

Study Protocol

Effectiveness and underlying mechanisms of Applied Relaxation as indicated preventive intervention in subjects at increased risk for mental disorders

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1 State of the art and preliminary work

Mental disorders are highly prevalent and associated with substantial impairment and tremendous societal costs (1-3). One third of the adult general population in Germany meets criteria for a 12-month diagnosis of any mental disorder; 44% of those suffer from more than one and 22% from three or more conditions (1). With 12-month prevalences of 15.3% and 9.3%, respectively, anxiety and mood disorders are particularly frequent (1) and rank among the top causes and risks for years lived with disability (3, 4). Mental disorders typically have an early onset, persist for many years and considerably increase the risk of unfavorable long-term outcomes (2, 5, 6). As described by symptom progression models, psychopathology develops from initially transient and circumscribed dysfunctions that accelerate with time and thus lead to manifest mental disorders, growing impairment and secondary comorbidities (**Figure 1**) (7, 8).

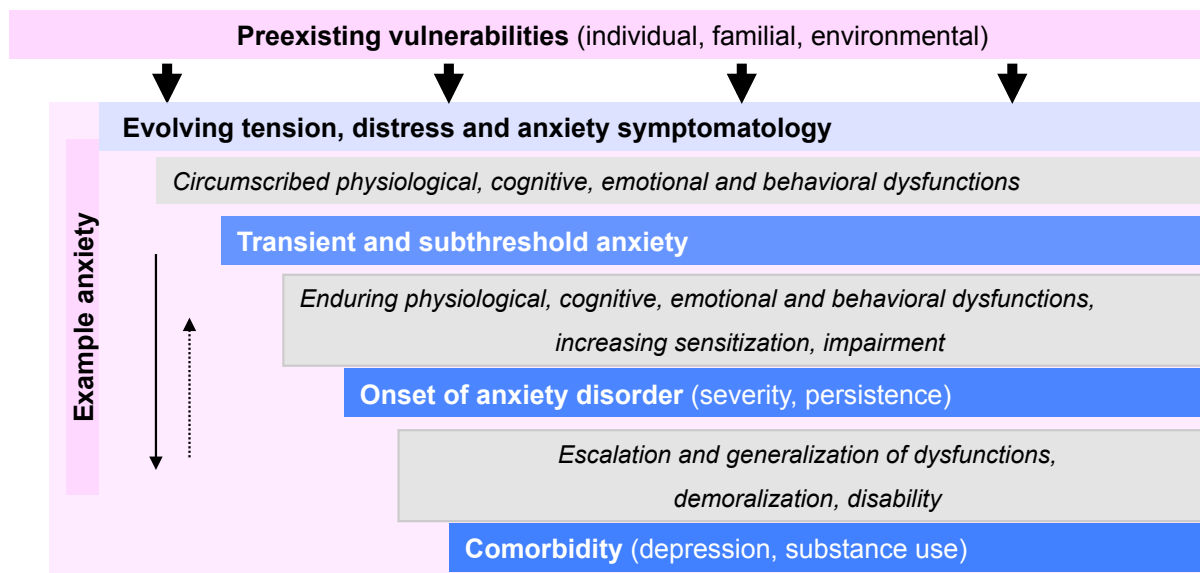


Figure 1: Symptom progression model, adapted from (8)

Thus, a major health challenge is to develop effective and feasible interventions that not only treat but also prevent mental disorders and associated complications at their early stages.

The fact that psychopathology mostly evolves from initially minor symptoms to full-threshold mental disorders (9-11) suggests that especially early interventions will

be effective that modify initial dysfunctional processes and thus prevent a subsequent cascade of psychopathology development including the onset of full-threshold mental disorders (**Figure 2**). Consistently, especially selective and indicated preventive interventions targeted to subjects with specific risk

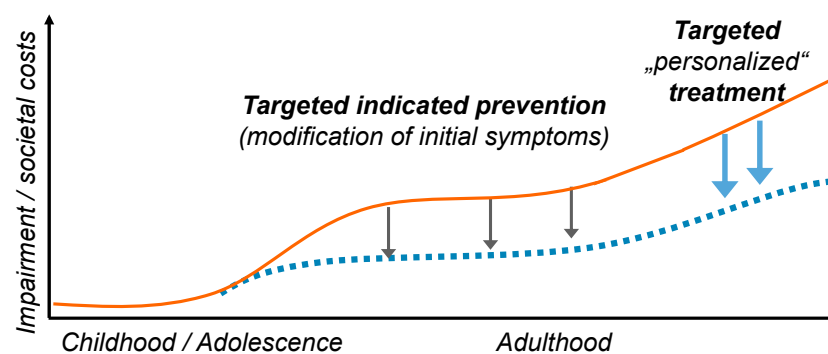


Figure 2: Principle of targeted preventive interventions, adapted from (8)

constellations or initial psychopathological symptoms have been proven effective (12). However, more work is needed particularly with respect to active intervention ingredients.

According to cognitive-behavioral models of mental disorders, **psychophysiological tension** plays a central role for psychopathology development and represents a central component within a vicious cycle of symptom escalation (13, 14): Respective models assume that elevated tension fosters subjective distress, promoting unfavorable emotional, cognitive and behavioral response patterns, hence reinforcing physiological tension and so on. Consistently, mental disorders including anxiety and depressive disorders have been linked to alterations on psychophysiological tension/distress indices such as higher heart rate (HR), lower heart rate variability (HRV) and higher salivary cortisol secretion (15-18).

