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Altered Neurobiology in Adolescent NSSI:
Examining the Influence of Psychopathology on a Continuum of Symptom Severity

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Abstract

Nonsuicidal self-injury (NSSI) is a complex phenomenon, particularly common in adolescence, with severe psychiatric sequelae. NSSI is most often used to regulate emotions and alleviate distress and aversive inner tension. Emotion dysregulation and low distress tolerance are common symptoms observed in most psychiatric disorders, potentially explaining the overlapping comorbidity with NSSI. Consistently, recent research indicates that increasing NSSI severity (e.g., frequency of NSSI) is associated with more severe emotion dysregulation and comorbid psychopathology. More transdiagnostic and dimensional approaches are imperative, to disentangle and clarify the existing literature on NSSI.

Moreover, further research investigating neurobiological systems that may underlie the psychosocial risk factors of NSSI as well as the engagement and maintenance of NSSI is warranted. Previous research indicates that individuals with NSSI perceive limited or no pain during NSSI episodes. Moreover, fronto-limbic neural systems (e.g., prefrontal cortex (PFC), amygdala, hypothalamus), neuroendocrinological systems such as the endogenous opioid system, the hypothalamic-pituitary-adrenal (HPA) axis, and the hypothalamic-pituitary-thyroid (HPT) axis, as well as physiological systems such as the autonomic nervous system (ANS) play a central role in pain perception as well as emotion and stress regulation. However, systematic research in the context of adolescent NSSI is still scarce. Therefore, the aim of this work is to extend theoretical and empirical knowledge on neurobiological correlates of NSSI while further examining the influence of dimensional NSSI severity and comorbid psychopathology.

Study I examined associations of altered basal beta-endorphin (BE) and pain sensitivity (PS) with experimental heat pain in female adolescents with NSSI compared to healthy controls (HC). Results indicated reduced PS and lower BE levels in adolescents with NSSI. Moreover, additional effects of comorbid psychopathology on BE and PS were observed. **Study II** investigated the effect of NSSI severity on the HPA axis and ANS response to experimental pain. Results revealed an increasing HPA axis response (e.g., cortisol) following pain with increasing NSSI frequency. Moreover, after adjusting for comorbid psychopathology, greater NSSI severity was associated with a decreased heart rate during pain and an increased heart rate variability following pain. Findings indicate an increased pain-related compensatory mechanism of NSSI on the HPA axis and ANS in individuals with NSSI, independent of comorbid psychopathology. **Study III** investigated resting-state PFC activity in adolescents with NSSI compared to HC. Results revealed a reduced PFC activity in adolescents with NSSI. Further, increased PFC connectivity was associated with greater severity of comorbid psychopathology but not with NSSI-specific behaviors (e.g., NSSI frequency). **Study IV** examined HPT axis functioning in adolescents with NSSI compared to HC. Results indicated a blunted HPT axis functioning in adolescents with NSSI, strongly associated with more severe borderline personality disorder symptoms, depressive symptoms, and symptomatic distress.

Content

List of scientific publications of the publication-based dissertation

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I. Manuscript

van der Venne, P., Balint, A., Drews, E., Parzer, P., Resch, F., Koenig, J., & Kaess, M. (2021). Pain sensitivity and plasma beta-endorphin in adolescent non-suicidal self-injury. *Journal of Affective Disorders, 278,* 199-208. https://doi.org/10.1016/j.jad.2020.09.036

II. Manuscript

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III. Manuscript

Koenig, J., Höper, S., **van der Venne**, P., Mürner-Lavanchy, I., Resch, F., & Kaess, M. (2021). Resting state prefrontal cortex oxygenation in adolescent non-suicidal self-injury–A near-infrared spectroscopy study. *NeuroImage: Clinical*, *31*, 102704. https://doi.org/10.1016/j.nicl.2021.102704

IV. Manuscript

Flach, E., Koenig, J., **van der Venne**, P., Parzer, P., Resch, F., & Kaess, M. (2021) Hypothalamic-pituitary-thyroid axis function in female adolescent nonsuicidal self-injury and its association with comorbid borderline personality disorder and depression. *Progress in neuro-psychopharmacology and biological psychiatry, 111,* 110345. https://doi.org/10.1016/j.pnpbp.2021.110345

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List of Abbreviations

ACC	Anterior cingulate cortex
ACE	Adverse childhood experiences
ACTH	Adrenocorticotropic hormones
ANS	Autonomic nervous system
BE	Beta-endorphin/β-endorphin
BPD	Borderline personality disorder
CNS	Central nervous system
CRH	Corticotrophin-releasing hormones
DSM-5	Diagnostic and Statistical Manual of Mental Disorders: fifth edition
HC	Healthy controls
HPA axis	Hypothalamic-pituitary-adrenal axis
HPT axis	Hypothalamic-pituitary-thyroid axis
HR	Heart rate
HRV	Heart rate variability
NSSI	Nonsuicidal self-injury
NSSID	Nonsuicidal self-injury disorder
PFC	Prefrontal cortex
PNS	Parasympathetic nervous system
PS	Pain sensitivity
RDoC	Research Domain Criteria Project
SNS	Sympathetic nervous system
T3	Triiodothyronine
T4	Thyroxine
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone

1 Introduction

Nonsuicidal self-injury (NSSI) is defined as the “deliberate, self-inflicted damage of body tissue without suicidal intent” (International Society for the Study of Self-Injury, 2022), including a range of methods, such as cutting, hitting and burning oneself (Ammerman, Hong, et al., 2019; Swannell et al., 2014). NSSI is most commonly observed in adolescence, and recent meta-analytic evidence indicates prevalence rates for single NSSI episodes of 17.2% in non-clinical samples (Swannell et al., 2014) and up to 50% in clinical samples (Plener et al., 2018). Importantly, NSSI is associated with significant impairments in psychosocial functioning (Ghinea et al., 2021; Washburn et al., 2015). Moreover, it has been shown to predict the onset of psychiatric disorders (Wilkinson et al., 2018) and suicide attempts later in life (Kiekens et al., 2018; Mars et al., 2019), making it a serious health threat.

NSSI has received increasing attention in recent years, both in clinical practice and research, vastly increasing our knowledge of this phenomenon. As such, although NSSI is a key symptom of borderline personality disorder (BPD) (American Psychiatric Association, 2013), high comorbidity rates were also reported for depressive disorders, anxiety disorders, posttraumatic stress disorders, and eating disorders (Kiekens & Claes, 2020; Nock et al., 2006; Plener et al., 2018; Zetterqvist, 2015). Moreover, NSSI also occurs independently (Glenn & Klonsky, 2013; Zetterqvist, 2015). In recognition of its transdiagnostic nature, NSSI was introduced in the 5th version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as a disorder warranting further research (NSSID; American Psychiatric Association, 2013). Prevalence rates for NSSID have been reported at 0.3-5.6% in non-clinical adolescent community samples and 46-80% in clinical adolescent samples (Plener, Allroggen, et al., 2016; Zetterqvist, 2015). Further, extensive evidence indicates that NSSI is driven by intra- and interpersonal functions. Individuals most often self-injure to regulate intense (negative) emotional states and distress, or to communicate distress and avoid unwanted social situations (Edmondson et al., 2016; Klonsky et al., 2015; Taylor et al., 2018). Consistently, affective instability was shown to be associated with NSSI (Santangelo et al., 2017), and emotion dysregulation, as well as low distress tolerance were found to predict the onset of NSSI (Ammerman, Olino, et al., 2017; Wolff et al., 2019).

The research field of mental health and the psychiatric sciences have been in transition for the past decade (for a brief review, see: Michelini et al., 2021). Clinicians and researchers alike have recognized the need for more dimensional approaches to psychiatric diagnoses and psychopathology, repeatedly highlighting problems of heterogeneity within classic diagnostic entities. Moreover, it is criticized that symptoms such as emotional lability and poor distress tolerance are shared across most traditional psychiatric disorders (American Psychiatric Association, 2013; Beauchaine, 2015a; Lass & Winer, 2020; Michel et al., 2016), leading to increasingly high comorbidity rates (Michelini et al., 2021). Consequently, a need for novel approaches has been acknowledged that are based on shared higher-order dimensions, beyond the classic categorical systems in psychiatric diagnostics, and that consider markers of severity (Brown & Barlow, 2009; Krueger et al., 2014). Such an approach seems pertinent to NSSI given its high rates of comorbidity and is in line with the proposed intrapersonal and

interpersonal functions (Nock, 2009, 2010). A dimensional approach could shed light on the pertaining question whether NSSI marks an independent disorder or is a transdiagnostic risk marker of psychopathology in general (Ghinea et al., 2020). Moreover, it may advance previous discussions on subtypes of NSSI (Brausch, 2019; Hooley et al., 2020). Extant studies suggest that individuals with NSSI significantly differ on the severity of comorbid psychopathology, psychosocial, cognitive as well as affective impairment as a function of increasing severity of NSSI behaviors, such as the frequency of NSSI and the number of methods used (Ammerman, Jacobucci, et al., 2017, 2019; Muehlenkamp et al., 2017). However, research is limited, and further studies are warranted.

Moreover, research on (adolescent) NSSI has, so far, seldom considered potential underlying neurobiological systems. In line with the need for more dimensional and transdiagnostic research, the assessment of pathophysiological mechanisms that may underlie psychopathological symptoms has gained increasing momentum (Insel et al., 2010). Advancing the knowledge on how normal and abnormal functioning of neurobiological processes relates to psychopathology and how these processes increase the risk for commonly observed symptoms holds great promise. For one, they offer an objective measure of psychopathology beyond observable symptoms and self-reports. Further, assessing underlying neurobiological mechanisms may reveal differences in (ab)normal functioning across different psychiatric disorders. This would support a better diagnostic differentiation of highly comorbid disorders based on underlying fundamental mechanisms, and their potential differential function in distinct disorders. Finally, a better understanding of underlying neurobiological systems could advance and support the development of targeted interventions.

Research on neurobiological systems in NSSI has started to emerge. But our understanding is still limited. The present work aims to extend the knowledge of the role of neurobiological systems in NSSI, while taking into account dimensional effects of NSSI severity (i.e., NSSI frequency) and comorbid psychopathology. First, the general functioning of neurobiological systems associated with NSSI and an overview of the current literature will be described in the context of existing theoretical models (Chapter 2). Second, existing gaps will be presented (Chapter 3). Third, the contributions of this thesis will be outlined (Chapter 4). Last, findings from four empirical studies will be discussed in the context of the theoretical background, and clinical implications as well as avenues for future research will be outlined (Chapter 5).

2 Theoretical background

The increasing interest in neurobiological systems underlying NSSI has created the need for theoretical models guiding clinicians and researchers. A promising approach is the *Research Domain Criteria project* (RDoC) launched by the National Institute of Mental Health (National Institute of Mental Health, 2022). The RDoC aims to improve knowledge about the fundamental biological mechanisms and cognitive processes underlying mental health, and the dimensions of disordered behavior associated with psychopathology beyond the boundaries of the categorical systems largely in use (e.g., International Classification of Diseases (ICD), DSM; Clark et al., 2017; Cuthbert & Insel, 2013; Insel et al., 2010). In line with the suggestion to use higher-order dimensions (Brown & Barlow, 2009; Krueger et al., 2014), the RDoC framework consists of a matrix with six major neurobehavioral domains: *negative valence systems*, *positive valence systems*, *cognitive systems*, *systems for social processes*, *arousal/regulatory systems* and *sensorimotor systems*. Each domain subsequently contains behavioral processes and mechanisms referred to as constructs (e.g., negative valence systems: *acute threat (fear)*, *sustained threat*; positive valence systems: *reward responsiveness*, *reward learning*; cognitive control: *perception*, *cognitive control*). Finally, the framework describes units of analysis, enabling a categorization of research approaches, methods and thus levels of observation, including genes, molecules, cells, circuits, physiology, behavior, self-reports and paradigms (Clark et al., 2017; National Institute of Mental Health, 2022).

Based on evidence that NSSI is often used to regulate negative affective states (Klonsky et al., 2015; Nock, 2010) and in line with the *negative valence* domain, an implication of fronto-limbic brain regions (e.g., prefrontal cortex (PFC), anterior cingulate cortex (ACC), amygdala, hippocampus, hypothalamus) seems likely (LeDoux, 2000; Lupien et al., 2009). The functionality of these brain regions significantly overlaps with the *cognitive systems* (e.g., perception, cognitive control) and *positive valence systems* (e.g., reward) domains (Williams, 2017). These domains may further be implicated in the engagement and maintenance of NSSI, since NSSI has been associated with a rewarding experience and altered pain perception (Nock, 2009, 2010). Consistently, meta-analyses found alterations in common neural circuits responsible for emotional, cognitive and reward processes across a range of psychiatric disorders (Janiri et al., 2020; McTeague et al., 2020) and in NSSI (Lai et al., 2021). Beyond the central nervous system (CNS), the RDoC contains peripheral neuroendocrinological and physiological systems that may play a role in the development and maintenance of NSSI. As such, peripheral stress response systems such as the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) may be key systems to consider (Kreibig, 2010; Lupien et al., 2009). Both systems are involved in emotion regulation and pain processing (Gilbert et al., 2017; Lewis et al., 2018; Li & Hu, 2016). While not included in the RDoC, the hypothalamic-pituitary-thyroid (HPT) axis is involved in the regulation of the metabolism and involved in the stress response (Ortiga-Carvalho et al., 2011) and may thus be another system to consider. Furthermore, as NSSI has previously been linked to a decreased pain sensitivity (Kirtley et al., 2016; Koenig, Thayer, et al., 2016), a potential role of the endogenous opioid systems, and β-endorphin (BE) in particular, has been postulated (Bresin & Gordon, 2013).

The RDoC is a promising approach to defining fundamental neurobiological mechanisms that underlie psychopathology; however, it is relatively unspecific regarding symptomatology previously associated with psychiatric disorders. For now, clinicians and researchers still primarily rely on the well-established diagnostic classification of psychiatric disorders. As such, for the time being, a more NSSI-specific model might prove more beneficial to categorize and evaluate biological systems related to the development and maintenance of NSSI.

2.1 Temporal Framework Model

Kaess and colleagues (2021) recently introduced the Temporal Framework Model of NSSI, which marks an important step in organizing the neurobiological basis of NSSI (see Figure I). Their model hereby divides biological markers into distal biological traits (e.g., genes, epigenetics, biological manifestations of childhood maltreatment), proximal biological traits (e.g., brain circuitry, stress response systems, and pain systems) as well as biological states (e.g., changes in brain circuitry, stress response systems, and pain processing preceding or following NSSI) (Kaess et al., 2021). Biological traits are hereby defined as relatively stable biological (dys)functions, playing an antecedent role in the pathophysiology of NSSI that might either be directly linked to NSSI, or indirectly increase the likelihood to engage in NSSI through subsequent functional abnormalities (e.g., emotion dysregulation, poor distress tolerance, altered pain sensitivity). Biological states are defined as the transient (abnormal) changes in biological markers, reflecting the situation- or stimulus-specific changes that occur immediately prior to, during or after engagement in NSSI, and are thought to represent the status of clinical manifestation. As such, the Temporal Framework Model is consistent with a dimensional assessment of NSSI. Moreover, it considers a range of different neurobiological systems that should be accounted for. This is in line with the RDoC's proposed units of analysis (Clark et al., 2017; Kozak & Cuthbert, 2016; National Institute of Mental Health, 2022). Finally, it reflects, to a certain degree, recent psychological models of NSSI, such as the Integrated Theoretical Model of Self-injury, from a biological perspective (Nock, 2009, 2010). Important to note, the Temporal Framework Model (Kaess et al., 2021) extends the proposed dimensional approach to assessing the biological risk factors and correlates of NSSI by placing the different biological systems in a temporal and developmental context with NSSI and each other. As such, distal biological traits are seen as predicting risk factors already present in childhood, before NSSI occurred, and may increase the vulnerability for developing NSSI. They might lead to altered biological functioning (proximal biological traits; i.e., altered stress response, pain perception) or increase the vulnerability for psychiatric sequelae associated with altered biological functioning, increasing the risk of engaging in and maintaining NSSI (Kaess et al., 2021). Thus, the Temporal Framework Model holds promise for clinicians and researchers, as it outlines a potential developmental biological pathway of NSSI that can advance the existing knowledge and marks the first step to a unified, truly integrated model of NSSI.

This thesis focuses on proximal biological correlates of NSSI. As such, distal risk factors will only be marginally addressed, where applicable. Following, the general functioning of neural circuits, neuroendocrine, and physiological systems will be described. Further, an overview of the existing research on these systems in the context of NSSI will be provided.

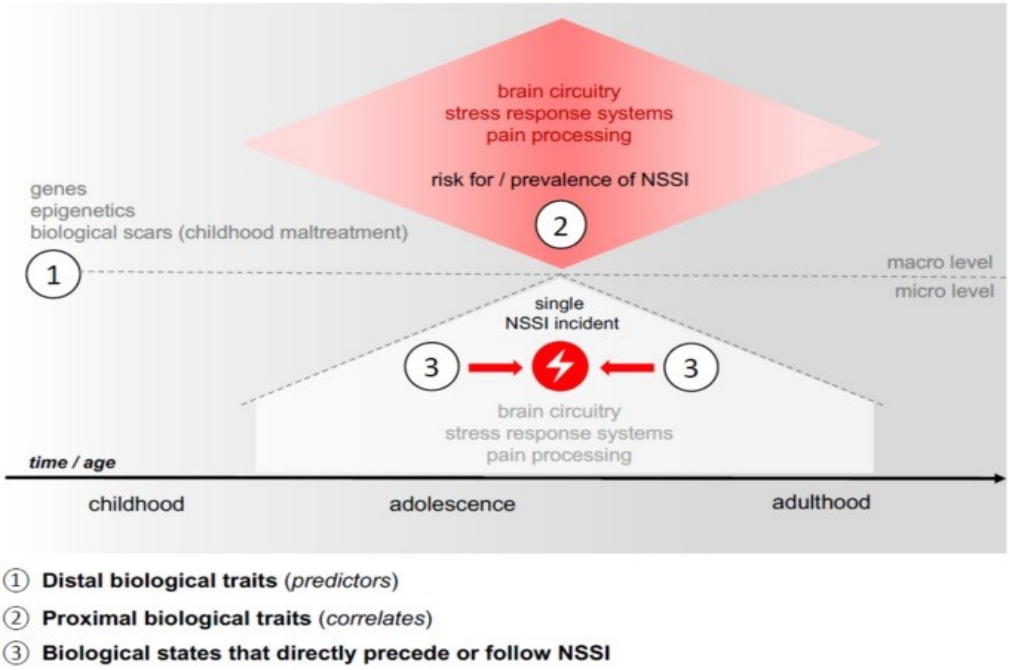

Figure I: A "Temporal Framework Model of NSSI" by Kaess et al. (2021), retrieved from https://doi.org/10.1016/j.neubiorev.2021.08.022, used under CC BY 4.0 license.

2.1.1 Central nervous system

In line with the RDoC domains (Kozak & Cuthbert, 2016; National Institute of Mental Health, 2022), fronto-limbic neural circuits are central to processes of affect, reward, arousal and cognitive control (for a brief overview, see: Williams, 2017), with significant overlap and strong interconnectedness for most processes. Processes of emotion generation and regulation are characterized by a continuous interaction of subcortical *bottom-up* and cortical *top-down* processes (Ochsner & Gross, 2007, 2014). As such, the amygdala and insula are core systems for processing emotions as a more automatic reaction to acute stimuli, such as perceived threat (*bottom-up*; Lindquist et al., 2016; Williams, 2017). Further, the hippocampus is associated with emotional memory and regulation of the neuroendocrinological as well as physiological stress responses through descending pathways via the hypothalamus (Lupien et al., 2009). Frontal neural circuits such as the PFC and ACC are implicated in higher cognitive functions (Lupien et al., 2009; Williams, 2017). They integrate information from the subcortical areas and subsequently influence and modulate emotion regulation through their influence on limbic neural systems (*top-down*; Ochsner et al., 2012). Successful emotion regulation requires the interaction of cortical and limbic areas as well as the normal functioning of the PFC (Ochsner & Gross, 2014). Consequently, alterations in fronto-limbic functioning, especially the PFC, ACC and amygdala, have been associated with emotion dysregulation as well as stimulus and response interference in BPD (Schulze et al., 2016; Sebastian et al., 2014), depression and anxiety disorders (Williams, 2017). As individuals with NSSI often report dysregulated emotion processing (Wolff et al., 2019), altered functioning of these neural systems may play an important role in the context of neurobiological NSSI research.

Extant neuroimaging studies assessing (ab)normal CNS functioning in NSSI and common comorbid disorders have reported reduced grey matter volumes in the ACC and other fronto-limbic areas in adolescents with NSSI compared to healthy controls (Ando et al., 2018; Westlund Schreiner et al., 2020), which were further associated with NSSI duration (i.e., how long individuals had been engaging in NSSI). Moreover, one study reported increased connectivity between the amygdala and the ACC at rest in adolescents with NSSI compared to HC (Westlund Schreiner et al., 2017). When faced with emotional pictures or social exclusion tasks, individuals with NSSI showed a greater PFC, amygdala and ACC activation (Brown et al., 2017; Groschwitz et al., 2016; Malejko et al., 2019; Plener et al., 2012), while a greater PFC activation was further reported in response to NSSI-related pictures (Plener et al., 2012). Finally, a study by Dahlgren and colleagues (2018) reported increased ACC and decreased dorsolateral PFC activation during an interference task in adult females with NSSI. Moreover, dorsolateral PFC activation was inversely correlated with emotional reactivity, indicating that decreased dorsolateral PFC activation was associated with poorer emotional control and increased impulsivity (Dahlgren et al., 2018). According to the Temporal Framework Model (Kaess et al., 2021), these studies indicate that altered fronto-limbic functioning is associated with an increased reactivity to perceived threat (i.e. social exclusion) and emotion dysregulation in individuals with NSSI and may thus constitute a proximal biological trait, increasing the risk to develop and maintain NSSI. However, several limitations need to be considered. As such, existing studies focused predominantly on adults. Further, most studies only included small sample sizes. Moreover, although emotion dysregulation and altered fronto-limbic functioning have been associated with a range of psychiatric disorders, the presence of comorbid psychopathology in NSSI and potential effects of symptom severity have thus far been insufficiently addressed. Extant studies often relied on the traditional categorical diagnoses, comparing NSSI samples with healthy control groups or clinical control groups (e.g., BPD, depression) with no NSSI, but often disregarded the presence of comorbid (sub-threshold) psychopathology in the NSSI groups. Moreover, only one study (i.e., Westlund Schreiner et al., 2020) covered the potential effect of NSSI severity, using the duration of NSSI as a severity marker.

Importantly, research indicates that fronto-limbic neural circuits also play a crucial role in pain processing (Garland, 2012; Leknes & Tracey, 2008). The areas involved include, among others, the PFC, the ACC, the amygdala, the insula, the hippocampus, and the hypothalamus (Garland, 2012; Navratilova & Porreca, 2014). Pain is a complex process involving *sensory-discriminative*, *motivational-affective*, and *cognitive* neural networks (Treede et al., 1999). These interconnected networks largely guide the perception of a noxious stimulus, based on the perceived intensity, quality and duration (*sensory-discriminative*), the attributed affective valence (*affective-motivational*) as well as the previously learned behavior associated with specific noxious stimuli (*cognitive*) (Ellison, 2017; Navratilova & Porreca, 2014). A pain-modulating role of these neural circuits has been suggested through ascending and descending pathways (Garland, 2012). As a significant functional overlap exists between these neural circuits for a vast array of processes, it has previously been suggested that pain and affect are closely associated. Consistently, negative affect was shown to increase, and positive

affect was found to reduce perceived pain (Finan & Garland, 2015; Garland, 2012; Wiech & Tracey, 2009). Conversely, pain has been shown to reduce negative affect (Bresin et al., 2018).

Schmahl and colleagues (2006) reported a greater dorsolateral PFC activation as well as deactivation of the ACC and amygdala during experimental pain in adult patients with BPD and NSSI compared to HC. This finding has been replicated in several studies (Niedtfeld et al., 2010; Reitz et al., 2015). Moreover, alterations in functional connectivity have been observed (Kluetsch et al., 2012; Reitz et al., 2015), leading to the assumption of altered affective and cognitive appraisal of pain as a potential mechanism for reduced PS (Kluetsch et al., 2012). Overall, the extant literature indicates the presence of altered fronto-limbic functioning in individuals with NSSI. With regard to the Temporal Framework Model (Kaess et al., 2021), these findings further suggest that altered fronto-limbic functioning can be considered a proximal biological trait. Task-specific and altered functioning at rest may be indicative of dysregulated emotion regulation and distress tolerance, which have previously been found to precede NSSI episodes (Ammerman, Olino, et al., 2017; Wolff et al., 2019). Moreover, the increased reactivity of fronto-limbic neural circuits in response to pain in individuals with NSSI may further highlight a maladaptive regulation mechanism, which subsequently drives the maintenance of NSSI. However, further research in adolescent populations controlling for potential moderating effects of comorbid psychopathology is warranted.

2.1.2 β-Endorphin

Extensive literature indicates that individuals engaging in NSSI report a reduced pain sensitivity, characterized by a greater pain threshold and tolerance, as well as lower perceived pain intensity (Koenig, Thayer, et al., 2016). Beyond fronto-limbic neural circuits, the modulation of pain perception is influenced by the endogenous opioid system. With reference to the RDoC (Kozak & Cuthbert, 2016; National Institute of Mental Health, 2022), endogenous opioids are centrally implicated in processes of the *negative valence systems* (e.g., acute threat), *positive valence systems* (e.g., reward) as well as *cognitive systems* (e.g., perception) domains. The release of endogenous opioids is associated with analgesic effects (Akil et al., 1984). Four groups of endogenous opioids are distinguished: endorphins, enkephalins, dynorphins and endomorphins. Each hormone binds to several receptor types (e.g., μ-, δ-, and κ-receptors) with varying affinity (Benarroch, 2012; Bresin & Gordon, 2013). Especially the activity of μ- and δ-receptors has been associated with the modulation of pain perception, thus emphasizing the role of BE. BE and μ- and δ-receptors are found in the peripheral and central nervous systems (Benarroch, 2012; Rachinger-Adam et al., 2011). The perception of a noxious stimulus triggers the release of BE in the hypothalamus and the pituitary glands, derived from its precursor proopiomelanocortin. Released BE subsequently binds to μ-receptors. In the peripheral nervous system, this results in the inhibition of tachykinin peptides which are responsible for the afferent transmission of pain (Sprouse-Blum et al., 2010). In the CNS, BE release has been associated with the descending pain modulatory system and changes in the affective valence of pain (Corder et al., 2018). Dense populations of μ-receptors have been reported in the amygdala, hippocampus, thalamus and insula (Browne & Lucki, 2019), which

are associated with social processes as well as processes of emotion regulation and reward. Here, the analgesic effect has been linked to an inhibiting effect of BE on the release of the inhibitory neurotransmitter GABA, which results in increased dopamine production (Sprouse-Blum et al., 2010). Dopamine plays a central role in mood and reward-motivated behavior (Navratilova & Porreca, 2014). A mutually inhibitory interplay between BE and dopamine in the CNS has previously been assumed, potentially explaining the role of BE in reward processing through a feedforward loop (Leknes & Tracey, 2008). This pathway underlines how pain and BE might influence mood and help to regulate stress in NSSI. As such, NSSI may stimulate the release of BE, subsequently producing analgesic effects and regulating emotion. These effects of BE release might be perceived as rewarding and, through its interaction with dopamine, might increase the motivation to repeatedly engage in NSSI. In their review, Browne and Lucki (2019) further outline how a dysregulation of endogenous opioids may contribute to the pathophysiology of depression and posttraumatic stress disorder, which are, similar to NSSI, characterized by emotion dysregulation and low stress tolerance. As such, BE is one potential neuroendocrine marker that should be assessed in NSSI.

The role of the endogenous opioid system in psychiatric disorders and self-injurious behavior has been assumed for decades. Regarding NSSI, Coid and colleagues (Coid et al., 1983) reported no significant differences in plasma BE levels in adult individuals with BPD and a history of NSSI. Since then, several studies mostly found decreased BE levels to be associated with self-injurious behavior. Stanley and colleagues (2010) reported lower basal levels of BE in cerebrospinal fluid in individuals with BPD and a history of NSSI compared to individuals with BPD without prior NSSI. Similarly, lower plasma BE levels have also been observed in animal studies of monkeys with self-biting behavior compared to monkeys without self-biting (Tiefenbacher et al., 2005). Further, several studies in individuals with developmental disorders that engaged in self-injurious behavior found elevated BE levels following self-injurious acts (Sandman et al., 1997, 2003; Sandman & Hetrick, 1995). These findings have led to the proposition of an opioid deficiency model, whereas individuals that engage in NSSI have low baseline levels of BE. It is proposed that this creates an imbalance due to a deviation from the homeostatic set point for opioids. Subsequently, it is suggested that individuals engage in NSSI in order to restore BE levels (Sher & Stanley, 2008; Stanley et al., 2010). According to the Temporal Framework Model (Kaess et al., 2021), altered basal BE may thus be considered a proximal biological trait, increasing the vulnerability to engage in NSSI. In a first study assessing salivary BE in daily life using ecological momentary assessments, the opioid deficiency model was partially supported (Störkel et al., 2021). BE levels were found to be significantly higher immediately after NSSI compared to assessments prior to an act of NSSI. No differences were reported during phases of high NSSI urge or on days without NSSI (Störkel et al., 2021). The authors concluded, that this potentially indicates a reinforcing mechanism for NSSI engagement (Störkel et al., 2021). Important to note, the study by Störkel and colleagues (2021) only included participants with recurrent NSSI. Thus, it remains unclear whether overall BE levels were altered in individuals with NSSI, as compared to healthy individuals or clinical control groups without NSSI. Overall, the existing literature indicates that BE constitutes a proximal biological trait, as alterations in BE levels have been associated with

emotion dysregulation and low distress tolerance (Browne & Lucki, 2019). Existing studies point to a potential role of lower BE levels in individuals with NSSI that can be regulated through self-injury. However, further case-control studies assessing BE levels in NSSI, especially in adolescent populations, are warranted. Further, given that most studies assessed an association between BE and NSSI in populations with comorbid psychopathology and varying definitions of NSSI, studies controlling for the influence of severity of comorbid psychopathology and NSSI are needed.

2.1.3 Hypothalamic-pituitary-adrenal axis

The physiological stress response is defined by responses of neuroendocrine and autonomic systems that guide an adapted physiological response. With reference to the RDoC (Kozak & Cuthbert, 2016; National Institute of Mental Health, 2022), the HPA axis is part of the *negative valence systems* domain, as it contains systems involved in adaptive responses to acute or sustained threat. The HPA axis response to stress is initiated by the secretion of corticotrophin-releasing hormones (CRH) and arginine vasopressin in the paraventricular nucleus of the hypothalamus. CRH, and to a lesser degree arginine vasopressin, then lead to the secretion of adrenocorticotropic hormones (ACTH) in the anterior pituitary, processed from proopiomelanocortin. The increase in ACTH subsequently triggers the secretion of cortisol and other glucocorticoids from the adrenal cortex, which act on a range of peripheral physiological systems (Gunnar & Quevedo, 2007; Sheng et al., 2021). The stress response of the HPA axis is terminated through a negative feedback loop, where increased circulating cortisol levels inhibit the production of ACTH and CRH (Sheng et al., 2021). Through this system, individuals can quickly adapt to situational demands. The HPA axis response to acute physical (e.g., pain) and psychological stress is defined by an increase in cortisol secretion (Ulrich-Lai & Herman, 2009). Sustained periods of stress (e.g., adverse childhood experiences (ACE)) have been shown to affect HPA axis activity, leading to chronic alterations in the HPA axis response to stress (Schär et al., 2022). This effect has been linked to a decreased capacity of glucocorticoids to inhibit the HPA axis, leading to increased CRH levels. In line with this, increased CRH levels have been reported in individuals with ACE (Lupien et al., 2009). Childhood and adolescence are sensitive periods for the development of the underlying biological systems that make up the HPA axis, leading researchers to suggest that these developmental phases might be especially sensitive to elevated levels of glucocorticoids and failures of the inhibitory feedback system (Lupien et al., 2009; Sheng et al., 2021). Conversely, dysregulations in HPA axis functioning, resulting from sustained stress (e.g., ACE), have been associated with the development of psychiatric disorders such as depression and posttraumatic stress disorder (Baumeister et al., 2014; McCrory et al., 2010; Shea et al., 2005). Moreover, the hippocampus, amygdala and the PFC have been reported to show reduced volume following sustained stress (McEwen, 2007). As described above (see chapter 2.1.1), these fronto-limbic structures are involved in a descending regulatory pathway of stress-induced activation of the HPA axis, thus potentially explaining alterations in behavioral and physiological responses (McEwen, 2007; Ulrich-Lai & Herman, 2009).

As NSSI is often preceded by interpersonal stress and subsequent distress, research on the stress-related HPA axis reactivity in adolescents with NSSI has seen increasing interest over the past decade, often relying on social stress tasks. In one of the earliest studies conducted, Kaess and colleagues (2012) reported that adolescents with a history of NSSI had a blunted salivary cortisol response to a standardized psychosocial stress task (*Trier Social Stress Test*) compared to a healthy control group (Kaess et al., 2012). This finding has since been replicated in two studies (Klimes-Dougan et al., 2019; Plener et al., 2017). Klimes-Dougan and colleagues (2019) could further show that the blunted salivary cortisol response was found in individuals with NSSI and a comorbid depression diagnosis compared to healthy controls and individuals with depression and no history of NSSI. Moreover, no differences were observed between healthy controls and individuals with depression only (Klimes-Dougan et al., 2019), suggesting an additive role of NSSI in the altered stress response in depression. Additionally, Reichl and colleagues (2019) reported an association between altered HPA axis activity and ACE in adolescents with NSSI, further supporting previous claims that ACE may be a distal risk factor for altered HPA axis functioning and subsequent psychiatric sequelae later in life. Overall, the extant literature indicates a blunted HPA axis response to stress in individuals with NSSI. Important to note, findings regarding a potential association with NSSI severity are sparse and mixed, warranting further research. On the one hand, the study by Kaess and colleagues (2012) found no association between lifetime NSSI acts and a blunted HPA axis response to stress. On the other hand, the study by Klimes-Dougan and colleagues (2019) reported a blunted HPA axis stress-response in patients with repetitive NSSI (4+ lifetime acts) but not with experimental NSSI (1-2 lifetime acts). The latter, however, used a categorical approach, artificially dividing individuals with NSSI into two groups based on NSSI frequency, thus failing to reveal more nuanced associations between increasing NSSI severity and HPA axis activity. Moreover, the chosen cut-off of 4+ lifetime acts of NSSI differs significantly from the NSSID criterion A (5+ acts of NSSI in the past year), which further limits the comparability with findings from research on psychopathology and NSSI severity. So far, only one study has systematically investigated the HPA axis response to experimental pain induction in adolescents with NSSI (Koenig, Rinnewitz, Warth, et al., 2017). This study assessed the HPA axis and ANS response to a cold pain stimulation in 30 female adolescents with at least five acts of NSSI in the past year and 30 healthy controls. Koenig and colleagues (2017) reported a significantly increased HPA axis response to pain in adolescents with NSSI. Interestingly, this association was further moderated by ACE, whereas more severe ACE earlier in life was associated with an increased HPA axis response following pain induction (Rinnewitz et al., 2018). This finding was inconsistent with the previously reported blunted HPA axis response to stress (Kaess et al., 2012; Klimes-Dougan et al., 2019; Plener et al., 2017). However, the authors argued that the increased HPA axis response to pain might be a compensatory mechanism for individuals with NSSI, likely resulting from the hyporesponsiveness of the HPA axis in stressful situations (Koenig, Rinnewitz, Warth, et al., 2017). In line with the Temporal Framework Model (Kaess et al., 2021), these findings support the assumption that dysregulations in the HPA axis may constitute a proximal biological trait, increasing the vulnerability to developing NSSI through an insufficient physiological stress response. The blunted HPA axis stress-response may be, at least in part, influenced by distal biological risk

factors (e.g., ACE) and countered by engaging in NSSI, resulting in increased pain-related cortisol secretion. This stimulus-specific reaction could subsequently influence the maintenance of NSSI. However, these findings warrant replication, and it remains unclear if the effects are moderated by the severity of comorbid psychopathology and NSSI, as preliminary evidence suggests (Fernando et al., 2012; Klimes-Dougan et al., 2019).

