The balance within

IMPACT PARAGRAPH

Research impact embodies real change in the real world and includes all the diverse ways that knowledge generated through research is applied to society and benefits individuals and specific target groups through increasing effectiveness of public services and policies, improving quality of life, overall health, or economic benefits. However, every-day routine may put up barriers between researchers, the research work itself and those who may benefit from it or can apply it to make change. Keeping research impact in mind, thus, helps keep us focused on the overall purpose, rather than the process, of research. A focus on impact can therefore help ensure the best possible return from the investments that societies are making in research.

Aim and key findings

The overall aim of this dissertation is to explore biological factors that objectively modulate central autonomic activity and reactivity to stress in humans using stress provocation challenges. In particular, the studies of this dissertation assessed the role of the central serotonergic and glutamatergic system, as well as the influence of the HPA axis on central autonomic reactivity. As stress reactivity is often influenced by subjective/cognitive factors, the following studies employed only objective stress challenges using endocrine and pharmacological stress provocation. Heart rate variability analyses were applied as a readout of central autonomic activity. In order to increase the translational comparability of the findings, both linear and non-linear heart rate variability measures were included, where possible.

In **Chapter 2**, our first study indicated enhanced sympathetic and/or diminished cardiac vagal activity and blunted autonomic reactivity to stress in subjects with the s/s genotype in comparison to the l/l genotype for the 5-HTTLPR. In **Chapter 3**, our second study indicated that long-term SSRI treatment with escitalopram shows no significant effects on baseline autonomic activity, but a significant increase of vagal tone and a blunted autonomic reactivity to stress. In **Chapter 4**, our third study indicated that $mGluR_{2/3}$ agonism with LY544344 shows no

significant effects on baseline autonomic activity, but a significantly enhanced autonomic recovery after stress. In **Chapter 5**, our fourth study indicated that HPA axis stimulation (metyrapone) is associated with reduced vagal tone, while HPA axis suppression (dexamethasone) has no effect on autonomic modulation of heart function. In **Chapter 6**, our fifth study indicated that positive history of prior episodes in patients with major depression showed no statistically significant effect of baseline autonomic state but distinct effects on autonomic reactivity to HPA axis stimulation (metyrapone) with inverse vagal response and lower vagal activity in comparison to first-episode patients.

Taken together, our results underline the complex functional balance of stress system activity and reactivity and highlight an important role of central serotonergic and glutamatergic activity, as well as of the vagal nervous system in the modulation of the CAN activity, and additionally show a vital importance of the interplay between ANS and HPA axis and the modulation of stress-related cardiovascular responsiveness, thereby confirming and extending previous studies. Finally, our results especially underline the utility of HRV as a transdiagnostic potential biomarker for stress system sensitivity and vulnerability to stress-related disorders and underline a much broader use in reach and clinical practice.

Individual impact

Stress research suggests that the individual ability for resistance to stress, rapid and effective rebound from stress and functional improvement after stress is crucial for healthy adaptation. Disrupted individual stress reactivity exerts profound debilitating effects on homeodynamic balance and adaptivity, development, and mental and somatic health of an individual and may have long-term, deleterious effects on mental and physical health by influencing disease development, course and outcome. Stress responsiveness and particularly autonomic reactivity has been linked to increased overall health risk as a measure of an individual's psychobiologic response to challenges in the environment and a mediator of psychosocial and behavioral risk factors. Respectively, in the last years, research provided robust

evidence that indeed reduced autonomic reactivity and slower recovery are associated with higher cardiovascular and overall (physical and mental) morbidity and mortality risk. However, although much research is taking place in the field of acute and chronic stress, there is still less known about individual biological factors that regulate stress reactivity and may help stress get "under the skin" to influence disease development. Thereby, the identification of distinct biological factors influencing individual stress reactivity is of vital importance for a personalized medical approach, as well as for the linkage of psychosocial and environmental stress factors on pathophysiology of disease development. The acknowledgement of such biological risk factors influencing stress reactivity could be used in individual risk assessment, as well as personalized prevention and treatment approaches. Our results support, for example, that the s/s 5-HTTLPR genotype might represent a genetic risk factor for developing stress-related, cardiometabolic and other chronic, non-communicable diseases, as well as the fact that pharmacological or endocrine serotonergic, glutamatergic and HPA axis modulation might have clinical utility for the individual stress reactivity and, respectively, for personalized treatment.

