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Annual Progress Report 2 (M13-M24)

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1. Document Information and Introduction

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Introduction

This document corresponds to the second Syn2Psy annual progress report, in which the activities carried out during the second year of this MSCA-ITN are described. Hereby, we provide information on the scientific and training progress of the Early Stage Researchers (ESRs), and of the action as a whole, in the light of a year strongly affected by the SARS-CoV-2 pandemic and subsequent confinement. In this report, we give details regarding the impact of the confinement on the ESRs' training and projects' progress, and describe the changes implemented in the network-wide events to adapt to the new circumstances. Furthermore, we describe the deviation from the original work plan, included in the Grant Agreement (GA) amendment, recently submitted.

We also provide information regarding Diogo Soares' resignation from ESR4 position and the steps taken to continue the work foreseen for ESR4 project, in order not to jeopardise the implementation of the action as per Annex I. Finally, we report on the communication activities performed during the second year and we end by a final comment regarding the implementation of the action.

2. Impact of SARS-CoV-2-related lockdown on the implementation of the action

This second year of the implementation of the action was marked by the SARS-CoV-2 pandemic and subsequent confinement measures, including a complete lockdown for an extended period, cancellation of events and impossibility to travel. This situation had a major impact on the projects' progression, on the ESRs' training and on the network-wide events.

2.1 Impact on ESRs' projects' progression and training

In March 2020, the ESRs faced the unprecedented situation of strict lockdown, during which they were prevented from going to the laboratories for 43 to 106 days (Table 1).

This represented a major disruption in the lab experiments, which are key to the ESRs' project progress, especially during the first year of their PhD. The majority of the ESRs had their working plan delayed and one ESR temporarily changed the research plan to adapt to the new circumstances. Besides the direct delay, some ESRs also faced an extra delay, caused by the additional waiting time needed to obtain the proper number and age of animals, upon re-opening of the animal facilities.

Some PhD courses and other training activities were postponed, including network schools 2 and 3, initially planned for June 2020 and January 2021, now planned to mid/late 2021 (see 2.2).

During this time, the ESRs worked on the aspects of the projects which were possible to carry out in confinement, such as data and image analysis, literature research, experiment planning, preparation of the thesis proposals, as well as work on the abstracts to submit to the journal Frontiers for Young Minds (FYM) and on the video scripts for the SimpleSci series. In terms of self-driven professional development, some of the ESRs attended virtual international conferences, joined webinars and took free online courses. Others used the time to do literature search in view of writing a review article.

Both ESRs and Principal Investigators (PIs) made considerable efforts to mitigate the impact of the quarantine. The mitigation measures included remote supervision and remote lab meetings (Table 1). Nonetheless, there is a general consensus that under the circumstances of not having access to the laboratories and other facilities, the measures that could be taken to mitigate the impact on the ESRs research and training, were rather limited.

Also, to alleviate the consequences of the postponement of the network-wide events, an online PhD day and an online private meeting between the Project Officer (PO) and the ESRs were organised (see 2.4 and 2.5).

In conclusion, despite the efforts to use this quarantine time to work on the research projects and complement training, the majority of the Syn2Psy projects were delayed as compared to the initial plan. The implementation of the action was not affected, nevertheless it currently has a substantial delay.

For more details regarding the impact of the confinement in the ESRs projects and training, including a description of progress by each ESR and PI, refer to the document "Syn2Psy report impact of confinement in research and training", submitted via formal notification on the EC Portal in August 2020.

Table 1: Overview of the impact of compulsory confinement on the implementation of the action. Detailed view on the time spent in compulsory confinement, impact on project progression and training; and mitigation measures taken.

ESR	Days in confinement	Impact on research	Change in working plan	Impact on courses	Outcome courses	Remote supervision	Remote lab meetings
ESR1 Orsolya Antal	>43	Yes	Delay	Yes	Cancelled/ Postponed	Yes	Yes
ESR2 Alessandro Chioino	>45	Yes	Delay	Yes	Cancelled/ Postponed	Yes	Yes
ESR3 Giuseppe Cammarata	>43	Yes	Delay	Yes	Postponed	Yes	Yes
ESR4 Diogo Soares	>69	Yes	Delay	Yes	Postponed	Yes	Yes
ESR5 Marcos Sintes	85	Yes	Delay	No	n/a	Yes	Yes
ESR6 Daniel Hunter	>67	Yes	Delay	Yes	Postponed/ Online	No	Yes
ESR7 Elisa Corti	>62	Yes	Delay	Yes	Cancelled/ Postponed	Yes	Yes – last 4 weeks
ESR8 Elizabeth Brockman	>56	Yes	Delay	No	n/a	Yes	Yes
ESR9 Laura Upton	>63	Yes	Delay	Yes	Postponed	Yes	No
ESR10 Flavio Tomasi	76	Yes	Delay	No	n/a	Yes	No
ESR11 Ágata Silván	98	Yes	Delay	No	n/a	Yes	Yes
ESR12 Manuela Rizzi	>98	Yes	Project temporary change	Yes	Online	Yes	Yes
ESR13 Loredana Cumpana	56	Yes	No	No	n/a	Yes	Yes
ESR14 Vanesa Salazar	>106	Yes	Delay	No	n/a	Yes	Yes

2.2 Impact on network-wide events

Network School 2, initially planned for Paris in June 2020, was postponed to the end of 2021, to be held on-site if the pandemic situation allows. If this is not the case, it will be held remotely on similar dates.

Mid-term meeting, initially planned for Paris in June 2020, then rescheduled to May 3-4 (2021) in Copenhagen, will be held online.

Network School 3, initially planned for January 2021 in Copenhagen, then rescheduled to May 5-7 (2021) in Copenhagen, will be held online on similar dates.

2.3 Impact on deliverables and milestones

The postponement of all network-wide events planned for 2020 and early 2021, led to the modification of the deadlines for several deliverables and milestones. Table 2 represents the deliverables submitted M13 to M24 and Tables 3 and 4 depict (respectively) the deliverables and milestones postponed due to SARS-CoV-2 related confinement measures.

Table 2: Deliverables submitted M13 to M24.

WP	Deliverable	Title	Lead Beneficiary	Receipt Date
WP4	D4.8 D37	Report on PhD courses	CNC	07-09-2020

Table 3: Deliverables postponed due to SARS-CoV-2 related confinement measures.

WP	Deliverable	Title	Lead Beneficiary	Deadline modification included in GA amendment
WP4	D4.3 D25	Report on network school 2	CNRS	Postponed from 30 Apr 2020 to 29 Feb 2022
WP4	D4.4 D26	Report on network school 3	H. Lundbeck AS	Postponed from 28 Feb 2021 to 31 Jul 2021
WP6	D6.4 D30	Report for the intermediate Syn2Psy meeting	CNC	Postponed from 31 Aug 2020 to 31 Jul 2021

Table 4: Milestones postponed due to SARS-CoV-2 related confinement measures.

Milestone	Title	Lead Beneficiary	Due date (in months) modification included in GA amendment
MS7	Syn2Psy intermediate meeting	CNC	Postponed from month 18 to 27
MS8	Project check	CNC	Postponed from month 15 to 27

2.4 Online PhD day

Due to the cancellation of all the Syn2Psy events planned for 2020 and early 2021, and given the uncertainty regarding traveling in the months that followed, the consortium members agreed to participate in an online PhD day (October 5th 2020, Zoom) to promote scientific discussion and interaction between the consortium members, as well as to give the opportunity to the ESRs to receive scientific feedback.

On this occasion, the ESRs described their progress (including training) in a 15 min presentation, followed by 15 min of discussion. 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 this event was fruitful and constructive.

The Training and Doctoral Studies Committee (TDSC) had an active role in assessing the ESRs' progress (based on the relevance of the scientific question, pertinence of experimental approach, project progress and training) and had the responsibility to prepare individual evaluation reports with feedback and recommendations, which were sent to each ESR.

2.5 Online private meeting PO-ESRs

For the same reasons described on 2.3, a remote meeting between the Project Officer (PO) and the ESRs, originally planned for the Mid-term meeting, was held on October 20th 2020.

This was a confidential and private meeting between the PO and the ESRs only, with the goal of ensuring that administrative and supervision aspects of the project are appropriately implemented. The PIs were allowed to join the introductory part of this meeting, as well as the Project Coordinator and Project Manager, who were also requested to stay for a feedback session.

During the feedback session, the PO reported the fellows to be happy with the projects, the institutions and their work. The issues raised regarded secondments, allowances, teaching hours and the impact of the current pandemic on their projects.

