larvae show social preference around the second week post-fertilization, but the moment this behavior appears is unclear. The aim of this internship is to characterize more precisely the developmental window of sociability by using a similar behavioral assay, as well as making sure this test can accurately measure social preference. We also want to identify which neuronal populations are involved in social behavior with immunofluorescence imaging.

Both GCaMP and wild-type (WT-TL) were used for the social preference assay. The WT-TL larvae's social behavior has previously been described, making this line useful as a control for the experiment. Social preference was measured by tracking free-swimming larvae in custom-made arenas at different timepoints during the first three weeks post-fertilization. Various social stimuli were used for control tests of the social preference assay.

Our results indicate that WT-TL larvae show clear social preference from 13 days post-fertilization (dpf) while the response from GCaMP larvae varies more between individuals. Freezing and other stress-induced behavior were more frequently observed in GCaMP larvae which might have an impact on their ability to show social preference. The causes for the greater variability in behavioral responses for social stimulus found in GCaMP will be further investigated. Moreover, individuals from both lines do not show interest in other moving non-social stimuli and do not seem to be influenced by place preference for any of the compartment, which confirms the assay's ability to correctly measure social behavior.

As we now know when social behavior appears, our next aim is to image the brain of social GCaMP larvae to observe the development of neural circuits involved in sociability. Immunolabelling protocols exists for 7 dpf larvae, but they need to be adapted to older fish with thicker brain tissue.

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Quantification of Dopaminergic Neuronal Density in Motor Learning

Megan Borelli, Christian Éthier

INTRODUCTION: Dopamine plays a crucial role in the learning of motor tasks by guiding cerebral plasticity. Therefore, it is of interest to investigate whether the motor cortical dopaminergic innervation undergoes changes following intensive training on a skilled motor task in rats.

METHODS: The study begins with a behavioral training phase where rats are trained to turn a handle or pull a lever under specific parameters to receive a reward. The protocol includes a test and a control groups. The test rat performs the motor task and receives a reward upon successful completion, while the control rat does not perform any task but still receives a reward at the same time as the test rat. After this training, the rats are injected in the ventral tegmental area (VTA) with biotin dextran amine (BDA), an anterograde tracer that allows visualization of VTA axonal projections to the motor cortex. Following tracer expression, the rats undergo perfusion to obtain 50 µm brain slices for performing immunohistochemistry. The preserved regions include the locus coeruleus (LC), the VTA, and the motor cortex. Primary antibodies, such as anti-TH, are used to label monoamine neurons. Indeed, TH (Tyrosine hydroxylase) is an enzyme essential for converting L-tyrosine into L-Dopa, a precursor to dopamine. In some samples, DAT (dopamine transporter) and NET (noradrenaline transporter) are used to label dopaminergic and noradrenergic neurons selectively. Finally, after the immunohistochemistry protocol, the images are analyzed using a laser scanner for Fiji image analysis.

RESULTS: During the intensive training phase, an increase in success rate is observed, stabilizing overall between 50% and 60%. The hit threshold also increases, showing that the rats have understood the task. Subsequently, image analysis reveals that only immunohistochemistry against TH seems to have worked. The experiment shows a higher monoaminergic neuronal density in trained rats. Similarly, the injected BDA shows no projections; it is only possible to visualize the cell bodies where the injection took place. This suggests that modifications are needed in the protocol, particularly regarding the incubation time of brain slices in the antibodies.

CONCLUSION: In conclusion, there is an increase in monoaminergic axon density in rats trained on the motor task compared to the control group. However, future work should focus on finding whether the labeled neurons are dopaminergic or noradrenergic by refining the protocol related to different tracers.

Customizable and low-cost mouse home cage system for automatic mouse weighing and tracking