2.1.4 Autonomic nervous system

As a second central stress response system, the ANS regulates the organ function of the body (i.e., heart (rate), respiratory system, metabolism, temperature) to guarantee an optimal adaptive physiological response to situational demands (Hall & Hall, 2020; McCorry, 2007). The ANS largely consists of two antagonistic, tonically active systems: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). Increased SNS activity results in an increased organ activity (e.g., increase in heart rate) through the secretion of noradrenaline, whereas increased PNS activity has inhibitory functions (e.g., decrease in heart rate) largely managed through the secretion of fast-acting acetylcholine. PNS control of the heart is hereby primarily regulated through the vagus nerve (McCorry, 2007). Heart rate (HR) and heart rate variability (HRV) have been extensively used as reliable, autonomic indicators of the ANS response to stress (Appelhans & Luecken, 2006; Mueller et al., 2022; Thayer et al., 2012). HR is the product of the interplay between SNS and PNS activity (Thayer et al., 2012). Especially vagally mediated HRV, described as the fluctuation in intervals between consecutive heartbeats, has seen increasing interest as an indicator of general adaptive capacity reflecting PNS activity (Thayer et al., 2010). High HRV has hereby generally been accepted as an indicator of normal cardiac function and good physiological reactivity, whereas lower HRV has been associated with an increased risk of cardiovascular and stress-related diseases (Weber et al., 2010), as well as psychiatric disorders in adults and adolescents (Hu et al., 2016; Koenig et al., 2014; Sgoifo et al., 2015). Beyond categorical diagnoses, low HRV has been associated with the severity of psychopathological symptoms found across internalizing and externalizing psychiatric disorders, most prominently emotion dysregulation (Beauchaine, 2015a, 2015b; Cattaneo et al., 2021). Previous research hereby indicates that the ANS can be influenced through descending neurologic pathways of fronto-limbic structures that are central for the generation and regulation of emotions (see chapter 2.1.1; e.g., PFC, amygdala, hippocampus) (Beauchaine, 2015b; Kreibig, 2010). Consequently, HRV has been proposed as a transdiagnostic physiological marker of psychopathology (Beauchaine & Thayer, 2015). It is important to note that the HPA axis and the ANS interact to regulate the physiological stress response, as the (re)activity of both is mainly regulated through descending pathways of the hypothalamus (Mueller et al., 2022). Accordingly, a study by Rotenberg and McGrath (2016) analyzed the interrelation between the HPA axis and the ANS in a sample of 201 children and adolescents. The authors reported that a higher sympathetic activation, combined with an increased cortisol awakening response were associated with greater perceived stress (Rotenberg & McGrath, 2016). Moreover, an inhibitory role of the vagus nerve in the regulation of the HPA axis has been assumed (Thayer & Sternberg, 2006). In line with this assumption, several studies reported an inhibiting effect of greater vagal activity on the HPA axis (Agorastos et al., 2019; Johnsen et al., 2012; Weber

et al., 2010). With reference to the RDoC (Kozak & Cuthbert, 2016; National Institute of Mental Health, 2022) and the Temporal Framework Model (Kaess et al., 2021), ANS functioning aligns with the categorizations of the HPA axis. The ANS is an adaptive system that guides the physiological response to acute or sustained aversive situations (negative valence systems). As dysregulations in ANS functioning indicate alterations in stress tolerance and are associated with psychopathology as well as the severity of psychiatric symptoms commonly observed in NSSI, ANS dysregulations may constitute a proximal biological trait, increasing the risk of engaging in NSSI.

Research on ANS (re)activity in NSSI has, so far, yielded conflicting results. Few studies have assessed vagally mediated HRV in individuals with NSSI (Crowell et al., 2005; Koenig, Rinnewitz, Parzer, et al., 2017; Koenig, Rinnewitz, Warth, et al., 2017). Crowell and colleagues (2005) reported a reduced vagally mediated HRV at rest and a greater vagally mediated HRV during a sadness induction task in adolescents with NSSI compared to a healthy control group (Crowell et al., 2005). In contrast, no alterations in resting HR and vagally mediated HRV were reported in a sample of adolescent outpatients with NSSI, compared to healthy controls (Koenig, Rinnewitz, Parzer, et al., 2017). However, an increased HR and decreased HRV were associated with BPD severity (Koenig, Rinnewitz, Parzer, et al., 2017), a finding which has since been replicated (Weise et al., 2020). Finally, Koenig and colleagues (2017) reported ultra-short-term variations in HRV related to experimental pain induction, indicating a delayed decrease in parasympathetic vagal activity 60 seconds before pain induction (e.g., increased HRV) and decreased HRV following painful stimulation for 30 seconds (Koenig, Rinnewitz, Warth, et al., 2017). This altered recovery from pain was associated with increased self-reports of body awareness, supporting a potential biological mechanism underlying the intraindividual functions of NSSI (Koenig, Rinnewitz, Warth, et al., 2017). Beyond assessments of HR and HRV, Nock and Mendes (2008) reported that adolescents with NSSI showed a greater physiological response during a distressing task compared to a control group without NSSI, measured using skin conductance level (Nock & Mendes, 2008) – another established marker of physiological arousal via SNS activity on the eccrine sweat glands (Dawson et al., 2007; Xu & Huang, 2020). In contrast, several studies found no differences in the ANS response, neither for assessments of HR nor skin conductance level, following a painful stimulation or psychosocial stress tasks, instead indicating a similar physiological reactivity (Bohus et al., 2000; Kaess et al., 2012; Tatnell et al., 2018; Tuna & Gençöz, 2021). In line with the Temporal Framework Model (Kaess et al., 2021), the existing literature indicates that altered ANS functioning may be considered a proximal biological trait of NSSI characterized by a dominating SNS activity associated with underlying comorbid psychopathology. However, further research is warranted to clarify the mixed findings of the existing literature, additionally addressing the influence of NSSI severity and disentangling the effects of comorbid psychopathology.

2.1.5 Hypothalamic-pituitary-thyroid axis

While not included in the Temporal Framework Model (Kaess et al., 2021) or the RDoC (National Institute of Mental Health, 2022) given an absence of studies up to date - another

neuroendocrine system that could be of importance in NSSI is the HPT axis. The HPT axis is vital for nervous system development and regulation of the metabolism (Hall & Hall, 2020; Ortiga-Carvalho et al., 2011). This influence is regulated through thyroxine (T4) and triiodothyronine (T3), which are released from the thyroid through stimulation by pituitary thyrotropin (thyroid-stimulating hormone, TSH). TSH production is stimulated by the hypothalamic thyrotropin-releasing hormone (TRH) (Hall & Hall, 2020; Ortiga-Carvalho et al., 2011). T4 and T3 subsequently control the secretion of TRH and TSH through a negative feedback loop to maintain optimal functioning of the HPT axis. The HPT axis has been found to react to stress, whereas a release of T3 and T4 entails a physiologic activation of the body, resulting in increased metabolism and HR (Ortiga-Carvalho et al., 2011).

For the past decades, HPT axis functioning has been assessed in the context of stress and stress-related disorders (e.g., posttraumatic stress disorder) as well as mood disorders (e.g., depression), albeit with inconsistent results. In one study, Fischer and colleagues assessed the effect of acute psychosocial stress on the HPT axis in healthy women (Fischer et al., 2019), reporting increases in TSH hormone but not T3 and T4. Prolonged or chronic stress has been associated with a blunted HPT axis response, leading to decreased metabolic activity in response to environmental demands (Helmreich et al., 2005). Moreover, the experience of ACE has been linked to lower peripheral T3 levels (Machado et al., 2015). This decreased activity has been linked to increased activation of the hypothalamic-pituitary-adrenal axis (Chrousos, 2007), a central neuroendocrinological system for the human stress response (see chapter 2.1.3.; Hall & Hall, 2020). Dysregulation of the HPT axis has previously been observed in individuals with depression (Duval et al., 2021; Stipčević et al., 2008) and with BPD (Sinai et al., 2014, 2015). This dysregulation has been linked to a reduced level of deiodinase, an enzyme responsible for converting T4 to T3 (Stipčević et al., 2008). In line with this, an influence of central HPT axis dysregulation on emotion dysregulation has been assumed through its effect on serotonin, which plays a role in depressive symptomatology (Yohn et al., 2017), and the presence of thyroid receptors on limbic structures associated with mood regulation (Bauer & Whybrow, 2001). Given the high comorbidity of NSSI with BPD and depression as well as other disorders (Kiekens & Claes, 2020; Nock et al., 2006), extensive evidence that emotion dysregulation is a common symptom across these diagnostic entities, and that ACE have previously been shown to increase the risk to engage in NSSI (Serafini et al., 2017), alterations in the HPT axis warrant a systematic assessment in adolescents with NSSI.

With reference to the RDoC (National Institute of Mental Health, 2022) and the Temporal Framework Model (Kaess et al., 2021), HPT axis functioning has not yet been introduced. However, given its response to and involvement in stress-regulation, altered HPT axis activity might be categorized among mechanisms of the *negative valence systems* and *arousal* domains. Within the Temporal Framework Model (Kaess et al., 2021), altered HPT axis functioning might be categorized as either a distal or proximal biological trait increasing the risk for NSSI, due to the proposed influence on emotion dysregulation.

3 Gaps in current research

Despite considerable efforts, the field of research on neurobiological risk factors and correlates of NSSI is still in its early stages, and comparatively few studies exist. While current findings indicate a role of altered neurobiology in NSSI, several limiting factors need to be addressed, as they affect the interpretability of findings. First, several biological systems (e.g., BE and HPT axis) have been studied almost exclusively in adult populations (Fischer & Ehlert, 2018; Sandman et al., 2003; Sinai et al., 2015; Stanley et al., 2010; Stipčević et al., 2008; Störkel et al., 2021). However, the prevalence of NSSI peaks in adolescence, around the age of 15 to 17 years (Plener et al., 2015; Swannell et al., 2014). Moreover, adolescence is a sensitive developmental phase for most biological systems (Gerber et al., 2009; Roberts & Lopez-Duran, 2019). Thus, assessing neurobiological mechanisms of NSSI in adolescent samples is critical to determine if biological alterations are present early in life and how they relate to the risk of developing and maintaining NSSI before a chronification of NSSI behaviors occurs. Second, there are considerable differences in the definition and assessment of NSSI across the extant studies. Earlier studies often examined associations between neurobiological systems and NSSI in the context of comorbid psychiatric disorders, particularly BPD. More recent studies have often relied on the diagnostic criteria of the proposed NSSID diagnosis introduced in the DSM-5 (American Psychiatric Association, 2013), comparing the subsequent NSSI samples with healthy and clinical control groups, often selectively recruited without self-injurious behavior. Many of these studies have reported one or several comorbid disorders in their NSSI samples, often without systematically assessing their respective impact on the outcomes of interest. However, this dramatically limits the interpretability of NSSI-specific pathophysiological mechanisms. A recent study conducted in a large sample of 464 help-seeking adolescents with NSSID reported that NSSID without comorbid disorders was rare and characterized by low illness severity and psychopathological distress, and relative diagnostic instability over a one-year period. This led the authors to conclude that NSSI should be considered a transdiagnostic precursor for psychopathological development (Ghinea et al., 2020). Moreover, research indicates that psychopathology follows a developmental pathway, whereas individuals may be diagnosed with different subsequent psychiatric disorders over their lifetime with shared psychopathological symptoms (Caspi et al., 2020). Extensive evidence hereby indicates that these psychopathological symptoms are largely influenced by shared, underlying pathophysiological mechanisms (e.g., Beauchaine, 2015b; Beauchaine & Thayer, 2015; McTeague et al., 2020). Consequently, analyzing the full functioning spectrum of neurobiological systems in heterogenous samples reporting NSSI and comorbid psychopathology might help to further disentangle whether previously reported alterations are associated with NSSI per se or primarily associated with comorbid psychopathology. Moreover, such analyses may further shed light on whether NSSI is best viewed as an individual diagnosis or a transdiagnostic precursor for future psychiatric diagnoses (Ghinea et al., 2020). Finally, mixed findings on neurobiological alterations in NSSI could be linked to the categorical conceptualization of NSSI groups. Recent studies have progressively relied on the proposed criterion A of the DSM-5 (American Psychiatric Association, 2013), including individuals with five or more days on which self-injurious acts were reported over the past year.

However, researchers have repeatedly criticized the respective cut-off as too low, reporting significantly higher frequencies of repetitive NSSI both in community samples and clinical samples (Andover, 2014; Washburn et al., 2015; Zetterqvist et al., 2020). Adding to this, several studies revealed discernible NSSI subgroups that varied as a function of the severity of comorbid psychopathology and psychosocial impairment with increasing NSSI frequency (Ammerman, Jacobucci, et al., 2017; Muehlenkamp et al., 2017; Zetterqvist et al., 2020). This reflects the proposition of the RDoC project that *"there are important similarities between those whose symptoms meet criteria for a disorder versus those who just miss the cut-off for diagnosis due to fewer and/or less severe symptoms."* (National Institute of Mental Health, 2022). As it becomes more apparent that psychopathology and its underlying pathophysiological mechanisms are naturally continuous (Michelini et al., 2021; Widiger & Edmundson, 2011), exclusively relying on specific, arbitrary thresholds may limit the reliability of previous scientific findings. In line with this, previous research indicates that even subthreshold presentations of psychiatric diagnoses were associated with psychosocial impairment and predictive of future psychiatric disorders (Shankman et al., 2009). As such, a dimensional approach to NSSI is warranted.

To sum up, future research will have to conduct studies in large adolescent samples with heterogenous psychopathological backgrounds to advance the field of neurobiological study on NSSI. Moreover, there is an urgent need for a thorough assessment of the severity of NSSI and (comorbid) psychopathology in general and how they may relate to altered neurobiological functioning.

4 Contributions of this work

As outlined in the previous chapters, previous research indicates the role of neurobiological systems in the development and maintenance of adolescent NSSI. However, the existing evidence is limited and sometimes mixed. Moreover, a systematic assessment of NSSI-related symptomatology, (comorbid) psychopathology, and their respective severity in relation to the neurobiological systems under investigation are often missing. The present work aims at filling some of these gaps, by reporting the results from four studies that systematically investigated the neurobiological functioning in adolescents with NSSI (aged 12 – 17 years), while assessing the influence of comorbid psychopathology and NSSI severity.

Data for all manuscripts were obtained from the research project "*Neurobiogenetische Prädiktoren der Entwicklung und des Verlaufs Selbstschädigender Verhaltensweisen bei Jugendlichen (AtR!Sk-Bio)*" (IRB ethical approval number: S-514/2015). This study assessed a broad range of potential neurobiological markers to gain new information on the neurobiological basis of risk-taking and self-harming behaviors. AtR!Sk-Bio was conducted from 2016 to 2021 and embedded in the larger "*Evaluation der Ambulanz für Risikoverhalten und Selbstschädigung (AtR!Sk)*" (IRB ethical approval number: S-449/2013) project. This longitudinal project evaluated the range of treatments offered at the special outpatient clinic while further aiming to gain new insights into the development and functions of risk behaviors and self-harm, personality development and psychiatric disorders in adolescence. Both projects were funded by the Dietmar Hopp Foundation and implemented at the Clinic for Child and Adolescent Psychiatry, University Hospital Heidelberg (Germany). The first manuscript investigated the association of pain sensitivity and basal plasma BE levels with experimental pain in female adolescents with NSSI compared to matched healthy controls (HC). The second manuscript examined the HPA axis and ANS response to experimentally induced pain in female adolescents with NSSI and matched HC. The third manuscript investigated PFC oxygenation, as an indicator of PFC activation, and functional connectivity within the PFC at rest in adolescents with NSSI compared to HC. The final manuscript assessed basal HPT axis functioning in female adolescents with NSSI compared to HC. Importantly, all studies investigated dimensional effects of NSSI severity (e.g., frequency of NSSI) and comorbid psychopathology (i.e., BPD and axis I disorders) on the neuroendocrinological and physiological systems.

All participants passed two appointments (see Figure II). The first appointment marked a detailed diagnostic interview with additional self-report questionnaires, evaluating a wide range of clinical and psychiatric symptoms, such as NSSI, axis I psychiatric disorders, BPD symptoms, depression symptoms, ACE, as well as participants' global functioning and severity of psychiatric symptoms. The neurobiological assessment started with a fasting blood draw as well as general health-related questions (i.e., height, weight, smoking behavior). Subsequently, the intelligence quotient was assessed using the *Hamburg Wechsler Intelligence Scale for Children IV* (HAWIK-IV) (Petermann & Petermann, 2010). Following a 5-minute resting phase, participants passed a cognitive test assessing neuropsychological dimensions such as attention and memory, using the Cognitive Basic Assessment Test (COGBAT; Vienna Test

System (VTS), Schuhfried, Mödling, Austria). Following, pain induction was administered using a heat plate. Subsequently, a second 5-minute resting phase and a debriefing marked the end of the second appointment. The manuscript-specific methodology will be addressed in more detail in the respective sections of each manuscript.

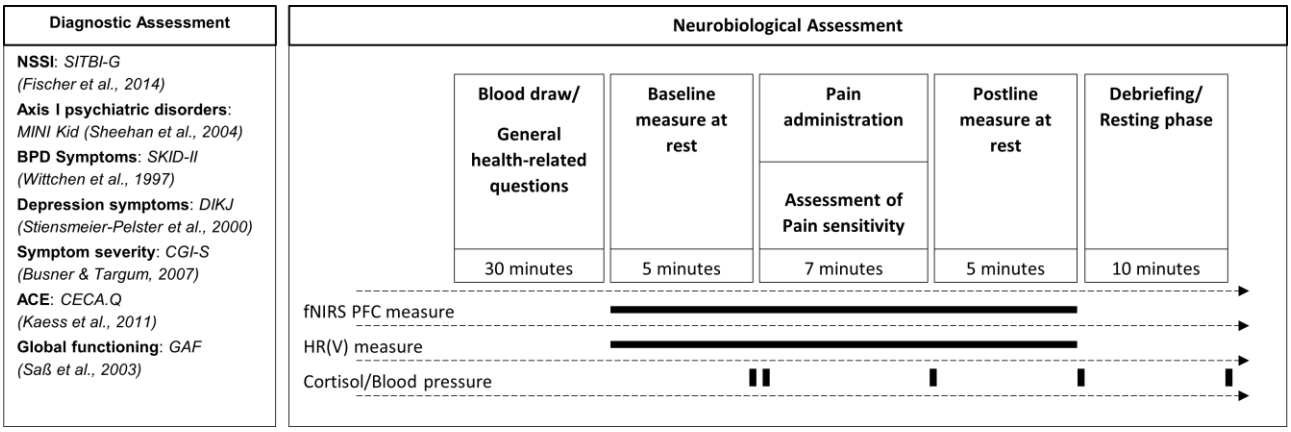

Figure II: Study design for the diagnostic and neurobiological assessments; adapted[1]

4.1 Manuscript I

van der Venne, P.; Balint, A.; Drews, E.; Parzer, P.; Resch, F.; Koenig, J.; Kaess, M. (2021). Pain sensitivity and plasma beta-endorphin in adolescent non-suicidal self-injury. Journal of Affective Disorders, 278, 199-208.

Reports consistently show that individuals with NSSI feel little or no pain when self-injuring (Nock & Prinstein, 2005). Several meta-analyses have confirmed significantly greater pain threshold and tolerance in adolescents and adults with NSSI (Kirtley et al., 2016; Koenig, Thayer, et al., 2016). Extant neuroimaging studies indicate a potential role of altered functioning in brain areas associated with affective and cognitive mechanisms of pain processing (Schmahl et al., 2004, 2006). As previous findings report a considerable overlap in brain regions serving affective/cognitive mechanisms of pain perception and emotion regulation (Garland, 2012; Leknes & Tracey, 2008), the influence of these circuits in the pathology of NSSI has been discussed. Indeed, decreased pain sensitivity (PS) has previously been found to correlate with the severity of BPD and depression symptomatology (Ludäscher et al., 2009; Thompson et al., 2016). Moreover, emotion dysregulation, a central risk factor for NSSI, BPD and depression alike (American Psychiatric Association, 2013), was shown to influence the association between NSSI and pain tolerance (Franklin et al., 2012). It is well evidenced that BE plays a major role in the modulation of pain perception and analgesia (Akil

[1] Figure II depicts an adapted form of the study design, containing only diagnostic tools and procedures relevant to the present thesis. For a detailed description of the AtR!Sk project see: Kaess et al., 2017. Regarding the neurobiological assessment, additional intelligence and cognitive functioning tasks using the Hamburg Wechsler Intelligence Scale for Children IV (HAWIK-IV) (Petermann & Petermann, 2007) and the Cognitive Basic Assessment Test (COGBAT; Vienna Test System (VTS), Schuhfried, Mödling, Austria) prior to and between the baseline assessment at rest and pain administration are not depicted (duration: ~120 minutes).

et al., 1984; Benarroch, 2012). Consistently, the potential role of BE in NSSI has gained increasing attention. With first support of lowered BE levels reported in animals and humans with a history of NSSI (Stanley et al., 2010; Tiefenbacher et al., 2005), opioid deficiency theories have been proposed (Bresin & Gordon, 2013). However, empirical data are still limited and often assessed the role of altered PS or BE levels in different psychiatric disorders, sometimes without considering NSSI (Prossin et al., 2010). Further, most studies solely included adult participants.

Therefore, this study aimed to systematically assess PS and basal BE levels in adolescents with NSSI compared to a group of HC. It was hypothesized that adolescents with NSSI report greater pain threshold and tolerance as well as lower pain intensity. Further, it was expected that adolescents with NSSI had lower resting plasma BE levels. Potential associations between PS and plasma BE levels were analyzed in exploratory analyses. Finally, associations of both PS and plasma BE levels with comorbid psychopathology (e.g., NSSI frequency, BPD and depressive symptom severity, trauma, and general psychiatric symptom severity) were investigated.

The sample consisted of n = 94 female adolescents with at least five acts of NSSI in the past year (as defined by the DSM-5 criterion A) and n = 35 female HC. The groups were matched for age, height, and weight. Concerning the diagnostic assessment (Figure II), the first manuscript used data on NSSI assessed with the *Self-Injurious Thoughts and Behaviors Interview* (Fischer et al., 2014). Axis-I psychiatric disorders were assessed using the *Mini International Neuropsychiatric Interview for Children and Adolescents* (Sheehan et al., 2004). BPD symptoms were assessed using the *Structured Clinical Interview for DSM-IV Axis II Personality Disorders* (Wittchen et al., 1997). Depression symptoms were assessed using the *Depression Inventory for Children and Adolescents* (Stiensmeier-Pelster et al., 2000), and the severity of psychiatric symptoms was assessed using the *Clinical Global Impressions-Severity* (Busner & Targum, 2007). ACE were assessed using the *Childhood Experiences of Care and Abuse Questionnaire* (Kaess et al., 2011). Severity of ACE was assessed using a dimensional score using four modules of the CECA.Q: antipathy, neglect, physical and sexual abuse. Each variable was dichotomized, and a mean score ranging from 0 (no trauma) to 1 (multiple trauma) was generated. BE levels were assayed using fasting blood draws at the beginning of the second appointment. Samples were analyzed at the central laboratories of the Heidelberg University Hospital using enzyme-linked immunosorbent assay (ELISA) by Cloud Clone (Houston, TX, US). PS was assessed using a heat plate. Participants were asked to put their non-dominant hand on the plate at a 32 °C baseline temperature. After a three-minute adaptation phase at 32 °C, the temperature rose to 50 °C, increasing linear over four minutes. The temperature at first pain sensation was defined as pain threshold (verbal feedback), while pain tolerance was defined as the temperature when pain sensation became intolerable (hand removal). Further, the evolution of perceived pain intensity was assessed via a visual analogue scale. Participants continuously rated the perceived intensity (range: 0-100) from the moment pain threshold was reached until they removed their hand (pain tolerance). For this study, pain intensity scores were calculated based on the rated intensity at pain tolerance, generating an average score for each participant within five seconds prior to and following pain tolerance.

Participants with NSSI showed a significantly greater pain threshold and a significantly lower pain intensity compared to the HC, while no significant differences were found for pain threshold. Moreover, participants with NSSI had significantly lower basal plasma BE levels compared to the HC. As previous literature indicates a potential influence of nicotine administration on BE levels (del Arbol et al., 2000; Tziomalos & Charsoulis, 2004), smoking behavior was controlled for. However, no significant influence was found. Comorbid psychopathologies were common in participants with NSSI (BPD: 32%; Depression: 58%), warranting a detailed assessment of individual associations with PS and BE levels. Correlation analyses revealed a significant positive correlation between pain threshold and BPD symptoms. Further, depression scores correlated negatively with pain intensity and BE levels. Subsequent stepwise regressions revealed that BPD symptoms significantly predicted pain threshold, while group (NSSI vs. HC) significantly predicted pain intensity and BE levels. Further correlation analyses revealed no significant association between NSSI frequency, trauma, and psychiatric symptom severity and PS and BE levels, respectively. Finally, no significant association was found between PS and BE levels.

In line with our hypotheses and previous findings, adolescents with NSSI reported a significantly lower PS (Kirtley et al., 2016; Koenig, Thayer, et al., 2016) and lower basal plasma BE levels (Sandman & Hetrick, 1995; Stanley et al., 2010). The findings on altered BE levels constitute an important advance to previous research in adults, as they are the first to report alterations in adolescents with NSSI. No significant association was found between PS and BE levels at rest. However, considering the previous theoretical background, pain experience might be modulated by higher-order top-down cognitive processes (Garland, 2012), resulting in central BE release that is uncoupled from peripheral BE levels (De Riu et al., 1997). Moreover, the opioid deficiency model proposes that BE release modulates pain experience, whereas lower basal BE levels may increase the sensitivity to analgesic effects (Bresin & Gordon, 2013). The findings of manuscript I thus mark preliminary evidence for lower basal BE levels in adolescents with NSSI, potentially indicating increased analgesic effects due to BE release following NSSI. Finally, beyond significant alterations in PS and basal BE levels in adolescents with NSSI compared to HC, BPD symptoms significantly predicted alterations in pain threshold, indicating a role of severity of comorbid psychopathological symptoms underlying NSSI in altered pain experience.

4.2 Manuscript II

van der Venne, P., Höper, S.; Koenig, J.; Kaess, M. (submitted) Physiological response to pain in female adolescents with nonsuicidal self-injury as a function of severity. Translational Psychiatry.

Adolescents with NSSI report deficits in emotion regulation and stress tolerance (Andover & Morris, 2014; Tatnell et al., 2017). Many individuals engage in NSSI as a maladaptive coping mechanism to regulate their emotions (Edmondson et al., 2016; Taylor et al., 2018) – a strategy which has since been proven successful (in the short run) in studies analyzing changes in affect following NSSI using ecological momentary assessment (Armey et al., 2011; Kranzler et al., 2018). The ANS and HPA axis are two central biological systems involved in the stress

response (Kreibig, 2010; Lupien et al., 2009). Several studies reported a blunted HPA axis and altered ANS response during social and emotional stress tasks in adolescents with NSSI (i.e., Crowell et al., 2005; Kaess et al., 2012). HPA axis and ANS reactivity are influenced by both external stimuli and top-down cognitive emotional processes.

Pain experience plays a crucial role in the development and maintenance of NSSI (Selby et al., 2019). Pain itself is a stressful experience that leads to an activation of the HPA axis (e.g., increased cortisol secretion) and ANS – indexed by a decreased PNS and increased SNS activity, derived from changes in vagally mediated HRV (Cowen et al., 2015; Goodin et al., 2012; Koenig et al., 2014; Tennant, 2013). However, several studies showed that experimental pain induction decreased subjective stress and aversive tension in individuals with BPD and a history of NSSI (Reitz et al., 2012, 2015; Willis et al., 2017). So far, only one study systematically assessed the ANS and HPA axis response to an experimental pain induction in adolescents with NSSI compared to HC (Koenig, Rinnewitz, Warth, et al., 2017). The authors reported a significantly greater cortisol response following pain induction in individuals with NSSI – which is inconsistent with previous findings of a blunted HPA axis response to psychosocial stress. Further, individuals with NSSI had a delayed decrease in parasympathetic vagal activity (greater HRV) prior to the pain induction and a prolonged recovery (reduced HRV) following painful stimulation (Koenig, Rinnewitz, Warth, et al., 2017). While these findings lend preliminary evidence for an altered, pain-specific physiological response in adolescents with NSSI, further studies replicating these findings are warranted. Moreover, research on the effect of NSSI severity and dimensional psychopathology severity is virtually missing. However, as the need for more dimensional approaches has been recognized (Michelini et al., 2021) and increasing NSSI severity has previously been associated with increasing psychosocial impairment and severity of comorbid psychopathology (Ammerman, Jacobucci, et al., 2017; Muehlenkamp et al., 2017; Zielinski et al., 2018), a similar approach for potential pathophysiological mechanisms seems warranted.

The second manuscript investigated the effect of NSSI severity on the HPA axis and ANS response to experimental pain induction in adolescents with NSSI. Based on the existing literature and acknowledging the ongoing debate for more dimensional assessments of NSSI symptomatology, it was hypothesized that increasing NSSI severity (e.g., higher NSSI frequency) would be associated with an increasing HPA axis response to pain and an ANS response characterized by an increased sympathetic and decreased parasympathetic activity. Further, to account for additional effects of comorbid psychopathology, analyses were adjusted for dimensional psychopathology severity (e.g., ACE, BPD, and depression).

The sample consisted of n = 164 female adolescents with at least one lifetime incident of NSSI, as defined by Criterion A of the DSM-5 (American Psychiatric Association, 2013), and n = 45 female HC. During the detailed diagnostic assessment (see Figure II), NSSI, axis I psychiatric disorders, BPD symptoms, depression symptoms, ACE, the severity of psychiatric symptoms and participants' global functioning were assessed using the interviews and questionnaires described above (see 4.1.). In addition, the second study additionally used data on participants' global functioning, assessed using the *Global Assessment of Functioning scale* (GAF; Saß et

al., 2003). During the biological assessment, the HPA axis response to pain induction was assessed using repeatedly collected salivary cortisol samples (see Figure II). Cortisol levels were assessed at five predefined time-points: a baseline measure following a 5-minute resting period (1), immediately before (2) and after (3) the painful stimulation, following a second 5-minute resting period (4) and ten minutes after the fourth sample (5). The cortisol samples were stored at -20 °C until assay with a chemiluminescence immunoassay at the TUD Biopsychology Laboratory in Dresden. The ANS response was assessed by repeated measures of blood pressure and continuous measurement of HR to quantify for HRV. Blood pressure was measured following the salivary cortisol samples at all five time-points. HR was continuously measured from the first resting period until after the second resting period (see Figure II). Experimental pain induction was again administered using the previously described heat plate and related paradigm (see 4.1., p.24).

The analyses revealed significant increases in cortisol levels, blood pressure and HR during or following the pain induction, indicating successfully increased physiological arousal. Regarding the effects of NSSI severity (frequency in the past six months), cortisol secretion increased as a function of increasing NSSI frequency following pain induction. Notably, robust significant effects only occurred if individuals reported 63 or more days with NSSI. Adjusting for effects of comorbid psychopathology further revealed a significant association between NSSI frequency and the ANS response to pain. As such, HR response decreased progressively with increasing NSSI frequency during pain induction when controlling for ACE. Moreover, after adjusting for depression severity, HRV response increased progressively following pain induction as a function of greater NSSI severity. Importantly, these effects were not moderated by the severity of comorbid psychopathology. However, greater severity of psychopathology was associated with overall increased HR and decreased HRV, as well as an increasingly blunted HPA axis response following pain induction.

This manuscript is the first to indicate a significant effect of NSSI severity on the ANS and HPA axis response to pain, systematically assessed in female adolescents with NSSI. These findings expand previous research on altered HPA axis and ANS activity, further supporting the assumption of a pain-specific HPA axis and ANS response in adolescents with NSSI, that extends to symptom severity. As such, they potentially indicate how increasing NSSI severity may function as a compensatory mechanism, serving an emotion-regulatory and stress-reducing function. Moreover, these findings underline the importance of more dimensional assessments of NSSI severity and comorbid psychopathology, which could increase our understanding of the pathophysiological mechanisms underlying the development and maintenance of NSSI.

4.3 Manuscript III

Koenig, J., Höper, S., van der Venne, P., Mürner-Lavanchy, I., Resch, F., & Kaess, M. (2021). Resting state prefrontal cortex oxygenation in adolescent non-suicidal self-injury–A near-infrared spectroscopy study. NeuroImage: Clinical, 31, 102704.