Relaxation interventions aim to break such a vicious cycle of symptom escalation by inducing relaxation and thus lessening psychophysiological tension, perceived distress and associated adversities (**Figure 3**). Relaxation can be defined as specific psychophysiological process moving along a continuum from activation towards deactivation (19). The physiological relaxation response involves several neuromuscular, cardiovascular, electrodermal, respiratory and central nervous changes, e.g. a decrease in skeletal muscle tone, HR, arterial blood pressure, respiratory rate and skin conductance, an increase in HRV and vasodilatation as well as changes in brain-electrical and neuro-vascular activity (20, 21). Relaxation interventions have been linked to improvement on psychophysiological tension/distress indices (e.g. lower HR, higher HRV and lower cortisol secretion) (19-24), emotional (e.g. improved mood, reduced anxiety) (23, 25) and cognitive (e.g. higher self-efficacy and higher internal control beliefs) (26-29) measures.

A well-established relaxation technique is **Applied Relaxation (AR)**. Originally developed as treatment for anxiety disorders, AR is viewed as a behavioral coping technique that teaches individuals to early recognize initial signs of tension/distress, to rapidly react with relaxation and thus to prevent an escalation of tension/distress, anxiety and associated symptoms (30). AR is a stepwise relaxation training in which participants first learn to perform Progressive Muscle Relaxation, followed by release-only, cue-controlled, differential and rapid relaxation (30 seconds). During imaginary and real life practice, participants finally train to rapidly relax in response to evolving distress symptoms as triggered by imaginal and real life stressors. AR can be applied individually or as group intervention and - as isolated treatment or part of more complex treatment modules - has been shown to reduce perceived tension/distress, anxiety, depressive and somatic symptoms in the context of treatment of a wide variety of clinically manifest mental disorders and physical illnesses: For instance, AR has been demonstrated to effectively reduce anxiety symptoms in patients with generalized anxiety disorder (31-33), panic disorder (34-36), agoraphobia (36-38), social phobia (39-41) and specific phobias (42, 43) as well as to lower pain intensity and duration in patients with chronic or recurrent headache (44-46), migraine (47, 48), neck / back (49, 50) or chronic pain (51-53). A series of study further evidenced the efficacy of AR in non-patient samples including pregnant women (54), students (55, 56) and athletes (57). For instance, Bastani et al. (54) demonstrated that pregnant women receiving AR training more strongly improved on state/trait anxiety and perceived distress from pre- to post-assessment compared to non-interventional controls. Hutchings et al. (56) found that students with elevated levels of anxiety and neuroticism receiving AR scored significantly lower on anxiety measures at post-assessment relative to controls. However, no changes from baseline- to post-assessment were considered and sample sizes (N = 10 per condition) were relatively small.

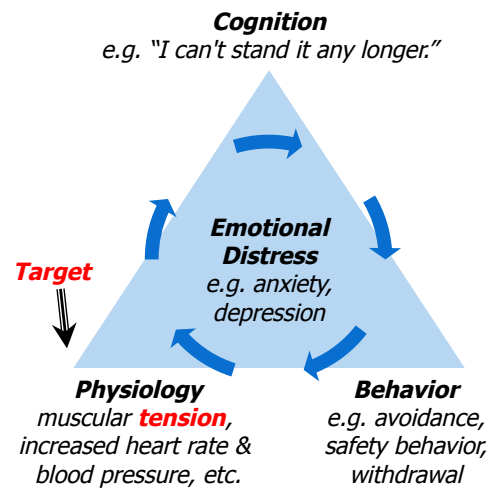


Figure 3: Vicious cycle of symptom escalation

In summary, the efficacy of AR has been primarily tested in patient samples and it is unclear so far whether AR can prevent a subsequent symptom escalation as well as the onset of full-threshold psychopathology in subjects with initial tension/distress, anxiety or depressive symptomatology but no manifest mental disorder yet (indicated preventive approach).

The fact that AR specifically promotes a successful transfer of AR into everyday life suggests that AR will be associated with considerably higher long-term efficacy than conventional Progressive Muscle Relaxation and hence constitute a particularly useful preventive tool (34). Scientifically and methodologically, it is particularly promising to test the preventive efficacy of AR in probands with initial tension/distress, anxiety or depressive symptomatology but no manifest mental disorder yet, as they are particularly likely to progress to full-threshold psychopathology and hence to profit from indicated preventive interventions (9-12).

It is additionally crucial to study which favorable physiological, emotional, cognitive and behavioral changes **mediate** the preventive efficacy of AR. Respective knowledge would foster an optimization of AR preventive programs and maximize beneficial outcomes (**Figure 4**). Based on previous research, it is conceivable that especially variables such as HR/HRV (24, 58, 59) and cortisol secretion (20, 21, 23), affectivity (23, 25), self-efficacy and internal control beliefs (27-29) as well as favorable cognitive/behavioral coping (60) function as mediators of the preventive efficacy of AR. For example, previous research on stress management training including Progressive Muscle Relaxation found that pre-session salivary cortisol decreased over the intervention course and that this decline was related to lower subjective distress and negative affectivity as well as more frequent relaxation practice at home (23). Other studies found cognitive-behavioral interventions including relaxation training to be associated with favorable changes such as decrease in HR and increase in HRV (24, 58, 59) as well as increase in self-efficacy and internal health control beliefs (27-29). No previous study, however, has tested the efficacy of AR with regard to physiological, emotional, cognitive and behavioral outcomes and examined whether respective changes are related to symptom improvement in subjects with increased risk for mental disorders.

