1. Raziskovalna organizacija:

Univerza v Ljubljani, Medicinska fakulteta

2. Ime in priimek mentorja:

Robert Zorec

3. Področje znanosti iz šifranta ARRS:

3.03 Nevrobiologija

4. Kontaktni e-naslov mentorja:

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5. Kratek opis programa usposabljanja:

Uravnavanje vzdražnosti astroglije, mobilnosti sekrecijskih mešičkov in izločanja prenašalcev glije iz astrocitov v kulturi.

Astrociti so najštevilčnejše celice v centralnem živčnem sistemu sesalcev. Zagotavljajo ključno metabolno podporo živčnim celicam, uravnavajo transport vode v možganovini in signalizirajo možganskim celicam z eksocitotskim izločanjem prenašalcev glije. Motnje oz. odpoved homeostatske funkcije astrocitov sodoloča potek in izid številnih nevrodegenerativnih bolezni. Možganski nevrotrofični dejavnik (BDNF) je eden od najbolj razširjenih rastnih dejavnikov v osrednjem živčnem sistemu sesalcev; je ključnega pomena za razvoj možganov in reorganizacijo sinaps (sinaptično plastičnost). Tradicionalno ga povezujejo z nastankom nevropsihiatričnih bolezni, kot so depresija, shizofrenija in odvisnosti. Njegovo izražanje v hipokampusu povečajo zdravila antidepresivi. BDNF z uravnavano eksocitozo izločajo živčne kot tudi celice glije, čeprav je malo znanega o izločanju iz glije. S tehnikami lipofekcije in/ali nukleofekcije astrocitov v kulturi bo kandidat/-ka izrazil plazmidno DNK z zapisom za fuzijsko beljakovino BDNF in DsRed (rdečo fluorescentno beljakovino), EGFP (okrepljeno zeleno fluorescentno beljakovino) ali pHSE (mutirano zeleno fluorescentno beljakovino občutljivo za pH), označil/-a posamezne sekrecijske mešičke v živih celicah in izvedel/-a fluorimetrične meritve (konfokalna mikroskopija ali mikroskopija s strukturiranim osvetljevanjem (SIM)) v katerih bo okarakteriziral spontano ter izzvano mobilnost mešičkov in ugotavljal/-a morebitno povezavo med velikostjo in mobilnostjo fluorescentno označenih mešičkov. V nadaljevanju bo testiral/-a vpliv sekretagogov, ki povečajo citosolno aktivnost kalcijevih ionov, hitro delujočih antidepresivov ter anestetikov na mobilnost in sekrecijsko aktivnost mešičkov. Ugotavljal/-a bo delež spontanih in izzvanih sekrecijsko aktivnih mešičkov ter kvantitativno okarakteriziral/-a izločanje njihove fluorescentne vsebine. Z meritvami fluorescence permeabilnega neraciometričnega kalcijevega indikatorja (Fluo-4-AM) bo kandidat/-ka ovrednotil/-a vpliv izbranih učinkovin na homeostazo prostih kalcijevih ionov in raziskal/-a morebitne interakcije med učinkovinami na ravni kalcijeve vzdražnosti v astrocitih. Nato bo ugotavljal/-a povezavo med spremembo mobilnosti mešičkov in aktivnostjo kalcijevih ionov v citosolu astrocitov. V preparatu fiksiranih astrocitov bo kvantitativno ovrednotil/-a kolokalizacijo BDNF z imunocitokemično označenimi markerji za sekrecijske in endosomske mešičke ter neodvisno ovrednotil/-a sekrecijski potencial mešičkov, ki pričakovano vstopajo v uravnavano, od kalcija, odvisno eksocitozo. Ugotavljal/-a bo tudi lokalizacijo različnih tipov molekulskih motorjev (dinein, kinezin, miozin) na astrocitnih mešičkih. Z izvedbo dvojne transfekcije astrocitov s plazmidi, ki kodirajo zapis za atrijski natriuretični peptid označen z mutirano zeleno fluorescentno beljakovino (ANP.emd) in mutirane preseniline (PS1Δ9, PS1 M146V in PS2 N146V), ki delujejo kot kanalčki prepustni za kalcijeve ione v membrani endoplazemskega retikuluma (ER), bo raziskal/-a povezavo med spremenjeno mobilnostjo mešičkov in homeostazo kalcija v lumnu ER. S transfekcijo astrocitov s plazmidno DNK za akvaporine AQP4e-GFP (AQP4e) in AQP4d-GFP(AQP4d) ter uporabo fluorescentnih

