Role of the blood-brain barrier in stress resilience: investigating new pathways towards pharmacological augmentation of stress resilience (a PHASR-PP project study)

Study Protocol (including Statistical Analysis Plan)

Date: 12-03-2025

NCT number: T.B.D.

Role of the blood-brain barrier in stress resilience: investigating new pathways towards pharmacological augmentation of stress resilience (a PHASR-PP project study)

Die Rolle der Blut-Hirn-Schranke in der Stressresilienz: Untersuchung neuer Wege zur pharmakologischen Förderung der Stressresilienz (eine Studie des PHASR-PP-Projekts)

3.1 STUDY SYNOPSIS

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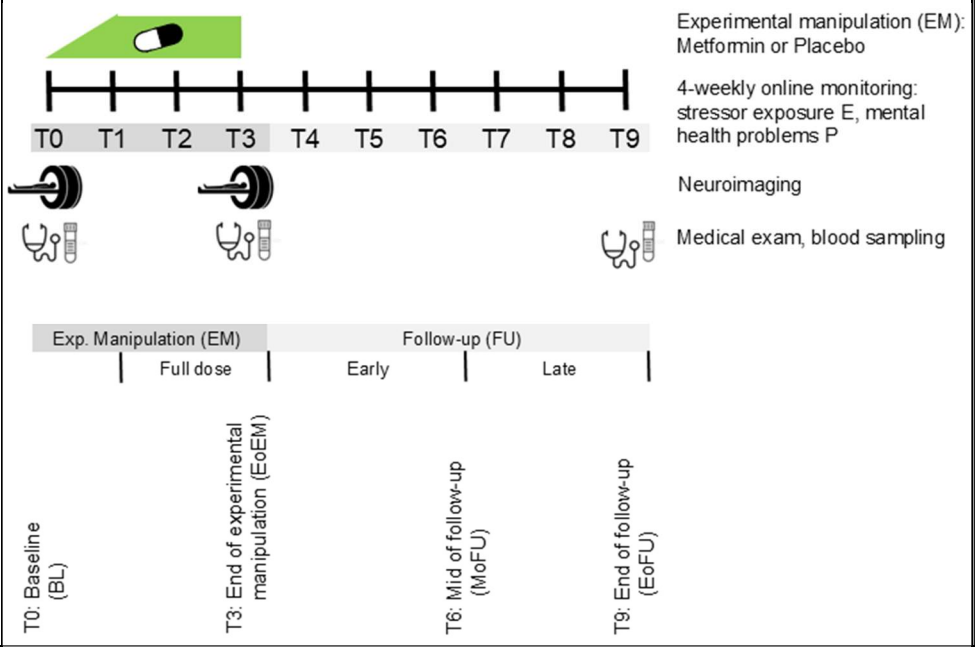
TITLE OF STUDY	Role of the blood-brain barrier in stress resilience: investigating new pathways towards pharmacological augmentation of stress resilience (a PHASR-PP project study)
CONDITION	Healthy university students aged 18-25 years

OBJECTIVE(S)	Overarching aim is to better understand the relationship between blood-brain barrier (BBB) function and stress resilience and to investigate a potential causal role for BBB function in stress resilience in humans <u>Primary objective (O1):</u> O1a) testing whether better BBB integrity, assessed with neuroimaging at whole-brain level, is prospectively associated with better resilience in the **short** term (reduced average stressor reactivity at time points T4-T6 during early follow-up). O1b) testing whether an experimental pharmacological manipulation, consisting of the administration of metformin from time points T0-T3, improves BBB integrity at time point T3 at end of experimental manipulation relative to time point T0 at baseline. O1c) testing whether the effect of the experimental manipulation on BBB integrity at time point T3 mediates a potential effect of the experimental manipulation on **short**-term resilience (reduced average stressor reactivity at time points T4-T6 during early follow-up). <u>Secondary objective (O2):</u> O2a) testing whether better BBB integrity, assessed with neuroimaging at whole-brain level, is prospectively associated with better resilience in the **long** term (reduced average stressor reactivity at time points T4-T9 during whole follow-up). O2b) testing whether the effect of the experimental manipulation on BBB integrity at time point T3 mediates a potential effect of the experimental manipulation on **long**-term resilience (reduced average stressor reactivity at time points T4-T9 during whole follow-up). 

INTERVENTION(S)	Experimental intervention: Metformin 850 mg twice daily Control intervention: Placebo Duration of intervention per participant: 12 weeks Follow-up per participant: 24 weeks Experimental and / or control off label or on label in Germany: off-label experimental intervention
KEY INCLUSION AND EXCLUSION CRITERIA	Key inclusion criteria (participants meeting all of the following criteria will be considered for enrolment in the study): • Absence of mental disorder diagnosis • University students • GHQ-28 score ≥ 20 • Three or more adverse life events (LEs) acc. to LE list in their past • Beck Depression Inventory (BDI) ≤ 14 & Columbia-Suicide Severity Rating Scale (C-SSRS) ≤ 1 (thereby excluding concurrent depression and suicidality) • Age 18 - 25 years • Able to understand character and individual consequences of the study (MMSE Folstein > 28) Key exclusion criteria (participants presenting 1 of the following criteria will not be enroled in the study): • Life-time and current diagnosis of any severe mental disorder determined by M.I.N.I. diagnostic interview • Known history of brain injuries or neurodevelopmental disorder • Evidence of neurodegenerative disorder (e.g., Parkinson) • Multimorbidity or significant organ (esp. liver or renal) dysfunction or manifest diabetes or substance abuse (esp. alcohol) • Contraindication to metformin such as renal insufficiency (Creatinin-Clearance <60ml/min), recent (<3 months) ischemic events (e.g., myocardial infarction or stroke) • Women of childbearing age, who do not practice a medically accepted contraception (i.e., systematic contraceptives, diaphragm, condoms with spermicide, sexual abstinence) during the study and during a 2 years post-study period and who do not present a negative pregnancy test (serum or urine) • History of hypersensitivity to the study drug, to any drug with similar chemical structure, or to any excipient present in the pharmaceutical form of the study drug • Diabetes type 2 (would result in interference with the experimental manipulation) • Participation in other studies employing a drug during the present study or within the last three months • Current use of antidiabetic, weight-loss, or psychoactive medication or substances • Pacemaker, implanted medical pumps, implanted cardiac catheters or acute or unstable heart disease (angina pectoris). • Intracranial implant (aneurysm clips, shunts, stimulators, cochlear implants or electrodes) or other metallic objects inside or near the head (mouth excluded) that cannot be removed. • Claustrophobia or another contraindication to MRI. • Insufficient language skills

OUTCOME(S)	Primary endpoint: Relating to O1a): Prospective association between participants' whole-brain water exchange rate (kw) from blood to brain tissue assessed with arterial spin labelling in neuroimaging (i.e., whole-brain BBB permeability as an inverse marker of BBB integrity) at time points T0 at baseline and T3 at end of experimental manipulation and participants' average stressor reactivity (SR score) at time points T4-T6 during early follow-up (as an inverse marker of **short**-term resilience), taking into account the experimental manipulation. Key secondary endpoints: Relating to O1b): Effect of the experimental manipulation between time points T0 and T3 (groups verum and placebo) on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation, taking into account BBB permeability at time point T0 at baseline. Relating to O1c): Mediation by the effect of the experimental manipulation on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation on participants' average stressor reactivity at time points T4-T6 during early follow-up. Further secondary endpoints: Relating to O2a): Prospective association between participants' whole-brain water exchange rate (kw) from blood to brain tissue assessed with arterial spin labelling in neuroimaging (i.e., whole-brain BBB permeability as an inverse marker of BBB integrity) at time points T0 at baseline and T3 at end of experimental manipulation and participants' average stressor reactivity (SR score) at time points T4-T9 during whole follow-up (as an inverse marker of **long**-term resilience), taking into account the experimental manipulation. Relating to O2b): Mediation by the effect of the experimental manipulation on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation on participants' average stressor reactivity at time points T4-T9 during whole follow-up. Assessment of safety: Frequencies of participants experiencing at least one adverse event (AE) will be displayed by body system and preferred term according to MedDRA terminology.
STUDY TYPE	Prospective, multi-center, double-blind, randomized, parallel-group placebo-controlled experimental study
STATISTICAL ANALYSIS	Population: The primary population for the analyses of efficacy is the intention to treat (ITT) population. Complementary analyses will be conducted in the per-protocol population (ITT population without major protocol violations). Significance testing: All hypotheses will be tested on a two-sided level of significance $\alpha=0.05$.

	The primary hypothesis (primary analysis relating to the primary objective O1a) will be tested separately and without correction for multiple testing. Correction for multiple testing will be applied family-wise to the key secondary hypotheses (two key secondary analyses relating to the primary objectives O1b and O1c), provided that the conditions to test the key secondary hypothesis relating to objective O1c are met. <u>Primary analysis:</u> Relating to O1a): To test for a prospective association of whole-brain BBB permeability with average SR at T4-T6 during early follow-up (SR(T4-T6)), a **linear mixed model** will be used to regress BBB permeability at baseline (BBB(T0)), BBB permeability at end of experimental manipulation (BBB(T3)), and experimental manipulation (EM; groups metformin and placebo) onto (SR(T4-T6)). <u>Key secondary analyses:</u> Relating to O1b): To test for an effect of the experimental manipulation between time points T0 and T3 (groups metformin and placebo) on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation (BBB(T3)), a **linear mixed model** will be used to regress EM and BBB(T0) onto BBB(T3). Relating to O1c): If BBB(T3) shows a significant association with SR(T4-T6) (see O1a) and if EM shows a significant association with BBB(T3) (see O1b): To test for mediation by the effect of the experimental manipulation on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation on participants' average stressor reactivity at time points T4-T6 during early follow-up, **mediation analysis** will be conducted using a Baron-Kenny approach with EM as predictor, BBB(T3) as mediator, SR(T4-T6) as outcome, and , BBB(T0) and earlier covariates as covariates. <u>Further secondary analyses:</u> Relating to O2a): To test for a prospective association of whole-brain BBB permeability with average SR at T4-T9 during whole follow-up (SR(T3-T9)), a **linear mixed model** will be used to regress BBB(T0), BBB(T3), and EM onto SR(T4-T9). Relating to O2b): If BBB(T3) shows a significant association with SR(T4-T9) (see O2a) and if EM shows a significant association with BBB(T3) (see O1b): To test for mediation by the effect of the experimental manipulation on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation on participants' average stressor reactivity at time points T4-T9 during whole follow-up, **mediation analysis** will be conducted using a Baron-Kenny approach with EM as predictor, BBB(T3) as mediator, SR(T4-T9) as outcome, and , BBB(T0) and earlier covariates as covariates. <u>Safety:</u> All summaries and listings of safety data will be performed for the safety population. Summary tables of AEs will present the number of participants observed with AEs and corresponding percentages. Laboratory values and vital signs will be analyzed by descriptive methods

	and exploratory p-values.
SAMPLE SIZE	To be assessed for eligibility: n = 122 (61 per group) To be allocated to study: n = 36-46 per study site To be analyzed: n = 109
STUDY DURATION	Time for preparation of the study (months): 5 months (M01-M05) Recruitment period (months): 10 months (M03-M12) First participant-in to last participant-out (months): 18 months (M06-M023) Time for data clearance and analysis (months): 9 months (M024-M032) Duration of the entire study (months): 32 (M01-M032)
PARTICIPATING CENTERS	To be involved (n): 3 actively recruiting centers (UM, UZH, UNIWARSAW)

A.3.2. RESULTS OF THE PREVIOUS FUNDING PERIOD

N.A.