3. Grant Agreement amendment: projects and secondments

During the first year of the implementation of the action, several beneficiaries requested to make modifications to the projects and/or secondments, which called for an amendment to the GA. The modifications in the network-wide events and the respective deliverables and milestones due dates described on 2.2 and 2.3, were also included in this amendment.

The changes in the direction of the projects were due to the natural progression of science, resulting from the completion and publication of the previously proposed projects and/or from new ideas being generated following the results of the previously proposed projects. The new projects are slight deviations from the original proposed projects, however, they still are part of the Syn2Psy topic and they do not affect the previously proposed ethical issues, work-packages, milestones, collaboration between ESRs, beneficiaries and partner organisations.

Similarly, the changes to secondments duration and/or host institution were agreed between ESRs and Principal Investigators as being more suited to the career development plan of the students and do not affect the implementation of the action. Importantly, the changes did not result in an exchange from academic partner to non-academic nor vice versa.

Overview of the changes requested by the beneficiaries:

Table 5: Changes in projects and secondments. Detailed view on the altered secondments (Modified secondment), comparing to the original proposal (Original secondment).

	Project change	Secondment change	Original secondments	Modified secondments
ESR1 – Orsolya Antal				
ESR2 – Alessandro Chioino	X	X	L. Groc - 4 months T. Knopfel - 6 months	L. Groc - 3 months E. Osterweil - 4 months
ESR3 – Giuseppe Cammarata		X	P. Kind/E.Osterweil – 6 months	P. Kind/E. Osterweil – 3 months CHUC – 3 months
ESR4 – Diogo Soares		X	AL. Carvalho – 3 months Eurotrials-CTI – 3 months	AL. Carvalho – 2 months Eurotrials-CTI – 2 months
ESR5 – Marcos Sintes				
ESR6 – Daniel Hunter		X	CHUC – 3 months	Lundbeck – 3 months
ESR7 – Elisa Corti		X	Lundbeck – 4 months V. De Paola – 6 months	Lundbeck – 1 month V. De Paola – 9 months
ESR8 – Elizabeth Brockman		X	D. Choquet/L. Groc – 4 months CHUC – 3 months	D. Choquet/L. Groc – 4 months CHUC – 0.5 months Lundbeck – 2.5 months
ESR9 – Laura Upton				
ESR10 – Flavio Tomasi		X	Eurotrials-CTI – 3 months	Eurotrials-CTI – 1 month
ESR11 – Ágata Silván	X	X	AL. Carvalho – 4 months T. Knopfel – 4 months	C Sandi – 3 months P. Kind/E. Ostwerweil – 4 months
ESR12 – Manuela Rizzi	X			
ESR13 – Loredana Cumpana	X	X	T. Knopfel - 6 months	T. Knopfel – 4 months
ESR14 – Vanesa Salazar	Change in project title only	X	Eurotrials-CTI – 3 months	Eurotrials-CTI – 2 months

Changes in projects requested by the beneficiaries included in the GA amendment: original versus modified project descriptions.

ESR2 – Alessandro Chioino (Host institution - BMI-EPFL; Host supervisors - Carmen Sandi, Simone Astori).

Original project description	Modified project description
Role of Neuronal Cell Adhesion Molecule polysialylation in neurodevelopmental trajectories of psychopathology	**Role of mitofusins in neurodevelopmental trajectories of psychopathology**
Objectives: Emerging evidence from psychiatric genomics indicates common trajectories for multiple neurodevelopmental disorders. Dysfunctions related to the Neuronal Cell Adhesion Molecule (NCAM) represent a trigger of multiple pathophysiological routes, as mouse models of NCAM deficit exhibit symptoms of both ASD and schizophrenia. In particular, deletion of the NCAM-related sialyltransferase ST8Sia2, which is highly expressed in the brain during the prenatal and early postnatal periods, produces a schizophrenia-like phenotype and alterations in the social domain that are typical as well for ASD, such as reduced sociability and increased aggressive behaviours. We recently found that ST8Sia2 ablation impairs the connectivity between the frontal cortex and the amygdala, key brain regions involved in emotional, social and aggressive behaviour. At the synaptic level, *St8sia2* deletion decreases the expression of N-Methyl-D-Aspartate (NMDA) receptors and impairs NMDA receptor-mediated synaptic plasticity at cortical inputs to the amygdala. This project proposal aims at testing the role of reduced NCAM polysialylation during early development in the emergence of specific schizophrenia and ASD-related behavioural deficits - caused by alterations in ST8Sia2 expression in the amygdala and its main target regions, such as the prefrontal cortex (PFC).	**Objectives**: Emerging evidence points to a critical role of mitochondrial dysfunctions in the pathophysiology of psychiatric disorders, including anxiety and depression, but the mechanisms mediating mitochondrial effects remain understudied. Recent findings highlight reduced brain expression of the mitochondrial GTPase Mitofusins 1 and 2 (Mfn1 and Mfn2; molecules critically involved in mitochondrial fusion and other functions) in depression and stress-related psychopathologies. The nucleus accumbens is a critical hub for motivated behavior and its function is altered in anxiety and depression. This project will use mouse genetics and gene delivery to examine the contribution of Mfn deficiency in specific accumbal medium spiny neurons for the production of motivated behaviors related to anxiety and depression. The final goal is to identify the circuitry engaged in dysfunctional behaviors produced by impaired mitochondrial dynamics in specific nucleus accumbens neurons and uncover relevant treatment targets for neuropsychiatric alterations.
Expected results: **Task 2.1 Excitation/inhibition (E/I) balance in the amygdala and PFC:** *In vitro* electrophysiological recordings will be performed to assess whether *St8sia2* deletion causes elevations in the E/I balance in the amygdala and PFC, a hallmark of network alterations linked to both schizophrenia and ASD. At early developmental stages, we will specifically examine the switch in the driving force of $GABA_AR$-mediated transmission, as an impairment in the shift from depolarizing to hyperpolarizing GABAergic currents, with a resulting E/I imbalance, has been recently linked to ASD. These experiments will be complemented with molecular analyses of the expression level of genes and proteins involved in excitation/inhibition, including neuroligins and other synaptic markers. **Task 2.2 Alterations in amygdala-cortex communication:** NMDA receptor-mediated transmission and plasticity in the lateral amygdala is reduced at cortical synapses, suggesting that behavioural impairments might be associated with input-specific deficits in synaptic plasticity at the entry site of the amygdala complex. As a further level of analysis, we will focus on the amygdalofugal projections to the cortex, and in particular on the glutamatergic afferents from the basolateral nucleus to the PFC. To selectively stimulate inputs from the amygdala in acute slices we will use optogenetic tools: upon viral expression of ChannelRhodopsin2 in the basolateral amygdala, AMPA receptor- and NMDA receptor-mediated transmission in PFC neurons will be evoked by brief LED flashes. In addition to AMPA/NMDA ratio and NMDA receptor-dependent plasticity, we will examine local feedforward inhibition in the PFC, which has been shown to be strongly driven by amygdalar inputs, and might contribute to imbalanced E/I in *St8sia2* KO mice. **Task 2.3 Region selective ablation and rescue of ST8Sia2 function***:* We will manipulate ST8Sia2 selectively in the amygdala – with a preferential targeting of the lateral/basolateral nuclei – and in the PFC by infusing viral constructs inducing a loss- and a gain-of-function of ST8Sia2 in wild-type and in *St8sia2* KO mice, respectively. To align our interventions with the endogenous perinatal expression of ST8Sia2, these manipulations will be performed at early developmental stages, through *in utero* electroporation of the viral construct. At adulthood, behavioural analyses of schizophrenia- and ASD-related phenotypes will unravel the impact of ST8Sia2 – and, thus, NCAM levels – in the aforementioned brain regions in the development of these pathologies. Molecular analyses (see Task 1) and *in vitro* electrophysiological recordings will be implemented to verify successful ablation/rescue of ST8Sia2 and the resulting changes in NMDA receptor-mediated transmission.	**Expected results**: **Task 2.1 identification of nucleus accumbens efferent targets for psychopathological impact of Mfn loss of function.** Fiber photometry and calcium imaging through mini-endoscopes will be applied in vivo to identify target brain regions involved in the impact of Mfns loss of function and psychopathological alterations (i.e., depression-like behaviors, dysfunctions in social behavior). To this end, we will use Mfn2loxp/loxp mice and Mfn1loxp/loxp mice combined infusion of adeno-associated virus (AAV) expressing Cre for specific accumbal cell types. Given the role of accumbal-ventral pallidum projections in the regulation of motivated behaviors, we will focus on this projection to disentangle the role of dopamine 1 (D1) and dopamine 2 (D2) receptor-containing neurons in the regulation of the different behaviors under study. These experiments will be followed up by optogenetic approaches to establish the causal involvement of specific projection neurons. Based on the preliminary evidence, we predict that reduced Mfn1 or Mn2 in the NAc will enhance depression-related behavior in a cell type specific manner. Although unknown, we hypothesize the involvement of accumbal-ventral pallidum projections in these effects. **Task 2.2 Excitation/inhibition (E/I) balance in target areas following accumbal Mfn loss of function in specific cell types.** The impact of Mfn deficiency in specific cell types for E/I balance will be monitored *in vivo* and its synaptic consequences *in vitro*. These experiments will be complemented with molecular analyses of the expression level of genes and proteins involved in excitation/inhibition, including neuroligins and other synaptic markers. **Task 2.3 Impact of accumbal Mfn deficiency for the regulation of single NMDA receptor dynamics.** Preliminary work indicates that accumbal Mfn2 deficiency leads to a reduction in medium spiny neuron dendritic complexity and reduced excitatory inputs. Here, we aim at understanding how Mfn2 loss of function in specific cell types may affect NMDA receptor dynamics. To this end, we will track NMDAR subtypes at the surface of accumbal medium spiny neurons in accumbal acute brain slices using single nanoparticle (Quantum Dot) tracking. Subsequently, we will examine the implication of specific findings by manipulating NMDA receptor surface dynamics in Mfn2 deficient mice in accumbal D1 or D2 receptor-containing neurons and assess the impact of this manipulation in neurotransmission and psychopathological (i.e., depression-like and dysfunctional social) behaviors.

