

Innovative Training Network

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1. Document Information and Introduction

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Introduction

This document corresponds to the third Syn2Psy annual progress report, in which the activities carried out during the third year (M26 to M35) of this MSCA-ITN are described. Hereby, we provide information on the scientific and training progress of the Early Stage Researchers (ESRs), the dissemination and communication activities carried out by the fellows, as well as the network-wide events organised by the consortium. We make an overall description of the progress of the action, in the light of a year marked by the delays caused by the COVID-19-related confinements and the procedures still in place in the laboratories.

Furthermore, we also provide information regarding the recruitment of ESR4, Veronica Villeri, following the resignation of former ESR4 Diogo Soares and the change in supervision of ESR5 Marcos Sintes.

We end by a final comment regarding the implementation of the action.

2. Project Progress

2.1 Note about COVID-19

This third year of the implementation of the action was marked by the aftermath of SARS-CoV-2 pandemic, during which lockdowns and confinement measures led to delays in the project progression, postponement of networkwide events and impossibility to carry out secondments, as well as delays in deliverables and milestones.

During this year, ESRs and supervisors made efforts to compensate for the delays: some secondments were transferred to an online format and others were readjusted, where needed. Network-wide events, such as Network School 3 and the Mid-term meeting, were held online. The implementation of the tasks as described in Annex I Part B is not affected, however, it has a considerable delay.

2.2 Scientific progress

ESR1 Orsolya Antal

Previous *in vitro* characterisation has suggested that the schizophrenia-associated stargazin variant has altered functionality. Recent work in this project has focused on the behavioural and morphological characterisation of the knock-in mouse model carrying this schizophrenia-associated CACNG2 variant.

Orsolya has recently started the characterisation of its behavioural phenotypes, through a battery of behavioural tests aimed at assessing locomotor activity, anxiety-related and depressive-like phenotypes, as well as cognitive changes. Animals carrying the disease-associated variant show no changes in locomotion or anxiety-like behaviours, assessed using the open field and elevated plus maze tests, and a tendency for increased depressive-like behaviour, assessed using the forced swimming test.

In the battery of cognitive tests, preliminary results suggest impairments in associative learning in a contextual fear conditioning and extinction paradigm, spatial memory in an object displacement test, spatial working memory in a T-maze test relying on spontaneous alternation.

Building on this, Orsolya has also started performing the morphological and functional characterisation of prefrontal cortex neurons. To achieve sparse labelling of neurons, tail-vein injections were performed with the AAV9.Syn.eGFP.WPRE.bGH viral vector. Ongoing work is focusing on the imaging of GFP-expressing neurons within the prefrontal cortex, the reconstruction and Sholl-analysis of these neurons to assess dendritic complexity, as well as the evaluation of dendritic spine density and morphology.

As mitochondrial alterations are emerging as a common feature of neuropsychiatric disorders and previous mass-spectrometry data suggested alterations in the expression of some mitochondrial proteins, Orsolya has also aimed to assess whether changes in mitochondrial structure and function could contribute to the phenotypes observed. To evaluate this, Orsolya has begun characterisation of mitochondrial function by evaluating mitochondrial respiration using the Seahorse XF extracellular flux analyser in mitochondrial extracts. ESR1 also aims to evaluate potential differences in mitochondrial structure using electron microscopy (EM), in the framework of a secondment with Professor Carmen Sandi at EPFL. Ongoing work and the secondment will focus on the acquisition and analysis of EM images to assess alterations in mitochondrial structure.

Finally, during the secondment with CTI, the aim was to design concept proposal for a clinical trial to test the effects of a hypothetical drug aimed at restoring synaptic function with a view to improve cognitive performance in patients suffering from schizophrenia. The general objectives through this were to understand the scientific and regulatory environment for clinical trials, to identify key assumptions, guidelines and key questions to address for a successful clinical trial.

ESR2 Alessandro Chioino

Alessandro has conducted genetic manipulations in mice to induce loss of function of Mitofusin 2 (Mfn2) in the Nucleus Accumbens (NAc) and assessed the impact thereof on motivated behaviour. Downregulation of Mnf2 in medium spiny neurons expressing the dopamine receptor 1 (D1-MSNs) induced an impairment in effort-related behavior, as shown by the adoption of a passive coping behavior in the forced swim test, indicative of depression-like behavior. Alessandro has performed cFos mapping of neuronal activation during the behavioral task, which revealed decreased engagement of NAc neurons. Based on these results, it was decided to first study the cellular and synaptic consequences of Mfn2 downregulation in accumbal cells, before exploring the impact in NAc downstream regions. Alessandro has performed electrophysiological recordings in mice with Mfn2 downregulation in D1-MSNs, which indicate aberrant intrinsic excitability, altered excitatory synaptic inputs and reduced dendritic complexity in D1-MSNs. To link the alteration of NAc D1-MSNs activity and the adoption of passive coping behavior, bulk calcium signals via fiber photometry during FST were recorded (analysis ongoing). Qualitative mitochondria evaluation shows alteration in organelles circularity in case of Mfn2 downregulation. To follow up, Alessandro planned electron-microscopy analysis of mitochondria morphology and spines in D1-MSNs.
Ribosome-associated mRNA (Ribotag) isolation from NAc D1-MSNs was implemented to perform an unbiased cell-type and region-specific RNA sequencing (analysis ongoing), that will provide Alessandro with candidates for the underlying molecular mechanisms leading to dendritic, synaptic and mitochondria defects induced by Mfn2 downregulation.

ESR3 Giuseppe Cammarata

During the last year, Giuseppe has been able to make big steps forward regarding his research project, which focuses on the effects of Valproic acid (VPA) chronic administration on the development of human brain organoids. This drug is a widely prescribed anticonvulsant and mood stabilizer. Its antiepileptic properties have been attributed to its inhibitory effects on the degradation of gamma-Aminobutyric acid and modulation of ion channels activity. VPA has also been shown to act as a Histone Deacetylase (HDAC) inhibitor, indirectly modulating the expression of transcription and growth factors. Nowadays, VPA use is restricted during pregnancy due to high risk of the unborn child later developing autism spectrum disorder (ASD) and mental disability. To date, little is known on the effects of VPA in the developing human brain, nor how the brain cellular architecture changes due to this drug. In line with what is observed in animal models, Giuseppe's preliminary calcium imaging results have shown that their model generates hyperactive networks.