Socioeconomic impact

Stress system dysregulation is considered endemic in contemporary societies, with about 2/3 of the population at the age of 55 years suffering from a "syndrome of chronic stress and inflammation". Acute and chronic stress system dysregulation with altered stress reactivity has been linked to a broad range of complex behavioural-psychological (e.g., anxiety, depression, eating disorders, post-traumatic stress disorder, sleep disorders, etc.), and psychosomatic and somatic diseases (e.g., chronic pain and fatigue syndromes, obesity, metabolic syndrome, chronic inflammation, diabetes type II, hypertension, atherosclerosis, cardiovascular diseases, body composition disorders, cancer, etc.), that all together constitute the so-called "chronic noncommunicable disorders", curtailing life expectancy. Interestingly, a chronically dysregulated stress system has been found as a common risk factor of 75-90% of all chronic, non-communicable diseases, excreting a huge

health-related socioeconomic burden on modern humanity. The high comorbidity of stress-related disorders and cardiovascular disease, in particular, suggests an important pathophysiological link between these disorders and autonomic control. Beyond these diseases, chronic stress particularly affects the immune system, with altered GC levels influencing all aspects of cellular, humoral, innate and adaptive immunity, thus contributing to increased susceptibility to infections (e.g., tuberculosis, common cold, and COVID-19), (auto-)immune and inflammatory disorders, allergies, and cancer. This huge burden of disease caused by stress-related pathophysiology desperately calls for further basic translational and clinical research in order to understand the biological background of risk, development and therapy of these disorders and their comorbidities, which actually affect all our lives from birth to older age. The understanding of the biological risk factors leading to chronic stress system malfunction may yield important insights into the etiopathology, course, prevention and treatment of the most important major public health concern of chronic, non-communicable diseases. Better understanding of biological factors affecting the development of stress-related disorders can further help in primary, secondary and tertiary prevention in the general population, in target groups at risk and in patients. Especially individuals with early-life stress and trauma experience (i.e., childhood abuse/neglect), individuals exposed to shift work, patients with stress-related disorders (e.g., depression, anxiety disorders, PTSD), and patients with chronic non-communicable diseases (i.e., cardiovascular, immune, autoimmune, metabolic, malignant) could greatly profit from targeted prevention and treatment alternatives tailored according to specific biological risk factors influencing their stress system reactivity. Alleviating this huge burden of disease through stress-related chronic non-communicable diseases would represent one giant step towards lower morbidity and mortality, lower health expenditure costs in every country and better societal productivity and prosperity.

Scientific impact

Due to its complexity and limited functional knowledge of the anatomically well described central stress system, the molecular and cellular basis for the normal and compromised brain-heart network in stress and stress-related disorders is still a widely unexplored area. Therefore, the National Institute of Mental Health (NIMH) has recently identified a set of priorities for stress biology research aimed at creating the basic and clinical knowledge bases for reducing and alleviating health burden across the lifespan. Accordingly, our studies provide relevant evidence in the better understanding of biological factors contributing to individual stress system reactivity through different objective stress paradigms, embrace different subsystems and their interaction to exploit the complexity of the stress response and apply translational methods (i.e., non-linear HRV analyses) that seek to test mechanistic hypotheses across species. Our studies combine expertise and methods from different experimental fields, including psychoneuroendocrinology, psychoneurophysiology, psychoneuropharmacology, experimental clinical research and clinical psychiatry to help zoom in on individual biological aspects of stress response in both health and disease and establish functional mechanistic links across different levels of stress response. For this purpose, our studies have followed very strict and timely precise methodological protocols and only objective stress challenges for an objective readout of central autonomic reactivity, in order to avoid cognitive, personality, circadian, and other influences that may affect individual stress responses. Our results underline the complex functional balance of stress system activity and reactivity and highlight an important role for serotonergic and glutamatergic signaling, as well as for HPA axis influence on CAN activity, thereby confirming and extending previous studies. These findings underscore the overlap of main regulatory systems of autonomic, affective and attentional regulation and the association between stress-related disorders, CAN dysregulation with compromised neuroautonomic control and somatic, in particular, cardiovascular morbidity and mortality in such patients. Autonomic imbalance may be a final common pathway to increased morbidity and mortality from a host of conditions

and diseases, while assessment of autonomic imbalance may provide a unifying framework in order to investigate the impact of risk factors, including biological, behavioral, psychosocial and environmental factors on health and disease. Thereby, measures of autonomic reactivity can be viewed as a transdiagnostic biomarker of self-regulation, cognitive control and overall health state. HRV assessment and especially utilization of nonlinear methods may improve our interpretations of autonomic dysregulation and serve as a sensitive clinical biomarker with potential prognostic value in the staging of chronic diseases and classification of morbidity and mortality risk.