It is further important to identify **moderators** of the preventive efficacy of AR to be able to specify subgroups of high-risk individuals who might particularly profit from AR courses. Previous research has evidenced relaxation training to be more effective in younger individuals, women and those attending courses with additional homework assignments (61). Studies have further shown that within-relaxation-session reductions of salivary cortisol secretion were associated with higher relaxation practice at home (23). Therefore, especially sex, age, symptom severity at baseline and homework adherence during the intervention course may be considered as moderators.

Ecological momentary assessments (EMA) have increasingly gained in importance, as they allow assessing a battery of psychological and physiological measures continuously in time to experience over a period of several days in participants' everyday life (e.g. via smart phone). Respective assessments are associated with several advantages. EMA enable (1) to minimize retrospective recall biases, hereby increasing accuracy, ecological validity and generalizability of findings, (2) to study physiological,

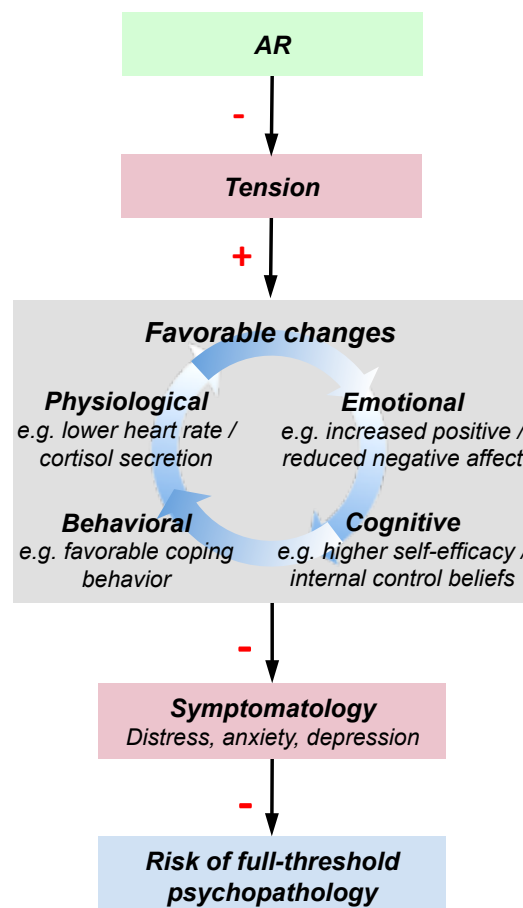


Figure 4: Hypothesized mechanism of action of AR preventive interventions

emotional, cognitive and behavioral long-term changes (including course patterns), (3) to combine subjective self-report with objective physiological data (multilevel approach) and (4) to examine complex reciprocal associations of multiple measures. Hence, EMA are predestined for studying the role of AR in everyday life for psychopathological symptom changes as well as physiological, emotional, cognitive and behavioral measures typically associated with tension/distress, anxiety and depression. Previous research based on EMA has shown that psychological distress, affect and physiological stress indices are closely interrelated (62-65). However, no previous study has tested the preventive efficacy of AR with respect to psychopathological symptoms using an EMA approach and thus, respective research promises important findings for preventional research and practice.

Summary and conclusions

As mental disorders are highly prevalent and associated with substantial individual and societal burden, increased research efforts are necessary to not only treat but also prevent the onset of full-threshold mental disorders in high-risk individuals. AR constitutes an established relaxation technique proven to effectively reduce tension/distress, anxiety, depressive and other psychopathological symptoms in the context of treatment of a wide variety of manifest mental disorders and somatic illnesses. However, its efficacy has been primarily evidenced in patient samples and randomized controlled trials are needed to test whether AR effectively prevents a symptom escalation as well as the onset of full-threshold psychopathology in persons with initial tension/distress, anxiety or depressive symptomatology but no manifest mental disorder yet (indicated preventive approach). From both a scientific and public health perspective, it is additionally crucial to identify mediators (favorable physiological, emotional, cognitive and behavioral changes) and moderators (sex, age, symptom severity at baseline, homework adherence during the intervention course) of the preventive efficacy of AR. EMA are predestined to measure the preventive efficacy of AR including mediators and moderators, as they allow capturing multiple measures in time to experience in real-world settings and hence enable studying the role of post-interventional AR use in everyday life for psychopathological symptom reduction as well as additional psychological/physiological measures typically associated with tension/distress, anxiety and/or depression.

2 Objectives and work program

2.1 Anticipated total duration of the project

Anticipated total project duration: 30 months.

2.2 Objectives

This randomized interventional study among high-risk individuals with elevated tension/distress, anxiety or depressive symptoms but no current full-threshold mental disorder (indicated prevention) aims to examine the following research questions:

1. Can AR effectively reduce current tension/distress, anxiety and depressive symptomatology (intervention efficacy) and prevent a subsequent symptom escalation as well as the onset of full-threshold mental disorders (preventive efficacy)?
2. Does AR induce favorable physiological, emotional, cognitive and behavioral changes (decrease in HR, increase in HRV, decrease in hair and salivary cortisol secretion, improved affectivity, higher internal locus of control and self-efficacy, more favorable cognitive/behavioral coping)?
3. Do respective changes mediate the intervention and preventive efficacy of AR?
4. Do sex, age, symptom severity at baseline and levels of homework adherence during the intervention course moderate the intervention and preventive efficacy of AR?