One of the scientific progresses that Giuseppe made, was to show similar results with electrophysiology recordings. Apart from confirming the results found in calcium imaging recordings, electrophysiology recordings have also shown that VPA-treated organoids generated neurons that were intrinsically highly excitable and less capable than the control organoids to produce trains of action potentials at high current injection.

Further progress was done in collaboration with Andre Salles Cunha in the analysis of calcium imaging recordings. Previously, Giuseppe was able to analyse mainly the activity of each cell in order to address the frequency of their activity. Now, Giuseppe may be able to perform cluster analysis of the cells recorded by comparing the fluorescence traces of different cells within the same recorded region. If finalized, this technique may give some hints to understand how the network develops in the human brain organoids and how chronic VPA administration may affect its maturation.

More recently, Giuseppe has performed a secondment at the Paediatric Department of Coimbra University Hostpital (Centro Hospitalar e Universitário de Coimbra - CHUC), at the Specialised Unit for Neurodevelopment and Autism (UNDA). Giuseppe assisted Dr. Frederico Duque in the evaluation of children's mental health. This evaluation was performed during consultations, and it was based on the identification of early manifestations of delayed or deviant neuronal development. This experience at CHUC has helped Giuseppe to expand his knowledge about the clinical aspect of autism spectrum disorder.

ESR4 Veronica Villeri

Veronica has started her Syn2Psy project in May 2021. From May 2021 until now, Veronica has learned the techniques that will be used for the development of her project by working on a related project in the laboratory. Specifically, Veronica has studied the effects of Long Term Depression on both SynGAP expression and SynGAP-AMPA co-organization in rat hippocampal primary neurons. To this aim Veronica has learned to master dual-color STORM imaging and to perform the related image analyses. At the same time Veronica has started to implement the culture of hESC-derived neurons bearing SynGAP mutations in the laboratory.

ESR5 Marcos Sintes

In 2021, Marcos has changed supervisor, laboratory and project (within Syn2Psy) and has started to work in his new project in March 2021.

Marcos redesigned the project to take advantage of the techniques developed in his current laboratory and based on the most recent scientific literature. The necessary collaborations with other research groups and industry partners were established to obtain the required cell lines and other materials for the project. Furthermore, Marcos received training in necessary animal work and in cranial window surgery on mice and got signed off, according to Imperial College regulations. Marcos also received training in cell culture techniques and two-photon microscopy. A first step for this new project is the characterisation of the expression of serotonin receptors in the cortical xenograft model, however, some of these receptors are not easy to detect due to lack of readily working antibodies. Marcos optimised different techniques for the detection of these receptors, including immunohistochemical techniques and the design and development of a fluorescence *in situ* hybridisation probe. Marcos performed cranial window surgery in several cohorts of control mice and imaged them to establish a baseline for the characterisation of neuronal plasticity. These animals will soon be used in drug-treatment studies.

ESR6 Daniel Hunter

Complementary to the investigations into the synaptic pathogenicity of NMDAR-Abs, with the novel identification of inhibitory signalling deficits, Daniel further investigated the intrinsic excitability of both excitatory and inhibitory cell populations of the hippocampus. Using both whole-cell current-clamp and cell-attached voltage-clamp approaches, new avenues to pathological network activity in NMDAR encephalitis (NMDARE) have been identified.

First, the network scale effects of these pathogenic antibodies in hippocampal slice cultures were investigated, which uncovered a significant increase in principal cell firing rates. Given that this phenotype is expected when considering the clinical features of patients afflicted with NMDARE, but is counterintuitive on the molecular scale – since the reduction of excitatory NMDAR-mediated signalling is unlikely to drive hyperexcitation directly in these cell populations – Daniel further explored the endogenous firing rates of inhibitory interneurons within this culture system. A dramatic and significant silencing of inhibitory cell firing was identified, which provides a novel mechanism relevant to the clinical scale of the disease, where seizure and other neurological disturbances are observed. To further this exploration, Daniel investigated the resting membrane potential of both excitatory and inhibitory neurons, after exposure to NMDAR-Abs, and found that - contrary to principal cells – inhibitory neurons are uniquely affected, demonstrating a hyperpolarising shift in resting potential. It can be anticipated that this is due to the removal of magnesium-insensitive GluN2D-containing NMDARs, selectively expressed in this cell population, which are known to play a role in setting of the resting potential.

Further, Daniel has also finalised a set of investigations, equivalent to those already explored with NMDAR-Abs, with another patient-derived monoclonal antibody against the GABAAR. These have focussed on understanding the impacts of these antibodies on spontaneous excitatory and inhibitory synaptic currents (sEPSCs and sIPSCs, respectively). Here, a decrease in both sEPSCs and sIPSCs was identified, after exposure to GABAAR monoclonal antibodies (GABAAR-Abs), similar to the effects previously observed with NMDAR-Abs. Daniel has also explored the impact of these antibodies at the cellular scale, uncovering an impact on both the resting membrane potential and intrinsic excitability of hippocampal neurons. These changes in synaptic and cellular function have also been examined at the network-level, where Daniel has demonstrated alterations in cell firing frequencies, congruent to those of NMDAR-Ab exposed hippocampal cultures.

ESR7 Elisa Corti

Brain-Derived Neurotrophic Factor (BDNF) is a mediator of synaptic plasticity and long-term potentiation. It has been found that the facilitatory effect of BDNF in glutamatergic synapses depends on local protein synthesis. A master regulator of this process in neurons is Fragile X Mental Retardation Protein (FMRP), that exerts translational control on a subset of neuronal transcripts, but it is still unclear whether FMRP is a mediator of BDNF effects. In the first part of the project Elisa showed that Fmr1 knock down (KD) abolishes the BDNF-induced upregulation of these GluN2A- and GluN2B containing NMDA receptors in the synaptic compartment.

During this year Elisa focused on the mechanism underlying this process, analysing some key players in the BDNF signalling cascade and observed that KD of FMRP does not affect the activation of the BDNF receptor TrkB, but it inhibits the activation of the tyrosine kinase Pyk2. Elisa is currently studying

if FMRP is involved in BDNF-induced local protein synthesis of Pyk2 and whether FMRP KD affects the lateral diffusion of NMDA receptors in the synaptic surface.