ESR11 - Ágata Silván (Host institution - H. Lundbeck A/S; Host supervisor - Kjartan Frisch Herrik).

Original project description	Modified project description
Improving cognition by pharmacological modulation of fast spiking interneurons	**Investigating the excitation/inhibition (E/I) imbalance hypothesis of neuropsychiatric disorders in the cortico-thalamic system with electrophysiological methods**
Objectives: Fast spiking interneurons have an important function for general inhibitory neuronal input and are essential for the synchronization of connected circuits in the brain. Dysfunctional interneurons have been associated with a number of brain disorders involving general hyperactivity, such as epilepsy and cognitive impairment. The overall aim of this project is to understand the role of fast spiking interneurons in animal model with chronically compromised interneurons, obtained either by pharmacology, optogenetics, by provoked epilepsies or in a genetically-modified model of schizophrenia (collaboration with Paulo Pinheiro/Ana Luisa Carvalho, CNC). A second aim is to identify pharmacological targets that could constitute new innovative entries into treatment of cognitive deficits associated to reduced interneuron function. To increase translatability to human conditions, chronic models of post-status epilepticus will be applied. The experimental assessment of the model will primarily be based upon electrophysiological recordings from prefrontal cortex and hippocampus. Experiments will include *ex vivo* slice recordings and *in vivo* single unit recordings, and will be conducted under normal physiological non-diseased control conditions and subsequently at progressive stages during repeated exposure to compounds like PCP or repeated episodes of status epilepticus. Remodelling as a consequence of the hyperexcitability will be addressed with electrophysiology and molecular biology/biochemical approaches to gain insight into the changes of protein expression as a consequence of the disease. The purpose is dual: to understand the underlying biology of cognitive impairment and to identify possible new targets for symptomatic treatments or disease modification by delaying or preventing hyperexcitability-induced remodelling. Since a common co-morbidity of epilepsy is cognitive impairment, the direct behavioural consequences of the pharmacological-induced and epileptogenic-induced phenotype will be investigated. We will investigate the cognitive consequences of the induced hyperexcitability using various behavioural assays of cognition.	**Objectives**: Understanding neural circuit function has recently been suggested as a pathway for developing diagnostics and therapeutics for neuropsychiatric disorders, as it is an intermediate level that links genes to behaviour (Krol et al., 2018). From this circuit perspective, it has been repeatedly hypothesised that neuropsychiatric disorders as schizophrenia (SCZ) and autism course with an imbalance between excitatory and inhibitory (E/I) networks (Gao and Penzes, 2015). Although the underlying mechanism remains elusive, an increased interest has been directed towards a dysfunction in the GABAergic interneurons (Ferguson and Gao, 2018). This is particularly important in the context of thalamocortical circuits, which play a fundamental role in sensory gating, memory consolidation and sleep (Manoach et al., 2016; Manoach et al., 2019). Importantly, the sleep spindle activity that is driven by the thalamic reticular nucleus (TRN) has been reported to be markedly reduced in schizophrenic patients and suggested to be an endophenotype of SCZ (Manoach et al., 2016; Merikanto et al. 2019). This reduction was correlated with thalamic hyperconnectivity in fMRI studies (Baran et al., 2019). Accordingly, postmortem studies reveal TRN abnormalities reductions in parvalbumin expressing neurons (PV) (Steullet et al. 2018). A consequent impairment of TRN-mediated inhibition of thalamocortical neurons could decrease the rebound bursting that generates spindles and impair the gating of information flow to the cortex. Treatments that target TRN interneurons would, therefore, be promising therapeutic strategies to restore E/I balance and could be relevant in the context of neuropsychiatric disorders as SCZ. The present PhD project aims to study the circuits and mechanisms that govern E/I balance in cortico-thalamic and thalamo-cortical networks, focusing in the interplay between TRN, mediodorsal (MD) thalamus and medial prefrontal cortex (mPFC). More specifically, the role of TRN neurons and ion channels involved in pacing thalamocortical activity will be addressed. We hypothesize that changes in TRN activity will lead to alterations in E/I balance in the thalamus and neocortex, with a strong implication in sleep and sleep-dependent memory consolidation. Using a variety of electrophysiological methods and pharmacological and chemogenetic tools, the E/I balance will be studied in both brain slices and awake and freely moving rodents. With access to both reduced (slice) and intact (freely moving) systems, the PhD student gets a unique opportunity to study E/I-mechanisms at a broad range of levels. The ultimate goal is to advance our understanding of the importance of upholding an E/I balance in the cortico-thalamic system and study both single neurons as well as population activity. The electrophysiological biomarkers studied in the freely moving rats, may further translate to human electroencephalographic and polysomnographic markers. The new Lundbeck supervisors as well as the supervisor from University of Copenhagen are experts in ex vivo slice electrophysiology (Hougaard, Kohlmeier), ion channel pharmacology (Hougaard, Kohlmeier, Herrik), rodent in vivo electrophysiology (Herrik) and polysomnography (Kohlmeier).
Expected results: Establishment of animal models with compromised interneuron function; •Identifying new targets that enables in vitro, ex vivo and in vivo modulation of interneurons; Elucidating whether pharmacological activation of fast spiking interneurons can increase cognitive performance; Understanding of cellular changes associated to decreased fast spiking interneuron function; Translational understanding of how modified interneuron function affects animal behaviour	**Expected results**: This project is experimentally designed in a comprehensive manner, where thalamocortical system will be studied at single neuron and ensemble activity levels. Besides, by combining in vivo and in vitro electrophysiological approaches, E/I balance will be studied in both reduced (brain slices) and intact (freely moving rats) systems. **Task 11: Identify ion channels as potential pacemakers of thalamocortical activity and its impact on E/I balance**. Pharmacological tools will be used to characterise those ion channels involved in pacing thalamocortical activity. Several compounds will be tested, targeting ion-channels that could potentially mediate E/I balance in the crosstalk between TRN and thalamus. **Task 11.2: Effect of chemogenetic modulation of TRN neurons on the corticothalamic loop.** Chemogenetics will be used to selectively enhance/silence activity of TRN neurons in order to study their contribution on oscillatory rhythms and E/I balance in the mediodorsal (MD) thalamus and medial prefrontal cortex (mPFC). **Task 11.3: Impact of TRN neurons modulation in thalamocortical rhythms during sleep and sleep-dependent memory consolidation.** Electroencephalographic (EEG) and

	electromyographic (EMG) recordings will be carried out during a behavioural task (place object recognition test) that is known to involve sleep-dependent memory consolidation (extended stay at BMI-EPFL in collaboration with Simone Astori and Carmen Sandi). Polysomnographic analyses will be crucial to characterize NREM, REM and intermediate sleep epochs and the underlying brain rhythms. Pharmacological and chemogenetic tools will be used in order to identify circuits, neuronal subtypes and ion channels involved in pacing thalamocortical activity. **Task 11.4: To study the thalamocortical system and E/I imbalance in a relevant model for SCZ and TRN dysfunction.** Ultimately, modulation of sleep spindles and corticothalamic networks will potentially be studied in a transgenic rat model of the disease in collaboration with Peter Kind (extended stay at UEDIN).