3.2 SUMMARY OF THE STUDY

3.2.1 SUMMARY

The project builds on findings from animal research indicating that the integrity of the Blood-Brain Barrier (BBB) is an important resilience factor. Stress and maladaptive stress-related behavioral alterations have been associated with low-grade peripheral inflammation and inflammation-associated impairments in BBB function that allow inflammation mediators to enter the brain parenchyma and adversely affect neural processes. BBB vulnerability to stress-related impairment, however, varies between individual animals, and individuals with a less vulnerable BBB also exhibit fewer stress-related behavioral alterations, that is, more stress resilience. Further, animal studies indicate that the mTOR pathway contributes to BBB vulnerability and that pharmacological inhibition of this pathway improves stress resilience. This effect is likely achieved via improvements in BBB function.

Translating these results to the human, the study aims to establish a prospective association between BBB integrity and stress resilience in stressor-exposed individuals at risk to develop stress-related mental health problems. It primarily builds on assumed natural inter-individual variation in BBB integrity, assessed with neuroimaging, which it attempts to relate to inter-individual variation in stress resilience in an observational approach. It is hypothesized that better BBB integrity predicts better stress resilience. Because the animal literature does not allow us to estimate the duration of the hypothesized effect, both a short-term and a long-term resilience outcome are employed. As a further measure to maximize sensitivity, we attempt to enhance inter-individual variation in BBB integrity in the study sample by administering the indirect mTOR pathway inhibitor metformin to half of the participants, using a randomized parallel-group placebo-controlled double-blind multi-center multimodal pre-registered experimental design. This experimental-interventional approach allows us to ask whether metformin improves BBB integrity and whether this in turn improves stress resilience.

In additional analyses, blood-based biomarkers are used as measures of BBB integrity, and the influence of immunological, psychological, and socio-demographic covariates on BBB function and stress resilience is also considered.

The study has the potential to establish for the first time a relationship between BBB function and stress resilience in humans and to yield hints at a potential causal role of BBB function in resilience. In the longer term, it may open the path towards a pharmacological method to augment BBB function and resilience, to be investigated in follow-up projects.

3.2.2 KEY WORDS

BBB integrity, stress resilience, at-risk, prospective, metformin, placebo-controlled

3.2.3 INTERVENTION SCHEME / STUDY FLOW

Design: Prospective, randomized, parallel-group, placebo-controlled, double-blind, multi-center experimental study to investigate the relationship between individual variation in BBB integrity and individual variation in stress resilience, making use of a 12-week treatment with orally administered metformin (850 mg twice daily) as an experimental tool in participants at risk for stress-related mental health problems in order to enhance individual variation in BBB integrity.

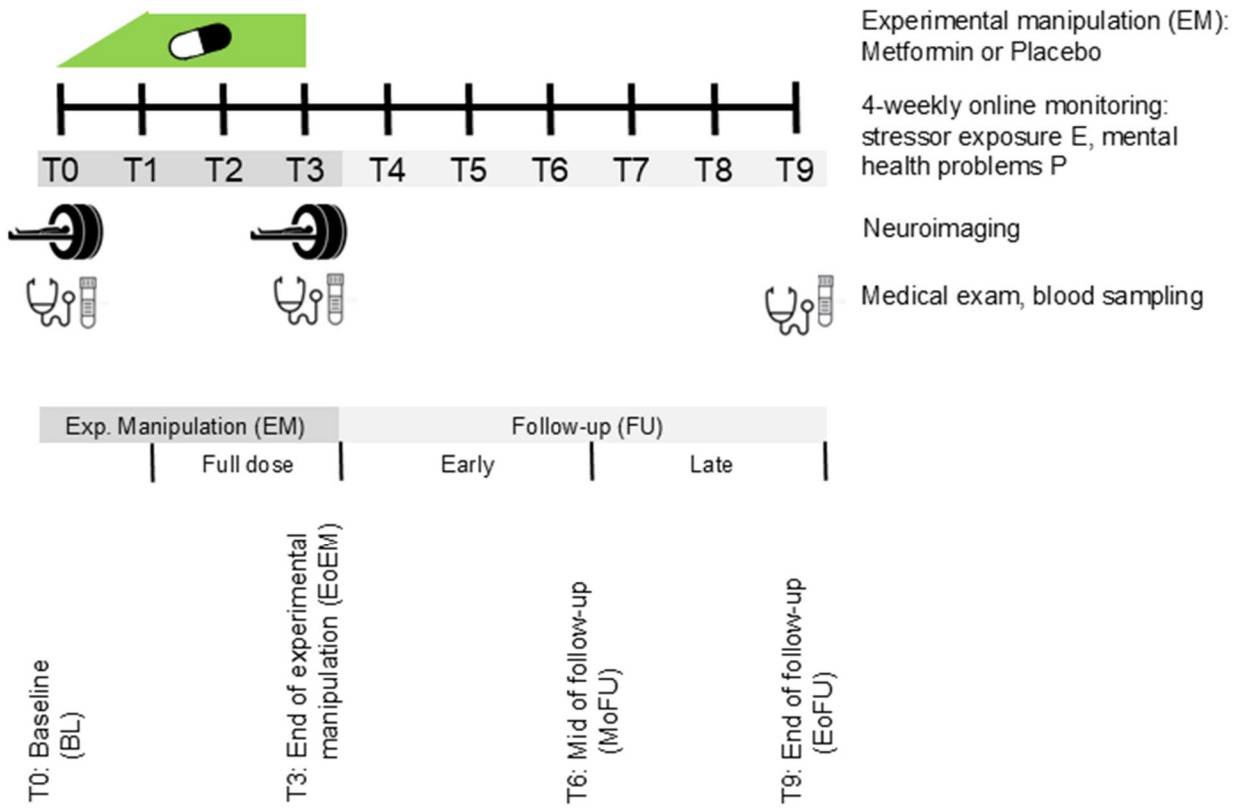


Figure 1. Schematic overview of study design. Online monitoring of stressor exposure (E) and mental health problems (P) as basis for the calculation of individual stressor reactivity (SR; endpoint) are conducted every four weeks (T0, T1, …). Neuroimaging-based assessments of whole-brain BBB integrity are conducted at time points T0 and T3. They bracket the experimental manipulation (metformin vs. placebo in a parallel-group design), expected to affect BBB integrity. Inter-individual variation in BBB integrity, both naturally occurring (spontaneous) and experimentally induced, can thus be related to SR. Additional laboratory-based assessments are shown below the time axis. Bottom: nomenclature of study phases and key time points.

Screening phase: Participants will be assessed at three different study sites (i.e. Germany, Switzerland, Poland). Potential participants will be screened and assessed for eligibility. If eligible, informed consent will be obtained.

Time point T0 (Baseline; week 1): Medical exam, blood sampling, neuroimaging, randomization, distribution of study drug starting dose (500mg once daily), start of monthly online monitoring.

Time points T0 to T3 (Experimental manipulation phase): Four weeks of dose increase (T0 to T1), i.e., first and second week 500 mg once daily, third and fourth week 500mg twice daily. Full-dose administration (850 mg twice daily) for eight weeks (T1 to T3). Monthly online monitoring.

Time point T3 (End of experimental manipulation; week 12): Medical exam, blood sampling, neuroimaging, end of full-dose administration. AE monitoring.

Time points T4 to T9 (Follow-up): separated into early (T4 to T6) and late (T7 to T9) follow-up period. Monthly online monitoring.

Time point T9 (End of follow-up; week 36): Medical exam, blood sampling, AE monitoring.

3.2.4 FREQUENCY AND SCOPE OF STUDY VISITS

Action / Visit	Screening	Baseline/ Visit 1 (at T0)	Visit 2 (at T3)	Visit 3 (at T9)
Study week		0	12	36
Participant information and written informed consent	x			
Demographics (e.g. sex, age, race)	x			
Vital signs (BP, pulse, temperature)	x			
Clinical signs of infection	x			
Physical and psychiatric examination	x			
Medical history	x			
Inclusion/exclusion criteria	x			
MMSE (according to Folstein)	x			
BDI	x			
CSSR-I	x			
M.I.N.I.	x			x
Medical examination, incl. blood samples	x	x	x	x
Online: life events, daily hassles, internalizing symptoms (every four weeks)		x	x	x
Neuroimaging: BBB assessment, anatomy and diffusion (T1, T2), resting-state fMRI		x	x	
Assessment of online battery of psycho-social resilience and risk factors		x	x	x
Distribution of study drug		x		
Randomization		x		
Adverse events		x	x	x
End of study (final visit)				x

3.3 THE MEDICAL PROBLEM

3.3.1 EVIDENCE

Blood-brain barrier function, inflammation, and stress

The central nervous system has traditionally been viewed as an absolute immune-privileged site[1]. However, there is increasing evidence that there are ways by which the brain extensively interacts with other organ systems[2]. The blood-brain barrier (BBB) tightly controls the bidirectional communication between the central nervous system and the circulation and is therefore vital for brain protection and function. This complex selective interface – the neurovascular unit – consists of several specialized cell types: non-fenestrated brain endothelial cells that are characterized by highly specific tight junctions sealing the para-cellular space, pericytes and smooth muscle cells that play a major role in controlling the cerebral blood flow, and astrocytic end-feet covering most of the vasculature[3]. The immune system and the BBB are tightly intertwined[3]. While under physiological conditions, most peripheral cytokines or immune cells cannot penetrate the BBB or depend on specialized transporters regulating their passage, **pathological conditions such as acute or chronic inflammatory states can lead to increased BBB permeability**[3,4], involving the influx of potentially neurotoxic proteins or factors, such as peripheral IL-6 into the brain parenchmya[5]. Given that **stress is associated with profound changes in the immune system**[6], one could hypothesize that BBB integrity and function might be also impaired in stress-related conditions. A recent seminal pre-clinical study showed that upon exposure to chronic social defeat (CSD), which is one of the best-validated stress models for the mouse, **stress-reactive (but not stress-resilient) mice showed increased BBB permeability**. This was caused by a stress-induced downregulation of the endothelial tight junction gene/protein Claudin-5 resulting in the influx of potentially neurotoxic proteins, e.g., peripheral IL-6[7]. In the same study, the Claudin-5 gene was also shown to be downregulated in postmortem tissue from patients with major depressive disorder (MDD). The link between stress and BBB permeability was further substantiated in a study showing that hippocampal BBB permeability was increased in mice that underwent the learned helplessness paradigm and that BBB permeability and behavioral abnormalities could be reversed after pharmacologically blocking the cytokine TNFα[7]. Areas of the brain where BBB function appears to be particularly sensitive to stress include the hippocampus (HPC), the prefrontal cortex (PFC), and the ventral striatum (VS), which are also important nodes in brain networks regulating stress and emotion[5,8].