Emotion dysregulation is a central characteristic of NSSI and a range of psychiatric disorders (Andover & Morris, 2014; Beauchaine, 2015a) and NSSI is most commonly used to regulate emotions (Taylor et al., 2018). Moreover, more severe emotion dysregulation has been associated with greater NSSI severity (Wolff et al., 2019). Converging evidence from existing neuroimaging studies emphasizes the role of fronto-limbic neural systems in generating, processing, and regulating emotions. Several regions of the PFC (i.e., dorsolateral, ventrolateral and orbitofrontal cortex) have been associated with successful emotion regulation through a modulating influence on limbic structures (Johnstone & Walter, 2014; Ochsner & Gross, 2014). However, so far, extant neuroimaging studies have often been conducted in individuals with BPD. As such, meta-analytic data reported an altered PFC activation during impulse control tasks and decreased PFC activation during the processing of negative emotional stimuli (Schulze et al., 2016; Sebastian et al., 2014). Similarly, a decreased PFC oxygenation, assessed with functional near-infrared spectroscopy (fNIRS), was found in adults with BPD and depression during a verbal fluency task (Husain et al., 2020). Regarding NSSI, greater activation of fronto-limbic regions (i.e., PFC) was reported in adolescents with NSSI during social exclusion tasks (Groschwitz et al., 2016; Malejko et al., 2019), while another study reported altered connectivity patterns between the amygdala and cortical regions at rest and during a negative emotion face-matching task (Westlund Schreiner et al., 2017). Contrary to this, a study on adult females with NSSI reported that decreased dorsolateral PFC activation was associated with greater emotion dysregulation and impulsivity during an interference task (Dahlgren et al., 2018). Overall, findings indicate task- and symptom-specific alterations in fronto-limbic neural regions. However, most findings were drawn from adult populations or individuals with BPD, with only a few studies addressing adolescent populations with NSSI.

As such, the third study investigated resting PFC activation, based on resting-state oxygenation, in adolescents with recurrent NSSI compared to HC. Based on the existing literature, it was hypothesized that adolescents with NSSI would show a decreased resting PFC activation, indexed by lower mean oxygenation and deoxygenation, compared to HC. Moreover, it was hypothesized that decreased PFC activation would be correlated with BPD symptom severity. Further, differences in PFC connectivity between adolescents with NSSI and HC were investigated in exploratory analyses.

The sample consisted of n = 170 adolescents with repetitive NSSI, and n = 43 HC aged 12 to 17 years. During the diagnostic assessment (see Figure II), NSSI, axis-I psychiatric disorders, BPD symptoms, depression symptoms, the severity of psychiatric symptoms, and ACE were assessed using the interviews and questionnaires described above (see 4.1. & 4.2.). Additionally, health-related quality of life was assessed using the KIDSCREEN-52 (The KIDSCREEN Group Europe, 2006). During the second appointment, PFC oxygenation throughout the first five-minute resting phase (see figure II) was assessed using a portable 8-

channel continuous-wave NIRS-system (OctaMon, Artinis, The Netherlands) attached to the participants' forehead. NIRS recordings are based on light within the near-infrared spectrum that penetrates the scalp and skull. In venous blood vessels, different wavelengths are strongly absorbed by oxygenated and deoxygenated hemoglobin (Ferrari & Quaresima, 2012). PFC activation can thus be assessed based on changes in reflected light over time, which has been shown to correlate with blood oxygenation level-dependent signals in fMRI (Alderliesten et al., 2014; Bulgarelli et al., 2018).

Adolescents with NSSI showed significantly decreased mean levels of oxygenated hemoglobin compared to HC, suggesting a decreased PFC activation at rest, while no differences were observed regarding deoxygenated and total hemoglobin. Moreover, mean levels of oxygenated hemoglobin correlated negatively with ACE – indicating a decrease in PFC oxygenation with more ACE – and positively with health-related quality of life – indicating an increase in PFC oxygenation with increased health-related quality of life. No associations were found with the severity of BPD, depression or NSSI, or with any other clinical measure. Regarding the exploratory analyses of PFC connectivity (i.e., connectivity between intra- and interhemispheric channel pairs), no differences were observed between adolescents with NSSI and HC. However, PFC connectivity was significantly correlated with the severity of psychopathology, indicating an increase in PFC connectivity with more severe BPD symptoms, depression symptoms, symptomatic distress, and ACE, and a decreased health-related quality of life.

This manuscript was the first to systematically assess PFC oxygenation at rest in a large sample of adolescents with NSSI compared to HC. Findings revealed a decreased PFC oxygenation already present early in life. Interestingly, PFC oxygenation was not associated with NSSI behaviors (e.g., NSSI frequency) per se. Moreover, decreased oxygenation was, except for ACE, not associated with psychiatric symptomatology, indicating a generally decreased resting-state oxygenation in the NSSI group that is not merely explained through comorbid psychopathology. Further, connectivity in the PFC was associated with psychopathology severity, but again not with specific NSSI behavior (e.g., NSSI frequency). Thus, these findings constitute an important advance to prior research that, embedded in the existing literature, might indicate an association between decreased PFC oxygenation and problems in emotion regulation, both in NSSI and general psychopathology. Increased connectivity might serve as a compensatory mechanism in individuals with greater psychopathology. However, the latter assumption is speculative and warrants further testing.

4.4 Manuscript IV

Flach, E.; Koenig, J.; van der Venne, P.; Parzer, P.; Resch, F.; Kaess, M. (2021) Hypothalamic-pituitary-thyroid axis function in female adolescent nonsuicidal self-injury and its association with comorbid borderline personality disorder and depression. Progress in neuro-psychopharmacology and biological psychiatry, 111, 110345.

NSSI is a highly heterogenous phenomenon often accompanied by comorbid psychiatric disorders, like BPD and depression. Evidence exists on psychosocial correlates that increase

the risk to develop and maintain NSSI (Taylor et al., 2018). Beyond psychosocial correlates, there has been a growing interest in endocrinological mechanisms. So far, hormones of the HPA axis have garnered the most interest, with some studies indicating that an altered HPA axis activity is already present in adolescence (Kaess et al., 2012; Koenig, Rinnewitz, Warth, et al., 2017). The HPT axis is closely related to the HPA axis. It is vital for nervous system development and regulation of the metabolism (Ortiga-Carvalho et al., 2011). Research so far has mainly been conducted in depression, where a blunted HPT axis functioning has been observed (Stipčević et al., 2008). Further, existing evidence suggests that prolonged or chronic stress can lead to a blunted HPT axis response (Helmreich et al., 2005; Olff et al., 2006). Moreover, some researchers have suggested an association between altered HPT axis functioning and emotion dysregulation (Bauer & Whybrow, 2001).

Based on the existing literature on high comorbidity rates of NSSI with depression and a central role of emotion dysregulation in NSSI, the fourth manuscript was the first to investigate HPT axis hormones in adolescent NSSI. It was hypothesized that participants with NSSI would show elevated TSH levels, as well as decreased fT3 and fT4 levels, and a decreased fT3/fT4 ratio. Further, this manuscript investigated if a stronger blunting of the HPT axis was associated with NSSI severity, the severity of comorbid psychopathology, notably BPD and depression symptoms, as well as symptomatic distress.

The sample consisted of n = 117 participants that reported at least five days with NSSI in the past year and n = 41 HC. All participants underwent the diagnostic and biological assessment as described above (see Figure II). During the detailed diagnostic assessment, NSSI, axis I psychiatric disorders, BPD symptoms, depression symptoms, ACE, general functioning, and severity of psychiatric symptoms (symptomatic distress) were assessed. Baseline thyroid function was analyzed based on TSH, fT3, fT4 and fT3/fT4 ratio gained from the blood draws during the second appointment. Samples were analyzed at the central laboratories of the Heidelberg University Hospital by immunoassays (ADVIA Centaur® Assay).

Participants with NSSI had a significantly lower fT3/fT4 ratio compared to HC. No group differences were found for TSH, fT3, and fT4. Additional analyses showed that BPD severity, depression severity and symptomatic distress correlated negatively with the thyroid markers (e.g., TSH, fT3 and fT3/fT4 ratio).

This manuscript was the first to systematically assess HPT axis markers in an adolescent sample with NSSI while controlling for a potential influence of comorbid psychopathology. The findings indicate that an altered fT3/fT4 ratio might be a biological correlate of NSSI. However, most thyroid markers were associated with comorbid psychopathology rather than NSSI behavior directly. Thus, the results from the fourth manuscript suggest that a dysregulated HPT axis function might be a non-specific mechanism for the development and maintenance of NSSI via an association with more severe psychopathological distress.

5 Discussion

Embedded in recent theoretical frameworks, the aim of the present work was to investigate neurobiological systems of adolescent NSSI, attempting to expand the existing knowledge on altered neurobiological functioning and inform on remaining gaps (see chapters 2 & 3). Consistent with previous research, alterations in neural, neuroendocrinological and autonomic functioning were observed in basal functioning (manuscript 1, 3 & 4) and response to pain induction (manuscript 1, 2).

Adolescents with NSSI were shown to report lower PS (e.g., higher pain threshold and lower perceived pain intensity) during experimental pain induction (manuscript 1), which is in line with the existing literature (Kirtley et al., 2016; Koenig, Thayer, et al., 2016). Moreover, the results of manuscript 1 revealed lower resting BE levels in adolescents with NSSI, albeit with no direct link to PS. As such, these findings partially support the proposed opioid deficiency model (Sher & Stanley, 2008; Stanley et al., 2010). It has previously been suggested that NSSI triggers BE release, which subsequently modulates pain experience (Bresin & Gordon, 2013). This is partially supported by recent findings from Störkel and colleagues (2021), who found that BE release following NSSI was positively associated with the severity of the injury. However, BE release and subjective PS were again not associated in the study by Störkel and colleagues (2021). In the context of the Temporal Framework Model (Kaess et al., 2021), the presented findings further support a potential role of decreased BE levels as proximal biological traits in NSSI and are among the first to do so in a well-characterized adolescent sample. However, as neither resting BE levels (manuscript 1) nor changes in BE levels (Störkel et al., 2021) were directly associated with PS, the exact mechanism of altered BE levels in the development and maintenance of NSSI remains an open question for future research. Störkel and colleagues (2021) proposed an additional pain-modulating role of top-down cognitive processes (Störkel et al., 2021). In line with the proposed domains of *arousal*, *negative valence*, and *positive valence* systems of the RDoC (Kozak & Cuthbert, 2016; National Institute of Mental Health, 2022), central BE release is implicated in the modulation of stress, affective pain experience, emotions and mood (Bresin & Gordon, 2013; Pilozzi et al., 2020; Zubieta et al., 2001). Previous research has linked altered BE levels and μ- and δ-receptor activity to increased negative affect (i.e., anhedonia) in individuals with depression (Browne & Lucki, 2019; Der-Avakian & Markou, 2012; Hegadoren et al., 2009). Interestingly, manuscript 1 revealed that depressive symptom severity was negatively correlated with BE levels and PS. As such, the present findings potentially indicate a joint mechanism where lower resting BE levels could be associated with more severe affective psychopathology (e.g., emotion dysregulation). NSSI could subsequently be used to regulate affect, triggering the release of central BE. This leads not only to a reduction in negative affect, but, also to a top-down regulated reduction in PS due to the considerable overlap in brain regions responsible for pain and emotion processing. Following this line of thought and considering the Temporal Framework Model, altered resting BE levels could be an underlying, proximal pathophysiological mechanism associated with the severity of general psychopathological symptomatology (e.g., emotion dysregulation, altered stress response) that is associated with

NSSI and its, often, comorbid psychiatric disorders, increasing the vulnerability to develop and maintain NSSI as a maladaptive coping mechanism.

Analyzing the effects of dimensional NSSI severity on the physiological response to pain induction, the second manuscript revealed an increasing HPA axis response as well as a greater PNS influence (e.g., decreasing HR and increasing HRV) during or following pain induction as a function of NSSI frequency. Importantly, these effects remained robust after controlling for additional effects of comorbid psychopathology. Moreover, in line with previous meta-analytic findings (Drews et al., 2019; Koenig, Kemp, et al., 2016), manuscript 2 revealed an increasingly blunted HPA axis response to pain and an overall increased HR and decreased HRV with increasing severity of comorbid psychopathology. The findings thus support the role of the HPA axis and ANS functioning in the proposed RDoC domain of *negative valence systems* (e.g., *acute threat*) (Kozak & Cuthbert, 2016; National Institute of Mental Health, 2022). Regarding the Temporal Framework Model by Kaess and colleagues (2021), the present findings highlight the importance of altered HPA axis and ANS functioning as proximal biological mechanisms of NSSI, potentially indicating an improved emotion-regulation and stress-reduction capacity as well as a decrease in physiological arousal in response to pain in adolescent NSSI. As such, the findings of manuscript 2 partially support previous findings of a pain-specific increased HPA axis and ANS (e.g., decreased PNS influence) response (Koenig, Rinnewitz, Warth, et al., 2017). Based on their findings, Koenig and colleagues (2017) proposed a stimulus-specific pathway for NSSI. They assumed that chronic exposure to stress might lead to a downregulation of the ANS and HPA axis response to environmental stress, which leads to difficulties in emotion regulation and stress tolerance (Koenig, Rinnewitz, Warth, et al., 2017), a notion, again, in line with the RDoC's proposed *negative valence system* (e.g., *sustained threat*). NSSI (e.g., pain experience) subsequently leads to an increased HPA axis response and an increased physiological arousal (e.g., decreased HRV), which enables a compensatory mechanism to regulate stress and emotions and improve body awareness (Koenig, Rinnewitz, Warth, et al., 2017). The findings from manuscript 2 support Koenig and colleagues' (2017) findings of an increased HPA axis response to pain, but, contrary to the previously reduced PNS activity following pain, indicate an increased pain-related PNS activity, which is in line with the affect-regulating and stress-reducing function of NSSI. Moreover, manuscript 2 revealed that the pain-specific HPA axis and ANS response extends to NSSI severity. Interestingly, Klimes-Dougan and colleagues (2019) reported that an increasing NSSI frequency was linked to a stronger blunting of the HPA axis response to a social stressor, which is in stark contrast to an increasing cortisol secretion with increasing NSSI frequency to pain induction in the second manuscript. The findings of manuscript 2 thus further highlight the importance of dimensional assessments of symptom severity in research on neurobiological systems associated with NSSI. Finally, cortisol secretion has been associated with analgesic effects. This has most frequently been observed in stress-induced analgesia (Butler & Finn, 2009). Stress leads to increased CRH secretion, which stimulates proopiomelanocortin production in the pituitary glands. Proopiomelanocortin is a common precursor for ACTH, which activates the stress response, and BE, which inhibits pain and the secretion of CRH (Charmandari et al., 2005; Ribeiro et al., 2005). Lower BE levels have been associated with

emotion dysregulation and low distress tolerance (Hegadoren et al., 2009) and were further shown to increase ACTH secretion (al'Absi et al., 2004). Thus, lower BE levels in individuals with NSSI (manuscript 1) could facilitate the increased reactivity of the HPA axis to pain (manuscript 2). Increased activation of the HPA axis, as an indicator for increased proopiomelanocortin secretion, could lead to BE release, potentially explaining the often-reported analgesia in individuals with NSSI. However, this speculative hypothesis would warrant systematic research in the context of adolescent NSSI.

The third manuscript revealed a decreased resting PFC activation in adolescents with NSSI compared to the HC. As mentioned above (see 2.1.1), the PFC is a key system for successful emotion regulation (Ochsner & Gross, 2014); successful emotion regulation has been associated with increased activation of the PFC, as well as reduced activation of limbic structures (e.g., amygdala, insula) through an inhibitory effect of the PFC (McRae et al., 2010). Existing literature points to a mechanism of reduced PFC activation and subsequent hyperactivity of limbic structures that underlies increased emotional reactivity, poor emotion regulation capacities and distress tolerance in individuals with BPD, depression and NSSI (Dahlgren et al., 2018; Plener et al., 2012; Schulze et al., 2016). Thus, in line with the domains of *negative valence systems* and *positive valence systems* proposed by the RDoC (Kozak & Cuthbert, 2016; National Institute of Mental Health, 2022), the finding of reduced resting PFC activation in adolescents with NSSI (manuscript 3) supports previous findings of altered neural functioning likely underlying altered emotion regulation and distress tolerance in individuals with NSSI (Nock, 2009, 2010). While no significant associations between a reduced PFC activation and severity of NSSI or comorbid psychopathology were found, resting PFC activation was inversely associated with the severity of ACE. Previous meta-analytic data indicates that experiencing ACE increases the risk of developing NSSI and other psychiatric disorders later in life (Ibrahim et al., 2018; Liu et al., 2018; Teicher & Samson, 2013) and is associated with altered biological functioning (e.g., HPA axis functioning, amygdala hyperreactivity) (Schär et al., 2022; Teicher & Samson, 2013). Further, findings of manuscript 3 showed that PFC connectivity was positively associated with the severity of comorbid psychopathology (e.g., BPD, depression, ACE). Together with previous findings of increased connectivity at rest between the amygdala and the ACC in adolescents with NSSI (Westlund Schreiner et al., 2017), increasing fronto-limbic connectivity might function as a compensatory mechanism to counteract the overactivation of limbic structures in individuals with greater psychopathology. Beyond emotion-regulation functions, fronto-limbic structures (i.e., PFC, ACC, amygdala, insula) are involved in pain processing. Previous research in adults with BPD reported that pain induction was associated with increased PFC activation, decreased amygdala and ACC activation, as well as increased inhibitory connectivity between dorsolateral PFC and the amygdala (Niedtfeld et al., 2010, 2012; Schmahl et al., 2006). Moreover, an experimental incision following stress induction was associated with a normalization of fronto-limbic connectivity in adults with BPD (Reitz et al., 2015). Embedded in the Temporal Framework Model (Kaess et al., 2021), manuscript 3 highlights several important findings. As such, they further support the notion that decreased resting PFC activation may constitute a proximal biological trait, potentially increasing the risk of developing problems of

emotion dysregulation and poor distress tolerance, commonly observed in a range of psychiatric disorders. Hypoactivation of the PFC may thus play a role in NSSI, but, as it was not associated with NSSI behavior per se, also be a transdiagnostic marker for general psychopathology. As emotion dysregulation and increased distress often precede NSSI episodes, NSSI may subsequently function as a maladaptive stress and emotion regulation mechanism, further reinforcing the maintenance of future NSSI. Moreover, the findings indicate a potential role of ACE as a distal biological trait, potentially increasing the risk of maladaptive maturation processes in neural systems prior to the development of NSSI (e.g., PFC, amygdala).

Finally, the fourth manuscript was the first to systematically assess endocrinological markers of the HPT axis in adolescents with NSSI. The findings revealed a lower fT3/fT4 ratio, indicating a disrupted conversion from T4 to T3 in adolescents with NSSI compared to HC. No other group differences could be observed (e.g., TSH, fT3, fT4). Importantly, findings revealed significant negative associations between most HPT axis markers (e.g., TSH, fT3 and fT3/fT4 ratio) and the severity of comorbid clinical psychopathology, but not the severity of NSSI per se (i.e., frequency of NSSI). As such, BPD severity, depression severity and symptomatic distress were all associated with a significant blunting of HPT markers. Decreased HPT axis functioning has been linked to the development of depression-like symptoms, such as emotion dysregulation (e.g., negative mood), cognitive impairment and fatigue. This has been attributed to the dense representation of thyroid receptors in fronto-limbic structures commonly involved in emotion regulation (Bauer et al., 2008; Bauer & Whybrow, 2001). Moreover, the effect of altered HPT axis function on mood and emotion regulation has been proposed to be linked to an interaction with neurotransmitters such as serotonin, whereas lower thyroid hormone levels may lead to increased serotonergic neurotransmission (Bauer et al., 2002). This is in line with previous findings of altered serotonergic function in BPD and depression alike (Beatson & Rao, 2013; Goodman et al., 2010). Conversely, increased serotonin levels have been shown to inhibit peripheral HPT axis functioning (Sullo et al., 2011). Painful stimuli (e.g., NSSI) increase serotonin release in a descending antinociceptive pathway (Wippert & Wiebking, 2018). Thus, repetitive NSSI could potentially result in a downregulation of the HPT axis through its effect on serotonin release. Further evidence stems from an animal study that found decreased T3 and T4 concentrations following repeated exposure to foot-shock stress (Helmreich et al., 2005). Interestingly, the authors reported that higher stress-induced HPA axis activity might partially inhibit HPT axis function (Helmreich et al., 2005). As adolescents with NSSI showed a significantly increased HPA axis response to pain with increasing NSSI frequency (manuscript 2), this could further indicate this inhibitory role of the HPA axis on the HPT axis. In light of the Temporal Framework Model (Kaess et al., 2021), manuscript 4 indicates that, while some altered HPT axis function can be observed in adolescents with NSSI, most thyroid hormones were associated indirectly with NSSI via comorbid psychopathology. As such, HPT axis dysfunction might be considered a distal biological trait that increases the severity of psychopathology in general, subsequently predisposing the development of NSSI as a maladaptive regulation mechanism. Following this line of thought, and with regard to the RDoC (National Institute of Mental Health, 2022), the HPT axis could thus play a role in the *negative*

valence, *positive valence*, and *cognitive* systems. However, this assumption needs to be tested in future research.

5.1 Strengths and limitations

Several methodological limitations need to be acknowledged when interpreting the results from the four manuscripts. First, the findings are based on a predominantly female sample. Only manuscript 3 included male participants, albeit not enough to analyze potential sex differences. As female sex has previously been reported as a risk factor for NSSI (Plener et al., 2015), the present findings are still highly valuable. Nonetheless, the generalizability of the findings to male individuals with NSSI is limited. This becomes even more relevant given previous reports on sex differences in neurobiological functioning (Koenig & Thayer, 2016; Kudielka & Kirschbaum, 2005; Lenroot & Giedd, 2010; Melchior et al., 2016). Second, findings were drawn from cross-sectional research only. As such, no statement can be made on how exactly these biological systems relate to the development of NSSI. Adolescence is a period marked by neural and biological maturation. Thus, it may be a phase characterized by increased susceptibility to the development of malfunctioning biological mechanisms due to environmental influences (e.g., early-life maltreatment). Longitudinal research is needed to assess whether alterations in biological functioning precede the onset of NSSI, how they influence its development and what factors contribute to their dysregulated functioning. A particular strength of the present work is that the findings are based on a relatively large and well-characterized adolescent sample. As prevalence rates of NSSI peak in adolescence (Plener et al., 2015), this marks a clear advancement from previous research, mostly relying on small and adult samples. However, as the sample consisted of help-seeking adolescents, further studies are needed to expand these findings to community samples, potentially increasing the generalizability of the present findings and further expanding our understanding of the role of altered biological mechanisms in NSSI. A second strength of the present work is the comprehensive assessment of associations between altered biological mechanisms and NSSI in a sample with heterogenous comorbid psychopathology. Finally, the present work systematically controlled for an influence of dimensionally assessed severity of NSSI and comorbid psychopathology, in line with recent demands for more dimensional assessments of psychopathology.

5.2 Directions for future research

In line with the proposed Temporal Framework Model (Kaess et al., 2021), the findings of the present thesis underline the importance and usefulness of assessing biological systems as proximal biological correlates of adolescent NSSI. At the same time, they offer avenues for future research to build on and, subsequently, further increase our understanding. As such, future research would benefit from repeated measurements of neuroendocrinological markers. While the present work found alterations in basal functioning of BE and the HPT axis (manuscripts 1 & 4), these systems are central to the physiological stress and pain response (see chapters 2.1.2 & 2.1.5). In line with the abovementioned reactivity of these systems, measuring neuroendocrinological markers prior to and following a stressful event, both in experimental settings and in real-life NSSI episodes, holds promise for a better understanding

of how these systems diverge from normal reactivity patterns and how they relate to the development and maintenance of NSSI. Moreover, the manuscripts of the present work focused on individual biological systems only. As mentioned above, most systems are strongly interconnected. Peripheral systems influence each other and the central nervous system and are, in turn, strongly regulated by the central nervous systems' descending pathways. Further, some evidence suggests that, at least for some neuroendocrine markers, peripheral and central levels are not correlated (e.g., BE, see De Riu et al., 1997; Kirtley et al., 2015). As such, the use of multiple *units of analysis*, as proposed by the RDoC (Clark et al., 2017; National Institute of Mental Health, 2022), would allow to further disentangle how exactly different systems are dysregulated and how they may interact.

Further implications arise from the analyses of dimensionally assessed psychopathology and NSSI severity (e.g., NSSI frequency). While several biological markers (e.g., BE, HRV, cortisol) were strongly related to NSSI (severity), most biological systems were further associated with comorbid psychopathology. Especially in the case of blunted thyroid markers, the severity of comorbid psychopathology was the main factor explaining variance (manuscript 3). As outlined above, altered functioning of biological systems has been associated with general psychopathology and the severity of psychopathological symptoms. The present work was based on a fairly large, heterogenous sample and considered the overall severity of comorbid psychopathology but not the severity of individual symptoms (e.g., emotion dysregulation). In line with dimensional approaches to psychopathology (Insel et al., 2010; Kotov et al., 2017; Michelini et al., 2021), future research should investigate these associations in more detail in large, heterogenous samples with different or multiple comorbid disorders. Moving beyond a mere assessment and comparison of individual categorical diagnoses, analyzing biological mechanisms in the context of shared symptom components and higher-order dimensions could vastly improve our understanding of neurobiological mechanisms' unique and individual role in NSSI, comorbid psychopathology and their underlying components. Of equal importance is the finding that ANS and HPA axis dysregulation was associated with NSSI severity (manuscript 2) over and above the presence of comorbid psychopathology. This underlines the importance of future studies to control for markers of NSSI severity. The use of NSSI frequency marks the first important step. However, previous research indicates that NSSI severity is best described using several descriptors (e.g., frequency, recency, number of methods, severity of injury, underlying psychopathology) (Ammerman, Jacobucci, et al., 2017; Anestis et al., 2015; Muehlenkamp et al., 2017; Zielinski et al., 2018). As such, future research will need to employ a more diverse set of NSSI-related severity markers when assessing neurobiological correlates of adolescent NSSI.

To sum up, the present work highlights the role of altered neural (e.g., PFC), neuroendocrinological (e.g., BE, HPA axis, HPT axis) and physiological (e.g., ANS) mechanisms as biological correlates of NSSI, in line with the recently proposed Temporal Framework Model (Kaess et al., 2021). Additionally, the findings indicate the need for dimensional assessments, taking into account markers of severity. In line with the central implementations of the RDoC, the present work indicates a need for a combined assessment of psychological, social, and biological correlates of NSSI across the full functioning spectrum.

Future research designs would greatly benefit from a model incorporating all these factors. Further, findings derived from such designs may subsequently improve efficient intervention programs.

5.3 Clinical implications

So far, successful treatment strategies for NSSI have been drawn almost exclusively from the well-understood psychosocial functions and risk factors of NSSI. Among the most prominent evidence-based interventions is Dialectical Behavioral Therapy (Linehan, 1993; Turner et al., 2014), but further NSSI-specific interventions (i.e., Cutting Down Program; Taylor et al., 2011) have been developed over the past decade (Calvo et al., 2022). At their core, these intervention programs often target emotion regulation, stress management strategies, and communication skills (Glenn et al., 2015; Plener, Brunner, et al., 2016). So far, treatments for NSSI based on or informed by neurobiological systems are virtually non-existent or lacking sufficient evidence. The findings of the present work, as well as the extant literature on neurobiological systems, could serve as the groundwork for such treatments. For one, the present work highlights alterations of neurobiological systems in adolescent NSSI and comorbid psychopathology, which have previously been associated with emotion dysregulation and poorer distress tolerance, as viable treatment approaches. Incorporated into psychotherapeutic treatments, assessments of neurobiological markers could serve as an objective marker for treatment success. In line with this, preliminary evidence suggests that stronger negative resting-state functional connectivity between the amygdala and the PFC was associated with a greater improvement in NSSI in adolescents following Dialectical Behavioral Therapy (Santamarina-Perez et al., 2019). Considering the findings of manuscript 2, assessing the HPA axis and ANS response to both psychosocial stress and NSSI episodes could reveal a potential normalization of the endocrinological and physiological stress response if newly acquired emotion/stress regulation strategies and communication skills enable a more adapted reaction, leading to a reduction in NSSI severity. In line with this, previous research has shown that altered neurobiological stress responses following ACE can be reverted, especially during childhood and adolescence (Beauchaine et al., 2019; McCrory et al., 2010). Moreover, relying on neurobiological markers could also shed light on the potential usefulness of pharmacological treatments in addition to psychological interventions. Findings on thyroid treatments and their influence on depressive symptomatology (Duval, 2018) as well as previous findings that antidepressants and opioid antagonists were associated with a reduction in NSSI (Bresin & Gordon, 2013; Westlund Schreiner et al., 2019), could serve as initial evidence. However, more systematic, longitudinal research on the usefulness of pharmacological treatments is warranted. Overall, the existing findings on neurobiological alterations in adolescents with NSSI point to a potential usefulness in treatments and should motivate future research to disentangle their role and treatment opportunities further.

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Pain Sensitivity and Plasma Beta-Endorphin in Adolescent Non-Suicidal Self-Injury

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Abstract

Background: Beta-endorphin (BE) has been suggested to play a central role as to why people engage in NSSI. To our knowledge, no study has systematically assessed this potential relationship in adolescents with NSSI.

Methods: 94 adolescents with NSSI (according to DSM-5 criteria) and 35 healthy controls (HC) were enrolled. All participants received heat pain stimulation, with pain threshold and tolerance measured in °C. Plasma BE levels were assessed. Sociodemographic and clinical characteristics were obtained via semi-structured interviews and self-report questionnaires.

Results: Adolescents with NSSI showed increased pain thresholds ($t_{(127)} = 2.071$, $p = .040$), lower pain intensity ($t_{(114)} = -2.122$, $p = .036$) and lower plasma BE levels ($t_{(127)} = -3.182$, $p = .002$) compared to HC. Groups did not differ on pain tolerance ($t_{(127)} = 0.911$, $p=.364$). Greater pain threshold correlated positively with borderline personality disorder (BPD) symptoms ($r = 0.182$, $p = .039$), while pain intensity ($r = -.206$, $p = .033$) and BE levels ($r = -.246$, $p = .007$) correlated negatively with depression severity. No significant relationship was found between pain threshold and plasma BE ($r = -0.013$, $p = .882$).

Limitations: Future studies should implement repeated plasma BE measures to assess BE release in association with pain in NSSI. Validity of plasma BE measures compared to central measures should be considered. Assessing the association between pain sensitivity (PS) and BE in a naturalistic setting presents a promising avenue for future research in NSSI.

Conclusions: Findings support both reduced PS and basal opioid deficiency as independent biological correlates and potential risk-factors for NSSI. Further longitudinal and experimental studies are needed to investigate the role of BE levels and PS as well as their potential association.

Keywords: *Non-suicidal self-injury; Plasma beta-endorphin; Pain sensitivity; Pain threshold; Pain analgesia*

1. Introduction

Non-suicidal self-injury (NSSI) is defined as the deliberate and self-inflicted hurting or destruction of one's own body tissue without suicidal intent (International Society for the Study of Self-injury, 2018; Nock, 2010). Commonly, NSSI has its onset in adolescence; with lifetime prevalence rates of 17.2% for adolescents (Swannell et al., 2014), it presents a serious threat to psychosocial development during adolescence. Despite being a key symptom of borderline personality disorder (BPD), NSSI frequently accompanies other psychiatric disorders, e.g. major depressive disorder (MDD), thereby affecting a heterogeneous clinical population (Glenn and Klonsky, 2013; Nock, 2009). Reasons for engaging in NSSI have been linked to negative affect, dissociation and emotion dysregulation, with NSSI serving as a dysfunctional coping mechanism (Klonsky, 2007). These phenotypes underlying NSSI are commonly found in both BPD and MDD. In addition to diverse types of psychopathology, the experience of adverse childhood experiences (ACE) has been defined as a central risk factor for the occurrence of NSSI (Cipriano et al., 2017; Glenn and Klonsky, 2013), with up to 80% of individuals with a history of NSSI reporting ACE (Gratz et al., 2002). However, NSSI also occurs independently (Zetterqvist, 2015) without comorbid psychiatric disorders (Wilkinson, 2013), supporting the notion of NSSI as an independent disorder (American Psychiatric Association, 2013). So far, research has focused on psychological functions and risk factors related to the occurrence of NSSI, yet its underlying biological mechanisms are still poorly understood.

Based on reports that individuals with a history of NSSI feel little to no pain (hypo-/analgesia) during or following self-injury (Nock and Prinstein, 2005), researchers proposed alterations in pain processing as a potential risk factor for NSSI (Nock, 2010). Pain perception and processing are complex, comprising sensory, affective and cognitive dimensions (Navratilova and Porreca, 2014), including the involvement of a number of brain areas (e.g. anterior cingulate cortex (ACC), amygdala, insula, prefrontal cortex) that are largely interconnected, serving somatosensory and affective/cognitive aspects of the holistic pain sensation (Garland, 2012; Price, 2000). While noxious stimuli are normally perceived as aversive, resulting in avoidance as a self-preserving measure, Nock hypothesized that individuals with lower pain sensitivity (PS), and subsequently no aversion to anticipated pain, are at greater risk to engage in NSSI (Nock, 2010). These assumptions were confirmed in two research reviews that reported significantly higher pain threshold and tolerance in individuals with a history of NSSI across all age groups (Kirtley et al., 2016; Koenig et al., 2016). Further support stems from recent neuroimaging studies showing that reduced PS seems to be related to altered pain perception, with altered activation patterns following painful stimuli in brain areas commonly associated with affective and cognitive processes of pain perception (Schmahl et al., 2006, 2004). Initial theories hypothesized processes of habituation as an explanation for lowered PS in individuals with NSSI (Joiner, 2007). However, with recent studies yielding no support for these assumptions (Koenig et al., 2017b), a potential role of comorbid psychopathology has been discussed. Consistently, PS was found to be negatively correlated with the

severity of BPD symptomatology (Ludäscher et al., 2009), as well as with MDD (McCoy et al., 2010; Thompson et al., 2016). Moreover, emotion dysregulation, self-criticism and neuroticism – central characteristics in BPD and depression alike (American Psychiatric Association, 2013; Bradley et al., 2011) – were found to be associated with lowered PS (Bunderla and Kumperščak, 2015; Franklin et al., 2012), with emotion dysregulation accounting for significant variance in the association between NSSI and pain tolerance (Franklin et al., 2012). Further, ACE have been related to lower PS (Fillingim and Edwards, 2005) and are central risk factors for BPD (Lieb et al., 2004), MDD (Bradley et al., 2011), dissociation (Korzekwa et al., 2009) and emotion dysregulation (Lieb et al., 2004). These findings support the assumption of a complex relationship between PS and comorbid psychopathology in NSSI. Nevertheless, more research is warranted to assess the nature of these associations.