ESR12 - Manuela Rizzi (Host institution - CDBS – UEDIN; Host supervisor - Emily Osterweil).

Original project description	Modified project description
Determining the role of proteostasis in *Fmr1*$^{-/y}$ and *Tsc2*$^{+/-}$ mouse models of autism	**Identifying convergent dysregulation of mRNA translation in multiple models of autism**
Objectives: Dynamic changes in synaptic strength rely on *de novo* protein synthesis and protein degradation by the ubiquitin proteasome system (UPS). Disruption of either of these cellular processes will result in significant impairments in synaptic plasticity and memory formation. Mutations in several genes encoding regulators of mRNA translation (i.e., *FMR1, TSC1/2, SYNGAP1*) and members of the UPS (i.e., *UBE3A*) have been associated with an increased risk for the development of ASD. It is possible that these mutations result in a similar imbalance in protein homeostasis (proteostasis) at the synapse. In mouse models of fragile X syndrome (*Fmr1*$^{-/y}$) and tuberous sclerosis (*Tsc2*$^{+/-}$), two of the most prevalent monogenic causes of ASD, synaptic protein synthesis is disrupted in an opposite fashion. Correcting protein synthesis by targeting mGlu5 and the associated ERK and mTOR signalling cascades will normalize a number of electrophysiological and behavioural disruptions throughout the brain, including altered hippocampal long-term depression (LTD), hyperexcitability in hippocampus and cortex, abnormal ocular dominance plasticity in visual cortex, increased susceptibility to audiogenic seizures, and deficits in contextual fear learning. This suggests that protein synthesis downstream of mGlu5 is a cellular disruption that is common to both the *Fmr1*$^{-/y}$ and the *Tsc2*$^{+/-}$ models, an insight that has consequences for designing targeted treatments. Interestingly, though the rate of protein synthesis is excessive in the *Fmr1*$^{-/y}$ brain and deficient in the *Tsc2*$^{+/-}$ brain, this does not appear to result in significant changes in overall protein accumulation. Based on this, we hypothesize that compensatory alterations in protein degradation must exist in these mutant models. Our preliminary evidence suggests that significant changes in UPS activity are present in both the *Fmr1*$^{-/y}$ and *Tsc2*$^{+/-}$ hippocampal slices. The goal of this project is to determine the extent of these changes, and determine whether they contribute to electrophysiological and/or behavioural phenotypes in the *Fmr1*$^{-/y}$ and *Tsc2*$^{+/-}$ mouse models of ASD. This will include (1) a thorough biochemical analysis of the kinetics of UPS function in acute brain slices, (2) analysis of stimulus-dependent localization and trafficking of UPS components in neurons and dendrites, (3) measurement of hippocampal mGluR-LTD in the presence of proteasome inhibitors (i.e., MG132) and modulators of specific E3 ligases, and (4) assessment of whether UPS modulators can ameliorate audiogenic seizures and deficits in contextual fear conditioning.	**Objectives**: Mutations in many genes that encode regulators of mRNA translation, such as *FMR1* gene encoding the translation repressor Fragile X Mental Retardation Protein (FMRP) and the *SYNGAP1* gene encoding the Ras-Erk 1/ 2 repressor SynGAP, are significant risk factors for ASD. Mutant models of these and other neurodevelopmental disorders exhibit excessive neuronal protein synthesis, suggesting the hypothesis that aberrant synaptic mRNA translation is a point of convergence for multiple genetic causes of Autism Spectrum Disorder with associated Intellectual Disability (ASD/ID). If dysregulated mRNA translation is a common contributor to ASD/ID, then the important long-term goal is to address how disruptions in local protein synthesis lead to cognitive and behavioural symptoms. An essential first step is to identify the mistranslating mRNAs that lead to altered function in brain circuits that are affected in ASD/ID models. In recent work we performed the first cell type-specific translation profiling screen of *Fmr1*$^{-/y}$ neurons using Translating Ribosome Affinity Purification and RNA sequencing (TRAP-seq, RNAseq). This study revealed over 120 mistranslating mRNAs in hippocampal CA1 neurons and identified muscarinic acetylcholine receptor 4 (M_4) as a novel target for the correction of biochemical, electrophysiological and behavioural phenotypes in the *Fmr1* $^{-/y}$ mouse. In this proposal, we will use TRAP-seq to interrogate mistranslating mRNAs in multiple circuits that contribute to pathological phenotypes in mouse models of FX and SYNGAP1 haploinsufficiency syndrome. Previous work shows that both protein synthesis and mGluR-LTD are excessive in the *Syngap*$^{+/-}$ hippocampus, similar to what is seen in the *Fmr1*$^{-/y}$ mouse and these phenotypes are corrected with mGluR5 antagonist or inhibition of Ras-ERK1/2. This correction will normalize a number of electrophysiological and behavioural disruptions throughout the brain, including altered hippocampal long-term depression (LTD), hyper excitability in hippocampus and cortex, increased susceptibility to audiogenic seizures, and deficits in contextual fear learning. In parallel to the ongoing cell-type specific approach through TRAP-seq, ESR12 will lead a new approach consistent in the establishment of a ribosome profiling protocol to monitor translation in *Fmr1*$^{-/y}$ and *Syngap* $^{+/-}$ rat models and get a new deep outlook of the translatome. This would possibly let new mistranslated RNAs emerge consistent and/or in opposite direction to what has been shown by our preliminary results. In addition, ESR12 will perform these experiments in rat models of disease. This will have high impact and will possibly enrich our knowledge about the topic. The working plan will include (1) a ribosome profiling analysis comparing the footprints derived from *Fmr1*$^{-/y}$ and the *Syngap*$^{+/-}$ rat models, (2) an assessment of the extent of mRNA mistranslation in behavioural phenotypes, performing paradigms such as fear conditioning, (3) possible validation of identified targets through means of viral injection leading to a possible rescue of the phenotype, (4) comparison of the ribosome profiling map of rat models and commercially available IPSCs derived from patients.
Expected results: **Task 12.1** Our preliminary data indicate that, similar to protein synthesis, UPS activity is disrupted in an opposing manner in the *Fmr1*$^{-/y}$ and *Tsc2*$^{+/-}$ hippocampus. ESR12 will explore the molecular mechanisms of this by	**Expected results**: **Task 12.1** Our preliminary data indicate that, TRAP-seq is a powerful approach that would allow us to identify mistranslating mRNAs in the *Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ mouse models. In this direction we would be very