Measurement of BBB function in humans

In humans, **BBB permeability can be assessed using** invasive methods, specifically contrast agent-enhanced **magnetic resonance imaging (MRI) of the brain as a direct indicator**[9] and measurement of plasma or cerebrospinal fluid (CSF) proteins as indirect indicators[10,11]. Recently, **a non-invasive MRI-based neuroimaging method without contrast agent** has been developed[12,13]. To date, however, **the evidence linking BBB dysfunction and stress-related disorders exclusively stems from studies that have used indirect measures like vascular markers** in circulation or ratios between CSF and blood proteins, such as the CSF/serum albumin quotient[14]. Because albumin is not synthesized in the CSF, albumin measured there stems from the circulation and can be used as a proxy to assess blood-CSF leakiness. In a study performed on elderly women, those with MDD had a higher CSF/serum albumin ratio[15]. In addition, an early study showed increased markers of permeability in MDD[16]. Another peripheral marker of BBB dysfunction is S100β. This calcium-binding protein, which is mainly expressed in glial cells, is normally not detectable in serum; however, it is elevated in the presence of BBB damage[17]. Several studies have reported increased levels of S100β in patients with MDD[18]. In addition, low-grade inflammation (assessed by CRP, SAA, ICAM-1, IL-6, IL-8, TNF-α) and endothelial dysfunction (assessed by VCAM-1, E-selectin, VWF, ICAM-1) were both associated with depression, while endothelial dysfunction was further associated with chronicity of depressive symptoms[19,20]. The anatomical localization of stress-related BBB leaks in the human brain cannot be established with these indirect marker methods.

In summary, in the context of a close interaction between peripheral inflammation and neurovascular dysfunction, there is increasing evidence that both contribute to the detrimental effects of stressor exposure on mental health, and improved methodology now allows for a more detailed and non-invasive investigation of the role of the human BBB in this interplay.

Stress-dependent regulation of behavioral and BBB function via the mTOR pathway

Recent animal work from LIR and UM (with PHASR-PP project partner Prof. S. Schweiger, Dept. Human Genetics) has identified the **mTOR (mechanistic target of rapamycin) pathway as a likely mediator of the detrimental effects of exposure to a chronic social stressor** on normal adaptive behavior in mice[21]. Specifically, pathway analyses of differentially expressed genes after CSD in hippocampal cells indicated mTOR pathway upregulation in stressor-reactive mice, whereas **mice that maintained behavioral functioning despite CSD (stress-resilient mice) showed relatively reduced expression of downstream mTOR effectors and enhanced expression of negative mTOR regulators** (MAPK and PI3K-Akt pathways). Importantly, administration of the **mTOR inhibitor** (and MAPK and PI3K-Akt upregulator) **rapamycin**[22] over several days following CSD prevented CSD-induced behavioral impairments, that is, **made animals more stress resilient**[21].

Interestingly, mTOR-related differential expression was observed not in neuronal, but mainly in glial, mural, and endothelial cells, pointing towards a role for the BBB in stress vulnerability vs. resilience. The mTOR signaling pathway is an evolutionarily conserved pathway that senses and integrates a broad range of environmental cues, e.g., growth factors or immune modulators, to regulate several homeostatic processes, including in the vasculature[23]. In various mouse models of neurological and inflammatory diseases that have been associated with impairments in the neurovascular unit and reduced BBB integrity, rapamycin had a **positive effect on BBB and vascular function**[24–27]. An additional BBB-protective effect of rapamycin beyond mTOR inhibition may be achieved by its anti-inflammatory actions[28].

Taken together, **pharmacological mTOR pathway inhibition and immunosuppression**, as effectuated for instance by rapamycin, presumably enhance resilience via **promotion of BBB integrity** and reduced exposure of the brain to mediators of inflammation.

Metformin as an experimental tool to enhance BBB function in humans

These combined insights from human and animal studies give rise to the hypothesis that better BBB integrity in stressor-exposed humans reduces their risk to develop stress-related mental health problems, that is, BBB integrity in the face of stress is an inter-individual resilience factor. Further, next to naturally occurring (spontaneous) inter-individual variability in BBB integrity, inter-individual differences may also be generated pharmacologically via mTOR inhibition (relative to a control pharmacological intervention). Improvement of BBB integrity as a result of mTOR inhibition should enhance resilience (mediation). Therefore, **an experimental design where natural and pharmacologically induced inter-individual variance in BBB function is combined should be highly sensitive to demonstrate effects of BBB integrity on stress resilience**.

To pharmacologically manipulate BBB function, rather than using the mTOR inhibitor and immunosuppressor rapamycin, it is likely advantageous to use the indirect mTOR inhibitor and immunosuppressor metformin. Rapamycin has already been employed in humans (as sirolimus), however, with an unfavorable side effects profile. Metformin inhibits mTOR via PI3K-Akt[29–31]. Orally administered metformin has been used for decades in millions of patients in the prevention and treatment of type 2 diabetes and has recently been suggested to have anti-aging and anti-cancer effects, among others[29–31]. **Metformin has a highly favorable safety profile and can be administered over extended times**; adverse effects can be avoided by excluding individuals with kidney dysfunction, liver disease, or vitamin B12 deficiency or in pregnancy and by gradual dosing[29,30]. The possibility to administer metformin over longer time periods is important since the beneficial effect on resilience (and presumably BBB function) of rapamycin in stressed mice was achieved through chronic treatment[21]. Metformin is off-patent and relatively inexpensive. Importantly, there are indications that metformin also has anti-inflammatory effects[31,32] and may protect against cardiovascular disease[29–31], and recent work in mice has shown that metformin attenuates BBB disruption following middle cerebral artery occlusion[33], suggesting that the **protection of BBB function is a plausible working mechanism of metformin**.

3.3.2 THE NEED FOR A STUDY

The medical problem: resilience factors

Mental disorders are one of the leading causes of the global burden of disease[34], with depression and anxiety being the most outstanding[35]. These disorders can have significant personal, economic, and societal costs and are often triggered by stressor exposure and the ensuing stress reactions during childhood and adulthood[36]. For various reasons (including the mostly arbitrary cut-off used to differentiate healthy from diseased persons in current diagnostic systems), research on stress-related and other mental health dysfunctions increasingly abandons diagnostic categories (such as Major Depressive Disorder) as research-relevant entities and instead focuses on continuous dimensions of psychopathology and impairments in the underlying functional systems, following a transdiagnostic approach[37,38]. In this logic, we here speak of **stress-related mental health problems**, which can be observed across different stress-related disorders but may also be found to a smaller or greater extent in individuals without a diagnosis, yet may cause significant burden. Accordingly, we understand the term mental health not as a binary construct, but in a continuous-multidimensional fashion. No good epidemiological information exists about the prevalence of the various known stress-related mental health problems, however, they can be safely considered a major societal challenge. It is assumed that factors or mechanisms that reduce the occurrence of stress-related mental health problems (resilience factors) will also reduce the occurrence of stress-related disorders. In the current project, we focus on factors conveying resilience to **internalizing problems** as psychopathological dimensions characteristic of depression and anxiety disorders.

Despite enormous efforts made in the last decades to understand stress-related pathophysiology and improve treatments, there has been no concomitant decline in disease prevalence. Insufficient investments into prevention research and prevention efforts ('prevention gap') have been identified as one major cause[39]. An approach **focusing on the maintenance of mental health despite stressor exposure (i.e., stress resilience)** is therefore an attractive complementary strategy to disease-oriented research to combat stress consequences, with the potential to prevent much individual suffering and societal burden[40]. This reasoning motivates research into the **mechanisms and factors underlying stress resilience (resilience factors)**. Better understanding resilience mechanisms may lead to new or improved prevention methods.

The study population: emerging adults

Stress-related mental disorders or problems often have their first onset during **adolescence or early adulthood**, where they tend to peak[41]. During the COVID-19 pandemic, youth and emerging adults were among the most strongly mentally affected groups[35,42]. The vulnerability of this age group may partly relate to the critical transition many of them undergo from life in a familiar environment (family, school, friends) into the unfamiliarity and challenges of professional life or higher education, often accompanied by geographical relocation. This is supported by reports of frequent stress-related problems specifically in university student populations[43]. These data and the observation that early-onset stress-related problems are often associated with life-long mental vulnerability, strongly suggest that **investment in the mental health of emerging adults is likely to yield lasting gains** and to be economically particularly efficient[41].