Moreover, a new protocol to obtain single-neurons growing in micro-islands (namely autaptic neurons) with hippocampal neurons from E18 Wistar rats has been established and a protocol to perform primary cultures from P0 WT and Fmr1 KO mice is currently being established. In future studies Elisa will investigate the role of FMRP in the development of the presynaptic terminal, using pseudo-explants, and on synaptic vesicle exocytosis using autaptic neurons.

At the end of 2021, Elisa performed her first secondment, at Lundbeck. The main aim of this secondment was to learn about the pre-clinical technologies used at Lundbeck for the development of novel therapeutics, focusing specifically on in vivo calcium imaging with miniscopes, EEG and single-unit recordings in awake freely moving rodents. Among all, Elisa joined an experiment aimed to measure visually-evoked activity of pyramidal neurons (PN) and parvalbumin positive interneurons (PV) located in the primary visual cortex (V1) by means of Calcium transients. One other experiment aimed to measure the levels of the neurotransmitters glutamate and dopamine in the striatum using microdialysis and fiber photometry in awake freely moving mice.

ESR8 Elizabeth Brockman

Since March 2021, Elizabeth has made significant progress. Briefly, a large number of craniotomy surgeries have been completed, labelling grafted neurons with both GFP and GCaMP fluorescent tags to visualize whole cell structure and calcium activity, respectively. Elizabeth has been performing experiments with GFP-labelled graft cells, evaluating how the structural development of boutons is altered in trisomy 21 cells compared to isogenic controls. These imaging sessions look at 3 timepoints each at both 3 months and 5 months post-transplantation. Over the past year, Elizabeth has brought this dataset to the brink of completion. It now requires only the addition of the final batch of animals, which is ongoing. Experiments with the GCaMP-labelled grafts to investigate spontaneous and oscillatory somatic activity in trisomic compared to disomic graft cells, have also been performed. This was just beginning at the start of 2021, and is now entering the final stages of data collection. Additionally, stainings of the graft cells have been performed post-mortem to quantify the cell types, which make up Elizabeth's current grafts. This post-mortem analysis is complete. Small additions are underway to add details. Finally, during this year, Elizabeth supervised a MSc student in her project. This involved working with novel lentiviruses and adenoviruses in an attempt to integrate those into Elizabeth's model. The MSc student's thesis was submitted and defended successfully in September, 2021. Time permitting, Elizabeth will expand on this preliminary data on in the months to follow in the hopes of developing a proof-of-concept of the use of these vectors in the xenotransplantation model.

Preliminary work on thesis writing has commenced and is now an ongoing part of Elizabeth's tasks, along with the experiments. The first draft of the methods section for the thesis is complete, as is a detailed outline of the introductory sections.

ESR9 Laura Upton

Male mice deficient in the X-chromosome linked gene Glra2 are a novel mouse model for autism spectrum disorder. In the past year, Laura characterised neuronal subpopulations present in the

prefrontal cortex of Glra2-deficient mice, using immunofluorescence and immunohistochemistry, in young and adult mice. Glra2-deficient mice show no difference in pyramidal neuron or calretinin & parvalbumin interneuron density, but show increased somatostatin interneuron density in both young and adult mice. These findings add to the original findings, which suggest that Glra2-deficient mice show altered prefrontal circuit assembly without gross alterations to overall cortical & neuronal density.

Previously, Laura's group observed a deficit in novel object recognition memory in Glra2-deficient mice, whilst anxiety behaviours, social behaviours, and spatial learning and memory were found to be intact. To identify which brain regions were implicated in this behavioural deficit, Laura studied neural activity following novel object recognition, using the neuronal activity marker c-fos. To do this, a protocol to measure short term novel object recognition memory in Glra2-deficient mice was adapted. In order to study the neural activity of Glra2-deficient mice following this behavioural test, Laura obtained brain tissue from mice immediately following these experiments and processed brain slices from relevant regions using immunohistochemistry. The analysis is focused on areas of the brain implicated in novel object recognition, such as the prefrontal cortex, hippocampus, perirhinal cortex and entorhinal cortex. Results from these experiments are still pending (the quantification is ongoing), but previous preliminary findings indicate hypoactivation of the prefrontal cortex in Glra2-deficient mice following novel object recognition. In further experiments using tissue from these same animals, Laura will be measuring colocalization of c-fos with neuronal subpopulation marker proteins, to determine whether any particular neuronal subpopulations show altered neuronal activation.

ESR10 Flavio Tomasi

During this last year, Flavio continued to study the effects of endocannabinoid system modulators on a mouse model of autism, the Shank3-deficient mouse.

Flavio tested the effect of chronic administration of the CB1 receptor antagonist rimonabant on the anxiety-like behaviour in heterozygous and KO Shank3-deficient mice and in wild-type littermates. Only KO males exhibited anxiety-like behaviour, which was unchanged after rimonabant. This was the last experiment to assess the behavioural effects of rimonabant.

A pilot study was performed to evaluate the effects of AEF0217, a new signal specific inhibitor of CB1 receptors, on the behavioural deficits of Shank3-deficient male mice with the same tests used for rimonabant. Since the treatment with AEF0217 showed promising results, Flavio is now performing a dose-effect study in KO males and females with 5 different doses. This experiment will be completed soon. Plasma and brains of treated mice were collected after the end of the experiments to measure the amount of drug absorbed. Plasma and brains from naïve male and female mice of the three genotypes were also collected, to evaluate the effect of the Shank3-deficiency on the endocannabinoid system. The levels of several endocannabinoids and CB1 and CB2 receptors will be measured in different brain areas.

The effect of CB1 receptor inhibition on the brain levels of the synaptic proteins Homer1 and mGluR5, which have been shown to be altered in the post-synaptic density of Shank3-deficient mice is being evaluated. These experiments are still ongoing and will be finished in the next few months.

Previous studies in Shank3 mice have shown variable effects on social behaviour. Flavio tested the social behaviour in male mice in a pilot study, and found that KO mice showed a deficit in social novelty, not preferring to interact with an unfamiliar mouse in comparison with a familiar mouse. Whether AEF0217 will rescue this deficit will be evaluated.

Furthermore, in October-December 2021, Flavio performed a remote secondment at CTI, a company specialised in clinical trials and consulting services. During this period, Flavio designed a hypothetical clinical trial to test the effects of an inhibitor of the endocannabinoid system in patients with Phelan-McDermid syndrome and collaborated with experts at CTI, who helped with the issues related to drug development for clinical use in a rare genetic neurodevelopmental disorder.