Levels of symptom reduction from baseline- to post-assessment will be used as measure of the **intervention efficacy**, while symptom changes from post- to 12-month follow-up-assessment and rates of psychopathology development (first incidence or recurrence) from entry exam to 12-month follow-up will be used as measure of the **preventive efficacy**.

Hypotheses:

1. Symptom reduction from baseline- to post- and post- to follow-up-assessment will be higher and rates of incident mental disorders from entry exam to 12-month follow-up will be lower in subjects of the AR interventional group compared to non-interventional usual care (UC) controls.
2. Compared to UC controls, AR subjects will show more favorable physiological, emotional, cognitive and behavioral changes from baseline- to post- and post- to follow-up-assessment (decrease in HR, increase in HRV, decrease in hair and salivary cortisol secretion, improved affectivity, higher internal locus of control and self-efficacy, more favorable cognitive/behavioral coping).
3. Respective changes will mediate the intervention and preventive efficacy of AR.
4. Sex, age, symptom severity at baseline and levels of homework adherence during the intervention course will moderate the intervention and preventive efficacy of AR, i.e. beneficial effects will be more pronounced in women, younger individuals, those with higher symptom severity at baseline and those with higher homework adherence during the intervention course.

2.3 Work program including proposed research methods

2.3.1 Design

Randomized controlled trial with one preventive interventional condition (AR) and one UC control group.

2.3.2 Recruitment, in- and exclusion criteria, sample size calculation

Participants will be recruited via personal contacts, flyers and advertisement in school, university, work and health care settings as well as public media in the Dresden area. Subjects will be screened for elevated levels of tension/distress, anxiety and depressive symptoms using a secured online webpage. Subjects who meet the initial screening criteria and agree to be contacted per telephone will be invited for further study participation. During the entry exam, more extensive diagnostic information will be assessed in personal interviews at the TUD. I will collaborate with other institutions to ensure an adequate sample size, including the Universitätsklinikum Carl Gustav Carus (TUD), a network of local general practitioners, public health insurances and other regional institutions (fitness centers, pharmacies, etc.).

Inclusion criteria will be age between 18 and 40 years (as first incident mental disorders rarely occur after the age of 40 (2)) as well as mild, moderate or severe levels of tension/distress, anxiety or depressive symptomatology (DASS-21 tension/stress: score 8 - 16, DASS-21 anxiety: score 4 - 9 or DASS-21 depression: score 5 - 13). Subjects with extreme symptomatology will be immediately excluded at screening stage, given their high probability of meeting 12-month criteria for any mental disorder (DASS-21 tension/stress: score > 16, DASS-21 anxiety: score > 9 or DASS-21 subscale depression: score > 13). Additional exclusion criteria (assessed at entry exam) will be the 12-month diagnosis of any mental disorder, lifetime psychotic symptoms, current psychological or psychopharmacological interventions

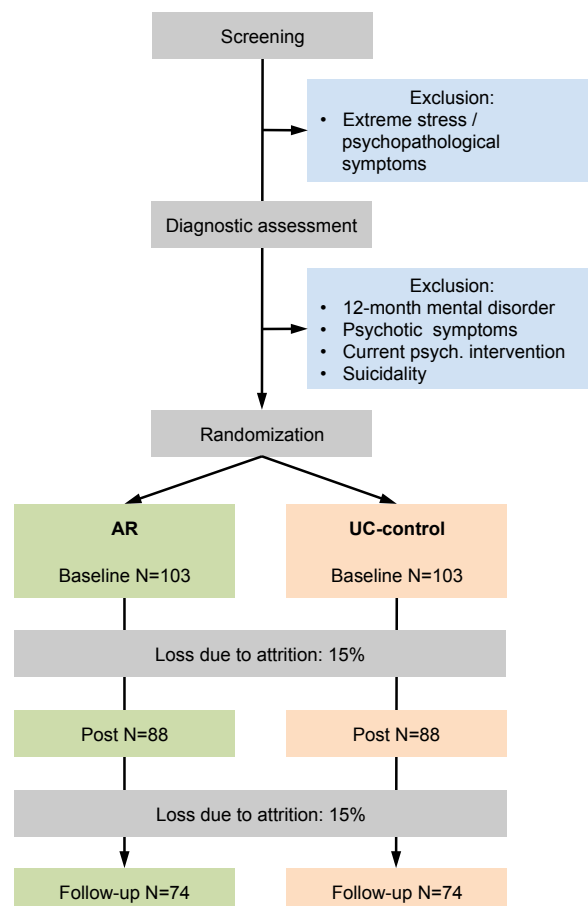


Figure 5: Sample size and screening/randomization procedure

and acute suicidality. Those with acute or developing suicidality or psychosis over the study course will be withdrawn from the trial, closely monitored and referred to treatment.

Subjects meeting the defined criteria will be randomized to the AR or non-interventional UC control group. AR individuals will be required not receiving any additional intervention during the study intervention; UC controls will be required being free of intervention at study entry but may or may not engage in pharmacological or psychological interventions over the study course (usual care). Main study subjects will receive a compensatory fee of 8.50 € per hour for participating in the main assessments at post- and follow-up-assessment as well as for participating in the EMA at baseline-, post- and follow-up-assessment.