In the ongoing longitudinal study MARP (Mainz Resilience Project) of LIR (coordinated by PHASR-PP project coordinator R. Kalisch) and UM (PI PHASR-PP group leader O. Tüscher)[44], we investigate young adults aged 18-19 years at study inclusion, who are faced with the challenges of transitioning into adulthood (>75% students). An additional inclusion criterion was a history of at least three significant adverse life events (LEs). A past or present formal mental disorder diagnosis was excluded. We observed an average 30-49% of participants at critical levels (above screening cut-off) of internalizing problems/symptoms (measured with the General Health Questionnaire, GHQ-28[45]), at any given study time point, and an average 4.8% symptom increase over the course of 3.5 years (unpubl.). In the EU H2020 project DynaMORE (Dynamic Modeling of Resilience, dynamore-project.eu), coordinated by LIR (R. Kalisch) and including partners UM, UZH (PI PHASR-PP project partner Prof. Birgit Kleim, Inst. of Psychology), and UNIWARSAW (PI PHASR-PP group leader D. Kobylinska), we have conducted a 9-months longitudinal observational study in 18 to 25 year-olds without current diagnosis, of which nearly all were

students and where we additionally required a GHQ score of 20 or higher and three past LEs as inclusion criteria. Participants were allowed to have a distant history of diagnosed stress-related disorders (multi-center study DynaM-OBS, incl. sites UM and UNIWARSAW[46]). In this study, we find 33-45% participants with critical GHQ levels at any time point and an average 2.5% increase over the study (unpubl.).
In both cohorts, we have conducted extensive regular monitoring via self-report of both mental health problems and stressor exposure. Mental health problems in the past two weeks were measured with the GHQ. Stressor exposure measurement was divided into macrostressors (adverse LEs since the last monitoring) and microstressors (daily hassles, DHs, in the past week). For the purpose of stressor monitoring, we have used tools specifically developed by LIR[47,48]. We find that LE and DH measures are typically highly correlated, and using either a combined stressor exposure score (expressing the occurrence of both LEs and DHs[48]) or also only the DH exposure score results in correlations of approx. r=0.25-0.48 of exposure with concurrent mental health problems P (GHQ) across time points and cohorts, in line with the literature (unpubl.). This suggests the presence of specifically stress-related internalizing problems in this population. See Figure 2.
These observations make an **emerging adult population suitable from a viewpoint of resilience research**, because such a population allows one to ask which factors underly the variance in internalizing problems or, more specifically, which factors and mechanisms are associated with fewer internalizing problems despite exposure (resilience factors). Further pragmatic advantages of working in this population are their easy recruitment and high compliance.

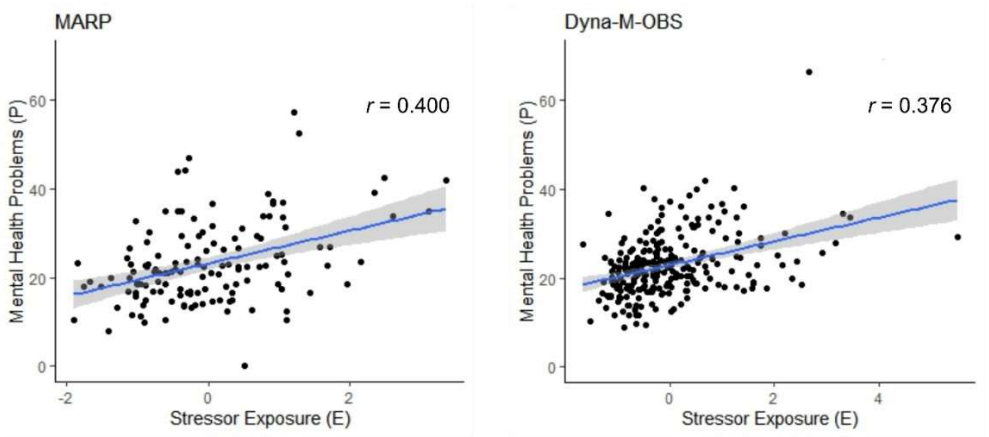


Figure 2. Relationship between stressor exposure (E) and mental health problems (P) in MARP and DynaM-OBS. In MARP, E is calculated as a z-score combining the occurrence (counts) of LEs and DHs, as reported each three months via online monitoring and averaged across the first three monitorings (first nine study months). N=133. In DynaM-OBS, E is a z-score of DH occurrence only, as reported at each bi-weekly monitoring and averaged across the whole 9-months study period. N=238. P is derived in both studies from the GHQ-28 over the respective monitorings. Blue line: regression with 95% confidence interval.

Operationalization of stress resilience: inverse stressor reactivity

Based on the observations in MARP and DynaM-OBS, we have defined a **stressor reactivity (SR) score** as a way to quantify resilience[48]. SR is the residual deviation of an individual from the population mean of the relation between stressor exposure (E) and mental health problems (P) (Figure 2). A positive residual indicates more mental health problems than expected based on the normative E-P relationship in the study sample. A negative residual (negative SR score) indicates less mental health problems than expected (normal). Hence, **SR is an inverse continuous expression of an individual's resilience.** To build SR, E is calculated as a z-score combining the average occurrence (counts) of LEs and DHs, as reported via online monitoring, over the study phase of interest. P, also obtained from online monitoring, is also averaged across a given study

phase. Hence, SR scores can be calculated for any observation time window, such as T4-T6 or T4-T9 in the current project.

Longer-term perspective: towards a pharmacological augmentation of stress resilience
If a role of the BBB in stress resilience can be shown, methods to enhance BBB function under stress could in principle be exploited for resilience promotion, that is, for the prevention of stress-related mental health problems in at-risk groups. Such methods may include pharmacological augmentation, for instance with metformin. Another future way to exploit the anticipated results is to use an assessment of BBB function, via MRI or blood-based markers, as a precision medicine tool allowing for identifying individuals with heightened risk and/or for targeting BBB-protective interventions specifically at individuals with impaired BBB function. Future follow-up work exploiting the anticipated results will require clinical efficacy trials.

3.3.3 PATIENT INVOLVEMENT

The DynaM-OBS study, on which the current study design is based, has been designed with the participation of target group members (young student focus groups). In another sub-study of the ERA-NET Neuron project PHASR-PP (qualitative research project on ethical, legal, and societal aspects (ELSA) of pharmacological augmentation of stress resilience, guided by LU), we will conduct stakeholder interviews with representatives of European patient, patient family, and student organizations, in order to obtain a broader picture of needs of at-risk individuals and families, including from different national and cultural perspectives. For this purpose, we will collaborate with umbrella organizations representing organizations across Europe to recruit interviewees, such as EPF, MHE, ESU, EUFAMI. Further, participants of the current study will be asked to give consent to be contacted for qualitative interviews by LU and to make their study data available to the ELSA study team.

3.3.4 STRATEGIES FOR DATA HANDLING AND THE DISSEMINATION OF RESULTS

Data collection and data management will be conducted in compliance with the principles of the declaration of Helsinki (1996) and relevant national and regional regulations and will only follow study protocols approved by the regional ethics committee at each partner site/country. The investigator at each partner site will be responsible for data management in accordance with these principles. The PIs are further responsible for keeping records of experiments and data collection in line with good laboratory practice.
We will collect and process MRI imaging data (digital: DICOM), biosamples (blood), biological data from the analysis of biosamples (digital: concentrations of plasma proteins/peptides, RNAs, metabolites, and other molecules), and computerized (digital: offline and online collection) and paper-and-pencil (analogue) questionnaire and interview data. Data will be made findable with a study description and metadata following fairsharing.org conventions. Data will be made interoperable by using common standardized formats (in particular, BIDS for MRI) and a detailed codebook. Open accessibility and data re-use are complicated by the sensitive personal nature of most data and the principled non-anonymizability esp. of MRI data. Participant consent forms will permit sharing of pseudonymized data and biosamples among the sites and with collaborators within the European Union and countries with an adequacy decision under Article 45 GDPR, under the supervision of a Data Use and Access Committee (DUAC). All anonymized data will be made openly accessible through public depositories (in particular, OSF) upon publication or at latest two years after the termination of the project and will remain accessible for at least ten years upon completion of the study. Data can be accessed and processed, for example, in R and Python, which are open-source tools that permit extensive documentation.
In general, data storage will be locally for raw data and on central data storage facilities for processed and quality-assured data. Online monitoring data will be collected on the secured SoSci Survey platform and exported directly to a secured server located at, and administered by, LIR. Biosamples will be shipped by courier to UM (Biobank) for central analysis. Full data security is assured by the participating institutions.
Results will be disseminated through publication in peer-reviewed English-language scientific

journals.
Resources are covered by the LIR, UM, UZH, UNIWARSAW, and LU (PhD students, infrastructure, staff) after project end.

3.3.5 GENDER ASPECTS

Because of the potential impact of gender, a comparable number of persons of the two dominating genders (male and female) in the study sample is desirable. Further, with the proportion of persons identifying as non-binary among younger individuals in European countries now reaching an approximate 4% (https://www.ipsos.com/sites/default/files/LGBT%20Pride%202021%20Global%20Survey%20Report%20%20US%20Version.pdf), representation of persons of non-binary gender appears necessary. We therefore plan to recruit at least 45% of participants of either male or female gender, leaving the remaining 10% to any gender, while explicitly also addressing non-binary persons in the recruitment campaign.

3.4 JUSTIFICATION OF DESIGN ASPECTS

3.4.1 CONTROL(S) / COMPARATOR(S)

Since the pharmacological experimental manipulation is not employed in order to test or achieve a clinical effect, placebo comparison is ethically acceptable.

3.4.2 DOSE, MODE AND SCHEME OF INTERVENTION

We have no specific information available on the optimal dose of metformin to achieve BBB effects. We therefore rely on experiences with the use of metformin for current indications (esp. diabetes), where dosages range from 250 to 3000 mg/day over months or years[36,37]. 850 mg twice daily, administered daily, is an average dose used for the treatment of Diabetes mellitus type 2. In a comparable setting (safety and tolerability of metformin for treatment of amnestic mild cognitive impairment), most participants tolerated dosages between 1000 mg and 1500 mg a day[15]. Similar regimens are used in trials of metformin in aging-related diseases. These data define a safe range, which to exploit enhances chances of finding an effect (sensitivity criterion). At the same time, it needs to be considered that our study participants are formally mentally healthy and that the motivation to participate and the adherence during the study may be negatively affected by a high dose (feasibility criterion). Minimization of participant burden is a further relevant aspect (safety criterion). In this trade-off, we choose a daily oral dose of 1700 mg (850 mg BID with meals) during the full-dose treatment (weeks 5 to 12) that is still well in the range of current dosage regimens and also well below the maximum recommended dose of 3000 mg, and we restrict administration to three months overall. We further opt for an initial titration starting comparably low at 500 mg daily the first two weeks, followed by 1000 mg daily during the third and fourth weeks.
Metformin is readily available as a generic drug.

3.4.3 ADDITIONAL TREATMENTS

Symptomatic treatment is allowed as long as there are no known interactions with metformin as documented in PSIAC. For other, especially for excluded, treatments see in-/exclusion criteria.