Nathan Bérubé, Daniel Côté

Drug testing and disease research, among other types of experiments, require animal models to improve our fundamental biological understanding of the disease and verify the drug viability. Daily mice weighting is sometimes required to assess the animal's health. However, measuring the animal's weight and evaluating the animal's behaviour, while essential, require the interaction of an experimenter, which could change the animal's behaviour by inducing stress and jeopardizing its wellbeing. Also, the whole process is time-consuming for the experimenter, preventing continuous data acquisition. Evaluating the behaviour in the animal's home cage, as well as continuously following the food intake, the water intake and the animal's weight, could provide more accurate information on its state of mind and habits. Automated mouse home cage systems already exist on the market, but they are not customizable and they are too expensive to be supplied in multiple copies for biological experiments done with many animals. In this study, we present an automated mouse home cage system that is easily adaptable to any home cage, low-cost and user-friendly for non-experts in electronics and programming. Our goal is to reduce the human interaction and obtain more data on the animal's behaviour. In addition, our approach complements any other already existing optical monitoring systems, such as video tracking, infrared beams and radio-frequency identification. We take advantage of an Arduino for its ease of implementation, cost-effectiveness and versatility. To weigh the animals, a load cell is attached to two 3D-printed platforms of the size of the experimenter's choice. When an animal is on the platform, its weight is measured according to the deformation of the load cell. A tiling of multiple load cells with their platform is placed in the mouse home cage to weigh the mouse at any time and at any location. The tiling is also used to assess the mouse movements in its home cage, which can indicate signs of hyperactivity or stress behaviour. The size of the platforms are variable; smaller platforms necessitate more load cells to cover the whole cage area, but also provides better resolution in the mouse movement. Furthermore, our system also measures the real-time mouse location with a tiling of capacitive sensing plates. The aluminium plate acts as a half-capacitor which can be completed by the animal's body to sense its presence. Also, we are currently working on estimating the water consumption with a load cell to measure weight variation of the water bottle through time. This method will be accompanied with a capacitive touch sensor at the tip of the water bottle to count the number of drinking events. We present a simple automated mouse home cage system that can be easily reproduced in any laboratory with non-expensive electronics and our open-source script.

Apprentissage machine pour mesure automatisée des mouvements chez le rat

Olivier Bourget, Christian Éthier

Dans l'étude des comportements moteurs en neurosciences, des marqueurs mis directement sur l'animal sont utilisés pour analyser les mouvements de différentes parties du corps de celui-ci. Cependant, cette méthode peut dénaturaliser les mouvements de l'animal et ainsi influencer les résultats obtenus dans l'analyse. C'est pourquoi il est important d'élaborer une méthode non-invasive pour l'animal. Avec des méthodes d'apprentissage machine, il est possible de faire apprendre l'emplacement de points précis sur une série d'images permettant à la machine de « comprendre » où sont ces derniers sur une autre image qui est similaire. C'est ce que fait DeepLabCut, un logiciel totalement gratuit et libre d'accès qui est utilisé pour le suivi de multiples animaux. La méthode non-invasive va permettre de comprendre le comportement moteur chez l'animal et, avec l'analyse de signaux neuronaux, il sera possible de déceler quelles parties du cerveau sont responsables de chacun des paramètres.

Pour débuter un projet avec DeepLabCut, il faut premièrement posséder de courts extraits vidéos d'une tâche quelconque. Dans notre cas, il s'agit d'attraper des pastilles. Une fois les vidéos prisent, on peut créer le projet. Ces vidéos seront utilisées dans le dessein de créer un ensemble de donnée robuste capable d'entraîner le modèle en bonne et due forme pour qu'il soit dans la capacité d'analyser à peu près n'importe quelle vidéo qui image un environnement et une tâche similaire.

On obtient plusieurs vidéos avec un marquage d'une qualité suffisante pour en extraire des paramètres sur le comportement moteur du rat. On possède également un modèle d'apprentissage profond qui permet de marquer d'autres vidéos recueillies dans l'environnement de capture vidéo sans qu'elles aient été dans l'entrainement du modèle.

Grâce à DeepLabCut, il est possible d'extraire les mouvements du rat avec une précision satisfaisante qui nous permet d'avoir différents paramètres moteurs chez le rat sans avoir à physiquement marquer le rat.

Longitudinal Brain Networks in Zebrafish Larvae Across Development

Pauline Marc Tudor, Paul de Koninck

The larval zebrafish, owing to its transparency, small size, and rapid development, has become a widely used model to study neurodevelopment and circuit function over the previous two decades. Progress in connectomics now allows the precise characterization of circuit maturation in nematodes, but these measurements remain elusive for larger models. During this internship, we performed whole-brain functional connectivity measurements in developing 4 to 8 dpf zebrafish larvae to quantify the functional and morphological growth of the brain. Head-restrained transgenic larvae expressing a panneuronal fluorescent sensor were imaged using resonant 2-photon microscopy under spontaneous and stimulated conditions, while monitoring their tail movements. We measured the brain volume and the length of the animals, observing a strong correlation between both growth parameters. We then extracted average fluorescence signals from atlas-defined brain regions, and computed correlation matrices, or functional networks. These functional networks will allow us to study the appearance and evolution of hubs, or highly connected nodes that facilitate the integration of anatomically separated neural systems. We will also study the modularity of zebrafish brain networks across development, which we expect to increase as functional networks become more specialized. Our work aims to identify the main functional and anatomical changes that occur in the larval zebrafish brain during development and highlights the potential of the zebrafish model for studying the integration of complex brain functions at both cellular and mesoscopic scales.