determining the kinetics of UPS activity during stimulation of mGluRs and other synaptic receptors using an assay of 20S proteasome activity that has already been set up in the laboratory. The components of the UPS will also be quantified in whole hippocampus and in isolated synaptoneurosome fractions using immunoblotting. We expect that changes in proteasome activity will continue to be observed in these mutants, and this may be accompanied by alterations in the expression of specific UPS components. **Task 12.2** An important regulation of protein breakdown in neurons involves the specific recruitment of UPS components to synapses upon stimulation. It may be that disruptions in overall UPS activity arise from specific changes in the localization of the 20S proteasome or related proteins. Thus, an important part of this project will be to assess the synaptodendritic trafficking of the proteasome in both models to determine if there are differences in the recruitment of the UPS upon stimulation. A virus containing GFP-tagged proteasome will be injected into *Fmr1*$^{-/y}$ and *Tsc2*$^{+/-}$ hippocampus, and ESR12 will perform live imaging on slices prepared from these animals using 2-photon microscopy. The movement of GFP-proteasome will be imaged under basal conditions and during stimulation of group 1 mGluRs using DHPG. To determine the synaptic localization of GFP-proteasome in these neurons with and without DHPG stimulation, we will use super-resolution imaging methods. **Task 12.3** Hippocampal mGluR-LTD is exaggerated in the *Fmr1*$^{-/y}$ mutant and deficient in the *Tsc2*$^{+/-}$ mutant. Although the role of protein synthesis has been explored with respect to these phenotypes, the role of the UPS has not been established. The ESR12 will explore this by performing field potential recordings of LTD in hippocampal CA1 of the *Fmr1*$^{-/y}$ and *Tsc2*$^{+/-}$ mutants in the presence of compounds that inhibit the proteasome (i.e., MG132, lactacystin, etc.) or other components of the UPS (i.e., E3 ligases). **Task 12.4** Both the *Fmr1*$^{-/y}$ and *Tsc2*$^{+/-}$ mutants exhibit deficits in contextual fear conditioning, which can be corrected by modulators of mGlu5. If UPS disruptions contribute to changes in neural function, we expect this would have an obvious impact on this phenotype. Thus, the ESR will examine whether UPS modulators can correct this learning deficit in the *Fmr1*$^{-/y}$ and *Tsc2*$^{+/-}$ mutants. As enhanced susceptibility of audiogenic seizures is also a robust phenotype in the *Fmr1*$^{-/y}$, this will also be assessed in our study.	interested to deepen this exploration through means of ribosome profiling in a rat model of disease (*Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ rats) and parallel it to the TRAP-seq approach. ESR12 will analyse the footprints derived by ribosome profiling and align them to the relative genome to understand the exact portions that are mistranslated, hopefully confirming the preliminary results obtained by TRAP analysis in mouse models and show a conservatory pattern in other species, or coming up with new targets to be validated. **Task 12.2** Alterations in context discrimination and learned fear are notable phenotypes in both *Fmr1*$^{-/y}$ and *Syngap*$^{+/-}$ mouse models. The mechanisms underlying these disruptions have not been established, however there is a long-standing observation that learned fear and the formation of memory require new protein synthesis. Interrogating the mRNAs translated in the underlying hippocampal CA1, layer 5 mPFC, and BLA neurons during fear conditioning would therefore yield important and novel information regarding the processing of fearful memory in general. Thus, an important part of this project will be to assess mRNA translation in a learning context to see if it shows consistency. ESR12 will perform sampling at different time points after behaviour: precisely 30 minutes, 4 hours and 24 hours after conditioning. **Task 12.3** The sequencing data derived from a naïve and a learning context will show if there is a convergence in the mistranslated mRNAs and this could be the starting point for a possible target validation strategy. The ESR12 will explore this by performing in vivo injections of a shRNA against a validated target to possibly see a rescue in the fear conditioning phenotype. **Task 12.4** Provided with patient-derived hIPSCs sent from collaborators, ESR12 will run a comparative ribosome profiling analysis and possibly show a reliability with the animal model. This will possibly convey outstanding insights in research

ESR13 - Loredana Cumpana (Host institution - BMI-EPFL; Host supervisors - Carmen Sandi, Simone Astori).

Original project description	Modified project description
Interaction of prenatal stress with genetic risk for neurodevelopmental disorders	**Effect of corticotropin-releasing hormone on a thalamic hub for neurodevelopmental disorders**
Objectives: Stress at prenatal and early postnatal stages impacts neuronal development and increases the risk of developing psychopathologies. Among the molecular targets affected by prenatal stress (PNS), we have identified the Neuronal Cell Adhesion Molecule (NCAM) as a key candidate associated with both ASD and schizophrenia. A mouse model harbouring a genetic deletion of the NCAM-related sialyltransferase ST8Sia2, which is highly expressed in the brain during the prenatal and early postnatal periods, exhibits alterations in sensory gating and social behaviour typical for these pathologies. Importantly, PNS generates alterations in the connectivity between the prefrontal cortex (PFC) and the amygdala, key brain regions involved in emotional, social and aggressive behaviour. Moreover, our recent data indicate that PNS induces an immediate decrease in St8sia2 expression in the same brain areas that are mainly affected by the genetic ablation, including the amygdala and frontal parts of the brain that comprise the PFC. This project proposal aims at studying the impact of combined PNS and genetic (*St8sia2* deletion) factors during early development on the emergence of schizophrenia- and ASD-related behavioural deficits, on alterations in PFC-amygdala connectivity and their synaptic substrates.	**Objectives**: Recent literature identifies a circuit endophenotype for neurodevelopmental disorders in the Thalamic Reticular Nucleus (TRN), the principal source of inhibition to the thalamus. TRN neurons express several disorder-relevant genes identified through GWAS, such as the schizophrenia-related genes Cacna1i and Grm3, and the autism spectrum disorder-risk gene PTCHD1, suggesting that this structure may reflect a source of common vulnerability across neurodevelopmental disorders (Krol et al., 2018). Notably, SZ patients display reduced density of sleep spindles (Manoach et al., 2016), a thalamocortical sleep rhythm that is initiated by TRN cell bursting (Astori et al., 2013). Besides its recognized role as a sleep pacemaker, TRN is being increasingly implicated in sensory and cognitive processing, such as attention selection, and TRN function is found to be altered in models of pathology (Krol et al., 2018). Whether the development and function of the TRN are affected by environmental factors, such as stress, has not been addressed. Notably, TRN cells express high levels the corticotropin-releasing hormone receptor 1 (CRHR1) (Pett et al., 2000; Bender et al., 2015), but how this stress-released neuropeptide affects TRN function is fully unexplored. By combining ex vivo cellular analyses and polysomnographic recordings we will assess how the CRH system impinges of TRN excitability and thalamocortical sleep rhythms. We will further examine whether chronic exposure to CRH at early postnatal stages alters the expression of SZ- and ASD-risk genes in the TRN.
Expected results: **Task 13.1 Impact of PNS on ST8Sia2 expression and brain connectivity**: We will first extend our recent data indicating a decrease in ST8Sia2 expression induced by PNS, by exploring expression levels at different developmental periods and including molecular markers for neuronal excitation and inhibition. Wild-type mice will be exposed to PNS (restrain stress in	**Expected results: Task 13.1 Impact of CRH on TRN cell excitability:** Our preliminary data from patch-clamp experiments indicate that exposure the CRH in acute slices alters TRN cell firing, with tonic firing being promoted over burst firing. We will extend these results and explore the molecular mechanisms whereby CRH modulates TRN intrinsic excitability. We will

gestational mothers). Structural analyses of connectivity between cortex and amygdala using magnetic resonance diffusion tensor imaging (MRI-DTI) will be performed at pubertal stages and at adulthood. **Task 13.2 Impact of PNS on cortico-amygdala synaptic transmission**: Studies carried out under Task 13.1 will be complemented by an electrophysiological description of amygdala and PFC neurons. In particular, NMDA receptor-mediated transmission and synaptic plasticity will be examined at cortical inputs to the lateral amygdala – a key entry site for amygdalar processing which we found to be affected by ST8Sia2 ablation. Additionally, a behavioural characterization of mice exposed to PNS will be performed to verify the emergence of schizophrenia- and ASD-related phenotypes. We expect the gravity of these symptoms to correlate with the decrease in the expression of ST8Sia2 in the amygdala and/or PFC, as measured in post hoc molecular analyses. **Task 13.3 Impact of combined PNS and ST8Sia2 ablation on neurodevelopment**: Wild-type, *St8sia2* heterozygous and homozygous KO mice will be subjected to a protocol of PNS. At pubertal and adult stages, a behavioural characterization of schizophrenia- and ASD-related phenotypes will be performed. We expect heterozygous animals to show higher predisposition to develop psychopathological traits than wild-type mice after PNS. We will further assess whether schizophrenic and autistic symptoms will be exacerbated or occluded by PNS in homozygous KO mice. These studies will be complemented by structural and connectivity analyses and *in vitro* electrophysiological recordings of synaptic function, as described in Tasks 13.1 and 13.2. **Task 13.4 Molecular reversibility of PNS-induced alterations**: In animals exposed to PNS, we will attempt at reversing the structural/synaptic changes at prenatal stages, through *in utero* electroporation of a viral construct over-expressing the ST8Sia2 gene. Alternatively, as the latter intervention might not be compatible with PNS manipulations, at early postnatal stages we will manipulate genes involved in excitation/inhibition balance, guided by the results from Task 13.1. Our current data from *St8sia2* KO mice (see also Task 13.2) indicate that GluN2A/B subunits of NMDA receptors are main targets of ST8Sia2 deficits at cortico-amygdala connections, and, thus, might be candidates for rescuing PNS-induced alterations.	examine whether cortical and thalamic glutamatergic inputs onto TRN are affected, as well as the intrathalamic spindle rhythmogenesis. **Task 13.2 Identification of the endogenous source of CRH onto TRN:** We will use retrograde labelling through viral infections in the TRN in order to identify the population(s) of CRH-releasing cells projecting to the TRN. The expression of opto- and chemogenetic tools in the identified cell population will allow us to examine the effects of endogenous CRH release on TRN excitability. **Task 13.3 Impact of CRH on thalamocortical sleep rhythms:** We will perform polysomnographic recordings in freely-behaving animals and examine the effects of CRH on sleep rhythms by gain-/loss-of-function experiments in which the TRN-projecting CRH-releasing cells will be activated/inhibited. **Task 13.4 Impact of stress on TRN gene expression profile:** We will assess whether chronic exposure to CRH or the repetitive stress exposure during early life affects the expression of SZ- and ASD-related genes in the TRN and alter the TRN cell development and function. We will attempt CRHR1-directed pharmacology to normalize the CRH-/stress-induced phenotype.