Based on previous findings from selective and indicated preventive interventional trials targeting perceived distress (71), anxiety (72, 73), or depressive symptomatology (74), I assume a medium effect size of 0.4 post-intervention. Assuming a correlation of 0.5 between baseline- and post-measures, while specifying the alpha level at 0.05 and the statistical power at 0.8 yields $N = 74$ individuals per group. Assuming a drop-out rate of 0.15 from baseline- to post- and post- to follow-up-assessment, $N = 103$ subjects per condition will be required at baseline (**Figure 5**). In addition to symptom reduction from baseline- to post-assessment, I will focus on symptom changes from post- to follow-up assessment as well as psychopathology development (first incident and recurrent sub-threshold and threshold mental disorders according to DSM-5) from entry exam to post-assessment. However, given that a 12-month follow-up is a relatively short time period to evidence group difference respecting the incidence of psychopathology on a categorical level, I (1) will consider subthreshold incidence cases from entry exam to 12-month follow-up (defined as falling short of one diagnostic criterion, e.g. time criterion) and (2) intend conducting a 5-year follow-up to evaluate the long-term preventive efficacy of AR. As this time span is beyond the funding period for this proposal, I plan to obtain separate funding for this additional assessment in due time.

2.3.3 Assessment

An idealized study scheme is presented in **Figure 6**. For organizational and practical reasons (availability of participants, staff personnel and instrumentation), participants will be continuously screened and randomized to both study groups. Participants will be studied progressively in subgroups à 10 persons. Subgroup assessments will approximately be staggered in weekly intervals. However, concrete time intervals between individual subgroup assessments may vary based on recruitment status.

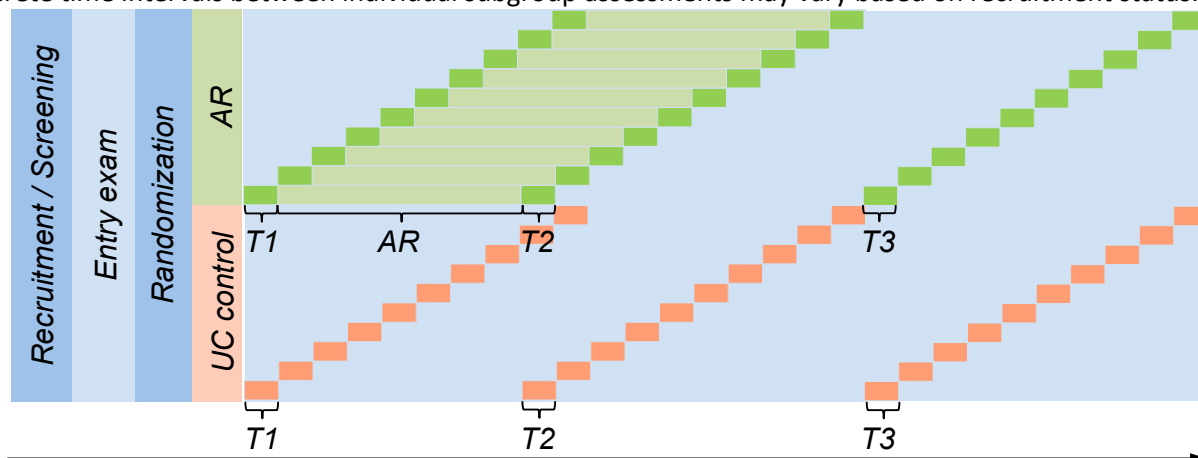


Figure 6: Study scheme

Note: T1: baseline assessment; T2: post-assessment; T3: 12-month follow-up-assessment; idealized schematic representation: participants will be recruited progressively over the study course. Time intervals between individual intervention courses may vary.

Assessment instruments applied at individual assessment waves are presented in **Table 1**. Participants will be initially screened for elevated levels of **tension/distress, anxiety or depressive symptoms** using the DASS-21. **Symptoms, syndromes and diagnoses of mental disorders** will be assessed face-to-face

at entry exam and 12-month follow-up using the fully standardized and computerized DSM-5 CIDI. Additional information on **psychopathological symptoms** (tension/distress, anxiety, depression, anger, somatic symptoms, and sleep disturbances), **emotional, cognitive and behavioral measures** (positive/negative affectivity, locus of control, self-efficacy, cognitive/behavioral coping strategies) and **volitional application of relaxation in everyday life** will be obtained at baseline-, post- and follow-up-assessment both via conventional questionnaire and EMA (abbreviated battery, selected items only). Conventional questionnaires will be applied via tablet computers at the respective main assessment point. For purpose of the EMA, participants will receive a smartphone at each wave and be instructed to answer a battery of scales and questionnaires five times daily in time to experience over a one-week-period. Individual EMA will be scheduled based on a time sampling scheme with variable assessment intervals. EMA will be combined with continuous HR/HRV monitoring and diurnal salivary cortisol sampling (see below) over the initial 48 h period (initial 2 days at each assessment point). The EMA approach will be chosen (1) to minimize retrospective recall biases, hereby increasing accuracy, ecological validity and generalizability of the assessed data, (2) to combine subjective self-report with objective physiological data (multilevel approach) and (3) to examine the role of AR application in everyday life for psychopathological symptom changes as well as additional physiological, emotional, cognitive and behavioral measures.