Exposure to blood serum of stress-susceptible vs resilient mice differentially alters the transcriptomic profile of cultured primary astrocytes.

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Major depressive disorder (MDD) is a severe neuropsychiatric illness affecting millions of people globally. Recent studies have revealed alterations in the blood-brain barrier (BBB) of individuals with MDD. The BBB is formed by endothelial cells, pericytes and astrocytes and its main function is to prevent potentially harmful substances circulating in the blood, such as inflammatory mediators, from entering the brain. Chronic stress exposure is the main environmental risk factor to develop MDD and it is used in rodents to mimic human depression. Mice subjected to chronic social defeat stress (CSDS) and characterized by depression-like behaviours display BBB alterations, such as increased permeability and loss of tight junction protein expression, changes also observed in human MDD. Exposure to chronic stress is also associated with increased circulating inflammation and high levels of proinflammatory cytokines which could fragilize the BBB. Here, we aimed to investigate whether the blood serum of stressed mice, passing through a leaky BBB, could influence gene expression and function of astrocytes, a main component of the BBB.

CSDS was carried out on adult male C57BL/6 mice. Briefly, mice were directly exposed to an aggressor (retired CD1 male breeder) for 5-10 minutes over 10 consecutive days. After the physical stress, the C57BL/6 are placed in a cage where they have visual and sensory contact with their aggressor through a plexiglass divider, without any physical contact. Each day, mice undergoing CSDS were exposed to a new aggressor. Following the CSDS paradigm, C57BL/6 males were subjected to the social interaction (SI) test to determine their phenotype: stress-susceptible (SS) or resilient (RES). Indeed, following CSDS only a sub-population of mice develops depression-like behaviours such as social avoidance while the other subgroup acts like unstressed controls (CTRL) and is thus considered resilient. Blood samples were taken from the three groups (CTRL, SS and RES), then spin to collect serum prior treatment of cultured primary astrocytes for 24h. Following RNA extraction and cDNA synthesis, patterns of genes related to astrocyte and BBB function were assessed by qPCR.

One-way ANOVA and normality tests revealed an increase of aldehyde dehydrogenase 1 family member L1 (*Aldh1l1*), a key marker of astrocyte expression, in SS-serum treated primary astrocytes vs control-serum treated cells. Interestingly, glial derived neurotrophic factor (*Gdnf*) was increased only in RES-serum treated astrocytes when compared to the other groups. No significant changes were observed for inflammatory pathway-related genes such as cAMP Response Element-Binding Protein (*Creb*), Interleukin-6 (*Il-6*) and Tumor necrosis factor α (*Tnfα*). Investigation of gene expression at earlier time points will be relevant in the future since unpublished data suggest transient inflammatory changes in other BBB-related cells.

Altogether, these results suggest a phenotypic specific profile in astrocyte reactivity induced by SS-serum treatment while RES-serum promotes an increase in gene expression of glial trophic factors. It may be deleterious vs protective for BBB function and underlie stress vulnerability vs resilience after exposure to CSDS. It will be important to evaluate if the same is true for females and if it can be replicated in human cultured astrocytes.

Relation entre le bilinguisme et la morphologie du cortex cingulaire antérieur au cours du vieillissement normal

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INTRODUCTION : Bien qu'il n'existe pas de consensus quant aux effets du bilinguisme sur le vieillissement du cerveau, une conclusion relativement constante est que les personnes âgées bilingues auraient un volume de matière grise plus grand dans des régions impliquées dans le contrôle exécutif. Cela a notamment été observé au niveau du cortex cingulaire antérieur (ACC) des deux hémisphères (Del Maschio et al., 2018). Étant donné que l'apprentissage et la maîtrise d'une seconde langue sollicitent grandement les réseaux reliés au contrôle exécutif afin d'éviter l'interférence entre les différentes langues (Abutalebi & Green, 2007; Green, 1998), et faciliter le passage d'une langue à l'autre (« code-switching ») (Hofweber et al., 2020), les régions impliquées dans le contrôle exécutif sont des cibles de choix pour examiner les effets du bilinguisme sur le vieillissement du cerveau. L'objectif de cette étude est d'examiner l'effet potentiellement protecteur du bilinguisme et du multilinguisme sur l'ACC des deux hémisphères au cours du vieillissement.