A well-established relationship exists between analgesia, pain perception and the endogenous opioid system, one of the innate pain-relieving systems (Akil et al., 1984). Especially the activity of μ- and δreceptors is linked to the reduction of pain perception (Benarroch, 2012; Bresin and Gordon, 2013), emphasizing foremost the role of beta-endorphin (BE) as a μ- and δ-receptor agonist. μ- and δreceptors, and BE are present in both the central and peripheral nervous system (Benarroch, 2012; Rachinger-Adam et al., 2011), and modulation of pain perception and analgesia are exerted through central and peripheral nervous BE release alike (Benarroch, 2012; Stein et al., 2009). Consistently, Zubieta and colleagues showed that increased μopioid receptor activity in the ACC is linked to lower self-reported levels of pain unpleasantness and that increased μ-opioid receptor activity in the amygdala is linked to lower perceived pain intensity (Zubieta et al., 2001). Further, Bruehl and colleagues showed that resting plasma BE levels predicted analgesic response to morphine in chronic pain patients (Bruehl et al., 2017). Based on the existing literature, the potential role of BE in NSSI has come to increasing attention. In line with the small number of existing studies, opioid deficiency theories suggest that people engaging in NSSI have lower resting levels of BE and, driven by an innate desire to restore a homeostasis, engage in actions, such as NSSI, that are likely to result in releases of BE (Bresin and Gordon, 2013; Hooley and Franklin, 2018). First support for these models was drawn from animal studies illustrating that rhesus monkeys with a history of self-biting behavior showed lower basal plasma levels of BE than a matched group of monkeys without such behavior (Tiefenbacher et al., 2005). Stanley and colleagues reported lower BE levels in cerebral spinal fluid (CSF) in participants with personality disorders, a history of suicide attempts and a history of NSSI (Stanley et al., 2010). Prossin and colleagues, using positron emission tomography (PET), found lower μ-opioid receptor activity in the orbitofrontal cortex and the left amygdala in individuals with BPD (Prossin et al., 2010). Despite some empirical support of these theoretical models, studies are limited mostly to animal models or assessed the role of changes in BE in different psychiatric disorders without assessing NSSI (Prossin et al., 2010). Additionally, most human studies solely included adults.

NSSI is most common in adolescents (Nock, 2010) and marks a serious risk factor for future suicide attempts, risk-taking behaviours, and potential long-term mental health problems, even beyond cessation into adulthood (Brown and Plener, 2017). While some evidence suggests altered biological mechanisms are already present in adolescence (Kirtley et al., 2016; Koenig et al., 2016), more research in adolescent populations is needed in order to increase the understanding of this phenomenon. A focus on younger samples further allows to rule out effects of long-term illness and secondary effects of chronification, as well as age-dependent differences in the endogenous opioid systems (Gibson and Farrell, 2004). Thus, in this study, we aimed to systematically investigate PS and basal BE levels in adolescents with NSSI compared with a group of healthy matched controls. We hypothesized that adolescents with NSSI show altered PS (indexed by higher pain threshold and tolerance, and lower pain intensity). In addition, we hypothesized that adolescents engaging in NSSI show lower resting plasma BE levels. Given the high comorbidity of NSSI, we further explored potential associations between PS and BE with BPD symptoms, depressive symptoms, trauma severity and general psychiatric symptom severity, respectively. Finally, our study is the first to assess the potential association between altered plasma BE levels and PS in adolescent NSSI.

2. Methods

2.1. Participants

Patients with NSSI were recruited from the outpatient clinic for risktaking and self-harm behavior "Ambulanz für Risikoverhalten und Selbstschädigung (AtR!Sk)" (Kaess et al., 2017) at the Department of Child and Adolescent Psychiatry, University of Heidelberg. Following an initial diagnostic assessment, patients are invited to participate in the nested AtR!Sk-Bio cohort, which is an ongoing study aiming at identifying biological correlates of risk-taking and self-harming behavior in adolescence. The Ethics Committee of the Faculty of Medicine, University of Heidelberg, approved the scientific evaluation of AtR!Sk (IRB approval number S-449/2013) and the add-on neurobiological assessments (IRB approval number S-514/2015).

Recruitment for AtR!Sk-Bio takes places within six weeks after the diagnostic assessment in AtR!Sk. Inclusion criteria comprise: age 12–17 years; completed diagnostic assessment; informed and written consent of adolescents and their caregivers. Patients showing acute psychotic symptoms or lacking speech comprehension are excluded. For the present analyses, only patients fulfilling the criteria for NSSI, defined as five or more incidents of NSSI in the last 12 months according to the DSM-5 (American Psychiatric Association, 2013), were included. Further, based on known sex-differences regarding the prevalence of NSSI and PS, only data from female patients were considered. Healthy controls (HC) were recruited via public advertisements and underwent an adapted form of the diagnostic assessment used in AtR!Sk. Eligibility criteria for HC comprised: age 12–17 years; no history of NSSI; no endorsement of any psychiatric disorder and no treatment for any psychiatric disorder prior to participation in the study.

All HC and their caregivers provided informed and written consent to participate in the study. Due to the ongoing nature of the AtR!Sk and AtR!Sk-Bio studies, August 2018 was chosen as cut-off for inclusion in the present study. All participants (patients with NSSI and HC) that had completed the neurobiological assessment until August 2018 were subsequently considered for analyses.

2.2. General procedures

The study comprised two separate appointments. The first appointment consisted of the diagnostic assessment for the patient group and the HC respectively, with relevant instruments described below (see *2.3.*). The biological assessment, marking the second appointment, started at 8 a.m. with measures of height and weight, as well as questions regarding participants' smoking habits; physical illness within the past three months; and regular medication intake. To account for potential interferences with the blood draw, participants were asked whether they were fasting as instructed, as well as to their cigarette consumption at the day of the assessment. Next, the fasting blood draw described in detail below (see *2.4.*) followed. After a resting period, PS was assessed as described below (see *2.5.*). All participants received 40€ upon completion of the neurobiological assessment.

2.3. Measures

NSSI and suicide attempts were measured using single items of the German version of *the Self-Injurious Thoughts and Behaviors Interview* (SITBI-G) (Fischer et al., 2014), a semi-structured interview for the detailed assessment of self-injurious thoughts and behaviours that was slightly modified to meet DSM-5 criteria for NSSI. The SITBI-G was carried out in its entirety and shows good psychometric properties (Fischer et al., 2014). BPD symptoms were assessed using the respective part of the German version of the *Structured Clinical Interview for DSM-IV Axis II Personality Disorders* (SCID-II) (Wittchen et al., 1997), with items showing good internal consistency (Cronbach's $\alpha = 0.83$). In addition, patients underwent the *Mini International Neuropsychiatric Interview for Children and Adolescents* (MINI-KID) (Sheehan et al., 2004), a short semi-structured interview designed to assess common axis I psychiatric disorders in children and adolescents aged 6–19 years. Self-reported depressive symptoms were measured using the *Depression Inventory for Children and Adolescents* (DIKJ) (Stiensmeier-Pelster et al., 2000). The 26 items of the DIKJ were constructed based on DSM-IV criteria for depression, showing excellent psychometric properties (StiensmeierPelster et al., 2000). Similarly, we found excellent internal consistency in the present study (Cronbach's $\alpha = 0.95$). Severity of psychiatric symptoms was rated based on the *Clinical Global Impressions-Severity* (CGI-S) (Busner and Targum, 2007) scale; This seven-point scale requires the clinician to rate how mentally ill a patient is, based upon observed and reported symptoms, behavior and function over the past seven days. To account for ACE, all participants completed the German translation of the *Childhood Experiences of Care and Abuse* questionnaire (CECA.Q) (Kaess et al., 2011), showing good psychometric properties. The CECA.Q items were taken directly from the interview version and adapted, covering modules for

parental care (antipathy and neglect), physical abuse and sexual abuse. To assess trauma severity, we created a dimensional trauma score using four modules of the CECA.Q, showing moderate to excellent internal consistency (Cronbach's α: *antipathy* (maternal α = 0.91; paternal α = 0.92), *neglect* (maternal α = 0.83; paternal α = 0.92), *physical abuse* (maternal α = 0.60; paternal α = 0.70), *sexual abuse* (α = 0.85)). Following dichotomization of each variable, a mean score ranging from 0 (no trauma) to 1 (multiple trauma) was generated for each participant. Smoking behavior and tobacco use were assessed during the first appointment using a short questionnaire, as well as during the second appointment using screening questions to account for potential influences on BE. While inconclusive, current literature indicates that acute nicotine administration could increase BE levels, while chronic exposure could eventually inhibit the biosynthesis of beta-endorphin, leading to a chronic decrease in beta-endorphin levels (del Arbol et al., 2000; Tziomalos and Charsoulis, 2004).

2.4. Blood Draws

Fasting blood samples were collected between 8:30 a.m. and 9:00 a.m. by trained medical personal via venepuncture from the crook of the arm, under sterile conditions. Blood samples were subsequently sent to the central laboratories of the Heidelberg University Hospital for further analyses. BE levels were determined using an enzyme-linked immunosorbent assay (ELISA) by Cloud Clone (Houston, TX, US), according to the protocol of the manufacturer. Blood samples were left to rest at room temperature for two hours, before being centrifuged for 20 min at 1000x*g* and stored at -18 °C within the central laboratories until assay. To determine BE levels, samples were thawed to room temperature and 50µL were added to each well of pre-coated 96-well strip plates. 50µL/well of detection reagent A were added and the plates were set to incubate for 1 hour at 37 °C. After incubation, the wells were washed and 100µL of detection reagent B were added to each well. Following incubation for 30 min at 37 °C, the wells were washed again and 90µL of substrate solution was added. The wells were incubated, protected from light, for 10 – 20 min at 37 °C. Subsequently, 50µL of Stop solution were added to each well. After the liquids mixed uniformly, absorbance was measured immediately at 450 nm. Samples were tested in duplicate. Central and peripheral BE play a role in the modulation of pain perception and analgesia (Benarroch, 2012; Stein et al., 2009), however plasma BE levels have been found to be sensitive to effects of stress (De Riu et al., 1997). Despite this sensitivity, studies assessing resting plasma BE levels across repeated sessions were able to show that resting plasma BE levels remained relatively stable (Bruehl et al., 2017; Leppäluoto et al., 2008), supporting the assumption that resting plasma BE levels are a reliable and relatively stable marker.

2.5. Pain Sensitivity

PS was assessed using an AHP-1800CPV Versatile Cold/Hot Plate (TECA Corp., Chicago, IL, USA) and a predefined programmed sequence for the temperature. Baseline adaptation temperature was set at 32 °C. Temperature was sealed off at 50 °C to avoid tissue damage. Participants were instructed to place

their non-dominant hand flat on the plate as soon as baseline temperature was reached. After a 3-minute adaptation phase, temperature raised up to 50 °C within 4 min, increasing linear by 1 °C over 13.3 s. Participants were asked to keep their hand firmly on the plate until the pain became intolerable. Temperature at the first pain sensation (pain threshold) and temperature at intolerable pain sensation (pain tolerance) was measured in °C. Pain intensity was assessed via visual analogue scale (VAS) using the Continuous Measurement System (CMS) software (Messinger et al., 2009). When pain threshold was reached, participants were instructed to continuously rate pain intensity on a scale from 0 to 100 using their dominant hand until pain tolerance was reached. If pain tolerance was not reached at 50 °C, the sequence ended automatically and participants were asked to remove their hand. For the present study, pain intensity scores were calculated for each participant based on the rated pain intensity upon reaching pain tolerance. To account for potential inaccuracies, an average score was generated, using all VAS ratings within five seconds prior to- and following attained pain tolerance.

2.6. Statistical Analysis

Prior to analyses, the main variables (NSSI, BE, pain threshold, and tolerance) were checked for missing values. BE data were additionally checked and corrected using sensitivity analyses. Sociodemographic and clinical variables were tested for between-group differences using two-sided *t*-tests and χ^2-tests respectively. Between-group differences regarding pain threshold and tolerance, as well as between-group differences regarding pain intensity and plasma BE levels were analysed using two-sided *t*-tests. To account for the potential influence of smoking behavior on BE, we calculated a regression model with BE as dependent variable and group, smoking prior to the neurobiological assessment and smoking during the last 12 months as independent variables. In additional regression models the potential influence of medication intake and body-mass-index (BMI) on BE and PS was assessed. Next, Pearson product-moment correlations were used to assess associations between pain threshold, intensity and tolerance, and BE. Finally, we conducted exploratory analyses, using Pearson product-moment correlations, to assess potential associations between clinical characteristics and pain threshold, intensity, tolerance, and BE respectively. Analyses regarding the number of NSSI episodes and severity of psychiatric symptoms were solely conducted for the patient group. All analyses were performed using Stata (Version 16; StataCorp LP, College Station, TX, USA) with the significance level set to $\alpha = 0.05$. Adjustments for simultaneously testing two directed hypotheses were not performed, as tests were two-sided. No a priori power analysis was conducted, as the studies, from which the data stem, are still ongoing and do not aim at recruiting up to an a priori fixed number of participants.

3. Results

3.1 Sample characteristics

For the present analyses, only data from participants up to August 2018 were included. Since recruitment for the AtR!Sk-Bio cohort started in August 2016, n = 203 patients passed the diagnostic assessment in AtR!Sk, making them eligible for the additional neurobiological assessments of AtR!Sk-Bio. N = 148 patients had completed the neurobiological assessments until August 2018 (participation rate: 72.9%). Of these, n = 31 (20.9%) patients were excluded due to not meeting DSM-5 criteria for NSSI. N = 10 patients (6.8%) were excluded due to male sex. Of the remaining n = 107 patients, n = 17 (11.5%) were excluded due to missing BE (n = 16; 10.8%) or pain data (n = 1; 0.7%). Data from one patient was excluded because this patient had, with 224.1 µg/ml, an extremely high BE value (the next highest value was 97.7 µg/ml). Sensitivity analyses showed that this patient had an undue influence on results. N = 40 healthy female adolescents were recruited as control group, with n = 5 (12.5%) being excluded from analyses for missing BE data due to an insufficient quantity of blood for assay. The final study sample consisted of n = 94 female patients with NSSI and n = 35 HC.

Sociodemographic and clinical characteristics of the study sample are presented in *Table 1*. No significant difference could be found regarding mean age, height, weight, or body-mass-index (BMI). Participants differed significantly regarding school type, ($\chi^2(3)=16.07$, $p=.001$). N = 20 (15.5%) participants were taking at least one form of medication (NSSI: n = 19, 14.7%; HC n = 1, 0.8%). Patients were significantly more likely to have smoked in the past 12 months compared to HC ($\chi^2(6)=16.62$, $p=.011$). Similarly, significantly more patients had smoked prior to the neurobiological assessment compared to HC ($\chi^2(1)=4.48$, $p=.034$).

Table 1. Sociodemographic characteristics of the study sample.

Variable	Group; mean ± SD or *N* (%)		Effect Size[b]	*P* [a]
	HC, (n=35)	NSSI, (n=94)		
Age (yr)	14.9±1.29	14.9±1.44	0.03	0.877
Height (cm)	164.5±5.39	165.9±6.26	0.25	0.211
Weight (kg)	57.6±10.91	61.7±14.06	0.31	0.116
BMI	21.2±3.29	22.37±4.79	0.27	0.183
School Type [c]			0.35	<0.001
Gymnasium	26 (74.3)	35 (37.2)		
Realschule	8 (22.9)	34 (36.2)		
Hauptschule	0 (0.0)	11 (11.7)		
Other	1 (2.8)	14 (14.9)		
Smoked past 12 months	9 (25.7)	50 (53.2)	0.36	0.011
Smoked prior bio. Assess.	0 (0)	11 (11.7)	0.19	0.034
ICD-10 Diagnoses				
Organic, including symptomatic, mental disorders	-	0 (0)		-
Mental and behavioural disorders due to psychoactive substance use	-	20 (21.3)		-
Schizophrenia, schizotypal and delusional disorders	-	0 (0)		-
Mood [affective] disorders	-	60 (63.8)		-
Neurotic, stress-related and somatoform disorders	-	42 (44.7)		-
Behavioural syndromes associated with physiological disturbances and physical factors	-	12 (12.8)		-
Disorders of adult personality and behaviour	-	35 (37.2)		-
Mental retardation	-	0 (0)		-
Disorders of psychological development	-	1 (1.1)		-
Behavioural and emotional disorders with onset usually occurring in childhood and adolescence/Unspecified mental disorder	-	21 (22.3)		-
DIKJ [d]	5.4±3.62	29.6±9.12	3.05	<0.001
CGI [d]	-	5.04±0.69		
CECA.Q [d]				
Experienced ACE	3 (8.6)	57 (65.5)	0.52	<0.001

BMI = body mass index; DIKJ = *Depressionsinventar für Kinder und Jugendliche*; CGI = clinical global impression; CECA.Q = *Childhood Experiences of Care and Abuse* questionnaire.

[a] Significance: Values refer to differences between groups, with t-tests for continuous variables and χ^2 tests for categorical variables.

[b] Effect Size; Effect sizes were calculated using Cohen's d for t-tests and Cramer's V for χ^2 tests.

[c] Hauptschule: secondary school terminating with a lower secondary-school level II certificate: Realschule: secondary school terminating with a secondary-school level I certificate; Gymnasium: secondary school terminating with the general qualification for university entry.

[d] Due to missing data, analyses were carried out with varying N for HC and NSSI-group: DIKJ (N = 34 HC & N = 84 NSSI); CGI (N = 92 NSSI); CECA.Q (N = 35 HC & N = 87 NSSI).

Patients reported on average 70.66 (*SD*=70.01) episodes of NSSI within the past 12 months. Mean age of onset for NSSI was 12.85 years (*SD*=1.36) and on average, patients reported engagement in NSSI for 1.9 years (*SD*=1.66). *N* = 42 patients (44.7%) reported at least one previous suicide attempt, with an average of 3.41 (*SD*=6.42) lifetime attempts. *N* = 89 patients fulfilled diagnostic criteria for at least one psychiatric disorder (MINI-KID). On average, patients endorsed 3.37 (*SD*=0.22) BPD criteria, with *n* = 30 (31.9%) patients fulfilling the diagnostic threshold for BPD diagnosis. Further, *n* = 52 (58.4%) patients fulfilled the diagnostic threshold for either a depressive episode (*n* = 35) or recurrent depressive disorder (*n* = 17). Patients scored significantly higher on depressive symptoms (DIKJ) compared to HC ($t(116)=14.993$, $p<.001$). Patients reported significantly higher ACE compared to HC ($\chi^2(1)=32.38$, $p<.001$).

3.2 Pain ratings

The NSSI group showed a significantly higher pain threshold compared to HC (NSSI: *M*=43.27, SD=3.77, HC: *M*=41.77, SD=3.36; $t(127)=2.071$, $p=.040$, 95% CI[-0.80, -0.02], Cohen's $d=0.41$; Fig. 1). No significant differences between groups were found on measures of pain tolerance (NSSI: *M*=47.16, SD=2.47, HC: *M*=46.73, SD=2.17; $t(127)=0.911$, $p=.364$, 95% CI[-0.57, -0.021], Cohen's $d=0.18$; Fig. 1). The NSSI group reported a significantly lower pain intensity compared to HC ($t(114)=-2.122$, $p=.036$, 95% CI[0.03, 0.84], Cohen's $d=0.44$).

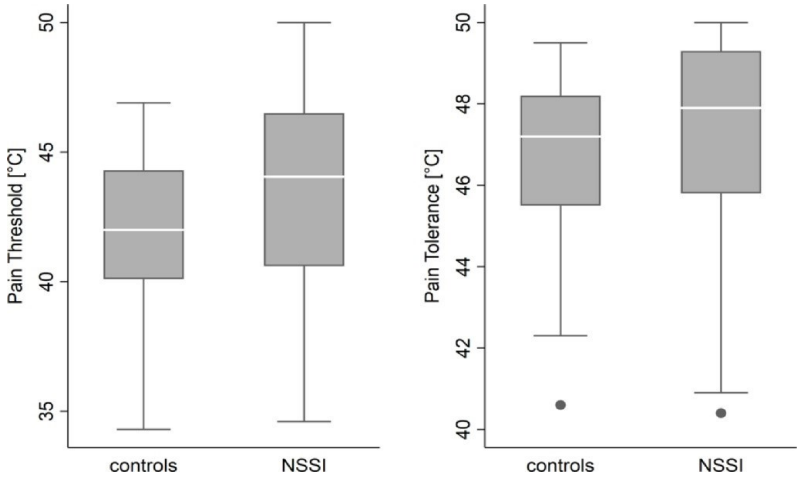

Fig. 1. Group differences in pain ratings. NSSI = Non-suicidal self-injury.

For exploratory analyses, we further assessed potential associations between clinical characteristics and PS (pain threshold, pain tolerance, and pain sensitivity respectively). Pain threshold showed a significant positive correlation with BPD symptoms ($r=0.182$, $p=.039$; Fig. 2). No significant relationship was found between pain threshold and the number of NSSI episodes in the past 12 months ($r=-0.064$, $p=.543$), depression scores (DIKJ; $r=0.091$, $p=.325$), severity of psychiatric symptoms (CGI; $r=-0.037$, $p=.729$) or severity of ACE ($r=0.101$, $p=.270$). No significant relationship was found between pain tolerance and BPD symptoms ($r=0.104$, $p=.239$), number of NSSI episodes in the past 12 months

(*r*=-0.098, *p*=.347), depression scores (DIKJ; *r*=0.003, *p*=.976), severity of psychiatric symptoms (CGI; *r*=-0.032, *p*=.763) or severity of ACE (*r*=0.037, *p*=.687). Pain intensity showed a significant negative correlation with depression scores (*r*=-0.206, *p*=.033). No significant relationship was found between pain intensity and BPD symptoms (*r*=-0.108, *p*=.250), number of NSSI episodes in the past 12 months (*r*=0.080, *p*=.474), severity of psychiatric symptoms (CGI; *r*=0.037, *p*=.743) or severity of ACE (*r*=0.049, *p*=.607). Additional exploratory analyses were conducted to determine what factors explain unique variance in PS. Sub-group analyses within the NSSI group revealed no significant differences for patients with BPD compared to patients without BPD (pain threshold: *t*(92)=-0.549, *p*=.585; pain tolerance: *t*(92)=0.437, *p*=.663; pain intensity: *t*(81)=-0.642, *p*=.523), patients with MDD compared to patients without MDD (pain threshold: *t*(92)=1.042, *p*=.300; pain tolerance: *t*(92)=-0.235, *p*=.815; pain intensity: *t*(81)=1.214, *p*=.228) and patients with previous suicide attempts compared to patients without previous suicide attempts (pain threshold: *t*(92)=-0.391, *p*=.696; pain tolerance: *t*(92) =-0.595, *p*=.553; pain intensity: *t*(81)=-0.497, *p*=.621). Subsequently, multiple regressions, using group, BPD symptoms and depression scores as independent variables, revealed a significant multiple regression model for pain threshold ($F_{(3, 114)}$=3.68, *p*=.014), with an adjusted R^2 of 0.06. However, no significant predictors could be found: group (β=0.29, *p*=.074), BPD symptoms (β=0.23, *p*=.053), depression scores (β=-0.29, *p*=.070). Finally, stepwise regressions were performed to assess best model fit for individual factors. BPD symptoms significantly predicted pain threshold, *b*=0.39, *t*(118)=2.65, *p*=.009, with the model explaining a significant proportion of variance in pain threshold, R^2=0.06, $F_{(1, 116)}$=7.02, *p*=.009. Group significantly predicted pain intensity, *b*=-9.66, *t*(118)=-2.20, *p*=.030, with the model explaining a significant proportion of variance in pain threshold, R^2=0.04, *F*(1105)=4.85, *p*=.030.

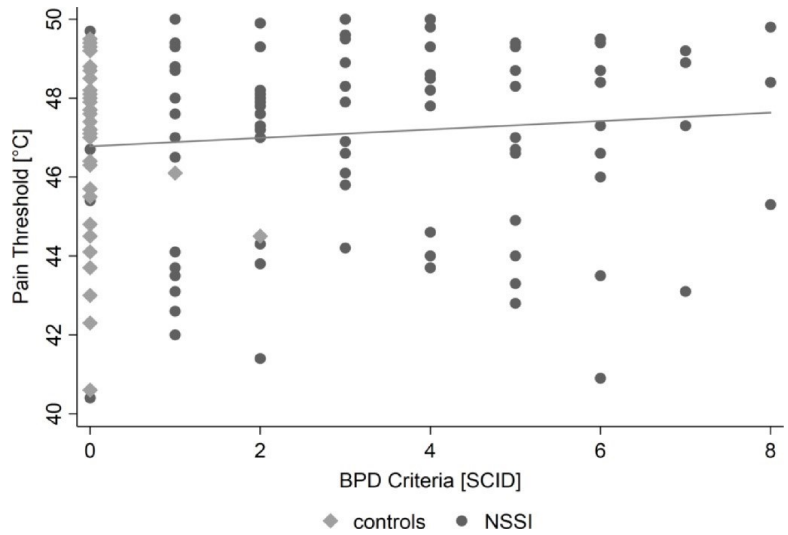

Fig. 2. Correlation scores between BPD symptoms and pain threshold. BPD = Borderline personality disorder.

3.3 Beta-Endorphin

The groups differed significantly on plasma BE levels ($t(127)$ =-3.182, p=.002, 95% CI[0.23, 1.03], Cohen's d=0.63): As depicted in Fig. 3, patients had significantly lower BE levels (M=26.21 pg/ml, SD=20.64) compared to the HC (M=39.02 pg/ml, SD=19.45). Regarding a potential influence of smoking habits, a significant regression equation was found for the multiple regression model ($F_{(3, 125)}$=4.25, p=.007), with an R^2 of 0.093. Group significantly predicted BE levels (β=-12.55, p=.004), while smoking prior to the neurobiological assessment (β=-11.57, p=.110) and smoking over the last 12 months (β=3.53, p=.460) had no significant influence.

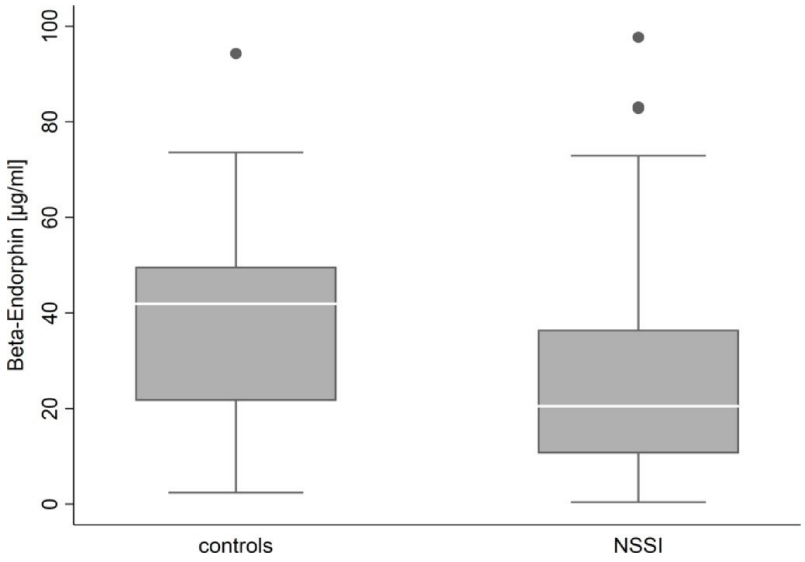

Fig. 3. Group differences in plasma beta-endorphin levels. NSSI = Non-suicidal self-injury; pg/ml = picogram per milliliter.

Further, in several exploratory analyses, we assessed potential associations between clinical characteristics and plasma BE levels. A significant negative correlation was found between BE levels and depression scores (DIKJ: r=-0.246, p=.007, Fig. 4). No significant relationship was found between BE levels and the number of NSSI episodes in the past 12 months (r=0.086, p=.412), BPD symptoms (r=-0.100, p=.258), severity of psychiatric symptoms (CGI: r=0.052, p=.621) or severity of ACE (r=-0.126, p=.168). Similarly, no significant relationship was found between BE levels and pain threshold (r=-0.013, p=.882), pain tolerance (r=0.053, p=.553) and pain intensity (r=0.012, p=.898). Again, additional exploratory analyses were conducted to determine what factors explain unique variance in BE levels. Sub-group analyses within the NSSI group revealed no significant differences for patients with BPD compared to patients without BPD ($t(92)$=-0.517, p=.607), patients with MDD compared to patients without MDD ($t(92)$=-0.411, p=.682) and patients with previous suicide attempts compared to patients without previous suicide attempts (t (92)=-0.507, p=.613). Subsequently, using group, BPD symptoms and depression scores as independent variables, we found a significant multiple regression

model for BE levels ($F_{(3, 114)}$=4.76, p=.004), with an adjusted R^2 of 0.09. Group significantly predicted BE levels (β=-0.40, p=.014), while BPD symptoms (β=0.15, p=.211) and depression scores (β=0.15, p=.915) had no significant influence. Finally, a stepwise regression was performed to assess best model fit for individual factors. Group significantly predicted BE levels, b=-13.84, t(118) =-3.56, p<.001, with the model explaining a significant proportion of variance in BE levels, R^2=0.10, $F_{(1, 116)}$=12.70, p<.001.

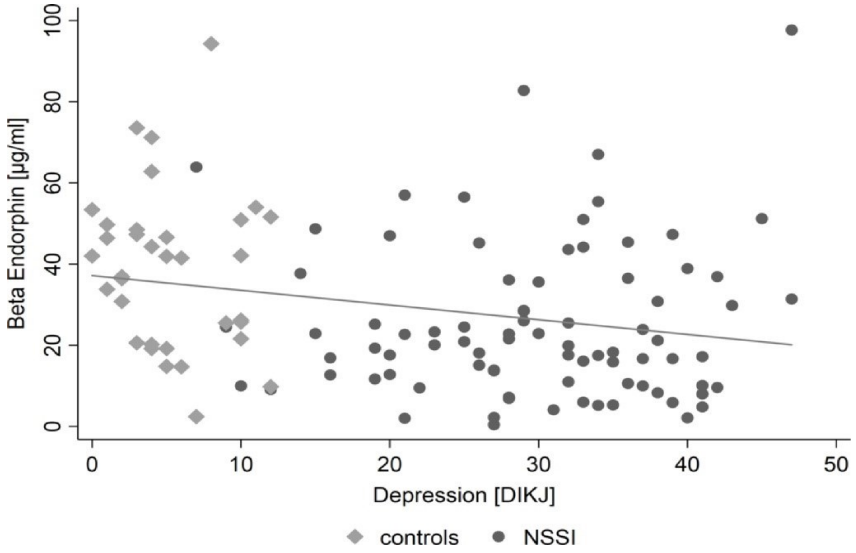

Fig. 4. Relationship between plasma beta-endorphin levels and depression scores.

4. Discussion

Adolescents with NSSI had significantly higher pain thresholds and reported lower pain intensities compared to HC, indicating lower overall PS. This finding is in line with previous research in adolescents (Koenig et al., 2017b) and findings on self-harm across all ages (Koenig et al., 2016). In accordance with more recent findings (Glenn et al., 2014; Koenig et al., 2017a), we found no association between a lower PS and NSSI frequency, yielding further support against previous theories on the effect of habituation (Joiner, 2007). In line with our second hypothesis, individuals with NSSI had significantly lower basal BE levels compared to HC. The endogenous opioid system is a key factor in the experience and processing of pain (Bresin and Gordon, 2013; Bruehl et al., 2012). In particular, BE release is linked to a reduction in pain unpleasantness and perceived PS (Zubieta et al., 2001). Previous research found that adults with a history of NSSI had altered basal BE levels (Sher and Stanley, 2008). Based on these findings a homeostasis model of NSSI was suggested (Stanley et al., 2010) – the opioid deficiency model. In line with this model, we demonstrate robust alterations of basal BE levels in adolescents with NSSI. However, we found no direct link between plasma BE levels and PS. Important to note, the opioid deficiency model postulated that BE release, rather than the baseline BE level, modulates pain

experience, with lower baseline BE levels potentially increasing the sensitivity for the analgesic effects of BE release (Bresin and Gordon, 2013), subsequently leading to a lower PS following NSSI. This assumption is supported by studies showing that increases in μ-opioid receptor availability lead to a heightened sensitivity for μ-opioid receptor agonists (Bresin and Gordon, 2013), with other studies showing that BE levels were elevated following intense physical sensations (Ribeiro et al., 2005; Sandman and Hetrick, 1995). Further, some evidence exists that BE levels are elevated following NSSI compared to baseline levels (Sandman et al., 2003; Sandman and Hetrick, 1995), but these studies did not assess related pain experience. Unfortunately, we did not assess BE release in this study. Therefore, while we provide evidence on the first assumptions of the opioid deficiency model in adolescent NSSI (lower basal BE level), we were not able to test for effects of BE release. Overall, the exact role of plasma BE for PS in adolescent NSSI has not yet been extensively researched. More studies combining assessment of basal BE and BE release in response to painful stimulation are warranted.

In additional exploratory analyses we assessed potential links between common comorbid psychopathologies in NSSI, and PS and plasma BE levels respectively. We found PS to be positively associated with BPD severity, further supporting assumptions that psychopathology underlying NSSI has a greater effect on PS compared to the behavior itself. Central functions of NSSI have repeatedly been associated with emotion regulation as well as ending dissociative states (Klonsky, 2007), with the latter seemingly representing a more specific function of NSSI in the context of BPD (Bracken-Minor and McDevitt-Murphy, 2014). Stress-related dissociative states as part of the diagnostic entity have been reported in up to 80% of individuals with BPD (Krause-Utz et al., 2017) and were found to be linked to lower PS (Ludäscher et al., 2010). More precisely, lower PS has been shown to be related to both state and trait dissociation (Ludäscher et al., 2007; Russ et al., 1992), with more pronounced effects during times of distress (Bohus et al., 2000; Stiglmayr et al., 2008). While these findings could explain our finding of a significant association between PS and BPD severity, we did not assess measures of dissociation in our study. Thus, we are unable to verify this assumption.

Further support for the effect of psychopathology underlying NSSI on PS derives from the significant association between PS and depression scores. Our finding partly supports current literature of altered pain processing in depression (McCoy et al., 2010; Thompson et al., 2016). However, relatively strong heterogeneity has been noted, with our findings being in line with experimental study designs using exteroceptive painful stimuli (Thompson et al., 2016). The association could be explained by referring to models of attentional pain processing, which state that pain might be perceived to a lesser extent if an individuals' attention is turned towards other stimuli (e.g. internal thoughts/emotions) (Eccleston and Crombez, 1999). Mood disorders are characterized by depressed moods, anhedonia and emotion dysregulation (American Psychiatric Association, 2013; Bradley et al., 2011). As such it seems plausible that, while engaging in NSSI subsequently helps to regulate emotions and resolve negative affect (Klonsky, 2007), the initial focus on depressed moods could decrease the subjectively perceived pain

intensity. However, this assumption and the overall association between PS and depression warrant further research.

An alternative explanation for altered PS could be related to ACE, either through a direct association between ACE and altered PS or through an indirect pathway via the role of ACE in the BPD specific pathology. ACE are a central risk factor for the occurrence of NSSI (Cipriano et al., 2017; Glenn and Klonsky, 2013) and BPD alike (Battle et al., 2004; Lieb et al., 2004) with high prevalence rates in both pathologies (Gratz et al., 2002; Lieb et al., 2004). Regarding the assumption of a direct association between ACE and PS, previous studies using experimentally induced pain showed that experiencing traumatic events during childhood was related to an overall lower PS (Fillingim and Edwards, 2005; Russ et al., 1993). In line with these findings, we found significantly higher ACE in our NSSI patients compared to our HC. However, we found no direct association between ACE and altered PS, thus again supporting the potential role of BPD specific pathology. Apart from its central role in the etiology of BPD, previous studies further reported ACE as an important risk factor for the occurrence of emotion dysregulation and dissociation (Korzekwa et al., 2009; Lieb et al., 2004). Studies also indicate that the severity of ACE is associated with the severity of BPD symptoms (Hébert et al., 2018; Silk et al., 1995; Zanarini et al., 2002). As such, it could be assumed that higher scores in ACE in our patient group are indicative of increased BPD severity. This could subsequently indicate more severe emotion dysregulation and more frequent and severe dissociative states, which modulate the PS (Bekrater-Bodmann et al., 2015).