ESR11 Ágata Silván

The results obtained from patch clamp recordings previously showed SK channel allosteric modulators are useful tools for modulating TRN firing by acting on the burst AHP. The next step on the project was to examine the effect of SK allosteric modulators in sleep architecture and particularly on sleep spindles. For that purpose, Ágata performed in vivo electrophysiology recordings in freely moving rats during sleep and wakefulness. Local field potentials were measured in thalamus, medial prefrontal cortex, and auditory cortex. An additional electrode was placed into muscle tissue to record from electromyographic activity. To differentiate between active/inactive states during sleep and wakefulness, an accelerometer was used. Ágata recorded for 22 hours, preserving the light/night cycle inside the recording boxes. The compounds were injected subcutaneously, single dose, after 1h of recording (at 10:00 am).

Regarding the analyses, Ágata is in the process of setting up a machine-learning based automated sleep scoring program that will allow to efficiently study sleep architecture and sleep spindles. The next steps of the project will be to analyse the recordings done so far and planning and performing those needed to assess the effect of SK modulation on sleep spindles.

In parallel Ágata has been working on the publication of the results gathered previously: writing the manuscript and receiving guidance and feedback from the supervisors.

Finally, as part of a future secondment in Peter Kind's lab at CDBS-UEDIN, Ágata has been working on how to design a point mutation in CACNA1i gene by using CRISPR-Cas9 system. CACNA1i is one of the major risk genes in schizophrenia and it is well known its relevance in TRN firing and sleep spindle density during NREM sleep.

ESR12 Manuela Rizzi

The main objective of Manuela's project is the identification of differentially translating mRNAs in *Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ models of disease. To this end, isolation of ribosomal cells from specific brain regions using TRAP technology is used to generate datasets of the translational and transcriptional activity.

The mainly explored brain region is CA1 since functional phenotypes such as exaggerated LTD downstream metabotropic glutamate receptors were observed in hippocampal slices from Syngap haploinsufficiency and Fragile X mice due to excessive protein synthesis (Barnes et al., J Neurosci, 2015). This explains the interest in studying translational activity to reveal targets of interest.

CA1 TRAP datasets were previously generated in the lab from Fmr1$^{-/y}$ and Syngap$^{+/-}$ models and Manuela started comparing them through bioinformatic analysis. The interesting observations were as follows:

- *Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ transcripts in CA1 TRAP reveal little overlap. If anything, there is a weak negative correlation between changes in *Fmr1*$^{-/y}$ CA1-TRAP vs *Syngap*$^{+/-}$ CA1-TRAP;
- GSEA (Gene Set Enrichment Analysis) of *Syngap*$^{+/-}$ CA1 TRAP shows upregulation of 63 GO terms related to transcription regulation and DNA replication and downregulation of 28 GO terms related to synapse and G-protein coupled receptors. Overlap of *Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ CA1-TRAP GO terms reveals differential regulation of transcription regulator terms, convergent upregulation of rRNA processing and polymerase activity and convergent downregulation of cell surface term;
- FMRP targets (transcripts binding to mRNA binding protein FMRP) and SFARI transcripts (autism risk transcripts), which are downregulated in *Fmr1*$^{-/y}$ CA1-TRAP, are upregulated in *Syngap*$^{+/-}$ CA1-TRAP

In conclusion, in hippocampal CA1 pyramidal neurons, there is an opposite profile of differentially translating mRNAs in *Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ models

In addition to CA1 region, Manuela is also interested in revealing the translating population of basolateral amygdala neurons. This region is highly involved in the association of fear memories. Alterations in context discrimination and learned fear are notable phenotypes in both *Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ mouse models (Guo et al. 2009; Zhao et al. 2005). The molecular characteristics that determine amygdala functions are still unexplored and to this end the strategy designed is the generation of a TRAP specific line for Basolateral Amygdala. This will allow to further analyse the translational profile of fear-associated memories by coupling Fear Conditioning Behavioural assay and TRAP technology. Progresses until now were the following:

- Generation and validation by immunohistochemistry *of Rasgrp1*-Ribotag lines (amygdala specific) for Syngap$^{+/-}$ and Fmr1$^{-/y}$ models;
- Optimization of Amygdala TRAP protocol looking at good enrichment of BLA specific genes and RNA quality;
- Optimization of Fear conditioning assay reporting upregulation of activity markers in CA1 neurons;
- Collection of RNA-seq samples from *Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ amygdala-TRAP to which will follow generation of sequencing libraries for amygdala translatome and transcriptome.
- Performing of contextual fear conditioning in amygdala-TRAP lines is starting and to that will follow: qPCR to examine differences in activation markers; Cfos+ staining to examine % activation in BLA neurons; TRAP-seq to identify differential translation and functional validation of specific targets using AAV-shRNA injection.

ESR13 Loredana Cumpana

The aim of the project is to investigate the role of a stress- and anxiety- related mediator, corticotropin-releasing hormone (CRH), on in the regulation of brain processes involved in sleep rhythms, through its action on the thalamic reticular nucleus (TRN). Ex-vivo electrophysiology experiments were conducted to investigate the effect of CRH on the excitability of neurons in the TRN. Loredana explored the ionic mechanism responsible for the previous finding, showing that CRH

decreases the TRN's neurons propensity to fire low threshold bursts. Loredana found that CRH negatively modulates the post-burst afterhyperpolarization, mediated primarily by the small conductance calcium-activated potassium channel 2 (SK2), which entrains repetitive burst firing. Additionally, it was found that CRH selectively increases the amplitude of cortical excitatory post synaptic potentials (EPSP). Viral tracing was used to map the CRH afferents to the TRN. CRH producing neurons from the anterior Baso-Lateral Amygdala, and dorsal Zona Incerta were found to project to the TRN in retrograde and anterograde tracing experiments. Other candidate regions such as the Medial Geniculate Nucleus, and the layer 6 neurons of the medial Entorhinal Cortex are being investigated. Loredana has started preparing to test the hypothesis in vivo, by using opto- and chemogenetic tools combined with EEG recordings. Additionally, using a newly developed CRH sensor (CRH-GRAB), Loredana is investigating the natural CRH fluctuations during sleep by combining fiberphotometry with EEG recordings.