3.4.4 INCLUSION / EXCLUSION CRITERIA

Inclusion criteria: (participants meeting all of the following criteria will be considered for enrolment in the study)

[1] Absence of mental disorder diagnosis.
[2] University students.
[3] GHQ-28 > 20
[4] Three or more adverse life events acc. to LE list in the past
[5] Beck Depression Inventory (BDI) ≤ 14 & Columbia-Suicide Severity Rating Scale (C-SSRS) ≤ 1. Thereby concurrent depression and suicidality are excluded.
[6] Age 18 to 25 years
[7] Ability of participant to understand character and individual consequences of the study (MMSE Folstein > 28)
[8] Signed and dated informed consent of participant

Exclusion criteria: (participants presenting 1 of the following criteria will not be enroled in the study)

[1] Life-time and current diagnosis of any severe mental disorder determined by M.I.N.I. diagnostic interview.
[2] Known history of brain injuries or neurodevelopmental disorder.
[3] Evidence of neurodegenerative disorder (e.g., Parkinson).
[4] Multimorbidity or significant organ (esp. liver or renal) dysfunction or manifest diabetes or substance abuse (esp. alcohol).

[5] Contraindication to metformin such as renal insufficiency (Creatinin-Clearance< 60ml/min), recent (<3 month) ischemic events (e.g. myocardial infarction or stroke).
[6] Women of childbearing age, who do not practice a medically accepted contraception (i.e., systematic contraceptives, diaphragm, condoms with spermicide, sexual abstinence) during the study and during a 2 years post-study period and who do not present a negative pregnancy test (serum or urine).
[7] History of hypersensitivity to the study drug, to any drug with similar chemical structure, or to any excipient present in the pharmaceutical form of the study drug.
[8] Diabetes type 2 (would result in interference with the experimental manipulation)
[9] Participation in other studies employing a drug during the present study or within the last three months.
[10] Current use of antidiabetic, weight-loss, or psychoactive medication or substances.
[11] Pacemaker, implanted medical pumps, implanted cardiac catheters or acute or unstable heart disease (angina pectoris).
[12] Intracranial implant (aneurysm clips, shunts, stimulators, cochlear implants or electrodes) or other metallic objects inside or near the head (mouth excluded) that cannot be removed.
[13] Claustrophobia or another contraindication to MRI.
[14] Insufficient German language skills.

3.4.5 MEASURES

Determination of primary, secondary, and tertiary measures

Primary endpoint/ outcome measure

- Relating to O1a): Average stressor reactivity (SR) score in study phase 'early follow-up' (time points T4-T6): SR(T4-T6)

Secondary endpoints/ outcome measures

- Relating to O1b): Whole-brain BBB permeability, assessed with neuroimaging, at time point T3 at end of experimental manipulation: BBB(T3).
- Relating to O1c): SR(T4-T6)

Tertiary endpoints/ outcome measures

- Relating to O2a): Average stressor reactivity (SR) score in study phase 'whole follow-up' (time points T4-T9): SR(T4-T9)
- Relating to O2b): SR(T4-T9)

Calculation of SR score

To build the SR score, first, the stressor exposure score E is calculated for every four-weekly monitoring time point T1 to T9 as the z-scored sum of occurrences of all daily hassles (DHs), assessed with the MIMIS[48]. Life event (LE) occurrence, assessed with a life-events list[48], is expected to be less frequent than DH occurrence but, if reported, to correlate with DH occurrence (see above). This will be used to corroborate the validity of the DH-based E score. For each monitoring time point, a mental health problem score P is also calculated. Unlike in the previous studies, we replace the measurement of internalizing symptoms with the GHQ-28 with the shorter (16 item) Patient Health Questionnaire – Anxiety and Depression Scale (PHQ-ADS)[49]. The PHQ-ADS is better suited for repeated measurements than the GHQ. It has been successfully used by us in four studies of the EU RESPOND consortium[50–53]. Its sum score provides a composite measure of depression and anxiety. P i the z-standardized sum score.

To quantify how strongly participants' mental health problems relate to stressor exposure, we then calculate the normative relationship between E and P in the per-protocol analysis sample for each endpoint by fitting a linear mixed model to predict P by E over all included participants and monitoring time points Tx, with random slopes and intercepts for participants. The E-P regression line is then determined by the fixed effect estimates for the E~P slope and intercept and serves as the normative E-P relationship for the analysis sample over the study phase of interest. The form of regression that explains most variance in P (linear or quadratic) will be used, as in prior work[54–56]. See also Figure 2. Based on experiences in DynaM-OBS, which has a comparable monitoring frequency and study duration in a comparable sample (see above), we expect an E-P

correlation of about r=0.27 to 0.43. Notably, exclusive reliance on a DH-based E score has been feasible in DynaM-OBS, with adding a measure of the (rare) LE occurrence not relevantly improving variance explanation (unpubl.).
On this basis, at each Tx, we enter participants' individual E scores into the normative E~P line equation, giving us their expected P score when assuming normal stressor reactivity. The SR score is the individual's average residual onto the regression line for the study phase of interest.

Calculation of whole-brain BBB permeability
A diffusion-prepared pseudo-continuous ASL (pCASL) MRI sequence will be used to estimate BBB permeability. By reading out pCASL signals at different post-labeling delays (PLD) under different diffusion gradients, perfusion signals in capillary and brain tissues can be reliably estimated and subsequently fitted into a model to estimate the water exchange rate (kw) from blood to brain tissue, an index quantifying BBB permeability. Analysis will use a toolbox provided by the sequence developer (http://loft-lab.org/index-5.html) and includes image reconstruction, preprocessing (motion-correction, co-registration, skull-stripping), and kw modeling. Whole-brain averaged kw values quantifying overall BBB permeability will be calculated.

Additional measures
For inclusion of covariates, socio-demographic and psycho-social variables will be assessed via questionnaire and scored acc. to standard procedures. For additional exploratory analyses, blood samples will be taken and used to determine blood-based molecular and cellular markers of immune system, BBB, and metabolic function (including proteins, peptides, RNAs, metabolites, HBA1C levels).

3.4.6 METHODS AGAINST BIAS

Randomization and blinding: Experimental manipulation groups are completely masked (double-blind study). A randomization list will be generated by the respective study center. The randomization ratio will be 1:1. One copy of the randomization list will be sent to the pharmacy of the respective site. At the randomization visit (T0), each participant eligible for study participation will receive the next consecutive randomization number from a block of randomization numbers per site. The randomization list will be kept in safe and confidential custody at each study center site. In addition to the study drug, the investigator will receive a set of sealed envelopes, one for each randomization number. These envelopes contain information on each participant's study drug and are to be opened only under circumstances in which it is medically imperative for diagnostic or therapeutic decisions to know what the participant is receiving.

3.4.7 PROPOSED SAMPLE SIZE / POWER CALCULATIONS

Because the association of BBB function in stressor-exposed individuals at risk for stress-related mental health problems with stress resilience (inverse SR score) has never been investigated, we base our power calculation for the primary endpoint on the sensitivity to detect a medium effect size of Cohen's d = 0.3. With a power of 0.8, a two-sided alpha-level of 0.05, and a linear mixed model with eight predictor variables (3 variables of interest and 5 covariates), we need n=109 participants.

Compliance / Rate of loss to follow up
Taking into account 10% attrition, we aim to recruit a total number of 122 participants (61 in each group). Adherence to online monitoring at a given online monitoring time point (T0-T9) will be based on a criterion of full completion of the DH and PHQ-ADS lists with reasonable scores (e.g., not all item scores in a list identical or zero) at that time point. Responses at online monitoring time points not fulfilling this criterion will be considered incomplete (missing). Adherence to the experimental manipulation will be based on a participant diary (paper and pencil) and the rendition of study drug blisters after the end of the experimental manipulation at study visit 2 (T3).
In order to promote adherence, a step-wise reimbursement scheme is implemented. Participants receive a first reimbursement of max. 90 € after study visit 1 (50 € for participation in the medical

exam and online questionnaires, 10 € specifically for blood draws, 30 € for neuroimaging), max. 290 € after study visit 2 (50 € for medical exam and online questionnaires, 10 € for blood draws, 30 € for neuroimaging, 200 € for study drug administration, where 10% missed administrations are allowed), and max. 240 € after study visit 3 (50 € for medical exam and online questionnaires, 10 € for blood draws, 180 € for online monitoring of stressor and internalizing symptoms, the latter proportional to completion). The total possible reimbursement is 620 €.

3.4.8 FEASIBILITY OF RECRUITMENT

We distribute participant recruitment across three different countries (D, CH, PL), since this will increase the heterogeneity of the study sample compared to a study in only one country and, consequentially, increase the likelihood that the results are generalizable (not only valid in a specific population). The recruiting centers UZH, UM, UNIWARSAW are all part of the former DynaMORE consortium and have proven to recruit participants from the target population (university students) in a variety of studies such as DynaM-OBS (UM n=39 participants; UNIWARSAW n=53 participants), DynaM-INT (UM n=27 participants; UNIWARSAW n=62 participants), and ecological momentary intervention trials (UZH many participants), each within periods of 12-18 months, including despite complications related to the COVID-19 pandemic.

3.4.9 INTERNATIONAL COLLABORATIONS

University Warsaw, Poland; University Zurich, Switzerland; University of Latvia, Latvia.

It is possible that the participant will be contacted during or after the study by the cooperation partner University of Latvia (LU) with the request to participate in interviews in which they will be asked about their opinions and attitudes towards the study topic. This will take place as part of another, separate study of the PHASR-PP project (Ethical, Legal, and Societal Aspects project part). Participation in the current study does not obligate participants to take part in this separate study.