Our studies also support a central role of the vagal branch of the ANS in the regulation of stress reactivity and also the fact that ANS resting activity and reactivity, although correlated, represent different regulatory processes with different functional and clinical impact. This model, thus, challenges the completeness of the sympathetic overactivation explanation of stress activation and anxiety. However, the functional understanding of stress reactivity has to be substantially improved through further preclinical and prospective research. Thereby, vagal activity and its normative increase from childhood to adolescence seem to hold a key role in the proper neurovisceral integration during neurodevelopment on a structural and functional level, subsequent psychological functioning and adaptive regulation. Thereby, factors as developmental timing, (epi)genetics, duration and nature of stressors among others play an important moderating and modulating role. An improved understanding of mechanisms underlying stress responses and the functional consequences of stress can and will speed translation from basic research to predictive markers of risk and to improved, personalized interventions for mental and chronic illness. For example, as neurocircuitry of stress-system and depression show a distinct overlap, many of the biological factors influencing stress reactivity could actually be responsible for prolonged or repeated dysregulation of brain regions in the pathophysiology of depression.

Clinical Impact

Despite that basic and clinical research have already offered great insights of stress system pathophysiology, there is still little recognition of the importance of the stress system within most medical disciplines, and only few stress-system-related implications flow into broad clinical practice. Novel approaches are needed for the proper neuroendocrine and neurophysiological assessment of stress system reactivity and efficacious management of stress system dysregulation in the individualized treatment of both mental and physical stress-related disorders, especially in view of the particular challenges of the evolving new lifestyle of modern societies. Thereby, our results especially underline the utility of HRV as a transdiagnostic potential biomarker for stress system sensitivity and vulnerability to stress-related disorders and underline a much broader use in research and clinical practice. In addition, simple strategies for autonomic function improvement and increasing cortical blood flow (i.e., regular moderate aerobic exercise) could be used to improve autonomic activity and reactivity in prevention and treatment. Further clinical intervention strategies could include more specific treatment alternatives, such as pharmacotherapy and somatic afferent stimulation (e.g., stroking skin, acupuncture, vagus nerve stimulation, HRV coherence training/Biofeedback), in order to restore autonomic balance. Instead of exclusively targeting sympathetic activation as in the past years, physicians should rather attempt to increase vagal tone. In particular, there has been increasing interest in treating a wide range of disorders with implanted pacemaker-like devices for stimulating the vagal afferent pathways for a broad range of diseases (e.g., obesity, depression, anxiety, epilepsy, migraine, chronic pain, etc.). In addition, drugs affecting CAN activity (e.g., SSRIs) and circadian rhythm, substances reducing oxidative stress or inflammation, or influencing stress-system dysregulation effects in the periphery (e.g., GR modulators), or even metabolism altering agents hold a potential of effectively disrupting the chronic vicious cycle of stress progression and its effects on the body.

Future directions

The results of this dissertation argue for a broader implementation of easily accessible stress-system biomarkers into clinical practice for the better assessment of chronic health risks, especially in the general and targeted prevention, monitoring and personalized treatment of patients with chronic non-communicable diseases and mental disorders. Nevertheless, more targeted research in broader patient groups is needed before such biomarkers can be applied in clinical routine settings. Healthcare insurers could play a facilitating role by including such examinations for patients in their refunding list and research foundations by enhancing biological stress research also outside the scope of mental health and psychiatry, but including it to all related disciplines.