ESR14 Vanesa Salazar

This study's first aim is to understand social interactions, and the habituation and immediate discrimination of conspecifics using a novel habituation dishabituation task in two rat models of SYNGAP1 haploinsufficiency. Briefly, in the Syngap+/ΔGAP rat, there is a 50% reduction of SYNGAP containing a Ras GTPase activating domain while total protein expression remains unaltered. Syngap+/- presents a heterogeneous deletion of the gene leading to half protein expression. Using two rat models allows delineation of the enzymatic and scaffolding functions of SYNGAP.
On the previous report, Vanesa showed how she had implemented a new habituation dishabituation to a conspecific task and two olfaction control tasks and the achieved preliminary results.

Since March 2021, Vanesa has replicated the results with more cohorts going through the habituation dishabituation to a conspecific task and the two tasks for olfaction (one for non-social odours and one for social odours using urine) to test their skill for the detection, habituation and discrimination of odours. The results suggest Syngap+/ΔGAP and Syngap+/- rats present less social exploration time with conspecifics (non-related age-matched wildtype rats) than the wildtype group. Interestingly, conspecific rats show similar social exploration time regardless of the genotype of the paired animal. Both, Syngap+/ΔGAP rats and Syngap+/- rats habituate and discriminate between conspecifics. Alterations in social behaviour are not due to olfaction deficits since animals can detect and discriminate both non-social and social odours. Since the needed N size has been reached, Vanesa is now in position to statistically analyse these results and extract definitive conclusions.
Moreover, Vanesa has worked on the analysis of the behavioural data by defining indexes to determine the ability of social habituation and discrimination, and odour detection and discrimination and also on the DeepLabCut video pre-processing to later obtain tracking information.
Vanesa has done the initial analysis of allogrooming and self-grooming in the context of the Habitat project. The Habitat allows a more natural and enriched behaviour in addition to the possibility of free social interactions with multiple individuals free of any experimenter intervention. Preliminary results don't show any difference on neither rat model. This might suggest that higher social and environmental stimulation may ameliorate the social phenotype observed on the habituation dishabituation task. Therefore, it is planned to run a pilot testing this hypothesis on the following months.

2.3 Training

Table I: Courses taken by the ESRs during M25 to M36.

ESR	Courses
ESR1 Orsolya Antal	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone.
ESR2 Alessandro Chioino	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Understanding statistics and experimental design – EPFL. RNAseq data analysis course – Edinburgh Genomics.
ESR3 Giuseppe Cammarata	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone.
ESR4 Veronica Villeri	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Scientific integrity in research – University of Bordeaux.
ESR5 Marcos Sintes	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Animal work – Imperial College.
ESR6 Daniel Hunter	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone.
ESR7 Elisa Corti	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone.
ESR8 Elizabeth Brockman	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Cryogenics Training Course – MRC LMS and Imperial College London. Careers: Job Search With A Difference – Imperial College London. Display Screen Equipment Online Assessment – Imperial College London. Fire Safety Training – Imperial College London.
ESR9 Laura Upton	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Research ethics and scientific integrity – Sorbonne Université.
ESR10 Flavio Tomasi	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Speaking in public – Sorbonne University, Paris Ethics in scientific research – Sorbonne University. Improve your communication skills in English – Sorbonne University. Autism and NeuroDevelopment Research Workshop – Center of Excellence on Autism and Neurodevelopmental Disorders and Autism and NeuroDevelopmental Disorders Scientic Interest Group. The First Annual SHANK2 Day Conference – SHANK2 Foundation.
ESR11 Ágata Silván	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Extracellular electrophysiology acquisition – Neurokit Cajal. *In vivo* pharmacology – University of Copenhagen. Communication and presentation in the academic context – University of Copenhagen.
ESR12 Manuela Rizzi	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone.

ESR13 Loredana Cumpana	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Understanding statistics and experimental design – EPFL.
ESR14 Vanesa Salazar	NS3 - Drug discovery and development: from idea to patient – Lundbeck. Training in Career Development – Chaperone. Bootstrapped Python – University of Edinburgh.

2.4 Secondments

Due to the impossibility to travel and the restrictions put in place in the laboratories due to COVID-19 pandemic, the secondments planned for 2021 did not take place and were postponed to late 2021 or 2022/2023. The Syn2Psy Supervisory Board decided that the secondments considered to be feasible online without a loss in content, should be held remotely. This included secondments with the partners CTI and CHUC.
Furthermore, to better adapt to the current circumstances, and in the cases that it was considered to benefit the fellow's training and the project's progression, other modifications to secondments will be implemented.

A document including all the details and justifications for the modification in secondments, is currently under preparation and will be submitted via formal notification on the EC Portal.

2.5 Deliverables and milestones

Table II and III include the deliverables and milestones due before M35. Some of the deliverables and milestones have a delay, which can be ascribed to the ESRs' loss of laboratory time due to the COVID-19 pandemic, notably the delays in the presentation of scientific data in peer-reviewed manuscripts (D1.1, D2.2, D2.4, D2.6, D3.1, D3.2, D3.3).

Network Schools 2 and 4 will be held in March/April (see below: 3.3 Network School 2 and 4) and the reports will be submitted shortly after.

Table II: Deliverables due during M25 to M36.

Deliverable	Title	Due date	Submission
D4.4 D26	Report on network school 3 (NS3)	31 Jul 2021	02. Jul. 2021
D4.6 D32	Report for the intermediate consortium meeting	31 Jul 2021	02. Jul. 2021
D4.3 D25	Report on network school 2 (NS2)	28 Feb 2022	Delayed: NS2 will be held on April 4-7, 2022
D4.5 D27	Report on network school 4 (NS4)	28 Feb2022	Delayed: NS2 will be held on March 28th to April 1st, 2022
D1.1 D1	Peer-reviewed manuscript: establishment of the role of stargazin in psychiatric disorders; development of targeted therapies	28 Feb 2022	Delayed
D2.2 D5	Peer-reviewed manuscript: characterization of cortical excitation/inhibition imbalance in vivo in models of synaptic dysfunction using mesoscopic methods	28 Feb 2022	Delayed

D2.4 D7	Peer-reviewed manuscript: identification of presynaptic defects and alterations in glutamate receptor dynamics in Fragile X syndrome	28 Feb 2022	Delayed
D2.6 D9	Peer-reviewed manuscript: characterization of the synaptic and neural correlates of spatial memory deficits in a glycine receptor knock-out model of ASD	28 Feb 2022	Delayed
D3.1 D10	Peer-reviewed manuscript: definition of the therapeutic potential of the endocannabinoid system in an ASD model	28 Feb 2022	Delayed
D3.2 D11	Peer-reviewed manuscript: identification of the role of the excitation/inhibition (E/I) imbalance in the cortico-thalamic system in the development of neuropsychiatric disorders	28 Feb 2022	Delayed
D3.3 D12	Peer-reviewed manuscript: identification of aberrant synaptic mRNA translation as a point of convergence for multiple genetic causes of ASD/ID	28 Feb 2022	Delayed

Table III: Milestones M25 to M36.