Table 1: Assessment instruments

| Construct | Instrument | Scr. | T0 | Int. | T1 | T2 | T3 |
|---|------------------|------|----|------|----|----|----|
| Sociodemographic and contact information | | X | | | | | |
| Psychopathological symptoms, mental disorders | | | | | | | |
| Tension/distress, anxiety, depression | DASS-21 | X | | | X | X | X |
| Anxiety | DSM-5 CCSM | | X | | X | X | X |
| Depression | DSM-5 CCSM | | X | | X | X | X |
| Anger | DSM-5 CCSM | | X | | X | X | X |
| Somatic symptoms | DSM-5 CCSM | | X | | X | X | X |
| Sleep disturbance | DSM-5 CCSM | | X | | X | X | X |
| Mental disorders (incl. exclusion criteria) | DSM-5 CIDI | | X | | | | X |
| Additional emotional, cognitive and behavioral measures | | | | | | | |
| Positive and negative affectivity | PANAS | | | | X | X | X |
| Locus of control | IE4 | | | | X | X | X |
| Self-efficacy | ASKU | | | | X | X | X |
| Coping strategies | SVF120 | | | | X | X | X |
| Physiological measures | | | | | | | |
| HR/HRV | HR sensors | | | | X | X | X |
| Salivary cortisol | Saliva samples | | | | X | X | X |
| Hair cortisol | Hair samples | | | | X | | X |
| Homework adherence (paper-pencil) | HRS | | | X | | | |
| Application of rapid relaxation in daily life (AR) | Additional items | | | | X | X | X |

Note: Scr.: screening; T0: entry exam; Int.: intervention; T1: baseline assessment; T2: post-assessment; T3: 12-month follow-up-assessment; DASS-21: Depression Anxiety Stress Scale; DSM-5 CCSM: DSM-5 Cross-Cutting Symptom Measures For Adults (LEVEL 2-Anxiety-Adult, LEVEL 2-Anger-Adult, LEVEL 2-Somatic Symptom-Adult, LEVEL 2-Depression-Adult, LEVEL 2-Sleep Disturbance-Adult); DSM-5 CIDI: DSM-5 Composite International Diagnostic Interview; PANAS: Positive and Negative Affect Schedule; IE4: Fragebogen Internale-Externale Kontrollüberzeugung (4-item version); ASKU: Allgemeine Selbstwirksamkeit Kurzskala; SVF120: Stressverarbeitungsfragebogen (120 item version; EMA will only include the relaxation subscale with 6 items); HRS: Homework Rating Scale.

As measures of general autonomic activity, **HR and HRV** will be assessed continuously over two consecutive weekdays at baseline-, post- and follow-up-assessment using commercially available chest straps with local HRV sensors (Firstbeat Bodyguard 2). Respective HRV measures are able to record a high-resolution pulse curve with a sampling rate of 1000 Hz. They are of low weight, convenient to wear and do not interfere with daily activities, thus allowing a reliable HR/HRV assessment in everyday life. HRV measures will be directly attached to the skin above the costal arch using costal electrodes to

continuously record HR/HRV over a 48 h period. After recording, signal will be cleared from artifacts; i.e. outliers (defined as values $M \pm 3$ SD) will be excluded and, if possible, missing data will be interpolated based on previous and subsequent data segments (depends on length of missing segments and data quality). R-waves will be extracted to calculate HR (number of R-waves per min) and HRV (variability of R-R intervals). High frequency component (HF-HRV, 0.15 - 0.4 Hz, measure of sympathetic activity), low frequency component (LF-HRV, 0.04 - 0.15 Hz, measure of parasympathetic activity) and LF-HF ratio will be analyzed using the software Matlab (MathWorks, USA).

To obtain **diurnal salivary cortisol profiles**, participants will be asked to provide six saliva samples (on awakening, + 30 min, 12:00 h, 16:00 h, 20:00 h, at bedtime) on two consecutive weekdays at baseline-, post- and follow-up-assessment using salivette devices (Sarstedt, Rommelsdorf, Germany). Subjects will be reminded via smartphone (EMA device) to deliver salivary samples. Participants will be told to refrain from eating, drinking (except for water) and smoking prior to sampling (30 min) and to avoid teeth brushing, abrasion and micro-vascular leakage. Subjects will be instructed to note the exact time of sampling, to place salivette devices in a MEMS 6 TrackCap container (Aardex Ltd., Switzerland; electronically records the time of opening and thus allows verifying participants' sampling adherence) and to store samples in a home freezer. Saliva samples will be delivered to the laboratory (by participants or staff personnel) and stored at -20 °C in a laboratory freezer. After thawing, saliva samples will be centrifuged for 10 min at 4000 rpm. Salivary cortisol concentrations will be determined using a commercially available chemiluminescence assay (CLIA, IBL-Hamburg, Germany).

As supplemental long-term measure of tension/distress, **hair cortisol concentrations** will be determined at baseline- and follow-up-assessment. Hair cortisol concentrations reliably reflect long-term systemic cortisol levels over a period of several months and are highly resistant against putative confounding factors (75). Hair strands (~3 mm diameter) will be taken scalp-near from a posterior vertex position to be able to reflect cortisol secretion within two months prior to the respective assessment point. Hair cortisol concentrations will be determined via liquid chromatography tandem mass spectrometry (for detailed information on analysis methods see (76)).