3.4.10 STOPPING RULES

Discontinuation criteria are

a) participant may discontinue participation due to any of the following reasons:
   - at their own request or at request of the legal representative
   - any medical condition demanding the immediate discontinuation of (potential) metformin treatment, e.g., need for urgent anaesthesia, renal failure etc.
   - for safety reasons at the request of the investigator or request of a regulatory agency
   - significant AEs related to the experimental manipulation (participants will be followed up for manipulation response and safety)
   - participant is non-compliant or not sufficiently compliant with the study procedures / study protocol
   - if, in the investigator's opinion, continuation of the study would be detrimental to the participant's well-being.
   - participant needs a medication not allowed in the protocol during the study
   - any clinically significant change in participant's pre-study medical condition
b) for the following reasons, a study site may be closed at the discretion of the investigator:
   - medical or ethical reasons that are detrimental to the continued performance of the study
   - difficulties in the recruitment of participants (i.e., which fail to include at least a third of estimated participants to be included by mid of the recruitment period (6.5 months after last participant-in))
   - critical protocol violations
   - violations of legal and ethical regulations
   - non-compliance of the study site investigators
c) for the whole study at the discretion of the investigator:
   - new risks for participants become known

- occurrence of AEs unknown to date in respect of their nature, severity, and duration or the unexpected increase in the incidence of known AEs
- medical or ethical reasons that are detrimental to the continued performance of the study
- difficulties in the recruitment of participants

3.5 STATISTICAL ANALYSES

Analysis populations
The primary analysis population will be the intention-to-treat (ITT) population (all participants who signed informed consent and were randomized). Secondary analyses will be conducted in the per-protocol population (ITT population without major protocol violations, not further described).
No interim analysis will be performed.
There is no replication sample.

Significance testing
All hypotheses will be tested on a two-sided level of significance $\alpha=0.05$. The primary hypothesis (primary analysis relating to the primary objective O1a) will be tested separately and without correction for multiple testing. Correction for multiple testing will be applied family-wise to the key secondary hypotheses (two key secondary analyses relating to the primary objectives O1b and O1c), provided that the conditions to test the key secondary hypothesis relating to objective O1c are met.

Method
All analyses will be conducted by means of linear mixed models. Depending on the pattern and extent of missing data, we will consider imputation approaches to ensure sufficient analysis power, especially in the analysis of SR at follow-up periods. We will perform autoregression-informed imputation, given that sufficient autocorrelation is present and if there is no evidence for non- randomly missing data.
Age, gender, site, and BMI will be used as covariates in all analyses. Selection of further covariates will follow a procedure used in earlier studies[55–57] such that all potential covariates with a p value <0.2 in univariate regression models on the endpoint will be included. We expect this to result in the selection of one additional covariate, most likely childhood trauma. Hence, in total, we expect to use five covariates.

Primary analysis
Relating to O1a): To test for a prospective association of whole-brain BBB permeability with average SR at T4-T6 during early follow-up (SR(T4-T6)), a linear mixed model will be used to regress BBB permeability at baseline (BBB(T0)), BBB permeability at end of experimental manipulation (BBB(T3)), experimental manipulation (EM; groups metformin and placebo), and covariates onto (SR(T4-T6)).

Key secondary analyses
Relating to O1b): To test for an effect of the experimental manipulation between time points T0 and T3 (groups metformin and placebo) on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation (BBB(T3)), a linear mixed model will be used to regress EM, BBB(T0), and covariates onto BBB(T3).

Relating to O1c): If BBB(T3) shows a significant association with SR(T4-T6) (see O1a) and if EM shows a significant association with BBB(T3) (see O1b): To test for mediation by the effect of the experimental manipulation on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation on participants' average stressor reactivity at time points T4-T6 during early follow-up, mediation analysis will be conducted using a Baron-Kenny approach with EM as predictor, BBB(T3) as mediator, SR(T4-T6) as outcome, and BBB(T0) and earlier covariates as covariates.

Further secondary analyses
Relating to O2a): To test for a prospective association of whole-brain BBB permeability with average SR at T4-T9 during whole follow-up (SR(T4-T9)), a linear mixed model will be used to regress BBB(T0), BBB(T3), EM, and covariates onto SR(T4-T9).

Relating to O2b): If BBB(T3) shows a significant association with SR(T4-T9) (see O2a) and if EM shows a significant association with BBB(T3) (see O1b): To test for mediation by the effect of the experimental manipulation on participants' whole-brain BBB permeability at time point T3 at end of experimental manipulation on participants' average stressor reactivity at time points T4-T9 during whole follow-up, mediation analysis will be conducted using a Baron-Kenny approach with EM as predictor, BBB(T3) as mediator, SR(T4-T9) as outcome, and BBB(T0) and earlier covariates as covariates.

Safety

All summaries and listings of safety data will be performed for the safety population. Summary tables of AEs will present the number of participants observed with AEs and corresponding percentages. Laboratory values and vital signs will be analyzed by descriptive methods and exploratory p-values.

3.6 ETHICAL CONSIDERATIONS

Risks and benefits
Individual participant risks are mainly associated with the potential adverse effects (AEs) of the study drug metformin, i.e., nausea, vomiting, stomach upset, diarrhea, weakness, or a metallic taste in the mouth may occur. In case of metformin accumulation lactic acidosis is possible as serious AE. There are no immediate benefits of this study for the individual participants aside from participant reimbursement. Participants will receive a reimbursement of 620€.

Care and protection for research participants
During the conduct of the study, the participant should not undergo clinical treatments except for cases of emergency. The participant is bound to inform the investigator immediately about any AEs and additionally drugs taken. According to Sect 40 AMG, the investigator is obliged to take out a participant insurance, which covers, in its terms and provisions, the legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards. The terms and conditions of the insurance will be delivered to the participant at the time of informed consent. Insurance provisions for this study are given in separate agreements.

Good clinical practice
The procedures set out in this study protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that all persons involved in the study abide by good clinical practice (GCP) and the ethical principles described in the Declaration of Helsinki. The study will be carried out in keeping with local legal and regulatory requirements. The requirements of the AMG, the GCP regulation, and the Federal Data Protection Law (BDSG) will be kept.

Patient information and informed consent
Before being admitted to the study, the participant must consent to participate after being fully informed about the nature, scope, and possible consequences of the study. The documents must be in a language understandable to the participant and must specify who informed the participant. A copy of the signed informed consent document must be given to the participant. The original signed consent document will be retained by the investigator. The investigator will not undertake any measures specifically required only for the study until valid consent has been obtained. If the participant has a primary physician and if the participant agrees to the primary physician being informed, the investigator should inform the participant's primary physician about the participant's participation in the study. After reading the informed consent document, the participant must give consent in writing. The participant's consent must be confirmed by the personally dated signature of the participant and by the personally dated signature of the person conducting the informed consent discussions.

Confidentiality
The name of the participants and other confidential information are subject to medical professional secrecy and the regulations of the EU and German Data Protection Acts (EU-DSGVO and Bundesdatenschutzgesetz). During the study, participants will be identified solely by means of an individual identification code (e.g., participant number, randomization number). Study findings stored on a computer will be stored in accordance with local data protection law and will be handled in strictest confidence. For protection of these data, organizational procedures are implemented to prevent distribution of data to unauthorized persons. The appropriate regulations of data legislation will be fulfilled in its entirety. The participant will declare in the written consent to release the investigator from the medical professional secrecy to allow identification of participant's name and/or inspection of original data for monitoring purposes by health authorities and authorized persons (monitors). The investigator will maintain a personal participant identification list (participant numbers with the corresponding participant names) to enable records to be identified.

3.7 QUALITY ASSURANCE, SAFETY AND MANAGEMENT STRUCTURE

3.7.1 QUALITY ASSURANCE / MONITORING

Requirements for investigational sites and staff
The investigator should be able to demonstrate (e.g., based on retrospective data) a potential for recruiting the required number of suitable participants within the agreed recruitment period. The investigator should have sufficient time to properly conduct and complete the study within the agreed study period. The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the study to conduct the study properly and safely. The investigator should ensure that all persons assisting with the study are adequately qualified, informed about the protocol, any amendments to the protocol, the study drug, and their study-related duties and functions.

Direct access to source data/documents
The investigator/institution must permit study-related monitoring and auditing by the study-external monitor, as well as inspections by the appropriate competent authorities and Ethics committees, providing direct access to source data/documents (confidentiality see 3.6).
The participants will be informed that representatives of the investigator, independent ethics committees (IEC), or competent authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

Investigator site file and archiving
The investigator will be provided with an investigator site file (ISF) at the start of the study. The investigator will archive all study data and relevant correspondence in the ISF. The ISF, all source data, and all documents will be kept filed according to the requirements of the ICH-GCP guidelines after termination of the study. It is the responsibility of the investigator to ensure that the participant identification sheets are stored for at least 15 years beyond the end of the study. All original participant files must be stored for the longest possible time permitted by the regulations at the hospital, research institute, or practice in question.

Monitoring
Monitoring will be done by personal visits from a study-external monitor. Pre-study visits will be conducted, and the reports will be reviewed by the investigator. To initiate the study, the monitor will visit all participating local study sites and study centers. The monitor shall ensure that the investigator and their staff understand all requirements of the protocol and their regulatory responsibilities. The monitor will ensure that the investigator will maintain a list of sub-investigators and other appropriately qualified persons to whom he or she has delegated significant study-related duties (personnel log). Each site will be visited by the monitor at regular intervals to ensure compliance with the study protocol, GCP, and legal aspects. The monitor will review the entries into the CRFs for completeness and correctness and verify the entries on the basis of the source documents. The presence of correct informed consents will be checked for every participant. Details will be specified in the monitoring manual for this study. The investigator must allow the monitor to look at all relevant documents and must provide support at all times to the monitor. By frequent communications (letters, telephone, fax), the monitor will ensure that the study is conducted according to the protocol and regulatory requirements.

Inspection by authorities and ethic committees
Competent authorities and by the investigator authorized persons (auditor) may request access to all source documents, CRF, and other study documentation in case of an inspection or audit. Direct access to these documents must be guaranteed by the investigator who must provide support at all times for these activities. Source data documents can be copied during inspection or audit in case the identity of the participant have been made unrecognizable.

Audits
No audits are planned for this study.

3.7.2 SAFETY / PHARMACOVIGILANCE

Assessment of safety
Adverse Event (AE) definition will be used according to GCP: an AE is defined as any untoward medical occurrence in a participant treated with a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to that drug.

Assessment of AEs by investigator
Participants must be carefully monitored for AEs by the investigator at each study visit. The intensity of the AEs and the causal relation to the study drug and/or procedures are to be assessed.

Period of observation
In this study, the period of observation for collection of AEs extends from the time the participant has signed the informed consent document up to the end of the 24 weeks follow-up period. If the investigator detects a serious AE in a study participant after the end of the period of observation, and considers the event possibly related to the prior study, they should contact the data and safety monitoring board (DSMB) to determine how the AE should be documented and reported.

Documentation of AEs and Follow-up
All AEs reported by the participant or detected by the investigator will be documented on the appropriate pages of the case report form (CRF). All participants who have AEs, whether considered associated with the use of the study drug or not, must be monitored to determine the outcome.