Milestone	Title	Achieved
M7	Syn2Psy intermediate meeting	04 May2021
M8	Project check	04 May 2021
M11	Provisional patents established	Delayed

3. Network-wide events

3.1 Mid-term meeting

The Syn2Psy intermediate meeting was held online on May 2 – 3, 2021. The 41 participants included ESRs, scientists-in-charge representing each beneficiary, representatives from each partner institution, the Project Officer (PO), the REA External Reviewer appointed by the PO, and one member of the Syn2Psy External Advisory Board.

The meeting started with a short presentation of all participants (except the ESRs) who described their role within Syn2Psy. This was followed by the Coordinator's report, during which Ana Luísa Carvalho reminded the consortium of the goals of Innovative Training Networks and the specific goals of Syn2Psy. The coordinator reported on the achievements of the project in terms of training, deliverables, communication and public engagement and provided information about the impact of COVID-19-restriction measures on the progress of the project.

Subsequently, during the Q&A session, the PO answered to questions from the consortium members, which revolved around the impact of COVID-19, the implementation of the secondments and clarifications regarding a possible project extension.
The first day of the meeting ended with the scientific presentations of the Syn2Psy fellows, which included their research progress, training and planned secondments and was followed by a short scientific discussion with the REA External Reviewer.
On the second day of the meeting the ESRs participated in a private and confidential session with the PO and the REA External Advisor to discuss administrative and supervision aspects of the project. Simultaneously, meetings were held for each of Syn2Psy committees: Dissemination and Communication Committee, Training and Doctoral Studies Committee, Research Coordination Committee and Supervisory Board. Furthermore, a scientific session under the theme "Synaptic dysfunction in neuropsychiatric disorders", with presentations from Syn2Psy Principle Investigators and the Syn2Psy External Advisor, was held in the afternoon.

3.2 Network School 3

Network School 3 (NS3), under the title "Drug discovery and development: from Idea to Patient" was held online on May 5 – 7, 2021. This school was organised by Kjartan Herrik (Principle Scientist – Lundbeck).
NS3 was a three-day intensive course focused on the principles of drug development. The ESRs gained theoretical understanding on how Lundbeck and other pharmaceutical companies develop scientific ideas into treatments to help patients with neuropsychiatric disorders. Through the seminars on Pre-Clinical, Clinical and Business Development and Licensing, given by Lundbeck's specialists, the ESRs were taken through the steps from generating ideas to treating patients.
Furthermore, NS3 was complemented with a workshop on the early signs and clinical variability of Autism Spectrum Disorders, given by PIN (Syn2Psy Partner) and with seminars about the pros and cons of working in academia and industry, as well as a round table discussion about this topic.
As an evaluation, the ESRs were asked to pitch their project in the *Dragon's Den*. This enabled the ESRs to communicate the potential value of their project for patients, commercial interests and the scientific community, as well as to understand what is needed from them to achieve these goals. Dragons were invited from Psychiatry, Industry and the Lundbeck Foundation.
To measure the overall course satisfaction, the students were asked to answer a satisfaction survey. The majority of the ESRs found the *Dragon's Den* to be a useful exercise, since it provided the opportunity to convert each project into a proposal for the drug development industry and to look for the potential applications of their research. Other considerations included the fact that the *Dragon's Den* allowed the ESRs to address their projects from a new perspective, different to what is usual in basic research, and to learn how to communicate with potential collaborators. Furthermore, several ESRs considered that it was valuable to get feedback on their specific projects from a non-academic jury.

3.3 Network Schools 2 and 4

After being postponed twice, due to COVID-19-related restrictions, Network Schools 2 and 4 will be held in-person in Spring 2022.
Network School 4 will be held in Bordeaux on March 28th to April 1st, followed by Network School 2 in Paris on April 4th to 7th (2022). The topics of these courses will be, respectively, "Advanced methods for imaging the brain: from nanoscopic to mesoscopic perspectives" and "Genetic approaches for the study of neuropsychiatric disorders", as described in Annex 1, Part B of the Grant Agreement.

3.4 2nd online PhD day

Following the success of the first Syn2Psy online PhD day, held on October 5th (2020), a second online PhD day was held on October 23rd to 24th (2021).
The aim of this yearly network-wide event is to promote scientific discussion and interaction between the consortium members, as well as to give the opportunity to the ESRs to receive scientific feedback from the experts in the field. This edition of the event was held in two days, to allow ample time for the scientific discussions: the ESRs described their progress in a 15 min presentation, followed by 20 min of discussion. Furthermore, this event counted on the presence of the two Syn2Psy External Advisors: Prof. Claudia Bagni from University of Lausanne, Switzerland and Prof. Gavin Rumbaugh from Scripps Research, Florida-USA, who had an active role in giving feedback and suggestions to the ESRs. The Training and Doctoral Studies Committee (TDSC) also had an active role in assessing the ESRs' progress (based on the relevance of the scientific question, pertinence of experimental approach and project progress).
Despite the delay in the projects as compared to the original plan, there was a unanimous opinion that the projects are on the right track and moving forward. The consortium members agreed that, once more, this event was fruitful and constructive, and a third edition is being planned for 2022.

3.5 Career development sessions

The promotion and organisation of advanced training in transferable skills and career development is an important topic within Syn2Psy. With this in mind, a series of online workshops were provided to ESRs by the company Chaperone, an online marketplace that offers personalized career development for scientists.

Based on the needs identified for Syn2Psy ESRs, the Supervisory Board approved a training programme that included eight online group sessions (four in 2021 and four in 2022) and one individual session for each ESR in a topic of their choice.

The four group sessions chosen by the ESRs for 2021 were as follows: How to write a paper; Preparing for a scientific conference; Project management; and Wellbeing & work-life balance.

The majority of the ESRs found the workshops to be relevant to their future and reported that it contributed to enhance their skills, rating the overall training sessions with 4/5. Of note, the "Wellbeing and work-life balance" was the workshop most appreciated by the students.

Individual sessions are ongoing.