BE release has previously been associated with analgesic effects, modulation of reward, emotion regulation, mood-enhancing and anxiolytic effects (Bresin and Gordon, 2013; Hegadoren et al., 2009). In line with these findings, Bandelow and colleagues proposed an intriguing theoretical model in which they discuss a potential role of alterations in BE in the pathophysiology of BPD (Bandelow et al., 2010). They propose that the frequently observed risk and attention-seeking behaviors could be explained by efforts to trigger the rewarding effects of human attachment, and that NSSI and sensation seeking behaviors might be direct attempts to artificially increase BE levels. Further, they assume that the regularly reported feelings of emptiness and anhedonia might be a result of the reduced availability of BE (Bandelow et al., 2010). Support for this model comes from experimental studies that reported lower BE levels in the CSF in individuals with a Cluster B personality disorder diagnosis with a history of NSSI and at least one suicide attempt (Stanley et al., 2010), as well as higher BE specific receptor availability in a number of brain regions in individuals with BPD (Prossin et al., 2010). However, we found no significant association between BE levels and BPD severity. As already mentioned by Bandelow and colleagues, “it may be argued that it is too simple to attribute so many behavioral dimensions to just one neurobiological system.” (Bandelow et al., 2010)[p.631]. Thus, assessing BE alone in the context of BPD might not be enough to elicit significant associations.

Interestingly, we found a significant negative correlation between BE and depression scores, indicating a link between lower basal BE levels and greater depression severity. Studies suggested BE as a key factor for the development and chronification of MDD, due to its role in emotion regulation (Daley, 2008; Hegadoren et al., 2009). Similar as for BPD (Bandelow et al., 2010), reduced BE availability has been linked to the occurrence of anhedonia (Der-Avakian and Markou, 2012) which could explain our association of greater depression severity with lower basal BE levels. Further, the association between BE and depression scores may be linked to our previously reported association between depression scores and PS. As such, we could assume that lower basal BE levels lead to more severe depression symptoms, prompting individuals to engage in NSSI. The increased depression severity could result in a stronger attentional focus on the internal emotions and states, resulting in a decreased PS while these individuals engage in NSSI (Bradley et al., 2011). Based on our present findings, one may speculate on a joint mechanism, linking affective symptomatology and basal BE in adolescent NSSI. While the mechanisms responsible for alterations in BE are still largely unknown, the experience of severe and continuous traumatic events has previously been associated with decreased levels of BE (Bremner and Pearce, 2016). However, we found no significant association between ACE and BE. This could be due to the fact that we assessed ACE as global score. As such, we did not account for severity or duration of experienced ACE. Future studies including more detailed assessments of ACE might be able to show an association with BE.

Overall, multiple regression analyses, stepwise regression analyses and sub-group analyses in the patient sample confirmed that group status explained most variance in measures of pain intensity and BE. However, BPD severity was the strongest predictor for pain threshold as further reflected by the reported correlation. While our exploratory analyses hint at potential associations between psychopathology, PS and BE levels respectively, further evidence is needed. As such, future research is needed to further investigate these associations in well-powered studies, using a confirmatory approach.

Finally, while the present paper mainly focusses on biological mechanisms of NSSI, it is important to consider psychological factors and their impact on pain perception and BE. Individuals engaging in NSSI often report an improvement in mood (e.g. decreased negative affect and increased positive affect) following self-injury, which subsequently could reinforce future NSSI (Nock and Prinstein, 2004). This phenomenon has been suggested to relate to brain circuits involved in emotion and pain processing (Navratilova and Porreca, 2014). Further, there is an extensive overlap between neural circuits that process physical pain and emotional pain (e.g. negative affect and emotional distress), with evidence indicating that the offset of physical pain results in an offset of emotional pain (Eisenberger, 2012). In line with this, several studies were able to show that the offset of pain was indeed associated with a subsequent reduction in negative affect (Franklin et al., 2010) and an increase in positive affect (Franklin et al., 2013b, 2013a). As such, it can be assumed that individuals engaging in NSSI learn to associate an improvement of mood with NSSI and more precisely the offset of experienced pain, subsequently

reinforcing NSSI due to its rewarding effects. This is supported by findings where pain relief correlated with the blood oxygen level-dependent response in brain areas commonly involved in processing pain and reward (Osuch et al., 2014). Further, studies also showed that the expectation of pain relief leads to alterations in μ-opioid receptor activity in brain regions again associated with emotion, pain and reward processing (Bushnell et al., 2013; Wager et al., 2007), potentially indicating that anticipated pain relief could potentiate pain-related BE release or elicit BE release itself (Wager et al., 2007). In line with the opioid deficiency model of NSSI and evidence for increased μ-opioid receptor sensitivity for BE release (Bresin and Gordon, 2013), it could be assumed that BE release following NSSI also has increased effects on reward circuitry. As such, it could be assumed that the anticipation of reward (e.g. improvement in mood) potentiates analgesic effects often observed in individuals with NSSI, subsequently reinforcing NSSI as behavior.

Further support for a potential impact of psychological factors stems from findings that individuals with a history of NSSI and high levels of self-criticism endured pain significantly longer and reported improvement of mood during the experience of pain, which was not found in individuals with low self-criticism (Fox et al., 2017). This led researchers to assume that mood is improved, because highly self-critical individuals believe they deserve to be punished and subsequently view NSSI and pain as self-affirming, thus they are willingly enduring pain for longer (Hooley and Fox, 2019). In line with the above-mentioned mechanisms, it can be assumed that high levels of self-criticism are distressing, creating emotional pain. The experience of physical pain might elicit BE release, which subsequently improves mood and reinforces NSSI through the effect of BE on reward circuitry. This assumption is further supported by a study that used a brief cognitive intervention to enhance self-worth in order to reduce self-critical beliefs, in individuals with NSSI (Hooley and St. Germain, 2014). Results showed that an increase in self-worth significantly reduced pain endurance, as well as willingness to endure pain in individuals with NSSI (Hooley and St. Germain, 2014). Again, in line with the above-mentioned mechanisms, increasing self-worth with a brief cognitive intervention could be an alternate pathway to reduce the emotional distress, induced by self-critical beliefs, which reduces the beneficial effect of pain on mood and subsequently reduces willingness to endure pain. Following this line of thought, it could be assumed that increasing self-worth, by reducing self-critical beliefs, is rewarding, potentially indicating a subsequent release of BE. As such, BE levels could already be increased, reducing the necessity to engage in NSSI to achieve the same effect. It seems evident that psychological factors impact biological mechanisms of NSSI (and vice versa), however these interactions are complex and often hypothetical, as more research is needed.

Our study is not without limitations. First, BE levels were solely assessed at rest. While findings in two studies indicate that resting BE levels are a relatively stable measure (Bruehl et al., 2017; Leppäluoto et al., 2008), future studies should implement multiple measures, especially before and after the pain stimulation, to enable a more precise investigation of the adaptive plasma BE response to painful stimuli

and its potential link to PS. Besides, BE measures were assessed from plasma. Studies have shown that plasma levels of BE are not correlated with central measures from CSF (De Riu et al., 1997; Kirtley et al., 2015). Yet, the exact role of plasma BE levels in pain processing remains unknown, and it has been argued that plasma BE levels, which are easily affected by stress factors (De Riu et al., 1997), could prove especially useful for studies interested in dynamic measures of BE release following painful stimuli (Kirtley et al., 2015). It is evident that further research is necessary to clarify the exact role of BE levels, central and peripheral alike, in NSSI. While our pain stimulation paradigm enabled us to systematically assess pain threshold and tolerance in an experimental setting, the paradigm itself is very different from self-inflicted pain associated with actual NSSI. Future studies should consider using pain stimuli more akin to methods used by individuals engaging in NSSI (Shabes et al., 2016). It might be worthwhile to investigate the use of blunted blade stimuli (Ammerman et al., 2018) as they elicit similar affective and sensory evaluations of pain compared to real incisions (Shabes et al., 2016). Further, the cross-sectional nature of the data marks an important limitation. Annual follow-up measurements could prove valuable to assess temporal changes, to investigate potential interconnections with other physiological- and psychological variables and the respective predictive value of altered PS and lowered BE levels for events of NSSI. Finally, although subjects were instructed to fast on the morning of testing, we cannot rule out the possibility that subjects took analgesics before participating in the laboratory session.

The large sample of well-characterized adolescents engaging in NSSI marks a clear strength of the present study. Furthermore, the present study is, to our knowledge, the first to systematically assess BE and PS in an adolescent sample.

4.1. Conclusion

The present study found significantly lowered PS (i.e.: increased pain threshold, lower reported pain intensity) and lower basal BE levels in adolescents with NSSI, that, in line with previous findings of alterations of the endogenous stress system (Koenig et al., 2017b), suggest that significantly altered biological processes are linked to the onset and maintenance of NSSI. No significant relationship could be found between increased pain thresholds and lower basal BE levels, potentially indicating that reduced PS and basal opioid deficiency might be independent biological correlates for NSSI. There is additional support of a modulating role of psychopathologies underlying NSSI for PS and basal opioid deficiency, respectively.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Physiological Response to Pain in Female Adolescents with Nonsuicidal Self-Injury as a Function of Severity

Running Title: Physiological Pain Response & Self-Injury Severity

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Abstract

Nonsuicidal self-injury (NSSI) is associated with altered pain sensitivity and alterations of the physiological stress response systems. Preliminary evidence indicates altered hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) response to experimental pain in NSSI. This study investigated effects of NSSI severity and severity of psychopathology on the HPA axis and ANS response to pain. *N*=164 adolescents with NSSI and *n*=45 healthy controls received heat pain stimulation. Salivary cortisol, α-amylase and blood pressure were repeatedly assessed before and after painful stimulation. Heart rate (HR) and heart rate variability (HRV) were assessed continuously. NSSI severity and comorbid psychopathology were derived from diagnostic interviews and self-reports. Regression analyses were conducted to examine main and interaction effects of time of measurement and NSSI severity, adjusted for severity of adverse childhood experiences (ACE), borderline personality disorder (BPD) and depression, on HPA axis and ANS response to pain. No effects of NSSI severity on pain sensitivity were observed. Increasing NSSI severity predicted an increasing cortisol response ($\chi^2(3)=12.09$, $p=.007$) to pain. After adjusting for comorbid psychopathology, greater NSSI severity predicted decreased α-amylase levels following pain ($\chi^2(3)=10.47$, $p=.015$), as well as decreased HR ($\chi^2(2)=8.53$, $p=.014$) and increased HRV ($\chi^2(2)=13.43$, $p=.001$) response to pain. Findings indicate an increased pain-related response of the HPA axis and an ANS response characterized by a reduced sympathetic and increased parasympathetic activity associated with NSSI severity. Results support extant claims for dimensional approaches to NSSI and its related psychopathology in association with shared, underlying neurobiological correlates.

1. Introduction

Nonsuicidal self-injury (NSSI), the deliberate and self-inflicted damage to body tissue in the absence of suicidal intent[1], typically peaks in adolescence[2], with lifetime prevalence rates of 13.4–17.2% among adolescents and young adults in non-clinical samples[3] and up to 80% in clinical populations[4,5]. Although being a key symptom of borderline personality disorder (BPD)[6], NSSI also occurs independently, and comorbid with a wide range of psychiatric disorders[5,7,8]. Consequently, NSSI was introduced in the 5th version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as a disorder requiring further research[6]. Adding to the high prevalence rates, adolescent NSSI has been linked to significantly reduced psychosocial functioning[9,10] and was shown to predict onset of psychiatric disorders later in life[11], as well as future suicide ideation and attempts[12–14]. Most individuals engage in NSSI as a dysfunctional strategy to regulate intense emotions, cope with distress, or to self-punish[15,16]. Consistently, an increased risk of NSSI was linked to emotion dysregulation[17,18], increased psychological distress[19] and lower distress tolerance[20].

While self-injurious acts are normatively inherently painful, a significant portion of individuals with NSSI report decreased pain perception or analgesia during self-injurious acts[21], as evidenced by higher pain thresholds and pain tolerances, lower overall perceived pain intensities[22,23], and a higher willingness to endure pain[24]. Moreover, experimental research revealed that pain is capable of reducing negative affect and aversive tension in individuals engaging in NSSI[25–31]. Indeed, the growing body of research indicates a complex association between pain and NSSI (severity), influenced by state-dependent psychological and physiological arousal[32]. Pain processes involve psychological and biological mechanisms, including sensory, affective and cognitive dimensions[33] and existing evidence suggests that altered biological mechanisms are associated with subjective NSSI-related pain perception[28,34–36].

Based on evidence that engaging in NSSI results in a reduction of self-reported negative affect and tension[37,38], research has started to assess changes in biological stress systems – the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis[39,40] – in the context of pain and NSSI. Generally, the physiological pain response is marked by a decrease in parasympathetic and an increase in sympathetic nervous activity, derived from changes in vagally mediated heart rate variability (HRV)[41,42], and an increased cortisol secretion[43]. In a first study, systematically assessing the ANS and HPA axis response to experimental pain in adolescents with a history of NSSI and healthy controls (HC), we found significant alterations in both the ANS and HPA axis response to pain in those with NSSI[25]. Individuals with NSSI had a delayed decrease in parasympathetic vagal activity (greater HRV) in anticipation of the painful stimulation and a prolonged recovery (reduced HRV) following painful stimulation compared to HC, which correlated with improved body awareness. Further, individuals with NSSI had a significantly greater cortisol response to painful stimulation compared with HC, which was positively associated with mood-improvement[25]. This hyperresponsiveness of the ANS and HPA axis to painful stimulation was further enhanced by the presence of greater childhood adversity[44]. While these

studies lend first empirical support for alterations of psychophysiological systems in NSSI-related pain experience, further studies replicating and expanding on these findings are needed.

Clinical research and practice have recognized the need for dimensional approaches of psychiatric diagnoses and psychopathology (see[45] for a review). Evidence suggests that categorical diagnoses, relying on arbitrary thresholds and cutoffs, fail to consider that psychopathology and the underlying mechanisms are naturally continuous, and psychiatric disorders are better defined along the continuum of several overarching dimensions (i.e., internalizing and externalizing) that largely influence the symptomatology of disorders[46,47]. Moreover, categorical classifications seldom consider underlying pathophysiological mechanisms, shared across most traditional diagnoses[48]. Dimensionally assessing the transdiagnostic symptomatology of NSSI could increase our understanding of how psychological processes and underlying biological mechanisms influence the development and maintenance of NSSI, NSSI severity and its relationship with comorbid disorders. Phenotypically, NSSI severity has often been conceptualized using the frequency of NSSI acts or days with NSSI[49]. When NSSI disorder (NSSID) was introduced in the DSM-5, five or more days of self-injurious acts over the past year have been considered as the clinically relevant cutoff (Criterion A of NSSID in DSM-5)[6]. However, the categorical nature of the NSSID diagnosis and its dichotomous frequency cutoff have been criticized[50]. First, researchers have deemed the cutoff as too low, reporting significantly higher frequency rates in community[51,52] and clinical samples[10,53]. Second, significant heterogeneity was found in the severity of comorbid psychopathology and psychosocial impairment with increasing NSSI frequency[54–56]. If low frequencies of NSSI are linked to overall lower levels of comorbid psychopathology, experienced traumata and impairment, alterations in underlying biological mechanisms ought to be analyzed in light of NSSI severity. Supporting this assumption, one study reported that, when faced with a social stressor task, individuals with 1-2 acts of NSSI did not show a blunted cortisol response compared to individuals with recurrent NSSI[57].

A growing body of literature indicates the presence of neurobiological mechanisms and correlates of NSSI[58], and the existence of altered pain sensitivity in individuals engaging in NSSI is well evidenced[23]. Yet, research on biological correlates of pain processing in NSSI is still scarce[25], and research on the effect of NSSI severity is virtually missing. Here we aimed to investigate the effect of NSSI severity on physiological responses to pain (focusing on the ANS and HPA axis) in adolescents with NSSI. Based on the existing evidence, we hypothesized that pain sensitivity would decrease as a function of NSSI severity. Further, based on previous findings and existing literature, we hypothesized that the HPA axis response to pain would increase and that the ANS pain response would entail an increased sympathetic and a decreased parasympathetic activity with increasing NSSI severity. Finally, in additional analyses, we controlled for effects of dimensional psychopathology severity on pain sensitivity, HPA axis and ANS responsivity.

2. Methods

2.1. Participants

Participants were recruited from the specialized outpatient clinic for risk-taking and self-harm behavior "Ambulanz für Risikoverhalten und Selbstschädigung (AtR!Sk)"[59], at the Clinic for Child and Adolescent Psychiatry, University of Heidelberg (Germany). The study consisted of two separate appointments: an initial diagnostic interview (AtR!Sk; IRB ethical approval number: S-449/2013) and a neurobiological assessment (AtR!Sk-Bio; IRB ethical approval number: S-514/2015) participants were subsequently invited to. While longitudinal data are still assessed, the present analyses will focus on data from the completed baseline assessments.

Recruitment took place between August 2016 and January 2020. Inclusion criteria for the patient group were a written informed consent by the adolescents and their caregivers, age between 12 and 17 years, and a completed diagnostic assessment prior to the neurobiological assessment. Exclusion criteria were a lack of speech comprehension (German), signs of acute psychosis, endocrinological or cardiovascular primary diseases likely interfering with the neurobiological assessments. For the present analyses, only patients with at least one lifetime incident of NSSI, as defined by the DSM-5[6], were included. Further, only female participants were considered, due to previously reported sex-differences regarding the prevalence of NSSI[3] and ANS/HPA activity[60–62]. HC were recruited through public advertisement. All HC and their caregivers provided written informed consent before being included in the study. Inclusion and exclusion criteria were mostly identical to the patient group. Additional inclusion criteria were no history of NSSI, no current psychiatric disorder, and no lifetime psychological or psychiatric treatment.

2.2. General Procedures

The first appointment marked an extensive diagnostic assessment, including an interview with trained personal and self-report questionnaires. Self-injurious and suicidal thoughts and behaviors were assessed using the German version of the *Self-Injurious Thoughts and Behaviors Interview* (SITBI-G)[63]. BPD was assessed with the respective parts of the German version of the *Structured Clinical Interview for DSM-IV Axis II Personality Disorders* (SCID-II)[64]. To assess axis-I psychiatric disorders commonly observed in adolescents aged 6 to 19 years, participants completed the *Mini International Neuropsychiatric Interview for Children and Adolescents* (MINI-KID)[65]. The severity of psychiatric symptoms was rated by the trained personal using the *Clinical Global Impression Scale* (CGI-S)[66], while participants' global functioning was rated using the *Global Assessment of Functioning* (GAF)[67]. Depressive symptoms were assessed using the *Depression Inventory for Children and Adolescents* (DIKJ)[68]. Furthermore, adverse childhood experiences (ACE) were assessed using the German version of the *Childhood Experiences of Care and Abuse* questionnaire (CECA.Q)[69].

HC received an adapted, shortened diagnostic assessment to assess potential current mental disorders as well as psychological or psychiatric treatments. To rule out any history of NSSI, screening questions from the SITBI-G[63] were used. Potential axis-I disorders were assessed using the *Structured Clinical Interview (non-patient edition)* (SCID-N/P)[70]. If clinical symptoms became apparent during the interview, the MINI-KID[65] was used to assess the presence of a psychiatric disorder in detail. If the criteria for any psychiatric disorder were met, participants were excluded from the study and not invited to participate in the neurobiological assessment.

The second appointment marked the assessment of neurobiological markers and variables, for which participants were invited within six weeks following the diagnostic interview. Starting at 8 a.m., participants' height and weight were measured. Participants answered questions about their handedness, physical illness, allergies, and medication intake. Subsequently, pain sensitivity was assessed using a thermal plate and a standardized procedure (see 2.3.). To assess the HPA axis response to the painful stimulation, saliva cortisol samples were collected repeatedly (see 2.4.). The ANS response was assessed by repeated assessments of blood pressure as well as continuous measurements of heart rate (HR) and HRV (see 2.5.). Participants received 40€ for their participation in the study.

2.3. Pain assessment

Three different aspects of pain sensitivity –pain threshold, pain tolerance and pain intensity– were assessed using a standardized preprogrammed sequence and an AHP-1800CPV Versatile Cold/Hot Plate (TECA Corp., Chicago, IL, USA). Participants were instructed to place their non-dominant hand flat on the plate with a baseline temperature of 32 °C. Following a 3-minute adaptation phase, temperature steadily rose to 50 °C, increasing linear by 1 °C each 13.3 seconds over four minutes. Participants were asked to keep their hand firmly on the plate until the pain became intolerable. The respective temperatures were noted in °C at first pain sensation (pain threshold) and when pain became intolerable (pain tolerance). Pain intensity was assessed using a visual analogue scale (VAS) ranging from 0 to 100, using the Continuous Measurement System (CMS) software[71]. Participants were instructed to rate pain intensity continuously, using their dominant hand, from the moment pain threshold was reached until the pain became intolerable. To avoid any damage to the skin due to long-term exposure, the sequence ended automatically at 50 °C and participants were asked to remove their hand if pain tolerance was still not reached. Pain intensity scores were calculated based on the rated pain intensity upon reaching pain tolerance. An average score was generated using the VAS ratings within five seconds before and after reaching pain tolerance to account for potential inaccuracies.

2.4. Endocrinological assays

The endocrinological pain response was assessed using salivary samples, collected at five different time-points following a standardized procedure and timeline: A baseline measure following a 5-minute resting period (1), immediately prior to (2) and after the painful stimulation (3), following a second 5-

minute resting period (4) and ten minutes after the fourth salivary sample (5). Participants were asked to chew on a cotton swap (Salivette®; Sarstedt, Numbrecht, Germany) for one minute. Samples were frozen at -20 °C until assay. Salivary α-amylase and cortisol were determined at the Biopsychology Laboratory at the Technical University of Dresden. Before analysis, samples were centrifuged at 3000 rpm to produce a clear supernatant of low viscosity. A-amylase concentration was determined using an enzyme kinetic method. Cortisol concentrations, as proxy of HPA axis responsivity, were determined with a commercially available chemiluminescence immunoassay (CLIA; IBL International, Hamburg, Germany), according to the protocol of the manufacturer. The reference range was 56–200 ng/ml, with inter- and intra-assay coefficients of variation between 2.9–6.0%.

2.5. Cardiovascular activity

HR was continuously recorded at 1024 Hz throughout the neurobiological assessment with an EcgMove 3 sensor (Movisens GmbH; Karlsruhe, Germany), attached to a chest belt with dry electrodes. Recording only started after participants were comfortably seated. Recordings lasted from the first resting period until after the second resting period. Raw electrocardiogram (ECG) data were first screened using UnisensViewer (Movisens GmbH; Karlsruhe, Germany). Subsequently, raw data were processed using the Kubios HRV Premium software (Version 3.0)[72]. R peaks were manually corrected, accounting for movement artifacts and potential extra systoles. HR in beats per minute and the root mean square of successive differences (rMSSD) of normal-to-normal intervals, as a measure of HRV, in milliseconds were derived. Diastolic (DBP) and systolic blood pressure (SBP) were assessed at five time-points, always following the salivary cortisol samples (see 2.4.), in a sitting position using an OMRON M500 sphygmomanometer (Omron Corporation, Kyoto, Japan).

2.6. Statistical analyses

Prior to analyses, pain sensitivity variables (threshold, tolerance, and intensity) were checked for missing values, with participants being excluded if at least one measure was missing. Values for pain endurance were generated by subtracting temperature at pain threshold from temperature at pain tolerance (see[24]). Clinical and sociodemographic variables were tested for between-group differences using χ^2-tests for categorical variables and one-way ANOVA analyses for continuous variables. Regarding effects of NSSI severity, associations were investigated using NSSI frequency (continuous) in the past six months preceding the assessments, independent of group assignment, to account for recent NSSI. Simple linear regression analyses were calculated to analyze whether NSSI frequency (continuous) in the past six months predicted clinical characteristics and pain sensitivity. Multilevel mixed-effects linear regression analyses were conducted to assess differences in the physiological pain response (cortisol, α-amylase, SBP, DBP, HR and HRV) with TIME (time of measurement or segment), NSSI FREQUENCY over the past six months (continuous), and their interaction as fixed effects, and the participants' ID as a random effect. Additionally, contrasts of marginal linear predictions on the main effects of TIME and NSSI

FREQUENCY as well as their interaction were derived. In additional analyses, the multilevel mixed-effects linear regression analyses were adjusted for ACE, BPD and depression severity (all continuous), to account for a potential influence of comorbid psychopathology on the physiological pain response. Similarly, additional multiple linear regression analyses were conducted with pain sensitivity measures as dependent variable and NSSI frequency as well as ACE, BPD and depression severity, and their respective interactions with NSSI FREQUENCY as independent variables, to account for a potential influence of psychopathology on pain sensitivity. All analyses were performed using Stata (Version 16; StataCorp LP, College Station, TX, US) with the significance level set to $\alpha = 0.05$.

3. Results

3.1. Sample characteristics

A total of n = 255 patients and their parents provided written informed consent to participate in the neurobiological assessments. N = 242 (94.9%) completed the baseline assessment. Of these, n = 43 (17.8%) were excluded due to male sex and n = 5 (2.1%) due to reporting no lifetime incidents of NSSI. N = 28 (11.6%) patients were excluded due to missing pain data. N = 58 adolescents provided written informed consent for the HC group, of which n = 49 (84.5%) completed the baseline assessment. N = 2 (4.1%) were excluded due to male sex and n = 4 (8.2%) due to missing pain data. The final sample consisted of n = 45 HC and n = 164 female patients with NSSI.

A detailed description of sociodemographic and clinical characteristics is provided in *Table 1*. Groups did not differ on age, height, and BMI. However, patients and HC differed significantly on weight ($F(1,203)=4.28$, $p=.040$) and school-type ($\chi^2(3)=13.44$, $p=.004$). Patients reported significantly more ACE ($F(1,190)=50.61$, $p<.001$), and scored significantly higher on depressive symptoms (DIKJ; $F(1, 186)=235.20$, $p<.001$) and BPD symptoms compared to HC ($F(1,206)=105.48$, $p<.001$). Overall, n = 46 patients (28.1%) fulfilled diagnostic criteria for BPD and n = 90 patients (55.0%) fulfilled diagnostic criteria for a depressive episode or disorder. On average, patients reported 60.7 (SD=70.32) episodes of NSSI within the past 12 months (range: 0–340), and 33.1 (SD=37.61) episodes within the past six months (range: 0–160). To assess continuous associations between clinical measures and NSSI frequency (past six months), simple linear regressions were calculated. The number of BPD criteria ($p<.001$), depressive symptoms ($p<.001$), ACE ($p<.001$), and the severity of psychiatric symptoms (CGI; $p=.025$) all significantly increased with more frequent NSSI in the past six months, while global functioning ($p<.001$) significantly decreased (see *SM Table 1*).

3.2. Pain sensitivity measures

Simple linear regressions were calculated with pain sensitivity measures as dependent variables and NSSI frequency (six months) as predictor (see *SM Table 1*). Analyses revealed no significant effect of NSSI frequency on pain sensitivity measures. As depicted in *Figure 1*, increasing NSSI frequency led to a non-significant increase in pain threshold ($\beta=0.002$, $p=.753$) and pain tolerance ($\beta=0.002$, $p=.694$) as well as a non-significant decrease in pain endurance ($\beta=-0.000$, $p=.951$) and perceived pain intensity ($\beta=-0.030$, $p=.478$). Additional regression analyses were conducted to control for a potential additional effect of comorbid psychopathology (e.g., ACE, BPD symptoms, depression symptoms). No significant main effects of NSSI frequency or comorbid psychopathology and no interaction effects on pain sensitivity measures were found (see *SM Tables 2-4*).

Table 1 Sample Characteristics

Variable	Group; mean ± SD or *N* (%)		*P*[1]
	HC, (n=45)	NSSI, (n=164)	
Age	14.8±1.28	14.8±1.48	0.727
Height	163.5±6.22	165.6±6.87	0.076
Weight	54.6±9.92	58.8±12.25	0.040*
BMI	20.4±3.24	21.4±3.93	0.127
School Type[2]			0.004**
Gymnasium	29 (64.4)	59 (36.0)	
Realschule	13 (28.9)	66 (40.2)	
Hauptschule	2 (4.4)	16 (9.8)	
Other	1 (2.2)	23 (14.0)	
BPD criteria	0.1±0.33	3.2±2.03	<0.001***
DIKJ	6.7±5.25	28.9±9.18	<0.001***
ACE	0.1±0.29	1.3±1.16	<0.001***
GAF	-	51.4±9.22	
CGI	-	4.8±0.83	
NSSI (past 12 months)	-	60.7±70.32	
NSSI (past 6 months)	-	33.1±37.61	
Suicide attempt (past 12 months)	-	72 (44.0)	

BMI = body mass index; BPD = Borderline personality disorder; DIKJ = Depressionsinventar für Kinder und Jugendliche; ACE = adverse childhood experiences; GAF= Global Assessment of Functioning; CGI = clinical global impression; NSSI = Nonsuicidal self-injury

[1] Significance: *p*-values refer to differences between groups, with χ^2 tests for categorical variables and one-way ANOVAs for continuous variables.

[2] Hauptschule: secondary school terminating with a lower secondary-school level II certificate; Realschule: secondary school terminating with a secondary-school level I certificate; Gymnasium: secondary school
terminating with the general qualification for university entry.

* $p < .05$. ** $p < .01$. *** $p < .001$

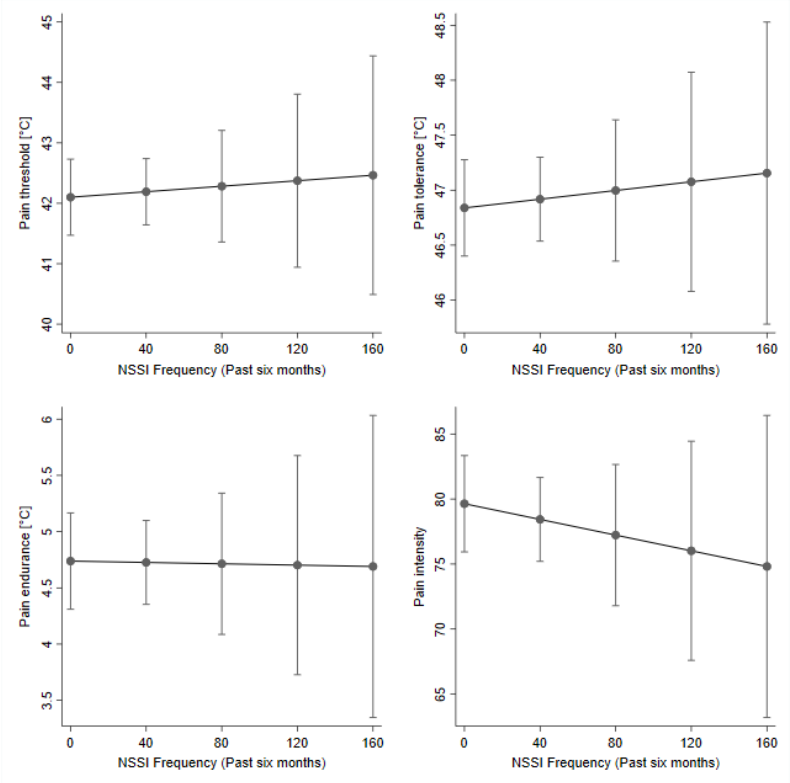

Figure 1 Pain Sensitivity as a Function of NSSI Frequency: depicted are mean pain threshold, pain tolerance, pain endurance (all in degrees Celsius), and pain intensity (0-100 visual analogue scale) and their 95% confidence intervals as a function of the frequency of non-suicidal self-injury (NSSI) in the past six months.

3.3. Endocrinological and autonomic measures

Results of the multilevel mixed-effects linear regressions with main and interaction effects of time and NSSI frequency (six months) modeled continuously are depicted in *Table 2*. Results of the additional, adjusted regression models with main and interaction effects of time, NSSI frequency (six months) and psychopathology (e.g., ACE, BPD and depression severity) modeled continuously are depicted in *SM Tables 5-7*.[2]

3.3.1. Cortisol

No significant main effects of TIME ($\chi^2(3)=1.56$, $p=.668$) nor NSSI FREQUENCY ($\chi^2(1)=0.00$, $p=.958$) on cortisol levels were found. However, the TIME*NSSI FREQUENCY interaction on cortisol levels was significant ($\chi^2(3)=12.09$, $p=.007$). As depicted in *Figure 2a*, cortisol levels increased significantly stronger following pain induction if individuals self-injured more frequently in the past six months. Post-

[2] Detailed results of these supplemental analyses can be provided upon request.

hoc analyses revealed that robust significant differences in cortisol response to pain occurred, if participants reported 63 or more incidents of NSSI in the past six months (see *SM Table 8*). Results were robust after adjusting for ACE, BPD, and depression severity, with no moderating effect of psychopathology on the TIME*NSSI FREQUENCY interaction.

3.3.2. α-amylase

Analyses indicated a significant main effect of TIME ($\chi^2(3)=26.92$, $p<.001$) on α-amylase, characterized by an increased α-amylase secretion following pain induction (see *Figure 2b*). No significant main effect of NSSI FREQUENCY ($\chi^2(1)=0.87$, $p=.350$) nor TIME*NSSI FREQUENCY interaction ($\chi^2(3)=5.32$, $p=.150$) was observed.

The main effect of TIME was robust after adjusting for potential effects of psychopathology (see *SM Tables 5-7*). However, adjusting for ACE revealed a significant main effect of NSSI FREQUENCY ($\chi^2(1)=4.40$, $p=.036$) not present in the unadjusted model, indicating overall increased α-amylase levels with increasing NSSI frequency when considering the presence of trauma. Adjusting for BPD severity revealed a significant main effect of NSSI FREQUENCY ($\chi^2(1)=17.66$, $p<.001$) and TIME*NSSI FREQUENCY interaction ($\chi^2(3)=10.47$, $p=.015$) not present in the unadjusted model, indicating overall higher α-amylase levels with increasing NSSI frequency and a more pronounced decrease in α-amylase following pain with increasing NSSI frequency. The TIME*NSSI FREQUENCY interaction was not moderated by BPD severity.