Immediate reporting of Serious AEs (SAEs) by investigator
SAEs must immediately (within 24 hours of the investigator's awareness) be reported to the DSMB. The initial SAE Report should be as complete as possible including the essential details of participant's identification (screening number, random number), the SAE (medical term, diagnosis), the study drug, and the assessment of the causal relationship between the event and the study drug. The SAE report must be reviewed and signed by the investigator. The investigator should provide related additional information on the clinical course and the outcome of each SAE as soon as possible (Follow-up report). The "Serious Adverse Event Form" is provided in the investigator site file (ISF). The investigator should also inform the study monitor in all cases.

Immediate Reporting of pregnancy by investigator
Any pregnancy diagnosed in a female participant or in the female partner of a male participant during the experimental manipulation must be reported immediately using the "Pregnancy Reporting Form" (provided in ISF) to the DSMB.

Safety evaluation and Reporting by investigator
The investigator will ensure that all legal reporting requirements are met. The investigator is responsible for the continuous safety evaluation of the study drug and the study. The investigator will conduct the management of SAEs and the expedited reporting as required by GCP regulation (GCP-V). Suspected unexpected serious adverse reactions (SUSARs) and safety issues as defined by GCP-V are determined for expedited reporting: The ethics committees should be notified as soon as possible but not later than 15 calendar days if the event is non-fatal and 7 calendar days if it was fatal. All co-investigators and sub-investigators will be informed, too. Work flow and procedures concerning SAE management will be described in a separate document (e.g., Safety manual). During the study, the investigator will submit the annual safety report including a list of all serious adverse reactions to the ethics committee(s) once a year.

Emergency procedures
During and following a participant's participation in the study, the investigator should ensure that adequate medical care is provided to a participant for any AEs including clinically significant laboratory values. The investigator should inform a participant when medical care is needed for intercurrent illness(es) of which the investigator becomes aware. Emergency treatment for overdose will be described. Emergency Unblinding: If it is medically imperative to know what study drug the participant is receiving, the investigator or authorized person should open the randomization envelope. The investigator or the person who breaks the blind must record the date and the reasons for doing so in the CRF, in the participant's medical record and on the randomization envelope. Unblinding within the scope of emergency treatment by third parties: For this double-blind pharmacological study, a 7 days/24 hours emergency unblinding will be established (for instance in case of an emergency hospitalization into an external hospital). It will be possible to contact the operator of the switchboard of UM Mainz and the on-call duty of the UM Pharmacy to organize such an emergency unblinding.

Other safety data
All observations pertinent to the safety of the study medication will be recorded on the CRF and included in the final report. Additionally to the registration of the AEs and serious AEs, we conduct further assessments of safety at every visit.

Prior and concomitant illnesses
Relevant additional illnesses present at the time of informed consent are regarded as concomitant illnesses and will be documented on the appropriate pages of the case report form (CRF).

Prior and concomitant treatments
Relevant treatments administered to the participants on entry to the study or at any time during the study are regarded as concomitant treatments and must be documented on the appropriate pages of the CRF.

3.7.3 MANAGEMENT STRUCTURE AND PROCEDURES

An independent data and safety monitoring board (DSMB; see list of proposed members) shall be established to supervise the progress of the study and to review relevant information from other sources to advise whether to continue, modify, or stop the study and provide the funding organizations with information and advice. The DSMB will meet twice: at mid of the recruitment period and end of the recruitment period. To monitor safety data and to ensure adherence to protocol will be responsibility of the study monitor.

3.7.4 REGISTRATION

The study will be registered with clinicaltrial.gov.

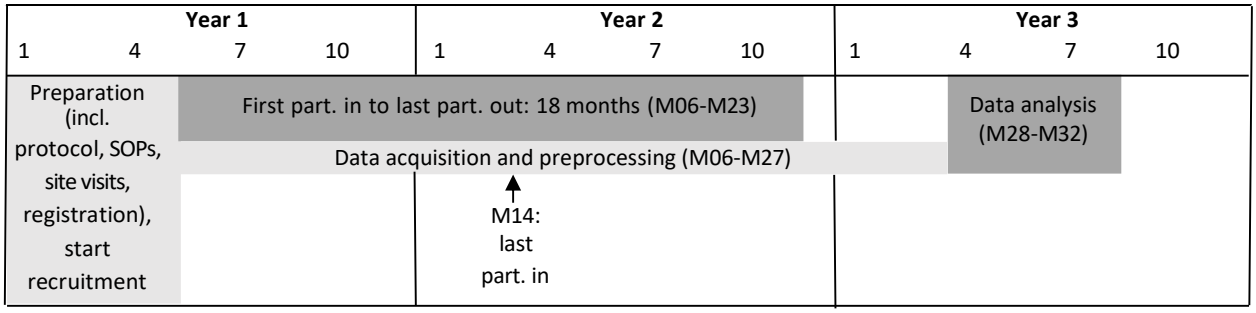
3.8 REFERENCES

1. Galea, I., Bechmann, I. & Perry, V. H. What is immune privilege (not)? *Trends Immunol.* **28**, 12–18 (2007).
2. Louveau, A., Harris, T. H. & Kipnis, J. Revisiting the Mechanisms of CNS Immune Privilege. *Trends Immunol.* **36**, 569–577 (2015).
3. Abbott, N. J., Patabendige, A. A. K., Dolman, D. E. M., Yusof, S. R. & Begley, D. J. Structure and function of the blood-brain barrier. *Neurobiol. Dis.* **37**, 13–25 (2010).
4. Danielski, L. G. *et al.* Brain Barrier Breakdown as a Cause and Consequence of Neuroinflammation in Sepsis. *Mol. Neurobiol.* **55**, 1045–1053 (2018).
5. Cathomas, F. *et al.* Beyond the neuron: Role of non-neuronal cells in stress disorders. *Neuron* **110**, 1116–1138 (2022).
6. Powell, N. D. *et al.* Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via β-adrenergic induction of myelopoiesis. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 16574–16579 (2013).
7. Menard, C. *et al.* Social stress induces neurovascular pathology promoting depression. *Nat. Neurosci.* **20**, 1752–1760 (2017).
8. Dudek, K. A. *et al.* Molecular adaptations of the blood-brain barrier promote stress resilience vs. depression. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 3326–3336 (2020).
9. Rebeles, F., Fink, J., Anzai, Y. & Maravilla, K. R. Blood-brain barrier imaging and therapeutic potentials. *Top. Magn. Reson. Imaging TMRI* **17**, 107–116 (2006).
10. Marchi, N. *et al.* Peripheral markers of brain damage and blood-brain barrier dysfunction. *Restor. Neurol. Neurosci.* **21**, 109–121 (2003).
11. Heye, A. K., Culling, R. D., Valdés Hernández, M. D. C., Thrippleton, M. J. & Wardlaw, J. M. Assessment of blood-brain barrier disruption using dynamic contrast-enhanced MRI. A systematic review. *NeuroImage Clin.* **6**, 262–274 (2014).
12. Shao, X. *et al.* Mapping water exchange across the blood-brain barrier using 3D diffusion-prepared arterial spin labeled perfusion MRI. *Magn. Reson. Med.* **81**, 3065–3079 (2019).
13. Joseph, C. R. Utilizing 3D Arterial Spin Labeling to Identify Cerebrovascular Leak and Glymphatic Obstruction in Neurodegenerative Disease. *Diagn. Basel Switz.* **11**, 1888 (2021).
14. Andersson, M. *et al.* Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *J. Neurol. Neurosurg. Psychiatry* **57**, 897–902 (1994).
15. Gudmundsson, P. *et al.* The relationship between cerebrospinal fluid biomarkers and depression in elderly women. *Am. J. Geriatr. Psychiatry Off. J. Am. Assoc. Geriatr. Psychiatry* **15**, 832–838 (2007).
16. Niklasson, F. & Agren, H. Brain energy metabolism and blood-brain barrier permeability in depressive patients: analyses of creatine, creatinine, urate, and albumin in CSF and blood. *Biol. Psychiatry* **19**, 1183–1206 (1984).
17. Kanner, A. A. *et al.* Serum S100beta: a noninvasive marker of blood-brain barrier function and brain lesions. *Cancer* **97**, 2806–2813 (2003).
18. Polyakova, M. *et al.* First evidence for glial pathology in late life minor depression: S100B is increased in males with minor depression. *Front. Cell. Neurosci.* **9**, 406 (2015).
19. Dion-Albert, L. *et al.* Vascular and blood-brain barrier-related changes underlie stress responses and resilience in female mice and depression in human tissue. *Nat. Commun.* **13**, 164 (2022).
20. Janssen, E. P. C. J. *et al.* Low-grade inflammation and endothelial dysfunction predict four-year risk and course of depressive symptoms: The Maastricht study. *Brain. Behav. Immun.* **97**, 61–67 (2021).
21. Vennin, C. *et al.* A Resilience Related Glial-Neurovascular Network Is Transcriptionally Activated after Chronic Social Defeat in Male Mice. *Cells* **11**, 3405 (2022).
22. Wullschleger, S., Loewith, R. & Hall, M. N. TOR signaling in growth and metabolism. *Cell* **124**, 471–484 (2006).
23. Liu, G. Y. & Sabatini, D. M. mTOR at the nexus of nutrition, growth, ageing and disease. *Nat. Rev. Mol. Cell Biol.* **21**, 183–203 (2020).
24. Lin, A.-L. *et al.* Chronic rapamycin restores brain vascular integrity and function through NO