3.6 Online psychology sessions

COVID-19 pandemic has had a major effect on mental health, globally. Within academia, early career scientists have been considered particularly vulnerable. In the case of Syn2Psy, several ESRs have manifested the need for mental health support.

As a response to this need, the Supervisory Board approved a series of online webinars, by the clinical psychologist and former researcher in Neuroscience, Desiree Dickerson. These webinars are aimed at providing the ESRs with tools to identify and prevent mental health issues and, ultimately, to be more resilient when facing the challenges of being a PhD student in the current circumstances.

Topics chosen by the ESRs: Navigating Uncertainty; Motivation and procrastination; Perfectionism and imposter syndrome; Rethinking productivity and Navigating supervision.

The series of webinars is ongoing.

4. Dissemination activities

4.1 Conferences

During this third year of the project, several ESRs had the opportunity to present their results in international conferences, both in-person and online, in the form of posters and oral communications.

Table IV: Conferences attended by the ESRs during M25 to M36.

ESR	Conference	Date	Place	Poster/ Oral	Title
ESR1 Orsolya Antal	The Synaptic Dimension of Brain Disorders – "Current Trends in Biomedicine" workshop	4-6 Oct 2021	Baeza, Spain	Poster + short talk	Cognitive behavioural deficits in a knock-in mouse model with a human schizophrenia-associated mutation in the CACNG2 gene
	XVII Meeting of the Portuguese Society for Neuroscience	1-3 Dec 2021	Coimbra, Portugal	Poster	Cognitive behavioural deficits in a knock-in mouse model with a human schizophrenia-associated mutation in the CACNG2 gene
ESR2 Alessandro Chioino	49th Meeting of the European Brain and Behavior Society	4-7 Sep 2021	Lausanne, Switzerland	Poster	Impact of Mfn2 in the Nucleus Accumbens on motivated behavior and underlying neurobiological mechanisms
	FENS regional meeting	25-27 Aug 2021	online	Poster	Impact of Mfn2 in the Nucleus Accumbens on motivated behavior and underlying neurobiological mechanisms
	Meeting of Neuroleman Network school	7 May 2021	online	Poster	Impact of Mfn2 in the Nucleus Accumbens on motivated behavior and underlying neurobiological mechanisms
ESR3 Giuseppe Cammarata	XVII Meeting of the Portuguese Society for Neuroscience	1-3 Dec 2021	Coimbra, Portugal	Poster	Neurodevelopmental effects of valproic acid exposure in human brain organoids
ESR7 Elisa Corti	XVII Meeting of the Portuguese Society for Neuroscience	1-3 Dec 2021	Coimbra, Portugal	Poster	Fragile X Mental Retardation Protein mediates BDNF-induced increase of synaptic NMDA receptors content
	RNA Mechanisms and Brain Disease	20-23 Oct 2021	Rungstedgaard, Denmark	Poster	Fragile X Mental Retardation Protein mediates BDNF-induced increase of synaptic NMDA receptors content

ESR10 Laura Upton	Scientific days ED3C	8-9 Apr 2021	online	Poster	Altered prefrontal cortex assembly in mice deficient in the autism-associated gene Glra2
ESR10 Flavio Tomasi	Scientific days ED3C	8-9 Apr 2021	online	Poster	Assessing the endocannabinoid system as a therapeutic target in autism using Shank3-deficient mice
ESR11 Ágata Silvan	NeuroGrad Winter School	20 Jan 2022	online	Oral	Gating modulation of SK channels in the Thalamic reticular Nucleus
	ENCODS	4-6 Jun 2021	online	Poster	Gating modulation of SK channels in the Thalamic reticular Nucleus
ESR13 Loredana Cumpana	49th Meeting of the European Brain and Behavior Society	4-8 Sep2021	Lausanne, Switzerland	Poster	The modulation of thalamic reticular nucleus neurons by corticotropin-releasing hormone
	FENS Regional Meeting 2021	25-27 Aug 2021	online	Poster	The modulation of thalamic reticular nucleus neurons by corticotropin-releasing hormone
	Neuroleman Network and Doctoral Schools Annual Meeting 2021	7.05.21	online	Poster	The modulation of thalamic reticular nucleus neurons by corticotropin-releasing hormone
ESR14 Vanesa Salazar	Social Behavior Symposium - Alicante	7-9 Oct 2021	Online	Poster	Reduced social interaction in two rat models of SYNGAP1 haploinsufficiency
	5th Annual SIDB (Simons Initiative for the Developing Brain) Retreat - (Hybrid)	30-31 Aug 2021	Edinburgh Hybrid	Poster	Reduced social interaction in two rat models of SYNGAP1 haploinsufficiency

4.2 Publications

During the third year of the project (M25 to M36), no original research articles or reviews have been published, however, one publication has been accepted in the journal Frontiers for Young Minds (see below: 5.1 Frontiers for young Minds).

5. Communication Activities

One of the major topics in the Syn2Psy project is the creation of proper science contents for multiple audiences. Within Syn2Psy, the ESRs have the opportunity to develop transferable skills in outreach, communication and public engagement, as well as in bridging the science-society gaps through several initiatives, including the creation of scientific articles for children (journal Frontiers for Young Minds); the development of a script for a theatre play on neuropsychiatric disorders; opinion articles for the general public (blog-posts); the creation of short-videos describing each of the fourteen projects (SimpleSci series) and the participation in scientific events that target the general public.

5.1 Frontiers for Young Minds

With the goal of writing communication articles shaped for younger audiences, Syn2Psy submitted seven abstracts to the journal Frontiers for Young Minds (FYM), which were all deemed suitable for publication in the Neuroscience section of this journal.

The ESRs, organised in seven groups, are currently in the process of writing the full articles, with the support and expertise of Syn2Psy's partner, PIN. One article has been endorsed for publication.

Table V: Articles for the journal Frontiers for Young Minds: groups, titles and status.