3.3.3. Blood pressure

The main effect of TIME was significant for both SBP ($\chi^2(3)=17.91$, $p<.001$) and DBP ($\chi^2(3)=15.40$, $p=.002$). As illustrated in *Figure 2c-d,* SBP and DBP increased significantly after pain induction compared to baseline (SBP: $\chi^2(1)=4.19$, $p=.041$; DBP: $\chi^2(1)=13.44$, $p<.001$) and decreased significantly after the second resting phase (SBP: $\chi^2(1)=14.89$, $p<.001$; DBP: $\chi^2(1)=6.50$, $p=.011$). Analyses showed no significant main effects of NSSI FREQUENCY nor TIME*NSSI FREQUENCY interaction (see *Table 2*).

Results on SBP were robust when adjusting for psychopathology. Regarding DBP, results of the unadjusted model were overall robust, except when the model was adjusted for depression severity (see *SM Tables 5-7*). Here, the main effect of TIME became non-significant ($\chi^2(3)=7.77$, $p=.051$). No significant main or interaction effects for psychopathology were observed.

3.3.4. Autonomic reactivity

Analyses revealed a significant main effect of TIME on HR ($\chi^2(2)=18.20$, $p<.001$), indicating an overall increased HR during painful stimulation ($\chi^2(1)=11.54$, $p<.001$) and the second resting phase ($\chi^2(1)=15.47$, $p<.001$) compared to baseline (see *Figure 2e*). The main effect of NSSI FREQUENCY

($\chi^2(1)=0.00$, $p=.976$) and TIME*NSSI FREQUENCY interaction ($\chi^2(2)=4.58$, $p=.101$) were not significant. The overall model fit for HRV was not significant ($\chi^2(5)=8.71$, $p=.121$).

Results on HR were largely robust when adjusting for effects of psychopathology (see *SM Tables 5-7*). However, controlling for effects of ACE revealed a significant TIME*NSSI FREQUENCY interaction ($\chi^2(2)=8.53$, $p=.014$) not present in the unadjusted model, indicating a progressive decrease in HR with increasing NSSI frequency during pain. No significant moderating effect of ACE was observed.

Regarding HRV, the overall model fit remained nonsignificant after adjusting for effects of ACE and BPD. Adjusting for depression severity revealed a significant model fit ($\chi^2(11)=24.62$, $p=.010$), with no significant main effects of TIME ($\chi^2(2)=1.02$, $p=.601$) nor NSSI FREQUENCY ($\chi^2(1)=0.05$, $p=.820$). However, the TIME*NSSI FREQUENCY interaction was significant ($\chi^2(2)=13.43$, $p=.001$), indicating that HRV increased significantly stronger with greater NSSI frequency following pain induction. No significant moderating effect of depression severity was observed (see *SM Tables 5-7*).

Table 2 Results of multilevel mixed-effects linear regressions for predictions of cortisol, α-amylase, systolic (SBP) and diastolic blood pressure (DBP), heart rate (HR) and heart rate variability (HRV) dependent on time and frequency of non-suicidal self-injury (NSSI) in the past 6 months (continuous).

Outcome	Model Fit		Main Effect Time		Main Effect NSSI Frequency		Interaction	
	X^2	*P*	X^2	*p*	X^2	*p*	X^2	*P*
Cortisol	22.77	0.002**	1.56	0.668	0.00	0.958	12.09	0.007**
α-Amylase	38.75	<0.001***	26.92	<0.001***	0.87	0.350	5.32	0.150
SBP	22.64	0.002**	17.91	<0.001***	0.08	0.773	1.42	0.701
DBP	19.61	0.007**	15.40	0.002**	0.63	0.427	0.90	0.827
HR	28.36	<0.001***	18.20	<0.001***	0.00	0.976	4.58	0.101
HRV	8.71	0.121	4.77	0.092	0.01	0.916	7.42	0.025*

Cortisol = Cortisol level in nmol/l; α-Amylase = α-Amylase level in U/ml; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; rMSSD = root mean square of successive differences.

* $p < .05$. ** $p < .01$. *** $p < .001$

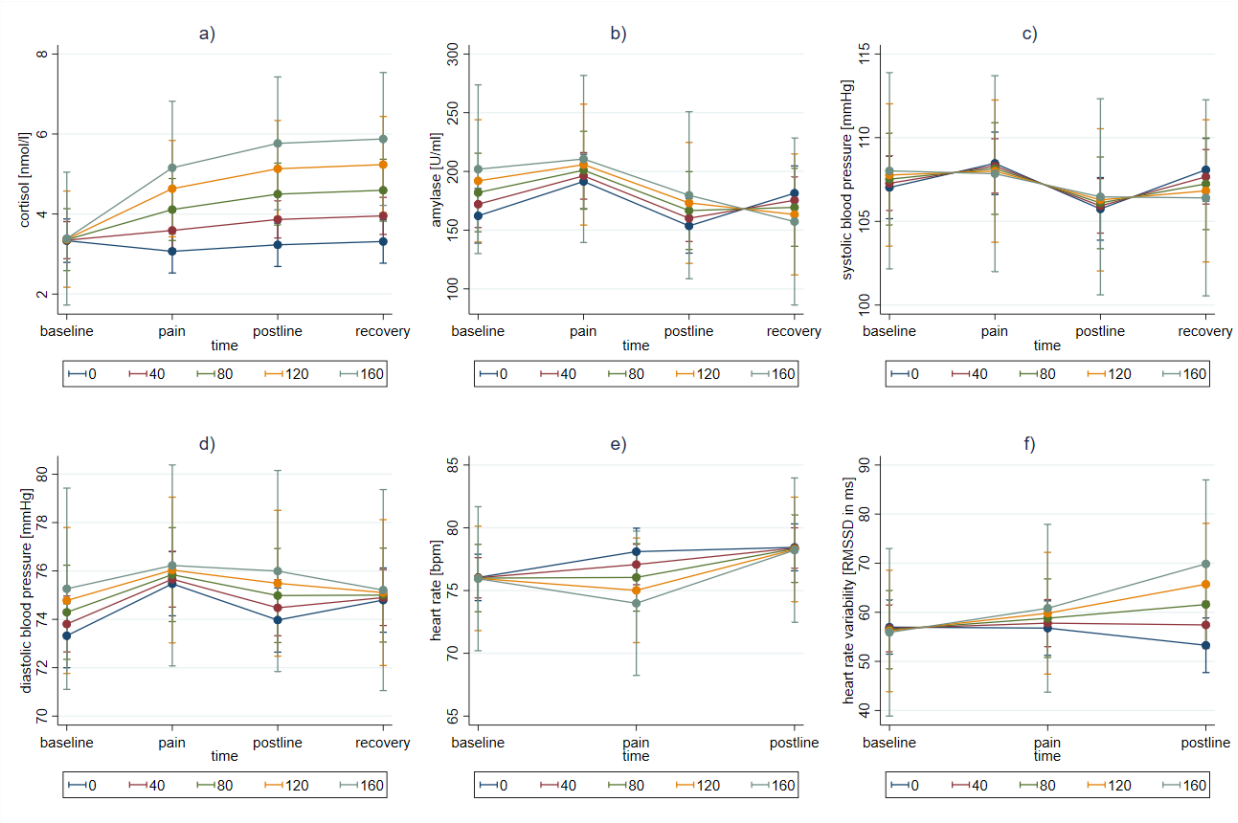

Figure 2 Endocrinological and autonomic response (cortisol, α-amylase, systolic and diastolic blood pressure, heart rate, and heart rate variability) by NSSI frequency (past six months) and time of measurement. Mean values and 95% confidence intervals of cortisol (a), α-amylase (b), systolic blood pressure (SBP) (c), diastolic blood pressure (DBP) (d), heart rate (HR) (e) and heart rate variability (HRV) (f) independent of group assignment. Times of measurement were during/immediately after a five-minute resting phase (baseline), during/immediately after heat pain stimulation (pain), during/immediately after a second five-minute resting phase (postline) and ten minutes after the postline (recovery).

4. Discussion

The present study examined the effect of dimensionally assessed NSSI severity on the physiological pain response in adolescents with NSSI. Contrary to our hypothesis, we found no significant association between pain sensitivity and NSSI frequency in general. In line with other recent studies, this may suggest that alterations in pain sensitivity are not a mere effect of habituation to constant or regular pain exposition[73,74]. Between- and within-person variance in pain experiences during NSSI episodes, that is known to be influenced by state-dependent psychological and physiological arousal[32], may account for variance in this regard. Future studies should assess affect and tension before and after pain stimulation to clarify the influence of state dependent affect and arousal.

Regarding the physiological response to pain, we found significant increases in cortisol and α-amylase levels, SBP, DBP and HR following pain across the whole sample, indicating successfully increased physiological arousal. In line with our hypotheses, we found increased pain-related cortisol secretion to vary as a function of NSSI severity (frequency in the past six months), independent of comorbid psychopathology. This expands previous findings by our group, further supporting the assumption of a pain-specific HPA axis response[25], that extends to symptom severity. A blunted HPA axis response to stress has previously been linked to more aversive emotional reactions in a range of psychiatric disorders[75,76]. In line with our previous reasoning[25], stressful situations might not elicit a sufficient HPA axis response in adolescents with NSSI[77,78]. Engaging in NSSI and the corresponding experience of pain might result in an increased cortisol secretion, which helps to cope with stress and reduce negative affect. Our findings suggest the presence of a compensatory mechanism of NSSI i.e., increased cortisol secretion following pain countering the blunted HPA axis response to stress[57]. Important to note, robust significant effects occurred in individuals reporting 63 or more days with NSSI in the past six months. This finding supports previous claims that the DSM-5 threshold for NSSI frequency is rather low[10,53] and that, especially for research addressing biological mechanisms, meaningful thresholds associated with alterations on the neurobiological level are supposedly significantly higher. Further studies are needed to replicate these findings.

Regarding ANS responsivity, unadjusted analyses revealed no significant effect of NSSI severity. However, further controlling for the influence of comorbid psychopathology yielded several important findings that are partly contrary to our hypotheses that derived from our prior study. As such, we found that HR response decreased with increasing NSSI severity during pain induction, when controlling for ACE. Concerning HRV, adjusting for depression severity revealed increased HRV response following pain induction as a function of greater NSSI frequency. Further, HRV was overall decreased with greater depression severity. Controlling for the potentially opposing effect of depression severity, which was positively correlated with NSSI severity, may have explained additional variance previously masking the TIMExNSSI FREQUENCY effect – potentially explaining that the unadjusted model failed to reach statistical significance. Finally, α-amylase, a sensitive biomarker of ANS responsivity[79], decreased

stronger with increasing NSSI frequency following pain induction, when controlling for BPD severity. Similarly, evidence suggests that changes in α-amylase levels indicate greater/reduced sympathetic activity[79,80], while changes in HR are the product of the interplay of sympathetic and parasympathetic activity, and vagally-mediated HRV is a measure of parasympathetic activity[81,82]. Consequently, our results potentially suggest that the ANS response to pain is associated with decreased sympathetic and increased parasympathetic activity as a function of greater NSSI severity. Previous data from our group pointed to an increased pain-inflicted autonomic arousal potentially counteracting reduced body awareness and dissociative states[25]. Our current results, however, point to decreased autonomic arousal after pain, which is in line with the affect-regulating and stress-reducing function of NSSI. While further research will need to investigate these conflicting findings, it is important to note that the current findings are based on a larger sample yielding increased statistical power. Overall, like our findings on HPA axis response, ANS related findings indicate more pronounced, stimulus-specific, physiological effects to pain (e.g., decreased α-amylase, HR and increased HRV response) with increasing NSSI frequency.

In line with previous meta-analyses[83–86], our findings indicate an overall increased HR and decreased HRV with greater severity of psychopathology. Lower resting HRV is associated with psychological dysfunctions such as emotion dysregulation[87,88]. NSSI is most often used to regulate emotions and aversive tension[89], and previous research indicates a pain-related decrease in tension, indexed by decreased HR and increased HRV response following pain[27–29]. Our findings support the existing evidence, potentially indicating a generally higher tension and more emotional lability associated with the severity of psychopathology – reflecting greater sympathetic dominance[58] – that are maladaptively regulated by means of engaging in NSSI.

The present study adds to the existing literature employing dimensional approaches to the neurobiological study of phenomena related to psychopathology[47,48]. First, our findings illustrate that NSSI severity is associated with a progressively altered physiological pain response, indicating that dimensional assessments of NSSI (symptom) severity and its underlying neurobiological mechanisms are warranted. Together with the previously reported heterogeneity in NSSI frequency and associated severity of psychopathology[50], our findings indicate that relying on diagnostic cutoffs, especially the one currently put forward in DSM-5 criterion A, may not adequately reflect how these psychological and physiological factors contribute to the development and maintenance of NSSI. To expand on our findings, longitudinal research is needed to examine the relationship between NSSI and altered neurobiological functioning, both at rest and in response to acute stressors, across adolescent development, and how changes in NSSI (symptom) severity relate to changes in underlying neurobiological mechanisms. Second, our findings indicate that altered HPA-axis and ANS activity are shared mechanisms underlying both NSSI and (comorbid) severity of psychopathology, while also revealing different pain-related working mechanisms. Alterations of the HPA-axis and the ANS have previously been linked to the severity of psychopathological symptoms that are central factors in many

psychiatric disorders (e.g., BPD, depression, NSSI)[76,88], likely contributing to the high comorbidity rates observed among these disorders. In line with the recently proposed dimensional approaches[47,48], our findings indicate that assessing HPA-axis and ANS functioning at rest and in response to acute stressors/pain dimensionally, across the entire spectrum of their functioning, in heterogeneous samples with varying degrees of NSSI severity and comorbid psychopathologies could further enable researchers to disentangle previously observed effects of comorbidity. While we assessed comorbid severity of psychopathology, future research should include more detailed assessments of underlying symptom severity (e.g., emotional lability, personality traits)[47].

The present study is the first to systematically assess effects of dimensional NSSI severity on the physiological pain response in a large, well-characterized sample of adolescents with and without NSSI. However, several limitations of the current study should be addressed. First, we used NSSI frequency as sole indicator of NSSI severity. However, as previously proposed[90–92], future research could rely on several indicators, potentially revealing more complex associations with the physiological response to pain. Second, while heat pain is a reliable method to assess the physiological pain response in individuals with NSSI, it differs considerably from actual NSSI methods. Assessing altered physiological responses to pain in individuals with NSSI during actual NSSI episodes might advance our understanding beyond effects observed in laboratory-based studies. Finally, our sample was limited to female adolescents, limiting the generalizability of findings. Future research should include male subjects to explore potential sex differences.

To conclude, the present study expands existing literature on altered physiological responses to pain in adolescents with NSSI and is the first to indicate a significant effect of NSSI severity. Following pain, increasing NSSI severity was associated with increasing cortisol secretion and an ANS profile characterized by reduced sympathetic (e.g., decreased α-amylase) and increased parasympathetic (e.g., increased HRV) activation – potentially indicating a pain-related decrease in physiological arousal and improved emotion- and stress-regulation capacity in NSSI. These effects remained after controlling for comorbid psychopathology. Our findings highlight the need for a dimensional assessment of NSSI severity and underlying psychological and neurobiological correlates that may play a role in its development and maintenance. Further research replicating and extending these findings in larger, more heterogenous samples are necessary.

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Supplemental Material

SM Table 1 Simple Regression analyses for frequency of non-suicidal self-injury (NSSI) in the past 6 months predicting sociodemographic and clinical characteristics as well as pain sensitivity.

Dependent variable	F	R^2	β	SE β	95% CI	*t*	*p*
Age	0.04	0.000	-0.001	0.003	[-0.01, 0.01]	-0.20	0.845
Height	0.12	0.001	0.005	0.013	[-0.02, 0.03]	0.34	0.732
Weight	0.53	0.003	-0.017	0.023	[-0.06, 0.03]	-0.73	0.467
BMI	0.96	0.005	-0.007	0.007	[-0.02, 0.01]	-0.98	0.329
BPD criteria	25.91	0.112	0.021	0.004	[0.01, 0.03]	5.09	<0.001***
DIKJ	56.37	0.233	0.168	0.022	[0.12, 0.21]	7.51	<0.001***
ACE	10.23	0.051	0.007	0.002	[0.00, 0.01]	3.20	0.002**
GAF	13.04	0.076	-0.067	0.019	[-0.10, -0.03]	-3.61	<0.001***
CGI	5.13	0.031	0.004	0.002	[0.00, 0.01]	2.27	0.025*
NSSI (past 12 months)	586.27	0.740	1.604	0.066	[1.47, 1.74]	24.21	<0.001***
Suicide attempt (past 12 months)	1.18	0.006	0.005	0.005	[-0.01, 0.01]	1.08	0.279
Pain threshold (°C)	0.10	0.001	0.002	0.007	[-0.01, 0.02]	0.32	0.753
Pain tolerance (°C)	0.16	0.001	0.002	0.005	[-0.01, 0.01]	0.39	0.694
Pain intensity	0.50	0.002	-0.030	0.042	[-0.11, 0.05]	-0.71	0.478
Pain endurance (°C)	0.00	0.000	-0.000	0.005	[-0.01, 0.01]	-0.06	0.951

F = F-statistic, β = Coefficient, SE β = Standard Error of Coefficient, CI = confidence intervals t = t-statistic
* $p < .05$. ** $p < .01$. *** $p < .001$

SM Table 2 Regression models of pain sensitivity adjusted for trauma.

	Sum of Squares	df	Mean Square	F	R^2	RMSE	B	SE_B	t	*p*
Pain threshold (°C)										
Model	26.026	3	8.675	0.63	0.010	3.713				.597
Residual	2591.738	188	13.786							
NSSI FREQUENCY (6 months)							-0.000	0.011	-0.01	.990
TRAUMA							0.301	0.303	0.99	.322
Interaction NSSI FREQUENCYxTRAUMA							0.001	0.006	0.08	.937
Pain tolerance (°C)										
Model	7.013	3	2.338	0.38	0.006	2.495				.771
Residual	1170.621	188	6.227							
NSSI FREQUENCY (6 months)							-0.001	0.007	-0.15	.882
TRAUMA							0.124	0.204	0.61	.543
Interaction NSSI FREQUENCYxTRAUMA							0.001	0.004	0.27	.788
Pain endurance (°C)										
Model	6.555	3	2.185	0.34	0.005	2.528				.795
Residual	1201.842	188	6.393							
NSSI FREQUENCY (6 months)							-0.001	0.008	-0.13	.897
TRAUMA							-0.177	0.207	-0.86	.393
Interaction NSSI FREQUENCYxTRAUMA							0.001	0.004	0.15	.881
Pain intensity										
Model	1920.534	3	640.178	1.38	0.022	21.548				.251
Residual	87294.293	188	464.332							
NSSI FREQUENCY (6 months)							-0.120	0.064	-1.87	.063
TRAUMA							-0.008	1.76	-0.00	.996
Interaction NSSI FREQUENCYxTRAUMA							0.051	0.037	1.37	.172

Note. df = degrees of freedom; F = F-statistic; RMSE = root mean square error; B = Coefficient; SE_B = Standard Error of the coefficient; t = t-statistic.
* $p < .05$. ** $p < .01$. *** $p < .001$

SM Table 3 Regression models of pain sensitivity adjusted for borderline personality disorder severity.

	Sum of Squares	df	Mean Square	F	R^2	RMSE	B	SE_B	t	*p*
Pain threshold (°C)										
Model	86.468	3	28.823	2.11	0.030	3.696				.100
Residual	2786.393	204	13.659							
NSSI FREQUENCY (6 months)							-0.018	0.015	-1.19	.236
BPD							0.193	0.143	1.36	.177
Interaction NSSI FREQUENCYxBPD							0.004	0.004	1.12	.264
Pain tolerance (°C)										
Model	22.556	3	7.519	1.12	0.016	2.590				.342
Residual	1368.273	204	6.707							
NSSI FREQUENCY (6 months)							-0.008	0.010	-0.80	.426
BPD							0.094	0.100	0.94	.347
Interaction NSSI FREQUENCYxBPD							0.002	0.003	0.83	.405
Pain endurance (°C)										
Model	21.073	3	7.024	1.10	0.016	2.531				.352
Residual	1306.886	204	6.406							
NSSI FREQUENCY (6 months)							0.010	0.010	0.92	.358
BPD							-0.099	0.098	-1.01	.312
Interaction NSSI FREQUENCYxBPD							-0.002	0.002	-0.78	.436
Pain intensity										
Model	991.894	3	330.631	0.68	0.010	21.984				.563
Residual	98593.612	204	483.302							
NSSI FREQUENCY (6 months)							-0.063	0.088	-0.72	.473
BPD							-1.056	0.849	-1.24	.215
Interaction NSSI FREQUENCYxBPD							0.014	0.021	0.65	.517

Note. Df = degrees of freedom; F = F-statistic; RMSE = root mean square error; B = Coefficient; SE_B = Standard Error of the coefficient; t = t-statistic; BPD = Borderline personality disorder.
* $p < .05$. ** $p < .01$. *** $p < .001$

SM Table 4 Regression models of pain sensitivity adjusted for depression severity.

	Sum of Squares	df	Mean Square	F	R^2	RMSE	B	SE_B	t	*p*
Pain threshold (°C)										
Model	38.454	3	12.818	0.92	0.015	3.723				.430
Residual	2549.885	184	13.858							
NSSI FREQUENCY (6 months)							0.023	0.027	0.87	.388
DEPRESSION							0.041	0.026	1.56	.121
Interaction NSSI FREQUENCYxDEPRESSION							-0.001	0.001	-1.01	.314
Pain tolerance (°C)										
Model	18.446	3	6.149	0.99	0.016	2.488				.397
Residual	1139.123	184	6.191							
NSSI FREQUENCY (6 months)							0.019	0.018	1.04	.300
DEPRESSION							0.028	0.018	1.57	.119
Interaction NSSI FREQUENCYxDEPRESSION							-0.001	0.001	-1.21	.227
Pain endurance (°C)										
Model	4.115	3	1.372	0.21	0.004	2.542				.888
Residual	1188.603	184	6.460							
NSSI FREQUENCY (6 months)							-0.005	0.018	-0.25	.803
DEPRESSION							-0.014	0.018	-0.75	.455
Interaction NSSI FREQUENCYxDEPRESSION							0.000	0.001	0.29	.769
Pain intensity										
Model	3242.523	3	1080.841	2.34	0.037	21.508				.075
Residual	85114.328	184	462.578							
NSSI FREQUENCY (6 months)							-0.318	0.156	-2.04	.043*
DEPRESSION							-0.297	0.153	-1.94	.054
Interaction NSSI FREQUENCYxDEPRESSION							0.009	0.005	2.03	.044*

Note. df = degrees of freedom; F = F-statistic; RMSE = root mean square error; B = Coefficient; SE_B = Standard Error of the coefficient; t = t-statistic.
* $p < .05$. ** $p < .01$. *** $p < .001$

SM Table 5 Adjusted Continuous Analyses: Results of multilevel mixed-effects linear regressions for predictions of cortisol, α-amylase, systolic (SBP) and diastolic blood pressure (DBP), heart rate (HR) and heart rate variability (HRV) dependent on time and frequency of non-suicidal self-injury (NSSI) in the past 6 months, adjusted for experienced trauma.

Outcome	Model Fit		Main Effect TIME		Main Effect NSSI FREQUENCY		Interaction TIMExNSSI FREQUENCY		Main Effect TRAUMA		Interaction TIMExTRAUMA		Three-way Interaction TIMExNSSI FREQUENCYxTRAUMA	
	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Cortisol	25.97	0.038*	1.62	0.656	0.10	0.749	10.94	0.012*	0.69	0.405	2.71	0.439	2.25	0.523
α-Amylase	43.59	<0.001***	17.38	<0.001***	4.00	0.036*	5.72	0.127	0.00	0.970	2.14	0.544	3.27	0.352
SBP	43.75	<0.001***	14.95	0.002**	0.23	0.632	2.07	0.557	4.72	0.030*	11.23	0.011*	2.67	0.445
DBP	28.19	0.020*	9.37	0.025**	0.14	0.706	2.18	0.537	1.89	0.170	2.82	0.420	1.97	0.578
HR	30.15	0.002**	8.92	0.012*	0.30	0.581	6.44	0.040*	0.40	0.525	1.45	0.484	0.52	0.771
rMSSD	12.73	0.312	1.34	0.512	0.00	0.962	8.53	0.014*	0.02	0.896	2.45	0.294	0.78	0.676

Cortisol = Cortisol level in nmol/l; α-Amylase = α-Amylase level in U/ml; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; rMSSD = root mean square of successive differences.

* $p < .05$. ** $p < .01$. *** $p < .001$

SM Table 6 Adjusted Continuous Analyses: Results of multilevel mixed-effects linear regressions for predictions of cortisol, α-amylase, systolic (SBP) and diastolic blood pressure (DBP), heart rate (HR) and heart rate variability (HRV) dependent on time and frequency of non-suicidal self-injury (NSSI) in the past 6 months, adjusted for borderline personality disorder severity (BPD).

Outcome	Model Fit		Main Effect TIME		Main Effect NSSI FREQUENCY		Interaction TIMExNSSI FREQUENCY		Main Effect BPD		Interaction TIMExBPD		Three-way Interaction TIMExNSSI FREQUENCYxBPD	
	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Cortisol	35.08	0.002**	4.09	0.252	0.18	0.670	10.32	0.016*	0.85	0.356	7.28	0.064	3.44	0.329
α-Amylase	67.21	<0.001***	12.35	0.006**	17.66	<0.001***	10.47	0.015*	0.00	0.999	8.68	0.034*	7.78	0.051
SBP	38.21	<0.001***	15.05	0.002**	0.53	0.467	4.48	0.214	2.07	0.150	13.33	0.004**	0.84	0.839
DBP	27.97	0.022*	11.03	0.012**	0.11	0.741	3.14	0.371	1.77	0.183	4.50	0.212	2.36	0.500
HR	32.53	<0.001***	12.12	0.002**	0.01	0.941	1.07	0.587	0.08	0.783	3.32	0.190	0.65	0.723
rMSSD	11.16	0.430	0.88	0.645	0.12	0.727	7.22	0.027*	0.01	0.930	1.72	0.423	0.41	0.814

BPD = Borderline personality disorder; Cortisol = Cortisol level in nmol/l; α-Amylase = α-Amylase level in U/ml; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; rMSSD = root mean square of successive differences.

* $p < .05$. ** $p < .01$. *** $p < .001$

SM Table 7 Adjusted Continuous Analyses: Results of multilevel mixed-effects linear regressions for predictions of cortisol, α-amylase, systolic (SBP) and diastolic blood pressure (DBP), heart rate (HR) and heart rate variability (HRV) dependent on time and frequency of non-suicidal self-injury (NSSI) in the past 6 months, adjusted for depression severity.

Outcome	Model Fit		Main Effect TIME		Main Effect NSSI FREQUENCY		Interaction TIMExNSSI FREQUENCY		Main Effect DEPRESSION		Interaction TIMExDEPRESSION		Three-way Interaction TIMExNSSI FREQUENCYxDEPRESSION	
	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Cortisol	28.94	0.016*	4.50	0.212	0.02	0.884	11.86	0.008**	0.79	0.374	11.24	0.011*	6.19	0.103
α-Amylase	43.39	<0.001***	12.50	0.006**	1.05	0.306	4.89	0.120	0.07	0.799	5.25	0.155	2.95	0.399
SBP	40.56	<0.001***	16.26	0.001**	0.88	0.348	5.47	0.140	2.77	0.096	10.18	0.017*	1.23	0.746
DBP	27.91	0.022**	7.77	0.051	1.21	0.642	1.10	0.777	1.21	0.271	1.04	0.791	1.59	0.663
HR	34.54	<0.001***	9.70	0.008**	0.10	0.755	1.32	0.518	4.92	0.027*	2.71	0.258	0.69	0.708
rMSSD	24.62	0.010*	1.02	0.601	0.05	0.820	13.43	0.001**	7.07	0.008**	4.88	0.087	3.18	0.204

Cortisol = Cortisol level in nmol/l; α-Amylase = α-Amylase level in U/ml; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; rMSSD = root mean square of successive differences.

* $p < .05$. ** $p < .01$. *** $p < .001$

SM Table 8 Differences in mean cortisol levels for the TIME*NSSI FREQUENCY interaction. Differences in cortisol levels over time as a function of increasing frequency of non-suicidal self-injury (NSSI) are depicted in incremental steps of ten across the entire range (0-160) of observed NSSI frequency (past six months) and increasing by one for the frequency range of 61 to 70 acts of NSSI (past six months). Times of measurement were immediately after a five-minute resting phase (baseline) and immediately after heat pain stimulation (pain).

Time	NSSI Frequency	Contrast	*SE*	*p*	95% CI	
	Full range (increasing by 10)					
Pain vs. Baseline	0	-0.27	0.24	0.262	-0.73	0.20
	10	-0.14	0.21	0.509	-0.55	0.27
	20	-0.01	0.19	0.955	-0.39	0.37
	30	0.17	0.19	0.542	-0.26	0.49
	40	0.24	0.20	0.229	-0.15	0.64
	50	0.37	0.23	0.101	-0.07	0.81
	60	0.50	0.25	0.053	-0.01	1.00
	70	0.63	0.30	0.035*	0.05	1.21
	80	0.75	0.34	0.026*	0.09	1.41
	90	0.88	0.38	0.021*	0.13	1.63
	100	1.01	0.43	0.019*	0.17	1.85
	110	1.13	0.48	0.017*	0.20	2.07
	120	1.26	0.53	0.016*	0.23	2.29
	130	1.39	0.57	0.016*	0.26	2.51
	140	1.52	0.62	0.015*	0.29	2.74
	150	1.64	0.67	0.015*	0.32	2.96
	160	1.77	0.74	0.015*	0.35	3.19
	Range 61 - 70 (increasing by 1)					
Pain vs. Baseline	61	0.50	0.26	0.053	-0.01	1.00
	62	0.51	0.26	0.051	-0.00	1.02
	63	0.52	0.27	0.048*	0.00	1.04
	64	0.54	0.27	0.046*	0.01	1.06
	65	0.55	0.27	0.044*	0.01	1.08
	66	0.56	0.28	0.042*	0.02	1.10
	67	0.57	0.28	0.040*	0.03	1.12
	68	0.59	0.28	0.039*	0.03	1.14
	69	0.60	0.29	0.037*	0.04	1.16
	70	0.61	0.29	0.036*	0.04	1.18

* $p < .05$. ** $p < .01$. *** $p < .001$

Resting State Prefrontal Cortex Oxygenation in Adolescent Non-Suicidal Self-Injury – A Near-Infrared Spectroscopy Study

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Highlights

- Resting prefrontal cortex (PFC) oxygenation is decreased in adolescents with non-suicidal self-injury (NSSI) compared to healthy controls
- Lower PFC oxygenation (full sample) is associated with greater adverse childhood experiences and less health-related quality of life (HRQoL)
- On the group-level, patients show no alterations of resting state functional connectivity within the PFC
- Among other clinical variables, increased PFC connectivity (full sample) is associated with greater borderline personality pathology

Abbreviations

BMI – body mass index

BOLD - blood oxygenation level dependent

BPD – Borderline Personality Disorder

(dl)PFC – (dorsolateral) prefrontal cortex

(f)NIRS – (functional) Near-infrared spectroscopy

fMRI - functional magnetic resonance imaging

HbR – deoxygenated hemoglobin

HbT – total hemoglobin

HC – healthy controls

HRQoL – health related quality of life

NSSI – non-suicidal self-injury

O_2Hb – oxygenated hemoglobin

PIN diode – positive intrinsic negative diode

Abstract

Introduction: Neural alterations in limbic and prefrontal circuits in association with self-injurious behavior have been studied primarily in adult borderline personality disorder (BPD). In adolescent patients, research is still sparse. Here, we used resting functional near-infrared spectroscopy (NIRS) to examine oxygenation of the prefrontal cortex (PFC) and its association with symptom severity in adolescents engaging in non-suicidal self-injury (NSSI) and matched healthy controls (HC).

Methods: Adolescents (12-17 years) with recurrent episodes of NSSI (n = 170) and healthy controls (n = 43) performed a low-demanding resting-state vanilla baseline task. Mean oxygenation of the PFC and functional connectivity within the PFC, were measured using an 8-channel functional NIRS system (Octamon, Artinis, The Netherlands). Various clinical variables derived from diagnostic interviews and self-reports were included in statistical analyses to explore potential associations with PFC oxygenation and connectivity.

Results: Adolescents with NSSI showed significantly decreased PFC oxygenation compared to HC, as indexed by oxygenated hemoglobin. Lower PFC oxygenation was associated with greater adverse childhood experiences and less health-related quality of life (HRQoL). While there was no evidence for alterations in PFC connectivity in adolescents engaging in NSSI compared to HC, increased PFC connectivity in the full sample was associated with greater adverse childhood experience, a greater BPD pathology, greater depression severity and psychological burden in general, as well as lower HRQoL.

Conclusion: This study is the first to examine PFC oxygenation using NIRS technology in adolescents engaging in NSSI. Overall, results indicate small effects not specific to NSSI. Clinical implications of these findings and recommendations for further research are discussed.

1. Introduction

Non-suicidal self-injury (NSSI) is defined by the *International Society for the Study of Self-Injury* as the deliberate physical damage of own body tissue without suicidal intent (International Society for the Study of Self-injury, 2018). It excludes culturally or spiritually accepted behavior. Most commonly, adolescents injure themselves to regulate intense emotions or to cope with distress (Taylor et al., 2018). NSSI has been associated with greater emotion dysregulation (Haid-Stecher & Sevecke, 2019; In-Albon et al., 2008; Wolff et al., 2019) as well as with higher impulsiveness (Glenn & Klonsky, 2010; Hamza & Willoughby, 2019; Hamza, Willoughby, & Heffer, 2015; You, Deng, Lin, & Leung, 2016). The prevalence of NSSI in clinical samples is reported as high as 50% (Plener et al., 2018). Meta-analytic research on non-clinical samples revealed lifetime prevalence rates for single events of NSSI of 17.2%-22.1% in adolescents, 13.4% for young adults, and 5.5% for adults (Lim et al., 2019; Swannell, Martin, Page, Hasking, & John, 2014). The high prevalence of NSSI even in nonclinical populations has made the behavior a major public health concern. In the DSM-5, NSSI has been included as a condition that requires further study and is therefore acknowledged as an entity worth consideration in clinical practice and research (American Psychiatric Association, 2013). Albeit concepts considering NSSI as stand-alone diagnostic entity (also see (Ghinea et al., 2020), recurrent NSSI is one of the diagnostic criteria for borderline personality disorder (BPD) in the DSM-5 (American Psychiatric Association, 2013) and the presence, severity and duration of NSSI are important predictors for BPD development (Ghinea et al., 2019; Groschwitz et al., 2015). BPD itself is characterized by instability in affect, identity, and interpersonal relationships as alongside increased impulsivity and a tendency for risk-taking and self-harm behavior (American Psychiatric Association, 2013). The onset of BPD is frequently reported in early adolescence with prevalence rates ranging from 0.9% in 14-year old adolescents to 3.2% in 22-year old young adults among the U.S. population (Johnson, Cohen, Kasen, Skodol, & Oldham, 2008). BPD has been associated with a host of comorbid psychiatric disorders such as mood, anxiety, or substance use disorders (Grant et al., 2008; Kaess, Fischer-Waldschmidt, Resch, & Koenig, 2017; Lenzenweger, Lane, Loranger, & Kessler, 2007), a lower health-related quality of life (HRQoL), and higher distress as a function of the severity of personality pathology (Kaess, Fischer-Waldschmidt, et al., 2017). In most cases, emerging BPD during adolescence is strongly associated with NSSI (Kaess, Brunner, Chanen, 2014). Taken together, while NSSI is one of the major symptoms of BPD, in particular in adolescents, there is ongoing debate whether NSSI disorder should be considered as an independent phenomenon and diagnostic entity, requiring further investigation.