ESR14 – Vanesa Salazar (Host institution - CDBS – UEDIN; Host supervisor – Peter Kind).
Only the project title was modified, the project and the tasks stayed unchanged.

For the details regarding the justifications for each change, refer to "Justification" on the amendment information section on the EC Portal (also included in Annex 1 of the present report).

4. ESR4: Diogo Soares' resignation and impact on the implementation of the action

On October 2020, Diogo Soares resigned from his position as Early Stage Researcher 4 at the laboratory of Daniel Choquet, under the (co-)supervision of Eric Hosy. This decision was taken by mutual agreement, the main motives being the divergent scientific views and misaligned perspectives between him and Eric Hosy.

The Supervisory Board (SB), as the ultimate decision-making body of the consortium, was given the task to decide whether the ESR4 funds should be kept where they were allocated to originally (in Daniel Choquet's laboratory), or if Diogo Soares should be given the chance to integrate another lab within the consortium, thus, transferring the funds to another Syn2Psy laboratory.

The SB decided that the funds should be kept in the laboratory they were allocated to originally, keeping Daniel Choquet's lab in the consortium and recruiting another student for ESR4.

ESR4 project will be resumed as planned on Annex 1 Part B, starting from where Diogo Soares left off, as soon as the new student is recruited (recruitment ongoing). Therefore, the recruitment of the new student for ESR4 will not jeopardise the implementation of the action as per Annex I.

Furthermore, Daniel Choquet committed to fund the successful candidate beyond the end of the MSCA-ITN.

For more details regarding Diogo Soares' resignation, including a description of the resignation process and current recruitment procedures, refer to the document "ESR4: Diogo Soares' resignation and plan for ESR4 project", submitted via formal notification on the EC Portal in January 2021.

5. Communication Activities

One of the major topics in the Syn2Psy project is the creation of proper science contents for multiple audiences, from general publics to target audiences. To fulfil this goal, the ESRs are working on communication pieces shaped for younger audiences and the general public. This includes several articles to be submitted to the journal Frontiers For Young Minds (FYM), the release of short-videos of their individual projects (SimpleSci series) and blog-posts for the website.

5.1 Frontiers for Young Minds

FYM is a scientific journal edited by and for children. The goal of this journal is to bridge between scientists' high-quality work and the children's scientific interest.

FYM was approached with a description of Syn2Psy consortium, which led to the invitation to submit several abstracts for the editors to judge the suitability of Syn2Psy topics for the Neuroscience section of this journal. All seven topics (abstracts in Annex 2) were deemed suitable and currently the ESRs are in the process of writing the full articles, with the support of PIN.

5.2 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 14 projects of this consortium.

The target audience is the general public. The main goal is that viewers with little to no scientific background can be able 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.

Three episodes have been released. They can be found on the Syn2Psy website (https://syn2psy.cnc.uc.pt/?page_id=4293) and on social media: Twitter - @Syn2Psy, Instagram - @msca_syn2psy, Facebook - @syn2psy and Youtube – syn 2psy:

Episode 1 – ESR12 Manuela Rizzi;

Episode 2 – ESR1 Orsolya Antal;

Episode 3 – ESR9 Laura Upton.

5.3 Blog-posts

The blog-post area of the website has the purpose of promoting project visibility through the creation of contents for the general public on various topics concerning the consortium. This includes information regarding dissemination activities generated within the project, consortium news and the posts of the Blog-Series, where the ESRs write about their PhD in a personal point of view.

During this second year of the project, the ESRs have actively participated in the creation of content for the Blog-Series. Many of the posts revolved around PhD life or the challenges of being a PhD student in a foreign country,

however, other topics were also covered, such as reflections regarding the current pandemic (loneliness, community, fake news), scientific book reviews and creative writing.

The plan is that the next round of blog-posts revolve around the ways in which science/research/neuroscience can solve societal challenges (present or future).

6. Final Remarks

This second year of the Syn2Psy project was marked by COVID19-pandemic, strict confinement measures and further restrictions, and by Diogo Soares' resignation from ESR4 position. Despite these setbacks, the implementation of the action has not been affected, although the tasks included in Work Packages 1-4 have a substantial delay as compared to the initial plan. Nonetheless, overall the action is on the right track and moving forward.

Annex 1 – Justifications for changes included in the Grant Agreement amendment

Justification for secondment length changes

ESR3 – Giuseppe Cammarata

Reason for change in secondments:
The project being developed by Giuseppe Cammarata was originally planned with secondments at the University of Edinburgh (6mo) and Lundbeck (3mo).
After a more detailed analysis on the experimentation and data analysis to be performed at the University of Edinburgh, it was concluded that the originally allotted timeframe was significantly overestimated in the initial drafting of the project. Additionally, the equipment necessary to perform the experiments at the University of Edinburgh was recently set up at University of Coimbra/CNC, and the ESR will benefit from the possibility of learning the techniques with experts and importing them to the institute in Coimbra, where he will help disseminate the tools and protocols he will acquire in the laboratory of Drs. Emily Osterweil and Peter Kind. This adjustment will not curtail the need for exchange with the University of Edinburgh but will rather enhance and allow for further integration of the ESR in the host institution.
Furthermore, after a careful appraisal of the project development, it would be beneficial for the student to engage in training at the University Hospital of Coimbra under supervision of Dr. Guiomar Oliveira. This will help accelerate the collection of additional samples for our dental stem cell biobank and help the ESR gain further training in the medical procedures that go along the diagnosis and treatment plan for children with autism.

ESR4

Reason for change in secondments:
ESR4 project was originally planned with two secondments of three months: Eurotrials-CTI and CNC (lab of Prof. Ana Luísa Carvalho). We would like to propose reducing each secondment by one month, resulting in two months at Eurotrials-CTI and two months at CNC.
A reduction in the length of the secondments is intended to benefit the ESR's project progression and training, in the light of the current circumstances, since this will give crucial extra time at the home-lab, allowing to better prepare and plan the experiments that will be carried out during the secondment at CNC, which ultimately will benefit the training purpose of this secondment. Some of the experiments planned for the lab of Prof. Ana Luísa Carvalho can be carried out at the ESR's home-lab in Bordeaux, thus, the reduction by one month, not only will not jeopardize the secondment itself, but will benefit the overall project by allocating more time to the proper preparation of the secondment.
We believe that reducing each secondment by only one third will not significantly hinder the training planned for each of the secondments, while benefiting the ESR and the progress of the project as a whole.

ESR7 – Elisa Corti

Reason for change in secondments:
The ESR Elisa Corti was supposed to spend four months at Lundbeck (Drs. Niels Plath and Kjartan Herrik) to perform electrophysiology experiments on brain slices from *Fmr1* KO mice, during the second year. Moreover, another period of six months in the Imperial College London (Prof. Vincenzo De Paola) was planned during the third year to perform *in vivo* imaging of presynaptic bouton dynamics in *Fmr1* KO mice.
We request to redistribute the period of work originally programmed to be carried out at Lundbeck as follows: one month at Lundbeck and the remaining nine months at the Imperial College London. The following reasons justify the present request:

- An electrophysiology rig was recently set up at CNC to perform electrophysiology on brain slices. One month is enough to get familiar with the technique at Lundbeck, using spare animals or animals that have been repositioned from other studies, while the experiments will be conducted in Coimbra. This excludes the need to move WT and *Fmr1* KO mice to Lundbeck, therefore avoiding the distress of the travel and avoiding wasting the time needed for animal acclimatisation. This period at Lundbeck will also allow the ESR to have the experience of working in a non-academic environment.
- At ICL the ESR will perform electrophysiology experiments with living animals. Becoming familiar with the mice and learning how to properly handle them to avoid distress and pain that may cause discomfort to the animals, are time demanding tasks, that are necessary to produce reliable and robust data. Nine months will allow a proper training in animal handling and in two-photon *in vivo* imaging.

These reallocations will be beneficial for the ESR and will ensure an appropriate training in order to produce a solid outcome.