synthase activation and improves memory in symptomatic mice modeling Alzheimer's disease. *J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab.* **33**, 1412–1421 (2013).
25. Lin, A.-L. *et al.* Rapamycin rescues vascular, metabolic and learning deficits in apolipoprotein E4 transgenic mice with pre-symptomatic Alzheimer's disease. *J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab.* **37**, 217–226 (2017).
26. Van Skike, C. E. *et al.* Inhibition of mTOR protects the blood-brain barrier in models of Alzheimer's disease and vascular cognitive impairment. *Am. J. Physiol. Heart Circ. Physiol.* **314**, H693–H703 (2018).
27. Chi, O. Z., Kiss, G. K., Mellender, S. J., Liu, X. & Weiss, H. R. Rapamycin decreased blood-brain barrier permeability in control but not in diabetic rats in early cerebral ischemia. *Neurosci. Lett.* **654**, 17–22 (2017).
28. Hadley, G. *et al.* Rapamycin in ischemic stroke: Old drug, new tricks? *J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab.* **39**, 20–35 (2019).
29. Chen, S. *et al.* Metformin in aging and aging-related diseases: clinical applications and relevant mechanisms. *Theranostics* **12**, 2722–2740 (2022).
30. Triggle, C. R. *et al.* Metformin: Is it a drug for all reasons and diseases? *Metabolism.* **133**, 155223 (2022).
31. Amin, S., Lux, A. & O'Callaghan, F. The journey of metformin from glycaemic control to mTOR inhibition and the suppression of tumour growth. *Br. J. Clin. Pharmacol.* **85**, 37–46 (2019).
32. Bai, B. & Chen, H. Metformin: A Novel Weapon Against Inflammation. *Front. Pharmacol.* **12**, 622262 (2021).
33. Liu, Y. *et al.* Metformin attenuates blood-brain barrier disruption in mice following middle cerebral artery occlusion. *J. Neuroinflammation* **11**, 177 (2014).
34. GBD 2019 Mental Disorders Collaborators. Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Psychiatry* **9**, 137–150 (2022).
35. COVID-19 Mental Disorders Collaborators. Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *Lancet Lond. Engl.* **398**, 1700–1712 (2021).
36. Calcia, M. A. *et al.* Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology (Berl.)* **233**, 1637–1650 (2016).
37. Cuthbert, B. N. & Insel, T. R. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. *BMC Med.* **11**, 126 (2013).
38. Kalisch, R., Müller, M. B. & Tüscher, O. A conceptual framework for the neurobiological study of resilience. *Behav. Brain Sci.* e92 (2015) doi:10.1017/S0140525X1400082X.
39. Jorm, A. F., Patten, S. B., Brugha, T. S. & Mojtabai, R. Has increased provision of treatment reduced the prevalence of common mental disorders? Review of the evidence from four countries. *World Psychiatry Off. J. World Psychiatr. Assoc. WPA* **16**, 90–99 (2017).
40. Kalisch, R. *et al.* The resilience framework as a strategy to combat stress-related disorders. *Nat. Hum. Behav.* **1**, 784 (2017).
41. Stelmach, R. *et al.* The global return on investment from preventing and treating adolescent mental disorders and suicide: a modelling study. *BMJ Glob. Health* **7**, e007759 (2022).
42. Penninx, B. W. J. H., Benros, M. E., Klein, R. S. & Vinkers, C. H. How COVID-19 shaped mental health: from infection to pandemic effects. *Nat. Med.* **28**, 2027–2037 (2022).
43. Tupler, L. A., Hong, J. Y., Gibori, R., Blitchington, T. F. & Krishnan, K. R. R. Suicidal ideation and sex differences in relation to 18 major psychiatric disorders in college and university students: anonymous web-based assessment. *J. Nerv. Ment. Dis.* **203**, 269–278 (2015).
44. Kampa, M. *et al.* A Combined Behavioral and Neuroimaging Battery to Test Positive Appraisal Style Theory of Resilience in Longitudinal Studies. *bioRxiv* (2018) doi:doi: https://doi.org/10.1101/470435.
45. Goldberg, D. P. & Hillier, V. F. A scaled version of the General Health Questionnaire. *Psychol Med* **9**, 139–45 (1979).
46. Wackerhagen, C. *et al.* Dynamic Modelling of Mental Resilience in Young Adults: Protocol for

a Longitudinal Observational Study (DynaM-OBS). *JMIR Res. Protoc.* **12**, e39817 (2023).
47. Chmitorz, A., Kalisch, R., Kubiak, T., Lieb, K. & Tüscher, O. Validation of a retrospective questionnaire for daily hassles with ecological momentary assessment. in ID 140 (2017).
48. Kalisch, R. *et al.* The Frequent Stressor and Mental Health Monitoring-Paradigm: A Proposal for the Operationalization and Measurement of Resilience and the Identification of Resilience Processes in Longitudinal Observational Studies. *Front. Psychol.* **12**, 710493 (2021).
49. Kroenke, K. *et al.* Patient Health Questionnaire Anxiety and Depression Scale: Initial Validation in Three Clinical Trials. *Psychosom. Med.* **78**, 716–727 (2016).
50. Mediavilla, R. *et al.* Effectiveness of a stepped-care programme of internet-based psychological interventions for healthcare workers with psychological distress: Study protocol for the RESPOND healthcare workers randomised controlled trial. *Digit. Health* **8**, 20552076221129084 (2022).
51. Purgato, M. *et al.* Effectiveness of a stepped-care programme of WHO psychological interventions in migrant populations resettled in Italy: Study protocol for the RESPOND randomized controlled trial. *Front. Public Health* **11**, 1100546 (2023).
52. Roos, R. *et al.* Effectiveness of a scalable, remotely delivered stepped-care intervention to reduce symptoms of psychological distress among Polish migrant workers in the Netherlands: study protocol for the RESPOND randomised controlled trial. *BMC Psychiatry* **23**, 801 (2023).
53. Melchior, M. *et al.* Addressing mental health problems among persons without stable housing in the context of the COVID-19 pandemic: study protocol for a randomised trial. RESPOND - France. *BMC Public Health* **23**, 2275 (2023).
54. Veer, I. M. *et al.* Psycho-social factors associated with mental resilience in the Corona lockdown. *Transl. Psychiatry* **11**, 67 (2021).
55. Bögemann, S. A. *et al.* Psychological Resilience Factors and Their Association With Weekly Stressor Reactivity During the COVID-19 Outbreak in Europe: Prospective Longitudinal Study. *JMIR Ment. Health* **10**, e46518 (2023).
56. Zerban, M. *et al.* What helps the helpers? Resilience and risk factors for general and profession-specific mental health problems in psychotherapists during the COVID-19 pandemic. *Front. Psychol.* **14**, 1272199 (2023).
57. Veer, I. M. *et al.* Psycho-social factors associated with mental resilience in the Corona lockdown. *Transl. Psychiatry* **11**, 67 (2021).

3.9 STUDY TIME FLOW



3.10 LIST OF PARTNERS INVOLVED IN THE STUDY

Study Management				
#	Name	Affiliation	Responsibility / Role	Signature
1	Prof. Dr. Oliver Tüscher	Dept. of Psychiatry, UM, and LIR	Applicant, Investigator	
Study Statistician				
#	Name	Affiliation	Responsibility / Role	Signature
2	Dr. Kenneth Yuen	Neuroimaging Center, UM	Statistical advice	

Study Supporting Facilities *(reference laboratories, pharmacies etc.)*			
#	Name	Affiliation	Responsibility / Role
3	Prof. Dr. Irene Krämer	Hospital Pharmacy, UM	Preparation of study drug
2	Dr. Kenneth Yuen	Neuroimaging Center, UM	Neuroimaging
4	Dr. Susanne Englisch	Studienzentrum Psychiatrie, Dept. of Psychiatry, UM	Study center services
5	Prof. Dr. Philipp Wild	CTH Biobank, UM	Biobank services
6	Prof. Dr. Karl Lackner	Inst. f. Laboratory Medicine, UM	Blood sample analyses

Recruiting centers			
#	Name	Affiliation	No. of participants to be recruited
1	Prof. Dr. Oliver Tüscher	Dept. of Psychiatry, UM, Mainz, Germany	36-46
7	Dr. Flurin Cathomas	Dept. of Psychiatry, Psychotherapy and Psychosomatics, UZH, Zurich, Switzerland	36-46
8	Dr. Dorota Kobylinska	Faculty of Psychology, UNIWARSAW, Warsaw, Poland	36-46
Total sum of participants to be recruited			**Σ = 122**

Data and Safety Monitoring Board (DSMB)		
#	Name	Affiliation *(only institution and city, no complete address)*
9	Prof. Dr. Henrik Walter	Dept. of Psychiatry and Psychotherapy, Charité Universitätsmedizin Berlin
10	Prof. Dr. Dominik Bach	Dept. of Psychiatry and Psychotherapy, University Hospital Bonn
11	Prof. Dr. Helen Morrison	Leibniz Institute on Aging – Fritz Lippmann Institute, Jena

Other participating groups / bodies			
#	Name	Affiliation	Responsibility / Role
12	Prof. Dr. Raffael Kalisch	LIR and Neuroimaging Center, UM	Co-Investigator

13	Prof. Dr. Signe Mezinska	Faculty of Medicine and Life Sciences, LU	Co-Investigator
14	Prof. Dr. Susann Schweiger	Dept. of Human Genetics, UM, and LIR	Project Partner
15	Prof. Dr. Birgit Kleim	Inst. of Psychology, UZH	Project Partner
Review of study protocol *(who will review and finalize the protocol? Please refer to numbers above and / or include others)*			
#	Name	Affiliation *(only institution and city, no complete address)*	
1	Prof. Dr. Oliver Tüscher	Dept. of Psychiatry, UM, and LIR	

3.11 FINANCIAL DETAILS OF THE STUDY

3.11.1 COMMERCIAL INTEREST

Metformin is a generic drug and this study is not an efficacy trial. There is, hence, no commercial interest of a company to sponsor this study.

3.11.2 FINANCIAL SUMMARY

ERA-NET NEURON Call 2023

Pre-proposal: Budget plan of the project

Project Acronym: PHASR-PP

	Coordinator	Partner 2	Partner 3	Partner 4	Partner 5	Partner 6*	
Name (group leader)	Kalisch	Tüscher	Cathomas	Kobylinska	Mezinska	N.A.	
Institution	LIR	UM	UZH	UNIWARSAW	LU		
Country	Germany	Germany	Switzerland	Poland	Latvia		
Funding organisation	BMBF (DFG)	BMBF (DFG)	SNSF	NCBR	LZP		
PROJECT COSTS (€)							**Total**
Personnel €	169,600	0	224,458 (225,029 CHF)	53,617 (252,000 PLN)	139,800		
Consumables €	0	28,600	24,905 (24,826 CHF)	10,000 (47,000 PLN)	0		
Equipment €	0	0	0	0	3,000		
Travel €[1]	10,800	2,400	10,800 (10,599 CHF)	10,800 (50,760 PLN)	10,800		
Other direct costs €[2]	0	101,218	21,000 (20,609 CHF)	73,165 (343,876 PLN)	3,500		
Overheads €[3]	16,960	26,444	0	23,104 (108,590 PLN)	39,275		
Total budget €[4]	197,360	158,662	281,163 (281,064 CHF)	170,686 (802,225 PLN)	196,375		**1,004,246**
Requested budget €[4,5]	N.A.	N.A.	N.A.	N.A.	N.A.		**N.A.**