Neurobiological mechanisms underlying various psychiatric entities and phenotypes have been under extensive investigation for the past decade, in the hope that the respective studies may contribute to a better understanding of predictors of treatment outcome, improved diagnostics, and the development of tailored interventions (Ehlis, Schneider, Dresler, & Fallgatter, 2014; Oldehinkel, Francx, Beckmann, Buitelaar, & Mennes, 2013). While the neurobiological underpinnings of NSSI have not been

extensively investigated, more research has been conducted on the neurobiology of BPD. In a review of existing neuroimaging findings, prefrontal dysfunctions during impulse control tasks in adult BPD patients have been mainly found in the orbitofrontal cortex, the dorsomedial PFC, and the dorsolateral prefrontal cortex (dlPFC) (Sebastian et al., 2014). During emotion regulation, alterations of limbic brain activity in adult BPD have been reported in the amygdala, the ventral striatum, the hippocampus and the posterior cingulate cortex. While the literature is consistent regarding the frontolimbic regions involved, there is mixed evidence regarding increases and decreases of brain activation (Dudas et al., 2017; Schulze, Schmahl, & Niedtfeld, 2016). Generally speaking, abnormalities in frontolimbic networks have been found to be characteristic of BPD in *adult* patients (Chanen & Kaess, 2012; Ruocco, Amirthavasagam, Choi-Kain, & McMain, 2013). Considering that BPD is associated with high comorbidity and burden, neuroimaging studies in BPD yield limited insight into NSSI per se and neglect a developmental perspective by exclusively focusing on adults. As neuroimaging research in NSSI is sparse, studies on BPD patients still serve as a helpful proxy and direction sign for studies on NSSI in adolescents.

The few existing studies, which examined brain activation in individuals engaging in NSSI, primarily focused on task-dependent alterations in neural activity. For example, one study addressing NSSI specifically found that decreased activation in the PFC and the cingulate cortex of young adults with NSSI (n = 15) during an interference task was associated with poorer emotion regulation abilities and increased impulsivity (Dahlgren et al., 2018). Alongside difficulties in emotion regulation and impulse control, alterations in pain sensitivity in patients engaging in NSSI are well documented (Koenig, Thayer, & Kaess, 2016). Further research showed that, pain sensation was associated with brain activation in the posterior insula in participants with NSSI and healthy controls (HC), but only HC showed greater neural activity as a function of increasing pain intensity. However, out of n = 14 participants of the NSSI group, only n = 6 reported actual incidents of NSSI during the past 12 months (Bonenberger, Plener, Groschwitz, Grön, & Abler, 2015). Hence, it might be questionable whether these findings are specifically related to NSSI or are driven by other factors. As emphasized in the definition of the *International Society for the Study of Self-Injury* (International Society for the Study of Self-injury, 2018), NSSI is often related to interpersonal difficulties. In line with this, adolescents with NSSI showed increased activation of the PFC compared to HC and depressed adolescents in a social exclusion paradigm (Groschwitz, Plener, Groen, Bonenberger, & Abler, 2016). Finally, aberrant amygdala connectivity with various cortical regions was found during resting state and an emotion task (n = 24 females with NSSI and n = 20 HC) (Westlund Schreiner et al., 2017). On a brain structural level, volumetric abnormalities of the insula and the inferior frontal gyrus in female adolescent NSSI patients have been reported to be similar to those observed in adult BPD patients (Beauchaine, Sauder, Derbidge, & Uyeji, 2019). Taken together, neuroimaging studies focusing on NSSI only showed neural alterations

in association with impulse control, pain sensation, and social exclusion. Unfortunately, it is unknown whether patterns of altered brain activation are due to task-specific demands or exist during rest. The finding of volumetric abnormalities might implicate that activation patterns at rest might differ between NSSI patients and HC.

Unfortunately, the majority of neuroimaging studies in adolescents engaging in NSSI lack statistically sufficient sample sizes. One alternative method with the potential to overcome difficulties associated with sample size due to its high acceptability in patients and relative ease in application, is near-infrared spectroscopy (NIRS). NIRS recordings are based on light within the near-infrared spectrum (650-950 nm). Human scalp and skull are penetrable for light at this wavelength (Ferrari & Quaresima, 2012) and the greatest light absorbing structure in this area is the hemoglobin in the venous vessels of the cortex. Functional NIRS (fNIRS) devices measure changes of oxygenated (O_2Hb) and deoxygenated (HbR) hemoglobin over time. In comparison with (f)MRI, fNIRS is conducted with smaller devices and tolerates body movement to a greater degree, which results in a high acceptance among patients undergoing respective recordings (Lai, Ho, Lim, & Ho, 2017) – especially in adolescents. Additionally, fNIRS has superior time resolution, although struggling with exact spatial resolution and measuring only changes in activation on the cortical surface (Koike, Nishimura, Takizawa, Yahata, & Kasai, 2013). During simultaneous fMRI and fNIRS assessment, it has been shown that fNIRS measurement correlates with blood oxygenation level dependent (BOLD) signals in fMRI, suggesting equivalence of both methods when examining activation on the cortical surface (Alderliesten et al., 2014; Bulgarelli et al., 2018).

To our knowledge, there are no previous studies investigating adolescent NSSI patients using NIRS technology. Even research on BPD using NIRS is sparse. In one of the few studies, adult BPD patients (n = 10) showed increased oxygenation in the left medial PFC during a social exclusion paradigm, related to ratings of rejection and fear of abandonment (Ruocco, Medaglia, Tinker, et al., 2010). A recent study reported hemodynamic alterations (i.e. decreased O_2Hb compared to HC during a verbal fluency task) in the frontal, parietal, and temporal cortices of adult (mean age = 32 years) BPD patients (Husain et al., 2020). Young BPD patients (n = 9) with a mean age of 20 years showed a reduced slope in oxygenation of left prefrontal channels when viewing emotional (sad) pictures (Ehlis et al., 2014; Ruocco, Medaglia, Ayaz, & Chute, 2010). As BPD emerges during adolescence, investigating young patients seems crucial to disentangle effects of chronicity and long-term illness. To our knowledge, PFC oxygenation has not been investigated in adolescents explicitly engaging in NSSI only.

Unfortunately, current evidence on NSSI is barely existent and research on fMRI in NSSI reports mainly task-dependent alterations in the PFC. When extending the focus to BPD, the reported studies above present mixed findings regarding the question whether activity and/or oxygenation levels in the PFC in BPD patients are decreased or increased compared to HC. While findings on brain functional correlates of BPD from task-based studies yielded somewhat inconsistent results due to the different tasks used,

the investigation of alterations in intrinsic brain activation during resting state seems important. Further, to detect cortical changes occurring already during the early course of BPD, it is important to examine adolescents engaging in NSSI across the spectrum of BPD pathology. As pointed out in the DSM-5 (American Psychiatric Association, 2013), NSSI is an entity which should be scrutinized in present research. Hence, this study focuses on brain alterations occurring in adolescents engaging in NSSI. In addition to that, the symptom severity of BPD should also be considered to control for effects solely relying on BPD. The aim of the present study therefore was to investigate resting state oxygenation of the PFC in adolescent NSSI patients compared to HC using NIRS. We hypothesized that (1) the mean oxygenation and deoxygenation is lower in patients compared to HC during a resting-state task and that (2) the relative decrease of oxygenation would be correlated with BPD symptom severity. Furthermore, we aimed to investigate differences in connectivity strength between patients and HC in exploratory analyses.

2. Methods

2.1 Participants

Participants were recruited from the outpatient clinic for risk-taking and self-harming behavior (AtR!Sk; *Ambulanz für Risikoverhaltensweisen und Selbstschädigung* (Kaess, Ghinea, Fischer-Waldschmidt, & Resch, 2017). The specialized outpatient clinic is part of the Clinic for Child and Adolescent Psychiatry at the Center for Psychosocial Medicine at the University of Heidelberg, Germany. The study was approved by the ethical committee of the University of Heidelberg (study ID S-449/2013; study ID S-514/2015) and consisted of two appointments, a diagnostic interview and a neurobiological assessment, at baseline. Adolescents and their caregivers provided written informed consent before inclusion in the study. The recruitment period for baseline assessments started in August 2016 and ended in January 2020. The general inclusion criteria were (1) presentation at our outpatient clinic, (2) written informed consent of the adolescents and their caregivers, (3) age between 12 and 17 years and (4) fluent German language skills. General exclusion criteria for study participation were: (1) acute psychosis, (2) pregnancy, and (3) neurological, endocrinological, or cardiovascular primary diseases, potentially interfering with the neurobiological assessments. For the present analyses, only patients reporting incidents of NSSI on at least five or more days during the past 12 months were included in the analyses. NSSI incidents were defined according to the definition of the *International Society for the Study of Self-Injury* (International Society for the Study of Self-injury, 2018) and included the intentional damage of own body tissue (e.g., via cutting, biting, hitting, burning).

HC were recruited via advertisement and matched to the patient sample according to age and sex. Exclusion criteria for HC were the same as for the patient group. Further exclusion criteria for the HC group only were: lifetime self-harming behavior, lifetime psychological or psychiatric treatment, or any

current psychiatric disorder. After completing the study, all participants received an allowance of 40€ for study participation.

2.2 Procedures

In a first appointment, participants completed an extensive diagnostic assessment. The presence of NSSI, suicidal thoughts and behavior were assessed using the German version of the *Self-Injurious Thoughts and Behaviors interview* (SITBI-G; (Fischer et al., 2014; Nock, Holmberg, Photos, & Michel, 2007). To assess BPD the respective sections of the *Structured Clinical Interview for DSM-IV Personality Disorders* were queried (SCID-II; (Wittchen, Zaudig, & Fydrich, 1997). Additionally, in patients, common axis-I disorders were assessed using the semi-structured *Mini International Neuropsychiatric Interview for Children and Adolescents* (M.I.N.I.-KID; (Sheehan et al., 1998). The interview ended with the investigator´s rating of the patient´s global functioning by means of the *Global Assessment of Functioning* (GAF; (Saß, Wittchen, Zaudig, & Houben, 2003) and rating of the severity of psychiatric symptoms on the basis of the *Clinical Global Impression Scale* (CGI-S; (Busner & Targum, 2007).

HC underwent a shortened clinical interview to make sure they did not meet the criteria for any current mental disorders and were not under psychological or pharmacological treatment. In a first interview via telephone, screening questions from the SITBI-G (Fischer et al., 2014) were used to ensure that there was no history of NSSI or suicidal behavior. The *Structured Clinical Interview (non-patient edition)* (SCID-N/P) was used to check whether there was any evidence for the presence of any axis-I disorder (First, Spitzer, Gibbon, & Williams, 2002). Whenever HC reported any symptoms indicative of the presence of a psychiatric disorder, the M.I.N.I.-KID was used as an additional diagnostic tool. Those participants meeting the criteria for any psychiatric disorder were compensated for their participation in the diagnostic assessment and excluded from further study appointments.

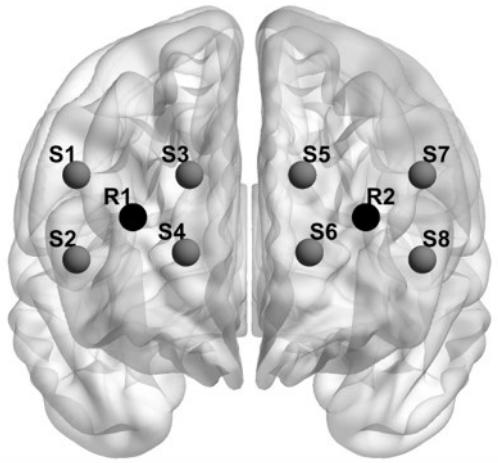
Furthermore, patients and controls answered questionnaires addressing depressive symptoms using the German version of the *Depression Inventory for Children and Adolescents* (DIKJ; (Stiensmeier-Pelster, Schürmann, & Duda, 1991). HRQoL was assessed with the *KIDSCREEN-52* (The KIDSCREEN Group Europe, 2006). Adverse childhood experiences (ACE) were assessed with the *Childhood Experiences of Care and Abuse* questionnaire (CECA-Q3; (Kaess et al., 2011). Lastly, general psychological burden was measured using the Symptom Checklist 90-Revised (SCL-90-R; (Franke, 1995).

The second part of the study was designed to investigate a host of neurobiological variables as well as the participants´ intelligence. All participants were invited for a second appointment within six weeks after their diagnostic assessment. In the beginning of the second appointment, participants´ weight and height were measured to calculate the body mass index (BMI). In addition, information about handedness, allergies, and diseases during the past three months were collected. Subsequently, the

intelligence quotient (IQ) was assessed using the *Hamburg Wechsler Intelligence Scale for Children IV* (HAWIK-IV) (Petermann & Petermann, 2007). Afterwards, prefrontal resting oxygenation in patients and HC was assessed using NIRS technology. For this purpose, a NIRS device was attached to the participants´ forehead. During a five-minute baseline task, prefrontal resting oxygenation was recorded. A detailed description of the measurement is provided below.

2.3 NIRS Measurement

PFC oxygenation was assessed using a portable 8-channel continuous-wave NIRS-system (OctaMon, Artinis, The Netherlands). NIRS is an optical neuroimaging tool consisting of light sources and receivers which are attached to the forehead of the participants with a headband. The light sources emit light in the near-infrared spectrum. The near-infrared light passes the skull cap and the cerebrospinal fluid before encountering the brain. The different tissues have unique light scattering and absorbing properties. The attenuation of light intensity is described by the modified Beer-Lambert law which calculates the absorption of light by different tissues and substances. As oxygenated hemoglobin best absorbs light with a wavelength greater than 800nm and deoxygenated hemoglobin has its highest sensitivity to light at a wavelength smaller than 800nm, the eight transmitters of the OctaMon emit light at two wavelengths, 760nm and 850nm, to detect both oxygenated and deoxygenated hemoglobin. The emitted light is recorded by two positive intrinsic negative (PIN) diode receivers with ambient light protection. The arrangement of receivers and transmitters (summarized by the term optodes) on the forehead is displayed in *Figure 1*. Inter-optode distance was fixed at 35mm. Optodes were placed onto the forehead of the participants according to the international 10-20 system for EEG electrodes placement (Jaspers, 1958). Estimated coordinates of optodes according to the Montreal Neurological Institute brain template are provided in *Figure 1*. When placing the NIRS headband, the investigator made sure that no hair was between optodes and skin impairing signal strength. The data acquisition values for signal strength should be between 3 and 97% of the intensity of the emitted light. If the percentage is close to 0%, light absorption in the tissue is so high that almost no signal reaches the receiver. A percentage close to 100% indicates that environmental light was received and measured. According to the general equation for the differential path length factor and in regard to current study protocols, the differential path length factor was set to six centimeters (Scholkmann & Wolf, 2013). A sampling rate of 50 Hz was set for each channel. The penetration depth of the NIRS light is around 17 millimeters. All participants were seated in front of a computer screen and performed a five-minute vanilla baseline task. Vanilla tasks are designed to be minimally demanding and have been shown to have consistent within- and baseline stability as well as generalizability between sessions (Jennings, Kamarck, Stewart, Eddy, & Johnson, 1992). The task in our study consisted of a colored rectangle which is presented in the middle of the screen. The rectangle contained one of the following colors: red, green, yellow, blue, purple, white. Every few seconds, it changed its color. Participants were asked to count the incidents of one specific color. After five minutes, they had to report the number of incidents.



	MNI coordinates		
	x	*y*	*z*
Receiver 1	33	61	20
Receiver 2	-35	57	22
Transmitter 1	49	45	32
Transmitter 2	49	53	8
Transmitter 3	17	69	32
Transmitter 4	18	78	10
Transmitter 5	-15	67	32
Transmitter 6	-17	77	10
Transmitter 7	-49	37	36
Transmitter 8	-57	45	8

Figure 1 Optode placement on the forehead; *Note*. R = receiver, S = source.

2.4 NIRS Data Preprocessing

Hemoglobin density values were recorded by the NIRS device and sent to a laptop via Bluetooth. There, the raw optical density measures were stored with the Oxysoft software version 3.0.103 (Artinis Medical Systems, 2016). NIRS data were segmented according to the start and end times of the color detection task. For analysis, they were imported to MATLAB (The Math Works Inc., 2015) using the oxysoft2matlab function and preprocessed with the HOMER2 toolbox (Huppert, Diamond, Franceschini, & Boas, 2009). During preprocessing, the raw optical densities were first converted to optical density (hmrIntensity2OD), which is recommended to detect and correct the data for motion artifacts (Cooper et al., 2012). Next, motion artifacts were rectified in a two-step process. First, wavelet-based motion correction with a probability threshold of $\alpha = .01$ was applied (hmrMotionCorrectWavelet). In a second step motion artifacts were corrected (hmrMotionArtifact). Additionally, high-frequent noise was removed with a Bandpass filter which removed frequencies greater than 0.5 Hz. As a final step, optical density rates were converted to hemoglobin concentration for O_2Hb, HbR and HbT ($HbT = O_2Hb + HbR$) and exported to Stata/SE software version 16.0 (StataCorp, 2019).

2.5 Statistical Analysis

All statistical analyses were conducted either in Stata/SE software version 16.0 (StataCorp, 2019) or in MATLAB (The Math Works Inc., 2015). In line with previous research (Alderliesten et al., 2014; Artemenko, Soltanlou, Ehlis, Nuerk, & Dresler, 2018; Niu et al., 2013, 2011; Pinti et al., 2018; Seidel, Carius, Kenville, & Ragert, 2017; Shi, Sakatani, Okamoto, Yamaguchi, & Zuo, 2014), we assessed the mean of O_2Hb, HbR and HbT across the standardized CDT, instead of applying event-related analyses

of NIRS data. For each hemoglobin variable (O_2Hb, HbR, HbT) mean values per channel and a grand mean value for oxygenated (O_2Hb), deoxygenated (HbR), and total hemoglobin (HbT) were calculated. For hypothesis one, t-tests were calculated for each hemoglobin variable to test for general differences between the groups. In a second step, the influence of potential confounding factors on PFC oxygenation was tested via t-tests (handedness, sex) and Pearson product-moment correlations (age, BMI, IQ). In a final step, regression models were calculated with group as predictor for differences in the hemoglobin variables of interest, while adjusting for those factors that showed significant influence on the variable of interest in step two. For hypothesis two, Pearson product-moment correlations were used to detect associations between PFC oxygenation and clinical variables, in case a respective hemoglobin variable showed significant group differences. First, correlations for variables of interest, assessed in both groups (number of BPD criteria, SCL-90-R, KIDSCREEN-52, CECA-Q3, DIKJ), were calculated in the full-sample. Second, analyses were repeated in the NSSI sample only – to avoid inflation of correlation coefficients - further including variables only assessed in patients (e.g. NSSI frequency, number of suicide attempts, CGI-S, GAF). For the exploratory analysis on connectivity within the PFC across all participants, cross-correlations between the n = 8 channels over the time of the CDT were generated with the FC-NIRS toolbox in MATLAB (Xu et al., 2015). Correlation coefficients from connectivity analyses for both groups were compared using Fisher´s z-transformation. In line with mean oxygenation parameters, associations between cross-correlation coefficients and clinical variables were assessed, following the approach outlined above. For all analyses, a significance level of $p = .05$ was applied. For visualization of the data, channel locations were provided as MNI coordinates and projected to a brain template using the BrainNet toolbox (see *Figure 1*) (Xia, Wang, & He, 2013). For visualization of results, differences in mean activation were assigned to the corresponding channels. A nifti-data format was generated in xjview (*XjView Toolbox*, 2019) and visualized using the software SurfIce (*Surf Ice*, 2019) and BrainNet (Xia et al., 2013). Visualization of the heat maps for the connectivity analyses was done within the FC-NIRS toolbox.

3. Results

3.1 Sample Characteristics

For the NSSI group, written informed consent was provided by $n = 257$ consecutive patients. Of these, $n = 242$ (94.16%) participated in the baseline assessment. $N = 227$ (88.33%) completed the NIRS assessment. $N = 195$ (75.88%) reported five or more events of NSSI during the past twelve months. $N = 25$ (9.73%) had to be excluded from the present analyses due to problems during the NIRS assessment (e.g. signal loss), resulting in a patient group of $n = 170$ (66.15%) participants. $N = 58$ adolescents provided written informed consent for the HC group. Of these $n = 49$ (84.48%) completed the baseline assessment. Reasons for not completing the baseline assessment were withdrawal of interest in study participation ($n = 7$; 12.07%), lifetime history of NSSI ($n = 1$; 1.72%), and present psychiatric disorder

(*n* = 1; 1.72%). *N* = 6 (10.34%) adolescents from the HC group were excluded from data analyses due to problems during the NIRS assessment resulting in a HC group of *n* = 43 (74.14%) adolescents. Eventually, the final study sample consisted of *n* = 170 NSSI patients and *n* = 43 HC. For a detailed description of sociodemographic and clinical characteristics of the current sample see *Table 1*. Groups differed on IQ ($t_{(211)}$ = 2.491; *p* = *.014*), with lower IQ in the patient group, and sex ($\chi^2(1)$ = 5.524; *p* = *.019*) due to the fact that the present sample only consisted of a sub sample (only those patients engaging in NSSI) and due to data loss during NIRS assessment. No significant group differences were found for age, school type, handedness, nor BMI.

Table 1 Sociodemographic and clinical characteristics by group

		NSSI	HC	*P*
N (% female)		170 (84.12)	43 (95.55)	.019
Age (SD)		15.04 (1.47)	14.74 (1.31)	.228
School type, *n* (%)				.086
	Hauptschule	19 (11.18)	1 (2.33)	
	Realschule	63 (37.06)	14 (32.56)	
	Gymnasium	61 (35.88)	27 (62.79)	
	Other	27 (15.88)	1 (2.33)	
IQ (SD)		100.83 (1.07)	106.56 (1.78)	.014
Right-handedness, *n* (%)		154 (90.59)	40 (93.02)	.617
BMI (SD)		21.58 (0.34)	20.34 (0.51)	.096
DIKJ (SD)		28.41 (9.94)	6.21 (4.03)	<.001
KIDSCREEN-52 (SD)		34.95 (6.93)	56.07 (8.43)	<.001
CECA-Q3 (SD)		0.35 (0.30)	0.02 (0.07)	<.001
SCL-90-R (SD)		1.55 (0.74)	0.21 (0.17)	<.001
CGI-S (SD)		4.96 (0.76)	-	
GAF (SD)		49.76 (8.97)	-	
Comorbidity (ICD-10), *n* (%)				
	F0X	0	-	
	F1X	40 (23.53)	-	
	F2X	0	-	
	F3X	99 (58.24)	-	
	F4X	69 (40.59)	-	
	F5X	18 (10.59)	-	
	F6X	60 (35.29)	-	
	F7X	0	-	
	F8X	2 (1.18)	-	
	F9X	54 (31.76)	-	
Number of suicide attempts (SD)				
	lifetime	10.81 (58.57)	-	
	past 12 months	1.60 (8.37)	-	
	past 6 months	1.71 (4.74)	-	
Number of NSSI events (SD)				
	past 12 months	75.56 (92.84)	-	
	past 6 months	38.37 (40.21)	-	

Note. NSSI = non-suicidal self-injury, HC = healthy controls, SD = standard deviation, IQ = intelligence quotient, BMI = body mass index, DIKJ = Depression Inventory for Children and Adolescents, KIDSCREEN-52 = Health-related quality of life questionnaire, CECA-Q3 = Childhood Experiences of Care and Abuse questionnaire, SCL-90-R = Symptom Checklist 90-Revised, CGI-S = Clinical Global Impression Scale, GAF = Global Assessment of Functioning.

N = 46 (27.06%) patients fulfilled criteria for BPD diagnosis (≥ 5 criteria). On average, NSSI patients met 3.14 (SD = 2.11) BPD criteria. Mostly, they met the criterion on recurrent suicidality and NSSI behavior (74.7%), followed by emotional instability (54.82%) and chronic feelings of emptiness (33.73%). The mean age of onset of NSSI was 12.81 years (SD = 0.13). *N* = 73 (42.94%) patients reported at least one suicide attempt. Number of suicide attempts and events of NSSI are also provided in *Table 1*. NSSI patients and HC differed significantly on all clinical measures of interest (including number of BPD criteria, depression symptoms, ACE, and HRQoL), indicating greater burden of psychopathology in adolescents engaging in NSSI (see *Table 1*).

3.2 PFC Activation

First, paired t-tests were calculated to investigate general group differences in PFC oxygenation. The NSSI group showed significantly decreased O_2Hb ($t_{(211)}$ = 2.333; p = *.021*) compared with HC. No significant group differences were found for HbR ($t_{(211)}$ = -0.654; p = *.514*) nor HbT ($t_{(211)}$ = 1.281; p = *.201*), see also (*SM Table 1*). In a second step, we aimed to investigate potential confounders of PFC oxygenation (handedness, sex, age, BMI, IQ). None of the tested variables was significantly related to O_2Hb (see *SM Table 2, SM Table 3,* and *SM Table 4*). Age and IQ were significantly correlated with HbR (age: $r_{(208)}$ = -.137, p = *.048*; IQ: $r_{(211)}$ = .141, p = *.040*) while IQ was also correlated with HbT ($r_{(211)}$ = .168, p = *.014*). None of the remaining variables (handedness, BMI, sex) was significantly related to PFC oxygenation. In a third step, regression models were calculated with group as predictor. Results from model one provided evidence that O_2Hb differed between groups ($F_{(1,211)}$ = 5.45, p = *.021*; NSSI: β = -0.353, $t_{(212)}$ = -2.33, p = .021). Model two yielded no evidence that HbR differed between groups, while adjusting for age and IQ ($F_{(3,204)}$ = 2.90, p = .036; NSSI: β = 0.144, $t_{(207)}$ = -1.21, p = *.227*; age: β = -0.063, $t_{(207)}$ = -1.91, p = .058; IQ: β = 0.007, $t_{(207)}$ = 1.97, p = .051). Finally, model three did not provide evidence of group differences in HbT, when adjusting for IQ ($F_{(2,209)}$ = 3.40, p = *.035*; NSSI: β = -0.182, $t_{(211)}$ = -0.84, p = *.400*; IQ: β = 0.015, $t_{(211)}$ = 2.29, p = *.023*). Further model characteristics are presented in *SM Table 5*. Mean PFC oxygenation and group differences for all hemoglobin variables are displayed in *Figure 2*.

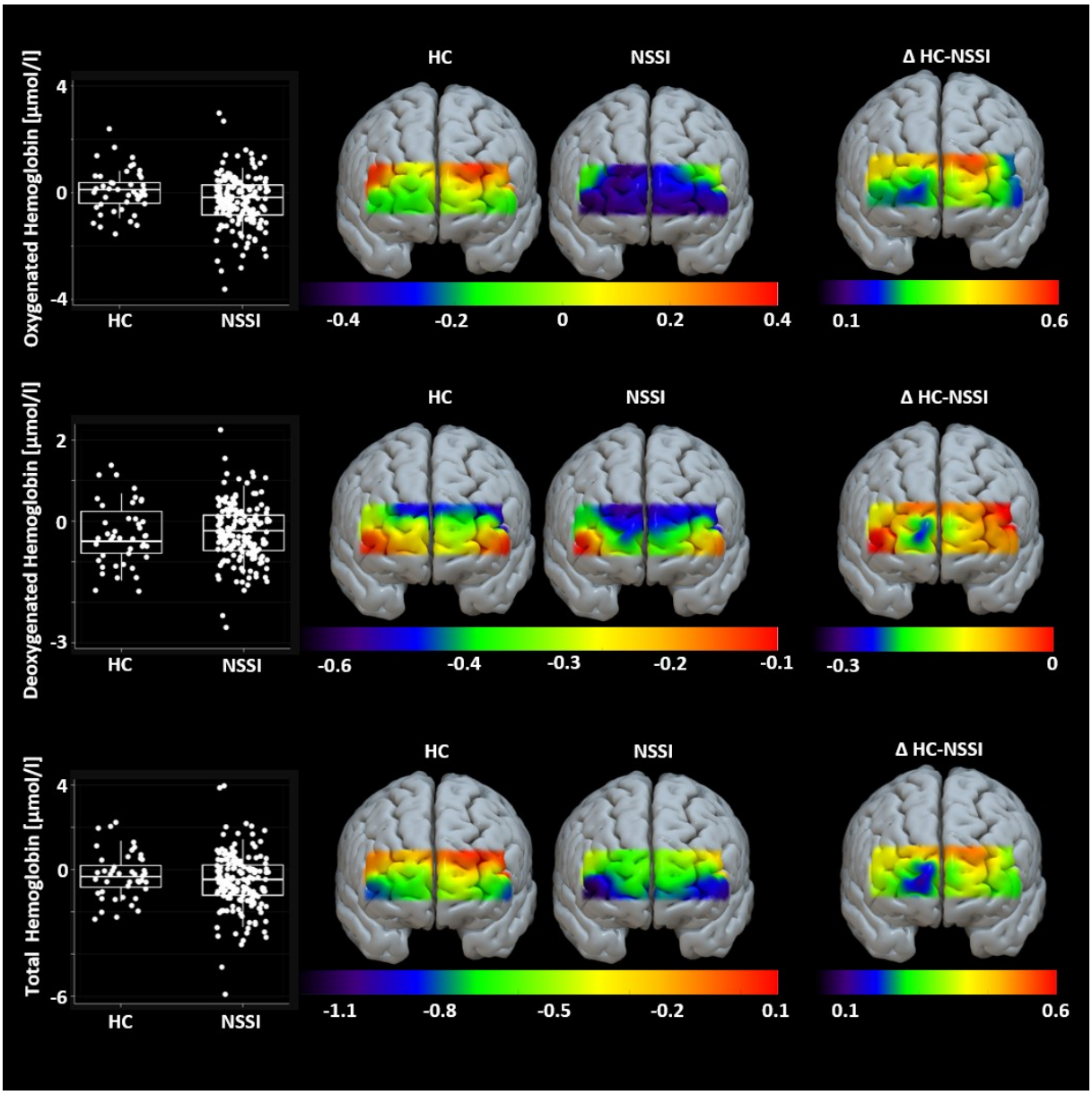


Figure 2 Differences in prefrontal brain activation across groups;

Note. Hemoglobin unit = µmol/l, HC = healthy controls, NSSI = non-suicidal self-injury group, ΔHC-NSSI = difference in hemoglobin concentration between HC and NSSI group.

3.3 Association between PFC Oxygenation and Symptom Severity

First, Pearson product-moment correlations between O_2Hb and clinical variables that were assessed in both groups were calculated. Here, significant correlations were found between O_2Hb and ACE (CECA-Q3: $r_{(187)}$ = -.155; p = *.034*) and between O_2Hb and HRQoL ($r_{(162)}$ = .154; p = *.049*; see also *Figure 3*). No significant correlations were found between O_2Hb and the number of BPD criteria met ($r_{(207)}$ = -.105; p = *.129*), general symptom severity (SCL-90-R; $r_{(188)}$ = -0.100; p = *.169*), or depression severity (DIKJ;

$r_{(186)}$ = -.100; p = *.175*). In subsequent calculations, only the patient group was included. In addition to the correlations above, the number of incidents of NSSI during the past 12 months and during the past six months, number of suicide attempts (lifetime), clinical global impression (CGI-S), and level of functioning (GAF) were included to the analysis. None of the Pearson product-moment correlations revealed a significant relationship to O_2Hb in the PFC (number of NSSI events during the past 12 months: $r_{(168)}$ = .062; p = *.420*; number of NSSI events during the past six months: $r_{(148)}$ = .050; p = *.544*; number of suicide attempts during lifetime: $r_{(70)}$ = .065; p = *.589*; clinical global impression: $r_{(160)}$ = -.113; p = *.154*; GAF: $r_{(160)}$ = .118; p = *.135*; number of BPD criteria: $r_{(164)}$ = -.022; p = *.783*; depression severity: $r_{(143)}$ = .030; p = *.719*; general symptom severity: $r_{(146)}$ = .014; p = *.870*; trauma severity: $r_{(145)}$ = -.109; p = *.189*; HRQoL: $r_{(122)}$ = .024; p = *.794*).

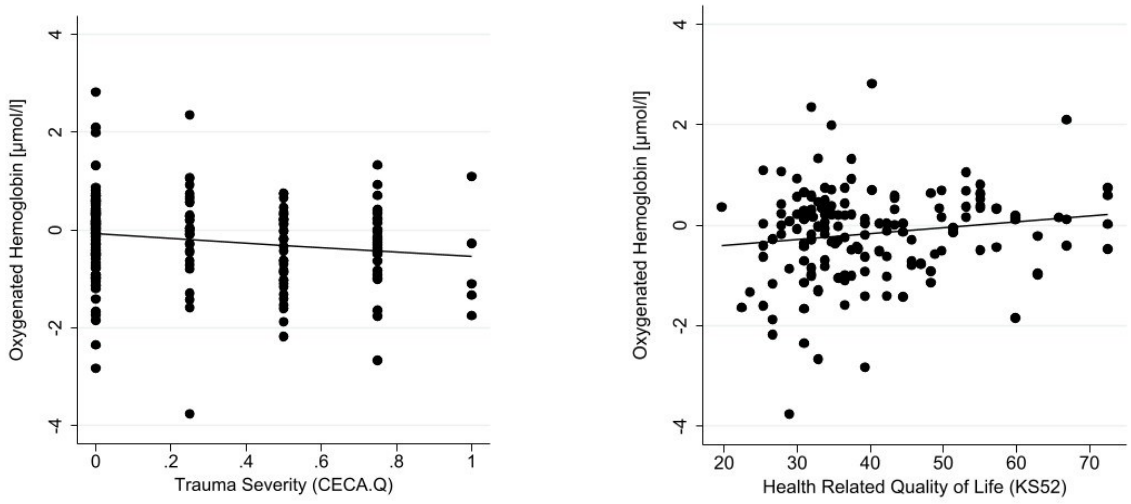


Figure 3 Significant correlation between O_2Hb and Adverse Childhood Experiences and Health-Related Quality of Life; *Note*. CECA = Childhood Experiences of Care and Abuse questionnaire, KIDSCREEN-52 = Health-related quality of life questionnaire.