I am experienced in clinical diagnostic assessments and integrated in a team that has built up experience in combined EMA and physiological measures. In preparation of this proposal, we have carried out a series of pilot tests and examined correlations of current mood and HRV in everyday life among 31 healthy students (age 19 - 34 years, 67.7 % female). Over a period of 3 days, current mood was assessed via smartphone app 8 times daily using a short form of the Multidimensional Mood Questionnaire (MDMQ). HR/HRV was recorded continuously using Firstbeat Bodyguard 2 HRV devices directly attached to participants' skin via chest electrodes. Findings revealed that subjective mood and HRV indices were moderately correlated. Associations between subjective vitality (tired-awake, full of/without energy) as well as overall mood (negative-positive) and HRV indices were particularly strong in a way that higher levels of subjective vitality and positive mood were associated with higher sympathetic activity as indicated by time and frequency domain of the HRV measures. Cortisol analyses will be conducted in collaboration with Prof. Clemens Kirschbaum and Dr. Susann Steudte-Schmiedgen (Chair of Biopsychology, TUD), who are highly experienced in hair and salivary cortisol analysis (75, 77, 78).

2.3.4 Intervention

AR intervention courses will be conducted in group-format (subgroups á 10 persons). Each course will comprise 10 sessions á 60 min,

Over the **intervention course**, participants will consecutively perform the following exercises under instructor guidance: (1) Information, AR rationale, Progressive Muscle Relaxation (long version), (2) Progressive Muscle Relaxation (short version), (3) release-only relaxation (direct muscle relaxation without prior tension), (4) cue-controlled relaxation, (5) differential relaxation I (cue-controlled relaxation in different positions, e.g., sitting, staying or walking), (6) differential relaxation II, (7) rapid relaxation (shortened cue-controlled relaxation), (8) AR - imaginal practice (rapid relaxation when noting first symptoms cued by an imaginal scenario), (9) AR - real life practice (transfer to real-life situations

typically associated with physical, emotional, cognitive or behavioral symptoms), (10) AR in real life and closing.

Course sessions will be accompanied by **psychoeducational elements** (information on nature and consequences of tension/distress, anxiety and associated symptoms as well as concept, aims and scopes of AR) and weekly **homework assignments** (daily practice and corresponding note in a relaxation practice diary). Subjects will be additionally instructed to document typical situations leading to key physical, emotional, cognitive or behavioral symptoms to identify early signs. Assignments will be prepared and discussed within each course session.

I am trained and experienced in conducting AR courses as well as integrated in a research environment familiar with the operations and logistics of behavioral interventions including relaxation techniques such as AR (32, 79-81).

2.3.5 Outcome measures

Primary outcome intervention efficacy: Reduction of tension/distress, anxiety and depressive symptoms from baseline- to post-assessment (DASS-21 tension/stress, anxiety and depression)

Secondary outcomes intervention efficacy: Other symptom changes from baseline- to post-assessment (DSM-5 CCSM anxiety, depression, anger, somatic symptoms, sleep disturbance); other clinical changes from baseline- to post-assessment (e.g. impairment, disability)

Primary outcome preventive efficacy: Rates of incident mental disorders from entry exam to follow-up-assessment (first incidence or recurrence of sub-threshold or threshold DSM-5 disorders; DSM-5 CIDI)

Secondary outcomes preventive efficacy: Changes of tension/distress, anxiety and depressive symptoms from post- to follow-up-assessment (DASS-21 tension/stress, anxiety and depression); other symptom changes from post- to follow-up-assessment (DSM-5 CCSM anxiety, depression, anger, somatic symptoms, sleep disturbance); other clinical changes from post- to follow-up-assessment (e.g. number of symptoms/diagnoses, impairment, disability)

Moderator variables: Age, sex, baseline symptom severity, homework adherence during the intervention course, application of rapid relaxation (AR) in everyday life after the intervention course

Mediator variables: Physiological, emotional, cognitive and behavioral changes (HR, HRV, salivary / hair cortisol, PANAS, IE4, ASKU, SVF120) from baseline- to post- and post- to 12-month follow-up-assessment

2.3.6 Statistical analysis

Statistical analyses will be conducted with Stata (82). Data will be analyzed on an intent-to-treat, per-protocol and full-information basis (last observation carried forward analysis, completer analysis and mixed model analysis saturated for a combined time X group effect on the outcome). Outcome measures at T1 vs. T2 and T2 vs. T3 will be related to assess temporal symptom changes from baseline- to post- and post- to follow-up-assessment.

For dimensional outcome measures (DASS-21, DSM-5 CCSM, other dimensional clinical measures), linear regressions adjusted for baseline-/post-assessment outcome values, respectively, will be applied to test associations between group (AR vs. UC control) and outcome at post- and follow-up-assessment (equivalent to ANCOVA). Effect sizes will be calculated as group differences in means at post- and follow-up-assessment (i.e. within- and between-differences divided by the pooled standard deviation of the respective outcome at baseline to be able to compare effects between differently scaled outcomes).

The alpha level will be specified at 0.05. No alpha adjustment will be made, as each pairwise comparison refers to an individual hypothesis. If necessary, the analyses will be repeated with robust standard errors (via the sandwich estimator) and robust linear regressions.