ESR8 – Elizabeth Brockman

Reason for change in secondment:
The time planned for Elizabeth Brockman's secondment in Bordeaux with in the groups of Drs. Daniel Choquet and Laurent Groc will remain the same as originally planned. This will be focused on studying the molecular synaptic mechanisms for the reduced synaptic turnover and network activity we recently found in Down syndrome (DS, Real *et al*. 2018). We will use advanced microscopic techniques to examine pre- and post-synaptic markers in our Ts21 and Ds21 brain tissue samples. These studies will also be expanded to include an evaluation of HCN1 and other potassium channels, which have recently emerged as candidate genes potentially involved in the synaptic and network phenotypes in DS. To get the full benefit of this secondment, we would like to distribute the 4 months over several visits. This will benefit the ESR's training and overall project progression.
The time planned for the Coimbra Hospital secondment has been shortened, as 0.5 months has been deemed to be sufficient time to observe and learn from care and research in a clinical setting. This secondment will be beneficial as it involves observing work in a unit of the hospital focusing on treatment of DS individuals and will allow the ESR to better understand the justification and uses behind finding treatments for problematic DS phenotypes.
The additional time originally allocated to Coimbra Hospital has instead been allocated to working at Lundbeck, a pharmaceutical company focusing on drug development for psychiatric disorders. We feel that in order to obtain an accurate understanding of how DS treatment can progress, and to obtain an accurate understanding of how our basic research can be applied to clinical settings, a mixture of

hospital clinical learning (Coimbra Hospital) and of pharmaceutical development learning (Lundbeck) would be optimal. Furthermore, as Lundbeck is a lab-based facility, this secondment would provide further learning opportunities, specifically regarding methodologies that would be useful for the PhD project. This includes electrophysiological techniques, which are currently being performed at Lundbeck and could be used as an alternative method for examining activity in Ds21 and Ts21 cells either in vivo, in vitro or both. This secondment will also allow for a deeper understanding of relevant work in the field of psychiatric and neurological research.

ESR10 – Flavio Tomasi

Reason for change in secondments:
Flavio had planned a 3-month long secondment at Eurotrials-CTI. The type of work that was designed for Flavio at Eurotrials-CTI, which consists of experience in Medical Writing activities, is feasible in 1 month. Eurotrials-CTI has previous experience with such internships. Given the short time the ESRs have to finish their PhD we agreed with Eurotrials for 1 month-long secondment. Both Eurotrials-CTI scientist-in-charge and the supervisors in Syn2Psy agreed to this period of secondment.

ESR11 – Ágata Silván

Reason for change in secondment length:
Ágata Silván, ESR11, started the planned secondment at EPFL in collaboration with Drs. Carmen Sandi and Simone Astori on the 9th of March 2020. Unfortunately, due to COVID-19 situation and EPFL shutdown, the experiments originally planned for this period, were postponed. As an alternative plan, Drs. Simone Astori and Carmen Sandi provided material to work remotely in sleep scoring and EEG analyses. During the 3 months of lockdown, Ágata learned about the Sirenia Sleep Pro software system and worked on these analyses. Although this was really beneficial for Ágata's training, going back in the future to perform the EEG experiments, will be a valuable opportunity for Ágata's training and will benefit her project. Due to this knowledge already acquired remotely, we consider that 3 months will be sufficient for the experiments.
Scientific basis and training purposes: due to the implication of thalamocortical networks in generation of sleep oscillations and in particular sleep spindles (which are known to be reduced in schizophrenic patients and therefore could represent a relevant biomarker), together with our common interests both at EPFL and Lundbeck in sleep and thalamic reticular nucleus, the collaboration between both labs points out to be very promising. The secondment at EPFL will be crucial to improve Ágata's learning of polysomnographic recordings and in freely moving rats. The experiments will include surgical electrode implantation for EEG and EMG recordings and, if possible, this will be combined with fiber photometry and/or behavioural tasks.

ESR14 – Vanesa Salazar

Reason for change in secondments:

Vanesa Salazar's initial secondment plan was 6 months at the laboratory of Drs. Laurent Groc and Daniel Choquet in Bordeaux, France and 3 months at Eurotrials-CTI, Lisbon, Portugal. After meeting with Ana Patacão (Eurotrials-CTI), it was decided that this secondment would be spent either working on a systematic review or a metanalysis. This project requires 2 months of work. Therefore, Eurotrials-CTI secondment would change from 3 to 2 months, remaining an extra secondment month to reallocate. However, if we take into account the current migration policies between countries, some weeks, if not a whole month in total may be spent on the collateral effects of travelling. For instance, France keeps a reciprocal quarantine with travelers from the UK; travelers entering Portugal may be required to take a COVID-19 test and self-isolate at their accommodation until the test results are known; entering the UK requires 14 days of self-isolation.

Additionally, a virtual secondment at Eurotrials-CTI may be considered, in case travelling policies do not change.

Further, to get the most out of the secondment in France, where Vanesa will focus on imaging, she must already be confident with electrophysiological techniques. Since the pandemic has prevented Vanesa from starting earlier this training, extra time at the host institution (University of Edinburgh) to develop those skills will be very valuable for her training.

All in all, a plan of 6 months in Bordeaux and 2 months at Eurotrials-CTI is a sensible option to avoid any further delays due to the coronavirus situation.

Justification for the project changes

ESR2 – Alessandro Chioino (Host institution - BMI-EPFL; Host supervisor - Carmen Sandi, Simone Astori)

Reason for the modification of ESR2 project:
Since the time when we wrote the Syn2Psy project application, the Sandi lab made significant progress on the goals of the previously proposed project. We have published a study (Bacq et al., Amygdala GluN2B-NMDAR dysfunction is critical in abnormal aggression of neurodevelopmental origin induced by St8sia2 deficiency, Mol Psychiatry 2019) and have another one in advanced state of publication (Bacq et al., Alterations in cortico-amygdalar connectivity in the polysialyltransferase ST8SIA2-deficient mouse model of schizophrenia, in preparation) that already covered in large extent the proposed goals. Therefore, we propose here a new project focusing on the role of specific mitochondrial genes for the development of psychopathologies. Given the emerging importance of mitochondria and metabolism for brain function and dysfunction, we believe that studying this new topic is highly timely and relevant for the scope of Syn2Psy.

ESR11 - Ágata Silván (Host institution - H. Lundbeck A/S; Host supervisor - Kjartan Frisch Herrik)

Reason for the modification of ESR11 project
Since the ESR11 project was described in the original Grant Agreement two of the three Lundbeck supervisors have been exchanged internally. The change in supervisor for ESR11 was approved by the supervisory board. To fully take advantage of their expertise the project has been slightly modified without changing the overall focus on the balance between excitatory and inhibitory neurons and how imbalances can lead to network dysfunctions. In the updated project description, the E/I balance will be studied in the thalamocortical network rather than in the hippocampus. This is advantageous for several reasons:

1) It allows to easily spatially differentiate excitatory (thalamic) from inhibitory (reticular) neurons, which may be an upside for both the planned ex vivo and in vivo studies.
2) It is possible to investigate mechanisms underlying thalamocortical network function using pharmacological and/or genetic models at the level of single E/I neurons and translate to freely moving rats. The electrophysiological biomarkers studied in the freely moving rats, may further translate to human electroencephalographic and polysomnographic markers.
3) Deficits in this circuit are also implicated in both epilepsy and schizophrenia.
4) Optimizing the project for the thalamocortical network allows even closer interaction with two other Syn2Psy groups. The extended stay at Simone Astori/Carmen Sandi will introduce the student to the art of polysomnography and the stay at Peter Kind/Emily Osterweil will give access to behavioural and later electrophysiological characterization of a relevant genetic rat model.
5) The new Lundbeck supervisors as well as the supervisor from University of Copenhagen are experts in ex vivo slice electrophysiology (Hougaard, Kohlmeier), ion channel pharmacology (Hougaard, Kohlmeier, Herrik), rodent in vivo electrophysiology (Herrik) and polysomnography (Kohlmeier).

Taken all together the optimized project plan with its moderate changes is tailored to fit the students wishes, the expertise of the supervisors and the Syn2Psy overall goals.

ESR12 - Manuela Rizzi (Host institution - CDBS – UEDIN; Host supervisor - Emily Osterweil)

Reason for the modification of ESR12 project

The shift twards new objectives for ESR12's project is due to the development of research in the lab that has come from recent TRAP-seq work on the fragile X mouse model and ongoing work in the Syngap1 happloinsufficiency mouse model, which suggests an exciting convergence in translating mRNAs. The lab now has access to rat models and human iPSC models of fragile X and Syngap1 happloinsufficiency that can be assessed to see if these changes are conserved in multiple species. Because this is an exciting opportunity that has arisen since the original application, the project has refocused on this work. In addition, this project will provide an excellent training opportunity for the ESR who will learn cutting edge techniques in ribosome profiling and RNA-seq. Furthermore, our laboratory received a new MRC grant to investigate Fmr1-/y and Syngap+/- mice, which will synergise with the new project using rat and human models.