MÉTHODE : Les données d'un échantillon de 109 personnes participantes âgées de 20 à 88 ans ont été analysées. Ces personnes participantes proviennent du projet PICCOLO (Projet de recherche sur les effets de la Pratique d'un Instrument ou du Chant sur la COgnition, le Langage et l'Organisation cérébrale). Celles-ci avaient été divisées en 3 groupes : 34 chanteurs, 38 instrumentistes et 37 individus pratiquant une activité psychomotrice non musicale. Toutes étaient en bonne santé, droitières, et parlaient couramment le français québécois. Les groupes ont été appariés pour l'âge, le nombre de langues parlées, la scolarité, le niveau de santé auto-rapporté, les symptômes de dépression (GDS), le fonctionnement cognitif général (MoCA), une mesure du risque de démence (Livingston et al., 2020) et les seuils auditifs. Un score d'expérience linguistique prenant en compte le nombre de langues parlées et la maîtrise (auto-rapportée) de chaque langue a été calculé pour chacune des personnes participantes. Les informations suivantes ont également été recueillies : l'éducation, le sexe et les facteurs du risque de démence. Des images IRM ont été obtenues dans un appareil Achieva TX Philips 3T incluant une séquence MPRAGE 3D pondéré en T1 (1mm3). Les images obtenues ont été traitées à l'aide du logiciel Freesurfer pour mesurer la superficie, le volume et l'épaisseur de la matière grise du cortex cingulaire antérieur des personnes participantes. Des modèles linéaires mixtes seront effectués à l'aide des logiciels R et R studio afin d'étudier la relation entre l'expérience linguistique et la morphologie du cortex cingulaire antérieur. L'âge, le score d'expérience linguistique et le groupe servent de variables indépendantes, tandis que la superficie, le volume et l'épaisseur de la matière grise de l'ACC servent de variables dépendantes. Les participants ont servi de variable aléatoire. Plusieurs covariables seront incluses dans les analyses. Les données sont en cours d'analyse. Lors de la présentation, des résultats préliminaires seront présentés. Enfin, les résultats de cette étude pourraient contribuer à mieux comprendre la relation entre le bilinguisme et la neuroplasticité.

Exposure to early life adversity alters gene expression of the blood-brain barrier tight junctions possibly contributing to stress vulnerability vs resilience in adulthood.

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Major depressive disorder (MDD) is the leading cause of disability worldwide. Only 30% of individuals with MDD completely recover suggesting that currently available antidepressants, all targeting neurons, may not treat all biological causes. Recent evidence highlights that changes at the blood-brain barrier (BBB) underlie stress responses and mood disorders. The BBB is formed by endothelial cells, astrocytes and pericytes and it prevents passage of circulating harmful molecules to the central nervous system. As for BBB tight junction complexes, they regulate paracellular permeability between endothelial cells. In mice, exposure to chronic stress, a major risk factor to develop MDD, leads to a loss of tight junction protein Claudin-5 (Cldn5), promoting passage of circulating inflammation and the establishment of depression-like behaviors. Interestingly, such effects are not observed in resilient animals. Early-life adversity (ELA) is another risk factor associated with MDD. However, it is yet unknown if it could have long-lasting impact on BBB properties and modulate stress responses in adulthood.

To address this knowledge gap, we evaluated the impact of ELA followed by chronic social defeat stress (CSDS) on key genes important for BBB integrity. Male and female mice were subjected to maternal separation and limited bedding and nesting from postnatal day (PD) 10 to PD19 for 4h/day. Later, during adulthood (PD61), animals went through 10 days of CSDS, a widely used mouse model of depression. Thereafter, a battery of depression- and anxiety-related behavioral tests was performed to establish behavioral profiles. 24h after the last behavioral test, brain punches from the prefrontal cortex (PFC), a brain area regulating mood, social behaviors, and decision making, were collected to assess gene expression of TJs genes, by real-time PCR.

First, we observed that ELA promoted anxiety and altered social interactions in males but not in females. Surprisingly, a second hit of stress in adulthood promoted resilience in both males and females. In line with behavioral adaptations to stress, ELA modulated BBB gene expression in the PFC of both male and female mice.

These findings contribute to a better understanding of the brain molecular mechanisms underlying the effects of ELA and stress susceptibility vs resilience in adulthood. Non-neuronal targets are receiving increasing attention in psychiatry and the BBB restrictive transport has been hampering the development of efficient therapeutics. Deciphering how ELA and stress exposure in adulthood impact this barrier biology could lead to innovative treatments or improve efficacy of currently available antidepressants for MDD.