Groups	Title	Status
ESR2 Alessandro Chioino ESR13 Loredana Cumpana	Reaching for the top: the role of mitochondria.	Ongoing
ESR9 Laura Upton ESR10 Flavio Tomasi	Fantastic beasts and where to find them: how can we use animals to study neuropsychiatric disorders?	Ongoing
ESR7 Elisa Corti ESR12 Manuela Rizzi	The play-doh brain: shapeable neurons make you learn!	Soon to be submitted
ESR1 Orsolya Antal ESR11 Ágata Silván ESR4 Veronica Villeri	Schizophrenia - what causes hallucinations and distorted thinking?	Ongoing
ESR3 Giuseppe Cammarata ESR5 Marcos Sintes	How to build a mini human brain**.**	Ongoing
ESR6 Daniel Hunter ESR14 Vanesa Salazar	A brain composed by multilingual neurons: A story of coded messages and crosstalking.	Ongoing
ESR8 Elizabeth Brockman	Exploring the living mouse brain using 2-photon microscopy	Endorsed for publication

5.2 Script for theatre play

Under the guidance of Syn2PSy partner Marionet, Syn2Psy fellows are in the process of writing a script for a play on the activities of Syn2Psy, to be performed at the Syn2Psy final meeting. Several group sessions have been held, during which the ESRs identified the characters, defined the main scenes and developed part of the script. Work on the full-script is ongoing.

5.3 Blog-posts

The blog-post area of the website has the purpose of allowing the ESRs to share their thoughts regarding science, their projects and/or the PhD experience in an informal and personal point of view. During this third year of the project, two posts were added to the blog: "Does free will exist?" by Elisa Corti and "The ethical emancipation of human neurodevelopmental studies" by Giuseppe Cammarata.

5.4 SimpleSci series

The SimpleSci Series is a collection of short-videos with the goal of describing, in a simple and attractive manner, each of the fourteen projects of this consortium. The target audience is the general public. The videos are created in a way that allows viewers with little to no scientific background to understand at least part of the contents, thus getting a general idea of what the main unanswered questions are, and the kind of research being done in the field of neuropsychiatric disorders. Furthermore, these videos also include a few elements for a general public with more scientific background or scientific interest.

Through the release of this video series, Syn2Psy also aims to promote the consortium and the project's mission, to create awareness about neuropsychiatric disorders and the need for research in this domain, as well as to contribute to improvement of the population's scientific knowledge.

Five episodes have been released, two of them during this third year of the project. They can be found on the Syn2Psy website and on the project's social media: Twitter - @Syn2Psy, Instagram - @msca_syn2psy, Facebook - @syn2psy and Youtube – syn 2psy.

Table VI. SimpleSci episodes released during M25 to M36.

SimpleSci Episode	ESR
Episode 4	ESR7 Elisa Corti
Episode 5	ESR2 Alessandro Chioino

5.5 Engagement with the public

An important aim Syn2Psy is to increase awareness of the progress and benefits of brain research by engaging with various audiences in European and global campaigns such as Brain Awareness Week and the European Researchers' Night.

Within the framework of the Brain Awareness Week, Giuseppe Cammarata and Elisa Corti participated in the online event *AperiNeuro: fai la tua domanda!* (AperiNeuro: ask your question!), organised by *Biologi per la Scienza* (Biologists for Science), a well-known Italian website dedicated to popularisation of science. The fellows participated in this event with the publication of an article in Italian and subsequent discussion with questions from the public. The topics of the articles and discussion were respectively "Organoidi di cervello – L'emancipazione etica degli studi sullo sviluppo cerebrale umano" (Brain organoids – The ethical emancipation of studies on human brain development) and "Ma la libertà di scelta esiste?" (But, does free will exist?).

Giuseppe Cammarata, Elisa Corti and Orsolya Antal also participated in the local European Researchers Night, in Coimbra, held in September 2021. The fellows hosted a stand with activities that aimed at promoting knowledge about neuroscience and the impact of climate change on mental health. The activities included the use the hardware Human-Human interface (Backyard Brains) with the goal of explaining how neurons can communicate with each other; and a fishing game for children associated to a *True of False* quiz for both children and adults, with statements that relate environment and mental health.

6. ESR4 recruitment

After the resignation of Diogo Soares in November 2020, and in order to resume promptly ESR4 project, as described on Annex 1 Part B of the Grant Agreement, ESR4 position was opened for applications. Details of the position announcement can be found in the document "ESR4: Diogo Soares' resignation and plan for ESR4 project", submitted via formal notification on the EC Portal in January 2021.

The recruitment procedures were carried out as follows:

The position was opened on the CNRS website on November 23rd (2020). At first, until December 14th (2020) and then, due to the reduced number of suitable candidates who applied, the deadline was

extended until January 31st (2021). It was further advertised through EURAXESS, Era Careers, FENS job market and mailing lists.

108 applications were received. The applications where pre-screened based on the following criteria: 1) Mobility: non-residence in France for more than 12 months in the past 3 years; 2) Adequate knowledge in Neuroscience. After this pre-screening, 8 candidates were retained and the 8 of them were invited for an interview. One of the candidates did not respond to the invitation.

Evaluation forms were produced for 7 candidates. The interviews took place during the first week of February and the final candidate had a second interview on February 8th (2021).

Final ranking of the interviewed candidates: 1st Veronica Villeri; 2nd Guglielmo di Molfetta; 3rd Olivia Romera Rincon; 4th Enrico Milizia; 5th Chiara Ballan; 6th Vasiliki Spatharioti; 7th Paritosh Jaiswal.

The Syn2Psy Research Coordination Committee analysed the application documents of the first ranked candidate, Veronica Villeri and approved this choice. The documents included the Evaluation Form, the Eligibility check table, the Application Documents (CV and Motivation Letter) and the Recommendation Letter.

Veronica Villeri is a Syn2Psy fellow ESR4 since May 2021.

7. ESR5 Marcos Sintes change in supervision

Marcos Sintes, ESR5, originally under the supervision of Thomas Knöpfel, was transferred to the group of Vincenzo De Paola, both at the department of Brain Sciences at Imperial College London (ICL).

This decision was taken and mandated by the Head of the Department of Brain Sciences at ICL after an internal investigation.

The Syn2Psy Supervisory Board (SB), as the ultimate decision-making body of the consortium agreed to abide to ICL's decision and transfer Marcos Sintes, and the corresponding ESR5 funds, to Vincenzo De Paola's group, who accepted to supervise the fellow.

A document with all the details of this process, including all the steps that led to the transfer, new project description and new secondment plan is currently under preparation and will be submitted via formal notification on the EC Portal.

8. Final Remarks

This third year of the Syn2Psy project was marked by the aftermath of COVID-19-related restrictions and the attempt of Syn2Psy fellows to recover from the delays accumulated over these two years of pandemic. Despite these setbacks, the implementation of the action has not been affected, Syn2Psy is on the right track and moving forward.