Future studies are needed to replicate our findings and further explore the role of autonomic stress reactivity and diurnal variability as potential biological mechanisms conveying an elevated risk for the development of stress-related disorders and physical comorbidity. Respectively, seriously challenging conditions, despite their ethical problems, should be explored more thoroughly, particular with respect to the recovery of the observed autonomic responses, and to determine any therapeutic efficacy also on autonomic responsiveness. Studies investigating HR measures should focus on both medicated and unmedicated patients and consider a range of important exclusion criteria that may otherwise impact on the results and its conclusions when investigating disease cohorts. In particular, since some studies suggest a non-linear gene dose effect of the 5-HTTLPR, inclusion of s/l subjects in further studies with a considerably larger sample size is necessary to precisely characterize genotype differences. In addition, HRV effects still need to be investigated in women, and in individuals of older age, since age-related changes in 5-HT transmission and SSRI effects have been reported. Future studies should also prospectively investigate putative mediators and their temporal sequence, while considering the potentially delayed time-frame for their phenotypical expression. Finally, the broader inclusion of HRV as a transdiagnostic measure of emotional and

autonomic biomarker into clinical research in further patient groups outside the psychiatric clinical context is of importance.

Furthermore, primary, secondary and tertiary prevention of stress-related effects on individuals could incorporate additional behavioral and life-style modification strategies to the assessment of biomarkers, such as stress management techniques, sleep hygiene, healthy nutrition, smoking cessation, positive psychology and emotional self-regulation strategies and social engagement/support strengthening strategies alone or in terms of a cognitive-behavioral psychotherapeutic process. Hereby, the implementation at a societal level supported by health policy makers is of crucial importance, in order to have an important impact at a general socioeconomic level. Policy makers could be involved and informed via expert groups that contribute to the development of policies. This suggests also that organizations with strong health-related or societal infrastructure should acknowledge and include stress-related applications and interventions into their functional algorithms, as technology-based interventions at nodal social hubs, are now scientifically and medically possible. Furthermore, training of the stress detection and management basics very early in life (e.g., school courses) and continuous training in important life stages (e.g., college, work, marriage, parenthood, etc.) could be fruitful as a general prevention strategy and increase the socio-psycho-somatic resilience to stress in societies, but also an individual level. The findings from this study could be used by policy makers to inform, to comprehend and to convince people that biological factors can alter their stress resilience and that improving lifestyle is not only good for their general health, but also specifically for their brain and mental health.

Dissemination of knowledge

Results of this dissertation were nationally shared at national and international congresses and symposia with colleagues in both research and clinical field (*12th World Congress of Biological Psychiatry 2015*, Athens, Greece; *27th ECNP Congress* 2014, Berlin, Germany; *DGPPN National German Psychiatry and Psychotherapy*

Congresses 2014-2016). Internationally, the results of this dissertation were published in internationally high-ranked peer-reviewed scientific journals of relevant fields [*J Psychiatr Res* 2020 (IF 2019: 3.74); *Psychoneuroendocrinology* 2019 (IF 2017: 4.73); *Int Clin Psychopharmacol* 2016 (IF 2015: 2.41); *Int J Neuropsychopharmacol* 2014 (IF 2013: 5.26); *J Psychiatr Res* 2014 (IF 2013: 4.09)], in which the quality of the studies is evaluated by experts in the field, and have been already cited over 62 times (23.09.2022). The working method and first results were disseminated within internal science meetings at the Department of Psychiatry and Psychotherapy, University Medical Center Hamburg-Eppendorf, Germany and deliberated on with international colleagues during work visits to the Center of Neurogenomics and Cognitive Research, VU University of Amsterdam. Results of these studies were used as basis for the candidate's granted application (PI) for one year's research scholarship through the Excellent Young Investigator Research Founds of the Medical Faculty of the Hamburg University, Germany (2014) and the candidate's granted application (co-PI) for a clinical research grant through the Werner-Otto Foundation, Germany (2015).

Conclusion

Biomedical research has shown that the impact of stress on human physiology and pathophysiology is pervasive and enormous. Thereby, individual differences in stress reactivity may vitally affect adaptive responses and possibly explain individual differences in stress resilience and, thus, deserve additional consideration by researchers, clinicians and policymakers as a target for early interventions to individually treat and prevent stress-related disorders. Identification of biological factors that influence stress reactivity is, thus, of major importance for the linkage of psychosocial and environmental stress factors to disease outcome and may yield important insights into the etiopathology, course, prevention and treatment of the most important major public health concern of chronic, non-communicable diseases and mental health disorders.