membranskih barvil (FM1-43FX, DiD) ali protiteles proti zunajceličnemu predelu akvaporinov izoliranih iz bolnikov z nevromielitisom vidnega živca bo raziskal/-a uravnavanje permeabilnosti za vodo na ravni uravnavanja prometa astrocitnih mešičkov, ki prenašajo vodne kanalčke h/od plazmaleme ter na ravni uravnavanja površinske gostote akvaporinov v plazmalemi v fizioloških in v hipoozmotskih razmerah, ki posnemajo možganski edem.

Regulation of astroglia excitability, vesicle mobility and release of gliotransmitters from cultured astrocytes

Astrocytes are the most abundant cells in the central nervous system of mammals. They provide key metabolic support to neurons, regulate water permeability in the brain parenchyma and signal to brain cells by the exocytotic release of gliotransmitters. The failure in homoeostatic functions of astrocytes determines the progress and outcome of most neurological disorders. Brain-derived neurotrophic factor (BDNF) is the most prevalent growth factor in the mammalian central nervous system. It is essential for development of the CNS and reorganization of synapses (synaptic plasticity). It is traditionally implicated in the neuropsychiatric disorders such as major depressive disorder, schizophrenia and addiction. The expression of BDNF in hippocampus is enhanced by fast acting antidepressants. BDNF is secreted via regulated exocytosis from neurons and from glial cells, although little is known about its release from astroglia. The candidate will transfect cultured astrocytes (by lipofection or nucleofection) to express plasmid DNA encoding fusion protein between BDNF and DsRed (red fluorescent protein), EGFP (enhanced green fluorescent protein) or pHSE ("super ecliptic" mutated green fluorescent protein sensitive for pH) and label individual secretory vesicles in living cells. In transfected cells, the candidate will perform confocal microscopic measurements or super-resolution structured illumination microscopy (SIM) measurements to characterize spontaneous and stimulated vesicle mobility, and to explore the relationship between the vesicle size and mobility of vesicles. The candidate will further examine the effects of secretagogues that elevate cytosolic calcium activity in astrocytes, the action of fast-acting antidepressants and anesthetics to mobility and secretory activity of labeled vesicles. The candidate will determine the fraction of secretory active vesicles in spontaneous and stimulated conditions and quantitate the release of fluorescent cargo from individual vesicles. By using permeable non-ratiometric fluorescent calcium indicator (Fluo-4-AM), the candidate will also examine the impact of selected agents to alterations of calcium homeostasis, and will explore the possible interactions amongst them at the level of astrocytic calcium excitability. The candidate will additionally explore the relationship between alterations in vesicle mobility and cytosolic calcium activity. To independently examine the secretory potential of vesicles, which are expected to enter regulated, calcium dependent exocytosis, the candidate will quantify colocalization of fluorescent BDNF with immunocytochemically labeled membrane markers of secretory vesicles and endosomes, and will also examine the localization of molecular motors (dynein, kinesin and myosin) in these vesicles. By double transfecting astrocytes with plasmids encoding atrial natriuretic peptide tagged with mutated GFP (ANP.emd) and plasmids encoding mutated presenilins (PS1Δ9, PS1 M146V in PS2 N146V) that act as calcium channels in the membrane of endoplasmic reticulum (ER), the candidate will explore the relationship between vesicle mobility and altered calcium homeostasis in ER lumen. By transfecting astrocytes with plasmids encoding aquaporins AQP4e-GFP (AQP4e) and AQP4d-GFP(AQP4d), and by using fluorescent membrane dyes (FM1-43FX, DiD) or antibodies isolated from patients with neuromielitis optica targeting extracellular domain of aquaporins, the candidate will explore the regulation of water permeability at the level of trafficking of vesicles carrying aquaporins towards/from the plasmalemma and the level of aquaporin surface density regulation in physiological and hypoosmotic conditions mimicking edema.