Moderators and mediators are defined causally. Moderator effects will be tested using linear regressions with interaction terms (group X moderator variable; age, sex, baseline symptom severity, homework adherence during the intervention course, AR use in everyday life after the intervention course), while adjusting for confounders. Mediator effects will be analyzed with bootstrapping confidence

intervals (bias-accelerated method with 2000 replications, HR, HRV, salivary / hair cortisol, PANAS, IE4, ASKU, SVF120), while adjusting for confounders, estimating which proportion of the mean difference is reduced when adding the putative mediator as predictor. Analogously, logistic regressions will be applied to test associations between group and disorder onset between entry exam and follow-up-assessment (first incidence or recurrence of sub-threshold or threshold DSM-5 disorders; DSM-5 CIDI). For dimensional outcomes/mediators/moderators assessed both via conventional questionnaire at the respective main assessment point and abbreviated EMA over the following 1-week period, analyses will be conducted twice using the conventional or person-centered EMA measure, respectively. In addition, EMA data will be analyzed separately using multilevel analyses. A 3-level approach will be chosen with level 1 being within-person variance, level 2 being between-person variance and level 3 being between-group variance. Data will be initially cleaned (e.g. duplicate reports and reports beyond the sampling interval will be eliminated). It will then be analyzed whether missing values might have occurred systematically. If appropriate, missing values will be estimated based on present data segments and replaced. Both basic and advanced multilevel models will be applied in order to capture dynamic associations between AR, psychopathological symptoms and psychological/physiological indices. For example, lagged effects of AR use on symptom and psychological/physiological changes as well as temporal associations between symptom changes and changes on psychological/physiological measures will be investigated.

2.3.7 Methods against bias

Subjects will be randomized to the AR and UC control group. Blinding of conditions is not feasible herein.

Extensive diagnostic information on symptoms, syndromes and diagnoses of mental disorders (current, 12-month and lifetime) will be assessed in face-to-face interviews using the fully standardized and computerized DSM-5 CIDI. Established dimensional instruments with proven psychometric quality will be applied both via conventional questionnaire (applied via tablet computer at the respective main assessment point) and via EMA (applied via smart phone, over a 1-week period, respectively, in participants' everyday life) to capture psychopathological symptoms and additional emotional, cognitive and behavioral measures. EMA minimize retrospective recall biases and hereby maximize accuracy, external validity and generalizability of the assessed data. HR/HRV and salivary cortisol secretion will be assessed continuously over a period of 2 consecutive weekdays in participants' everyday life (diurnal profiles) to ensure maximum reliability and validity. Participants will be reminded via smartphone to punctually provide saliva samples; moreover, sampling adherence will be monitored with MEMS 6 TrackCap containers (by recording the exact time they were opened). Salivary will be accomplished by hair cortisol analyses, as hair cortisol levels represent reliable indices of long-term distress over several months.

AR courses will be highly structured, manualized and conducted by psychologists only. Study personnel will be trained and certified initially (including manual adherence) and closely supervised throughout the trial. Homework adherence during the intervention will be continuously assessed; putative problems and barriers with respect to AR will be addressed within each course session.

To consider putative attrition biases, data will be analyzed on both an intent-to-treat and per-protocol basis. The analyses will be adjusted for putative confounders and, if necessary, repeated with robust standard errors (via the sandwich estimator) and robust linear regressions. Subjective self-report data will be linked with objective physiological measures (multilevel approach). Measures from conventional assessments and EMA will both be considered and findings will be compared.

2.3.8 Time schedule

A time schedule for the proposed research project is presented in **Figure 8**.

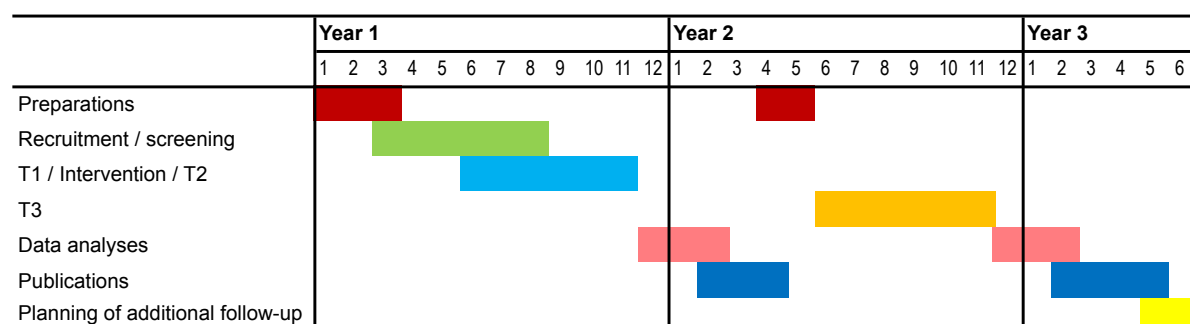


Figure 8: Time schedule

2.4 Data handling

Data will be handled and stored in accordance with established rules to safeguard good scientific practice (83) and national data protection acts. Data will be pseudonymized; codes will be kept securely by the PI. Data (including metadata) will be stored on a central data basis (secured file servers). Anonymized data will be made available for collaborators and other researchers upon request.

2.5 Other information

Not applicable

2.6 Descriptions of proposed investigations involving experiments on humans, human materials or animals

Research will be conducted in accordance with national data protection acts, the revised declaration of Helsinki and Good Clinical Practice Guidelines. After complete description of the study, written informed consent will be obtained from all participants.

Study approval (with respect to study proceedings, diagnostic assessment and AR interventions) will be requested from the ethics committee of the TUD. Both relaxation courses constitute minimal risk interventions (behavioral intervention only). Individuals requiring immediate psycho- or pharmacological treatment due to acute suicidality or severe psychopathology will be withdrawn immediately from the trial and referred to treatment (see 2.3.1).

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