ESR13 - Loredana Cumpana (Host institution - BMI-EPFL; Host supervisor - Carmen Sandi, Simone Astori)

Reason for the modification of ESR13 project

The project originally planned for ESR13 aimed at investigating the interaction between prenatal stress and *St8sia2* gene for the emergence of schizophrenia- and ASD-related behavioural deficits. Unfortunately, preliminary data collected in the lab during the months before the Syn2Psy consortium was granted indicated that prenatal stress leads to negligible modifications in schizophrenia- and ASD-related behavioural readouts. Thus, this recent data indicates that **the original project would not have served for the purpose of targeting neurodevolpmental disorders, the scientific question at the core of Syn2Psy. Performing experiments planned upon the previously envisioned project would have led the ESR13 researcher to significantly deviate from the scope of this consortium, and into a non-promising path.**

Alternatively, we propose now a project highly relevant to the Syn2Psy goals; namely, to explore the interaction between the anxiogenic and stress-related system mediated by the corticotropin releasing hormone (CRH) and the functioning of the thalamic reticular nucleus (an increasingly recognized thalamic hub of neurodevelopmental disorders) for the production of behavioral and neural circuit aberrations. Importantly, **the new project proposal follows the original aim of the ESR13 project in examining the effects of the stress system in predisposing individuals to neurodevelopmental disorders, but it refines the animal model and the specific brain circuit under investigation**. The questions addressed in this project are perfectly aligned with the endeavours of this consortium. Notably, the brain area under examination, an important thalamic pacemaker of sleep rhythms, is also at the centre of the ESR11 project. The interaction with the group hosting ESR11 will include the exchange of technical expertise (e.g., polysomnographic recordings combined with behavioural task involving for sleep-dependent memory consolidation) and will thus be beneficial to both laboratories and ESRs. Further objectives of the study will entail the impact of stress on the expression of key genes related to schizophrenia and ASD, an endeavour shared by other members of the consortium, e.g. ESR14 and Emily Osterweil. For all these reasons, we envision that this project proposal will be highly beneficial for both the ESR13 and the Syn2Psy network.

ESR14 – Vanesa Salazar (Host institution - CDBS – UEDIN; Host supervisor – Peter Kind)

The project title was modified, however, the project and the tasks stay unchanged. Reason for the modification of ESR14 project title from "Determining critical periods for therapeutic interventions in models of fragile X syndrome and SYNGAP1haploinsufficiency" to "Circuit deficits associated with ASD/ID":

The new title includes the study of neural networks in the models of interest. Whereas the study of therapeutic targets and critical periods remain in the project pipeline (time-permitting in the current circumstances), the previous title did not include the interest in studying circuits which is highly relevant for the project goals, as well as to understand the potential of therapeutic targets/treatments. The new title also adjusts better to the project interests than the previous one and it is more flexible for the natural course of scientific progress.

Annex 2 – Abstracts sent to the journal Frontiers for Young Minds

Authors: Alessandro Chioino and Loredana Cumpana

Title: Reaching for the top: the role of mitochondria.

Abstract: Humans, like most animals, live in social groups that naturally form ranks, following an order from dominant to subordinate that we call social hierarchy. What makes individuals more likely to fall into a certain rank? To study brain functions that contribute to this, social hierarchies are studied in laboratory rodents and identified, through social competition tests. Recent studies have shown that mitochondria, the powerhouse of the cells that produce the energy necessary for cells and organisms to work, play a role in social behaviour. Subordinate rats have lower mitochondrial capacity than dominant rats in a brain region -the nucleus accumbens- important for effort and motivation. If subordinate animals are energized with a booster of mitochondrial function, their behaviour can shift from subordinate to dominant. We will discuss what this animal research means for humans and how it may help us find new ways of treating troubled social behaviour.

Authors: Laura Upton and Flavio Tomasi

Title: Fantastic beasts and where to find them: how can we use animals to study neuropsychiatric disorders?

Abstract: A psychiatric disorder is a mental illness that disturbs a patient's behaviour, mood or ability to think, and can get in the way of them leading a normal life. Even though studying human patients might seem like the most obvious way to understand these illnesses, doing so is not always practical, affordable, or even possible. Because of this, other more roundabout methods have to be found. Fortunately, we can create animal models of some psychiatric disorders, to better understand their biological causes. Genetic differences are a common cause for these kinds of diseases. Because of this, genetic animal models are a very common way to study how these illnesses modify brain function and behaviour, and can even help us to find treatments for these disorders. Did you know that mice can imitate the symptoms of depression, schizophrenia or autism? In this article, we will discuss what kinds of information we can obtain from animal models, and what are the pros and cons of using these models to understand human diseases.

Authors: Elisa Corti and Manuela Rizzi

Title: The play-doh brain: shapeable neurons make you learn!

Abstract: Imagine your mum just baked pizza and you immediately eat a piece of it. Bad decision, it is really hot! Now you will wait for it to cool down before continuing to eat. The burning sensation reaches the brain and the neurons communicate with each other so that you stop eating. Next time your mum will cook pizza you will have learnt to be more patient. This is possible thanks to a process called synaptic plasticity, in which neurons increase the connection already formed in the first communication and prevent you from hurting yourself again.

In this article we will describe some properties of neurons, as well as what happens to these cells when you create new memories in a more detailed way. We hope that you will learn something new by reading this piece, and share it with others, as neurons would do!

Authors: Orsolya Antal, Diogo Soares and Ágata Silván

Title: Schizophrenia: what causes hallucinations and distorted thinking?

Abstract: Imagine living without being sure if what you see, hear or think is real or not. Schizophrenia is a mental disorder characterized by distortions in thinking, perception, emotions, language, sleep, and behaviour. It affects 20 million people worldwide, and typically becomes evident during late adolescence or early adulthood. While medication can be used to control hallucinations, most symptoms are resistant to treatment. As a result, patients and families have serious difficulties in their daily lives. But what do we know about what is happening in the brain of people afflicted with this disorder? Which parts of the brain may be altered and how can we develop better treatments based on this knowledge? In this article we will explore how the brains of people with schizophrenia are different and how gaining more knowledge can help them.

Authors: Elizabeth Brockman

Title: Exploring the living mouse brain using 2-photon microscopy.

Abstract: The human brain is a highly complex system comprised of billions of cells, called neurons, interconnected to each other in functional neural circuits. Normal brain function is dependent on effective communication between neurons, a process that requires synapses, the point of contact between nerve cells. Several diseases result from impairments in the communication between neurons, which is why it is essential to study synapses in the living brain. Since the human brain is not accessible, scientists use simpler organisms like mice.

To see mouse neurons, a piece of skull is replaced with a transparent glass window. Powerful microscopes called 2-photon microscopes, can then be used to capture snapshots of neurons deep inside live brains! Clever use of lasers to excite tags placed inside the cells make neurons emit fluorescent light and glow in the dark. Join us as we illuminate the inner workings of neuron communication in the living brain.

Authors: Vanesa Salazar and Daniel Hunter

Title: A brain composed by multilingual neurons: A story of coded messages and crosstalking.

Abstract: Everyday, we communicate with each other using language. To do this effectively requires two things: 1) the speaker to be clear and precise, and 2) the listener to understand the language and respond.

Similarly, in our brains, neurons communicate with each other, at a location called the synapse where one neuron "talks" to another neuron by releasing a particular chemical message. The “listening” neuron understands this message by receiving the chemical at receptors on their surface. Every neuron speaks to hundreds of others, simultaneously and using different languages. The brain works as a social network which integrates information and responds to hundreds of “chats” arriving at the same time.

This article focusses on synapses that use a chemical messenger (or language) called glutamate and two particular receptors, called AMPARs and NMDARs, that decode the message. This language is key to how we interpret the message and for learning and memory.

Authors: Giuseppe Cammarata and Marcos Sintes

Title: How to build a mini human brain.

Abstract: Growing miniature brains in a laboratory may sound like science-fiction, but advances in technology have made it possible. Scientists can collect human cells from different tissues, like the skin, culture them in a dish and reprogram them to make stem cells, like the ones in an embryo. These reprogrammed stem cells have the ability to multiply and convert into all the kinds of cells that form our body, which lets us study the development of human organs. Under the right conditions, stem cells can aggregate and grow into small brain-like structures, called brain organoids. The study of brain organoids has allowed us to expand the possibilities of recreating the human brain and can reproduce reliably many features of its function and development. In this article, we would like to explore how these brain organoids are created and how they can help us make new discoveries on diseases related to the development of the brain, such as autism.