3.4 Analysis of Functional Connectivity

Due to technical problems, n = 2 NSSI patients had to be excluded from the connectivity analyses resulting in n = 168 patients and n = 43 HC that were subject to the analysis. Connectivity analyses within the PFC were calculated only for O_2Hb, as this measure differentiated the NSSI and HC group in the preceding analyses. Cross-correlation coefficients were determined between all channels for each group (see *SM Table 6* and *SM Table 7*). Subsequently, Fisher´s z-transformation was conducted, and PFC connectivity values were compared between groups. Whereas almost all channels revealed a significant connectivity between channels on the within-group level (see *Figure 4*), no differences between groups were found (see *SM Table 8*). As illustrated in *Figure 4*, connectivity measures were

descriptively higher in NSSI patients (range: $r_{(166)} = .488 – r_{(166)} = .680$; all $p<.0001$) compared to HC (range: $r_{(41)} = .285 – r_{(41)} = .613$; $p = .064 – p < .001$). Both groups showed especially high connectivity between channels covering the orbitofrontal cortex area and slightly lower connectivity in the dorsolateral cortex areas.

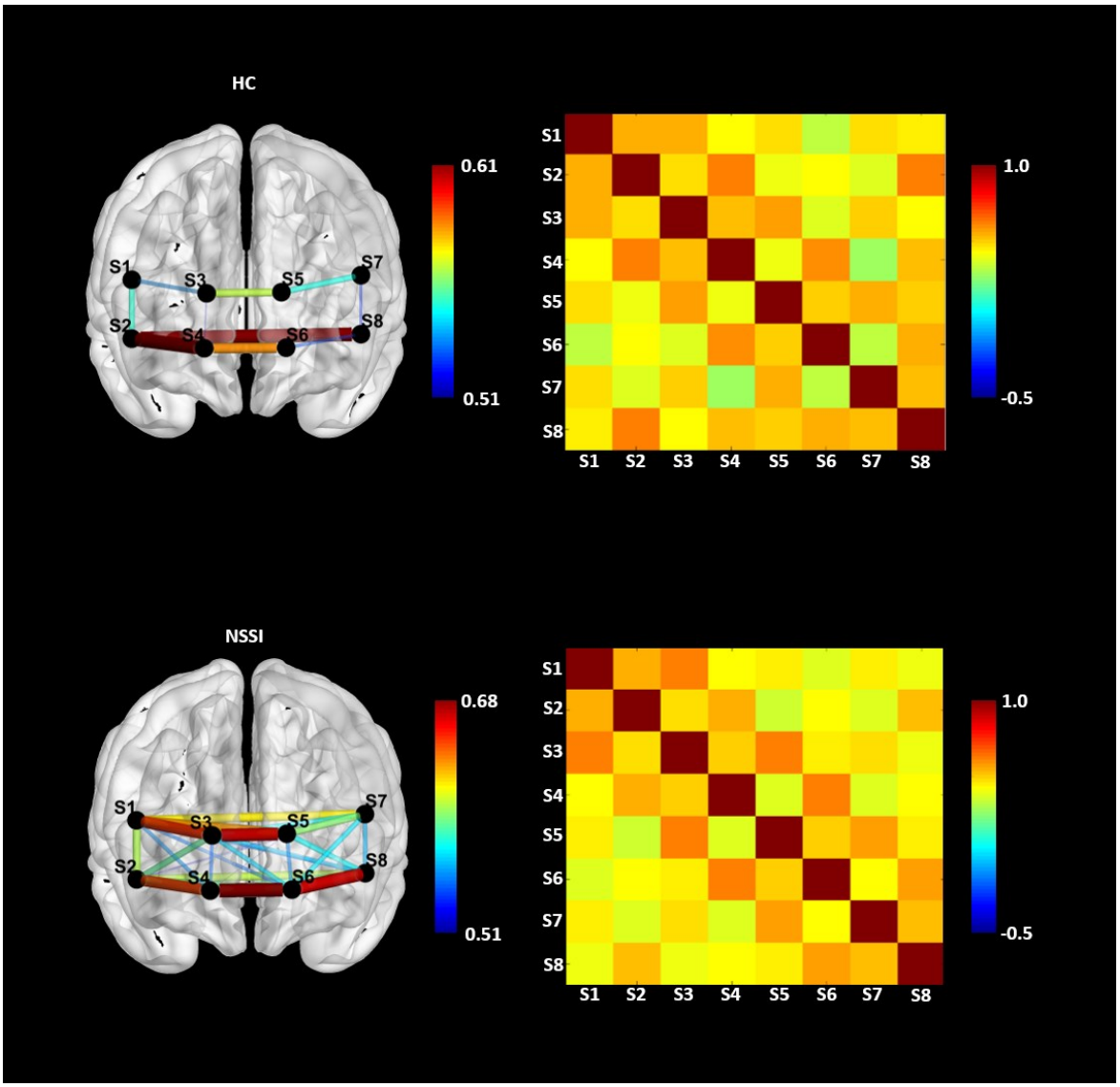


Figure 4 Brain connectivity pattern of cross correlations between HC and NSSI patients;

Note. In the left only the topmost correlations with correlation coefficients greater than r = .50 are displayed.

When examining associations between PFC connectivity and clinical variables across all participants, positive correlations were found between the number of BPD criteria and O_2Hb connectivity in n = 6 inter- and intrahemispheric channel pairs (range: $r_{(205)} = .138$, $p = .047 – r_{(205)} = .177$, $p = .011$), depressive symptoms and connectivity in $n = 3$ mostly left hemispheric channel pairs (range: $r_{(184)} = .148$, $p = .045 – r_{(184)} = .179$), general psychological burden (SCL-90-R) and connectivity in $n = 3$ channel pairs (range: $r_{(187)} = .154$, $p = .035 – r_{(187)} = .166$, $p = .023$), and ACE (CECA-Q3) in $n = 4$

channel pairs (range: $r_{(186)} = .155$, $p = .034$ – $r_{(186)} = .184$, $p = .012$). Negative correlations were found between HRQoL (KIDSCREEN-52) and connectivity between $n = 8$ channel pairs (range: $r_{(161)} = -.157$, $p = .046$ – $r_{(161)} = -.228$, $p = .004$). We did not find evidence of associations between global clinical impression (CGI-S) and PFC connectivity. Associations are illustrated for the number of BPD criteria, ACE and HRQoL in *Figure 5*. All correlations are provided in the *Supplemental Material* (*SM Table 9* to *SM Table 14*).

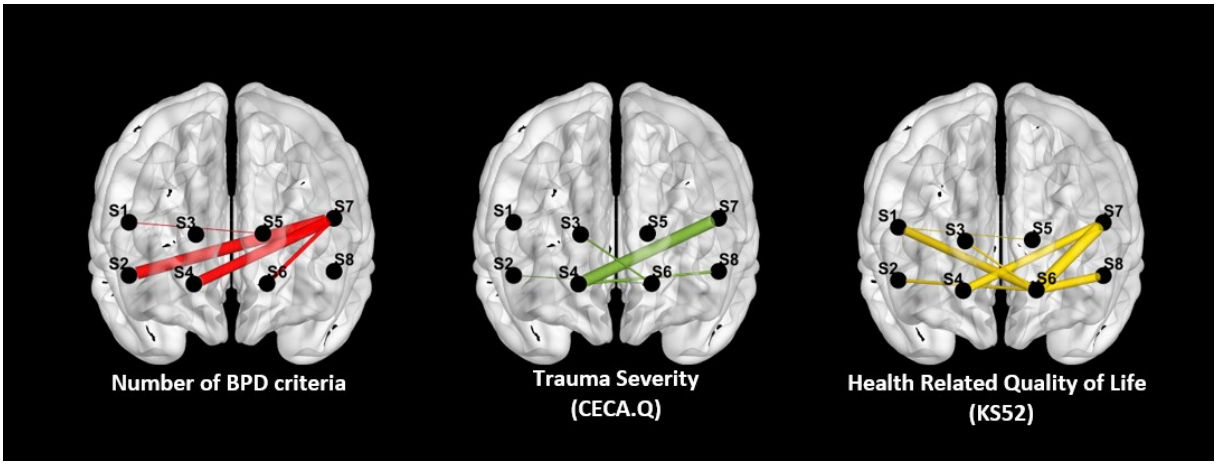


Figure 5 Significant correlations between Connectivity Measures and Clinical Measures; *Note*. Only significant correlations with $p < .50$ are displayed. Positive correlations between number of BPD criteria and adverse childhood experience and negative correlations between health-related quality of life with prefrontal cortex connectivity.

4. Discussion

To our knowledge, this is the first study to investigate resting-state PFC oxygenation in adolescents with NSSI. Analyses revealed significantly decreased O_2Hb concentration in the PFC in the NSSI group compared to HC. No group differences were found for HbR and HbT. Thus, hypothesis one was partially confirmed. For hypothesis two, we found decreased O_2Hb concentrations associated with ACE as well as lower HRQoL. Unlike hypothesized, there was no association with BPD pathology. Exploratory analyses on PFC connectivity revealed no significant group differences. However, correlations of the connectivity measures with clinical variables revealed that stronger connectivity was associated with greater BPD pathology, more depressive symptoms, higher general psychological burden and ACE as well as a lower HRQoL.

Our finding of reduced PFC oxygenation in adolescents engaging in NSSI adds some clarity to previous findings on brain functional correlates of NSSI and BPD that were somewhat inconsistent. Several studies using task-based fMRI found *increased* activation of different parts of the PFC, including dlPFC (Dudas et al., 2017), orbitofrontal PFC (Vega et al., 2018), and dmPFC (Malejko et al., 2019) in adults with NSSI and/or BPD. In contrast, meta-analytic research in BPD patients (Schulze et al., 2016) as well as one of the few studies in younger patients with NSSI (n = 15) found *decreased* activation of the PFC during an interference task (Dahlgren et al., 2018). Our finding is in line with the sparse existing literature in adolescents and potentially illustrates a developmental effect; we suggest that activity of the PFC might switch from under- (adolescence) to over- (adult) activation as a function of age and development. Importantly, however, most of the existing studies – in adults and adolescents - have been conducted in relatively small samples, did not carefully distinguish NSSI and BPD, and group differences in brain activation have been found in task-based designs only. Our study is one of the first to show decreased resting-state PFC oxygenation in a relatively large sample of well-characterized adolescents engaging in NSSI.

As our study examined differences in resting-state prefrontal activation and not of task-dependent alterations, a comparison to findings on structural changes in the PFC seems warranted. For example, studies using structural MRI to characterize brain alterations in patients with NSSI and BPD have shown reduced grey matter volume of the bilateral dlPFC as well as the left orbitofrontal cortex (Brunner et al., 2010) and the right orbitofrontal cortex (Chanen et al., 2008). In the same vein, a meta-analysis found reduced grey matter volume of the right inferior frontal gyrus in BPD patients, with greater alterations in older samples (Schulze et al., 2016). A recent study in adolescents with NSSI, however, found decreased brain volume of the insula and the anterior cingulate cortex but no differences in PFC volume compared to healthy adolescents (Ando et al., 2018). Finally, while not caption by our current investigation using NIRS, alterations of deeper brain structures, indexed e.g. by a decreased volume of the pituitary gland or decreased activity of the limbic system, were shown in adolescent and adult BPD patients (Chanen & Kaess, 2012; Dudas et al., 2017; Groschwitz & Plener, 2012; Jovev et al., 2012;

Ruocco et al., 2013; Whittle et al., 2009). Taken together, research consistently shows grey matter volume losses in patients with NSSI and/or BPD. These structural differences might contribute to decreased PFC oxygenation during a rest. Furthermore, it might explain prefrontal overaction during specific tasks, as described in the studies above, to compensate structural deficits. However, further research, integrating structural and functional measures, is needed to support these assumptions.

We have shown that NSSI patients and HC differed significantly on mean O_2Hb only. Most studies that investigated alterations in activation using NIRS focused on O_2Hb only and did not report values for the other two hemoglobin variables or only in the respective supplemental material (Husain, Tang, et al., 2020; Ruocco, Medaglia, Ayaz, et al., 2010; Ruocco, Medaglia, Tinker, et al., 2010; Ruocco et al., 2016). Generally speaking, differences between groups are easier to detect when focusing on O_2Hb (Ferreri, Bigand, Perrey, & Bugaïska, 2014). This phenomenon is routed in the physiology of brain activity and reactivity. NIRS technology measures O_2Hb and HbR concentration changes in the venous vessels. After O_2Hb has arrived in the cells through arterial vessels, the oxygen is partially consumed by the cells resulting in O_2Hb and HbR in the venous vessels. Whenever more oxygen is demanded, the cells are overflooded with O_2Hb. As the consumption of O_2Hb rises, the amount of HbR in the venous vessels rises simultaneously. But because of the overflow of O_2Hb, the ratio of O_2Hb in the venous vessels increases even more (Obrig, Rossi, Telkemeyer, & Wartenburger, 2010). Hence, it is important to report both O_2Hb and HbR and related to this its sum, HbT. But as differences in O_2Hb are bigger than in HbR, it is easier to detect significant results. Hence, the reported group differences in O_2Hb – also of small effect size - support the assumption that PFC activation is decreased in NSSI patients compared to HC during resting-state. Surprisingly, we did not find evidence of associations between PFC oxygenation and specific NSSI behavior. However, there was some evidence that decreased oxygenation was associated with ACE and HRQoL. Longitudinal treatment studies are warranted, assessing the clinical relevance of these associations. In a very first study, the influence of psychotherapy on cortical activation in BPD patients was examined (Ruocco et al., 2016). An increase in cortical activation in patients (n = 18) that reduced their self-harming behavior the most over the course of dialectical behavioral therapy was found. This finding indicates that changes in prefrontal oxygenation may help to monitor treatment progress and outcome.

Recent efforts in neuroimaging research, aimed at elucidating neural concomitants of psychiatric symptomatology, have been directed at studying not only regional brain activation, but rather the interplay between different regions, i.e., brain connectivity. While we found no group differences in PFC connectivity comparing NSSI and HC, O_2Hb connectivity was associated with a range of clinical measures, indicating higher connectivity in case of worse outcome - a seemingly counterintuitive finding. However, the only study examining resting-state connectivity in NSSI, has found greater connectivity between amygdala and the supplementary motor area as well as dorsal anterior cingulate to be associated with NSSI (compared to HC) (Westlund Schreiner 2017). Associations between

connectivity and other clinical variables have not been investigated, limiting the integration of the present findings. Potentially, higher connectivity between prefrontal regions might act as a compensatory mechanism in those patients with greater psychopathology in order to counteract overactivation of the limbic system (Niedtfeld et al., 2010; Plener, Kapusta, Kölch, Kaess, & Brunner, 2012), a speculative hypothesis that remains to be tested in future fMRI studies in NSSI and adolescent BPD.

Albeit the known advantages of NIRS technology, including high feasibility and acceptance by patients, facilitating recruitment of larger samples, this method is limited to the study of cortical brain oxygenation - in the present study even limited to PFC brain oxygenation. While the literature mentioned in the present report, as well as the data, speak for the relevance of the PFC for neurobiological research on NSSI and BPD, findings from MRI studies have also emphasized the importance of limbic structures. In fact, the interplay between the limbic and frontal regions has been demonstrated to be crucial for emotion regulation (e.g.,Etkin, Büchel, & Gross, 2015) and as such might be a promising target when investigating neurobiological mechanisms underlying NSSI. Therefore, more research on brain connectivity including prefrontal and limbic regions underlying NSSI as a precursor of BPD, is needed.

Studying resting-state PFC oxygenation may provide insights in basic physiological structures that may determine the phenotype of NSSI and BPD. The current study provides evidence that resting-state NIRS technology is able to differentiate between healthy and psychiatric samples. A critical question in resting state analysis is the required length of the paradigm to be sure that activity levels have stabilized. Research using NIRS technology has shown that signal stabilization occurs within the first minute of application, readily captured by our five minute paradigm (Geng, Liu, Biswal, & Niu, 2017). Further, research has illustrated the general suitability of NIRS devices to index resting-state activation (Niu et al., 2011). Traditionally, connectivity analyses are based on MRI technology or electroencephalogram. When comparing BPD patients either during resting-state or during tasks, altered connectivity was found with fMRI (Das, Calhoun, & Malhi, 2014; Zhu et al., 2017). Further, lateral asymmetries were illustrated using electroencephalogram (Beeney, Levy, Gatzke-Kopp, & Hallquist, 2014; LeBoeuf, Guilé, Labelle, & Luck, 2016). In the present study, no significant differences in connectivity between the two groups were found. However, it should be emphasized that this sample did not consist of BPD patients solely but of adolescents engaging in NSSI, across the spectrum of BPD pathology. This might explain why no significant differences were detected although patterns of connectivity differed.

As most research on PFC alterations in association with self-injurious behavior is limited to adult BPD patients, the present research extends our understanding of adolescent NSSI. It shows that findings from adult BPD samples are comparable to adolescent NSSI samples. In both groups, alterations in the PFC were found with decreased activation in patients compared to HC regardless of the imaging modality (MRI or NIRS) (Brunner et al., 2010; Chanen et al., 2008; Dudas et al., 2017; Husain, Tang, et al., 2020; Ruocco, Medaglia, Ayaz, et al., 2010; Ruocco, Medaglia, Tinker, et al., 2010; Ruocco et al., 2016;

Schulze et al., 2016). Hence, we suggest that decreased PFC activation is a potential feature of NSSI, independent of BPD. As these alterations are readily evident in adolescents, it can be assumed that prefrontal hypoactivation is not a consequence of the long-term course of comorbid psychopathology (such as BPD) or psychotropic medication intake for years - although an increase of functional aberrations in limbic systems in BPD patients has been found with older age (Schulze et al., 2016). One core feature of BPD and NSSI are difficulties in emotion regulation (Carpenter & Trull, 2012; Dixon-Gordon et al., 2015; Glenn & Klonsky, 2009). Successful emotion regulation requires increased activity of the PFC (Fusar-Poli et al., 2009; Golkar et al., 2012; Kim, Cornwell, & Kim, 2012; Kober et al., 2008; Ochsner, Bunge, Gross, & Gabrieli, 2002). Our finding of reduced PFC oxygenation at rest might be one contributing factor underlying these deficits in executive function. However, difficulties in emotion regulation have been shown in various psychiatric disorders, including depression, anxiety, substance use and eating disorders (Sloan et al., 2017). For example, adult depressive patients showed lower integral values over the course of a verbal fluency task compared to controls (Husain et al., 2020). Studies including clinical controls and explicit measures of emotion regulation are warranted to address the specificity versus generalizability of findings. Hence, the hypoactivation of the PFC found in this study might not represent a specific feature of BPD but supports the assumption that impaired emotion regulation plays a crucial role in NSSI as well as general psychopathology. The finding that self-perceived HRQoL decreased with a decline of PFC activation emphasizes that there might be a relationship between general well-being and prefrontal oxygenation. Further research is required to investigate the relationship between well-being, symptomatology and brain activation in detail utilizing longitudinal designs.

The present study has several limitations that should be considered when interpreting the results. First, although NIRS devices have a great spatial resolution, exact optode placement could only be estimated as we were not able to determine exact positioning with a 3D digitizer. Therefore, exact interpretation of region of interests for connectivity analysis was not possible. Second, as previously mentioned, the focus of this study relied on the PFC surface only. The PFC is known as the neural control center for integrating information from different brain regions. Hence, connectivity and activity analyses covering different regions of interest would be very informative and should be considered in future studies examining BPD and NSSI in adolescents. Furthermore, activation patterns and connectivity analyses could have revealed more distinctive results when using a fNIRS device with a higher density of channels covering the PFC, such as those used in other studies with up to 48 channels covering prefrontal areas (Zhang et al., 2020). Additionally, long-term effects of therapeutic interventions will help to provide more knowledge on the relationship of certain symptoms with neural alterations as well as the durability of these improvements. A further limiting factor of the study sample is that it mainly consisted of female, help-seeking adolescents. Hence, sex differences in PFC oxygenation could not be examined. When considering previous research on sex differences in brain activation, it becomes clear that differences may be related to certain tasks. For example, sex differences in brain activation have been

found for working memory tasks (Li, Luo, & Gong, 2010; Schmidt et al., 2009), for the processing of emotional stimuli (Stevens & Hamann, 2012) as well as for visuo-spatial, memory and emotion tasks (Al Ryalat, 2017). Therefore, it would have been interesting to investigate sex differences in adolescent NSSI patients, especially as self-harming behavior often differs between sexes (Andover, Primack, Gibb, & Pepper, 2010). Lastly, the study sample consisted of a NSSI group and a HC group only. To disentangle whether hypoactivation of the PFC in the NSSI group is specifically related to NSSI or general psychopathology, future studies in the field should include an additional clinical control group.

5. Conclusion

In conclusion, the present results emphasize the importance to investigate neural alterations in young patients with NSSI and provide some insight on underlying etiological factors that may contribute to impaired emotion regulation, associated with NSSI, comorbid psychopathology, and decreased health related quality of life. Future studies are needed to investigate the specificity of the reported neural alterations in patients with NSSI, including samples of clinical controls, and their long-term clinical trajectories in association with comorbid psychiatric diagnoses.

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Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplemental Material

SM Table 1 T-tests of the prefrontal oxygenation between groups

	NSSI		HC				
	M	SD	M	SD	t-statistic	df	p
Oxygenated hemoglobin	-0.290	0.940	0.064	0.625	2.333	211	.021
Deoxygenated hemoglobin	-0.273	0.699	-0.350	0.663	-0.654	211	.514
Total hemoglobin	-0.562	1.344	-0.287	0.852	1.281	211	.201

Note. Hemoglobin in µmol/l, M = mean, SD = standard deviation, df = degrees of freedom.

SM Table 2 T-test of the prefrontal oxygenation for handedness of participants

Handedness	right		left				
	M	SD	M	SD	t-statistic	df	p
Oxygenated hemoglobin	-0.221	0.878	-0.193	1.089	0.128	211	.899
Deoxygenated hemoglobin	-0.288	0.681	-0.299	0.803	-0.067	211	.947
Total hemoglobin	-0.508	1.219	-0.492	1.695	0.054	211	.957

Note. Hemoglobin in µmol/l, M = mean, SD = standard deviation, df = degrees of freedom.

SM Table 3 T-test of the prefrontal oxygenation for the sex of participants

Sex	female		male				
	M	SD	M	SD	t-statistic	df	p
Oxygenated hemoglobin	-0.188	0.897	-0.420	0.874	1.284	211	.201
Deoxygenated hemoglobin	-0.263	0.704	-0.455	0.578	1.368	211	.173
Total hemoglobin	-0.451	1.273	-0.875	1.151	1.662	211	.098

Note. Hemoglobin in µmol/l, M = mean, SD = standard deviation, df = degrees of freedom.

SM Table 4 Pearson product-moment correlation between prefrontal oxygenation and age, BMI, and intelligence

	Age			BMI			IQ		
	Obs.	r	p	Obs.	r	p	Obs.	r	p
Oxygenated hemoglobin	209	-0.013	.854	210	-0.128	.064	212	0.128	.064
Deoxygenated hemoglobin	209	-0.137	.048	210	0.089	.201	212	0.141	.040
Total hemoglobin	209	-0.084	.228	210	-0.043	.540	212	0.168	.014

Note. Hemoglobin in µmol/l, Obs. = number of observations, M = mean, SD = standard deviation, df = degrees of freedom, BMI = body mass index, IQ = intelligence quotient.

SM Table 5 Regression models of the prefrontal oxygenation

	Sum of Squares	df	Mean Square	F	R^2	RMSE	Coefficient	Standard Error	t	p
Oxygenated hemoglobin										
Model	4.278	1	4.278	5.45	0.025	0.886				.021
Residual	165.737	211	0.785							
Group (NSSI)							-0.353	0.151	-2.33	.021
Deoxygenated hemoglobin										
Model	4.044	3	1.348	2.9	0.041	0.682				.036
Residual	94.851	204	0.465							
Group (NSSI)							0.144	0.119	1.21	.227
Age							-0.063	0.033	-1.91	.058
IQ							0.007	0.004	1.97	.051
Total hemoglobin										
Model	10.577	2	5.289	3.4	0.035	0.032				.035
Residual	324.93	209	1.555							
Group (NSSI)							-0.182	0.216	-0.84	.400
IQ							0.015	0.007	2.29	.023

Note. df = degrees of freedom, F = F-statistic, RMSE = root mean square error, t = t-statistic, IQ = intelligence quotient.

SM Table 6 Mean values and cross correlation coefficients for O_2Hb between channels in NSSI patients

Channel	M	SD	df	1	2	3	4	5	6	7	8
1	0.345	1.376	166	1.000							
				-							
2	-0.155	1.411	166	.603	1.000						
				< .00001	-						
3	-0.011	1.902	166	.651	.588	1.000					
				< .00001		-					
4	-0.191	1.127	166	.553	.649	.545	1.000				
				< .00001	*< .00001*	*< .00001*	-				
5	0.380	1.759	166	.631	.517	.668	.511	1.000			
				< .00001	*< .00001*	*< .00001*	*< .00001*	-			
6	0.042	1.767	166	.548	.591	.564	.680	.550	1.000		
				< .00001	*< .00001*	*< .00001*	*< .00001*	*< .00001*	-		
7	0.250	1.584	166	.619	.521	.570	.488	.596	.567	1.000	
				< .00001	*< .00001*	*< .00001*	*< .00001*	*< .00001*	*< .00001*	-	
8	-0.151	1.281	166	.548	.605	.562	.589	.573	.670	.560	1.000
				< .00001	*< .00001*	*< .00001*	*< .00001*	*< .00001*	*< .00001*	*< .00001*	-

Note. Italic values indicate p-values. M = mean value; SD = standard deviation; df = degrees of freedom.

SM Table 7 Mean values and cross correlation coefficients for O_2Hb between channels in HC

Channel	M	SD	df	1	2	3	4	5	6	7	8
1	-0.106	1.516	41	1.000							
				-							
2	-0.424	0.974	41	.554	1.000						
				.000	-						
3	-0.459	1.297	41	.539	.483	1.000					
				.000	*.001*	-					
4	-0.348	0.622	41	.429	.613	.517	1.000				
				.004	*.000*	*.000*	-				
5	-0.205	1.551	41	.466	.395	.569	.399	1.000			
				.002	*.009*	*.000*	*.008*	-			
6	-0.390	1.176	41	.338	.416	.385	.586	.491	1.000		
				.027	*.006*	*.011*	*.000*	*.001*	-		
7	0.000	1.144	41	.467	.372	.499	.285	.553	.344	1.000	
				.002	*.014*	*.001*	*.064*	*.000*	*.024*	-	
8	-0.382	0.813	41	.454	.613	.436	.513	.490	.532	.528	1.000
				.002	*.000*	*.003*	*.000*	*.001*	*.000*	*.000*	-

Note. Italic values indicate p-values. M = mean value; SD = standard deviation; df = degrees of freedom.

SM Table 8 Z-values for cross correlation coefficients in O_2Hb between NSSI patients and HC

Channel	1	2	3	4	5	6	7	8
1	-							
	-							
2	0.420	-						
	.675	-						
3	0.990	0.840	-					
	.322	*.401*	-					
4	0.930	0.340	0.220	-				
	.352	*.734*	*.826*	-				
5	1.350	0.880	0.910	0.800	-			
	.177	*.379*	*.363*	*.424*	-			
6	1.500	1.340	1.320	0.890	0.460	-		
	.134	*.180*	*.187*	*.374*	*.646*	-		
7	1.230	1.060	0.560	1.360	0.360	1.610	-	
	.219	*.289*	*.576*	*.174*	*.719*	*.107*	-	
8	0.710	1.330	0.960	0.620	0.660	1.240	0.260	-
	.478	*.184*	*.337*	*.535*	*.509*	*.215*	*.795*	-

Note. Italic values indicate p-values; degrees of freedom = 207.

SM Table 9 Pearson correlation coefficients between prefrontal connectivity for O_2Hb and number of BPD criteria

Channel	1	2	3	4	5	6	7	8
1	1.000							
2	0.031	1.000						
	.655							
3	0.051	0.098	1.000					
	.463	*.162*						
4	0.067	-0.001	0.006	1.000				
	.335	*.994*	*.933*					
5	0.138	0.007	0.038	-0.019	1.000			
	.047	*.924*	*.584*	*.787*				
6	0.080	0.112	0.038	0.094	-0.047	1.000		
	.254	*.109*	*.592*	*.178*	*.506*			
7	0.091	0.177	0.077	0.171	0.091	0.149	1.000	
	.193	*.011*	*.273*	*.014*	*.193*	*.033*		
8	0.069	0.036	0.042	0.072	-0.022	0.020	0.015	1.000
	.326	*.606*	*.551*	*.303*	*.757*	*.774*	*.826*	

Note. Italic values indicate p-values; degrees of freedom = 205.

SM Table 10 Pearson correlation coefficients between prefrontal connectivity for O_2Hb and Clinical Global Impression Scale (CGI-S)

Channel	1	2	3	4	5	6	7	8
1	1.000							
2	-0.031	1.000						
	.696							
3	-0.007	0.052	1.000					
	.927	*.514*						
4	-0.043	-0.122	0.046	1.000				
	.590	*.124*	*.566*					
5	0.017	-0.108	0.007	-0.031	1.000			
	.831	*.173*	*.932*	*.700*				
6	0.043	0.035	0.018	0.040	0.104	1.000		
	.586	*.664*	*.827*	*.614*	*.190*			
7	-0.016	-0.014	0.039	-0.099	0.010	0.022	1.000	
	.839	*.858*	*.623*	*.214*	*.904*	*.785*		
8	0.021	-0.028	-0.008	-0.035	0.005	0.058	0.059	1.000
	.795	*.725*	*.924*	*.665*	*.954*	*.466*	*.461*	

Note. Italic values indicate p-values; degrees of freedom = 158.

SM Table 11 Pearson correlation coefficients between prefrontal connectivity for O_2Hb and depression ratings (DIKJ)

Channel	1	2	3	4	5	6	7	8
1	1.000							
2	0.019	1.000						
	.796							
3	0.115	0.013	1.000					
	.118	*.858*						
4	-0.008	-0.079	0.030	1.000				
	.918	*.283*	*.686*					
5	0.102	0.044	0.093	0.020	1.000			
	.166	*.551*	*.209*	*.792*				
6	0.118	0.131	0.108	0.090	-0.011	1.000		
	.109	*.075*	*.141*	*.223*	*.879*			
7	0.093	0.086	0.100	0.148	0.030	0.165	1.000	
	.205	*.243*	*.176*	*.045*	*.689*	*.024*		
8	0.013	-0.021	0.085	0.115	0.002	0.179	0.017	1.000
	.858	*.773*	*.248*	*.119*	*.983*	*.014*	*.820*	

Note. Italic values indicate p-values; degrees of freedom = 184.

SM Table 12 Pearson correlation coefficients between prefrontal connectivity for O_2Hb and Symptom Checklist 90 Revised (SCL-90-R)

Channel	1	2	3	4	5	6	7	8
1	1.000							
2	0.039	1.000						
	.597							
3	0.076	-0.003	1.000					
	.302	*.963*						
4	0.018	-0.038	0.030	1.000				
	.812	*.602*	*.680*					
5	0.101	0.047	0.023	-0.012	1.000			
	.167	*.523*	*.753*	*.866*				
6	0.154	0.166	0.127	0.059	-0.028	1.000		
	.035	*.023*	*.083*	*.417*	*.705*			
7	0.072	0.099	0.074	0.086	0.061	0.162	1.000	
	.328	*.176*	*.313*	*.241*	*.408*	*.026*		
8	-0.003	-0.031	0.040	0.037	-0.075	0.127	-0.006	1.000
	.963	*.671*	*.584*	*.616*	*.303*	*.083*	*.934*	

Note. Italic values indicate p-values; degrees of freedom = 187.

SM Table 13 Pearson correlation coefficients between prefrontal connectivity for O_2Hb and trauma severity (CECA.Q)

Channel	1	2	3	4	5	6	7	8
1	1.000							
2	-0.075	1.000						
	.307							
3	0.073	0.075	1.000					
	.322	*.306*						
4	-0.031	-0.085	0.043	1.000				
	.678	*.244*	*.558*					
5	0.094	-0.016	0.041	0.066	1.000			
	.200	*.824*	*.573*	*.367*				
6	0.122	0.155	0.158	0.086	0.041	1.000		
	.097	*.034*	*.031*	*.241*	*.573*			
7	0.057	-0.011	0.122	0.184	0.052	0.090	1.000	
	.441	*.877*	*.095*	*.012*	*.476*	*.222*		
8	0.075	0.031	0.138	0.160	0.059	0.098	-0.055	1.000
	.306	*.676*	*.059*	*.028*	*.436*	*.180*	*.457*	

Note. Italic values indicate p-values; degrees of freedom = 186.

SM Table 14 Pearson correlation coefficients between prefrontal connectivity for O_2Hb and health-related quality of life (KIDSCREEN-52)

Channel	1	2	3	4	5	6	7	8
1	1.000							
2	-0.056	1.000						
	.474							
3	-0.124	-0.077	1.000					
	.114	*.331*						
4	-0.076	0.047	-0.043	1.000				
	.338	*.553*	*.584*					
5	-0.161	-0.110	-0.109	-0.092	1.000			
	.040	*.164*	*.167*	*.245*				
6	-0.208	-0.175	-0.169	-0.067	-0.051	1.000		
	.008	*.026*	*.031*	*.396*	*.522*			
7	-0.116	-0.129	-0.046	-0.207	-0.044	-0.228	1.000	
	.142	*.101*	*.559*	*.008*	*.574*	*.004*		
8	-0.077	-0.013	-0.157	-0.141	-0.025	-0.208	-0.016	1.000
	.331	*.865*	*.046*	*.073*	*.756*	*.008*	*.839*	

Note. Italic values indicate p-values; degrees of freedom = 161.

Hypothalamic-Pituitary-Thyroid Axis Function in Female Adolescent Nonsuicidal Self-Injury and its Association with Comorbid Borderline Personality Disorder and Depression

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Highlights

- Female adolescents with NSSI display blunted fT3/fT4 ratios compared to HC
- BPD severity correlates negatively TSH and fT3/fT4 ratio
- Depression severity correlates negatively with fT3 and fT3/fT4 ratio
- Symptomatic distress correlates negatively with TSH, fT3 and fT3/fT4 ratio
- Implications should be addressed by dynamic and longitudinal research designs

Abstract

Objectives: Behavioral disturbances in adolescence are potentially linked to aberrant functioning of the thyroid gland. Accordingly, alterations of the hypothalamic-pituitary-thyroid (HPT) axis might impact psychopathological development. Yet corresponding research in adolescents with nonsuicidal self-injury (NSSI) and comorbid mental disorders is scarce. **Methods:** The present study examined HPT axis functioning in adolescents with NSSI compared to healthy controls (HC) using blood-based assays of thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), and the ratio of these hormones (fT3/fT4 ratio). Cortisol was additionally examined to contrast HPT axis functioning with a well-established biomarker of stress responsivity. Moreover, associations between clinical characteristics, HPT axis and HPA axis functioning were investigated. Female adolescents meeting NSSI criteria according to DSM-5 criteria (n=117) were compared to adolescent HC (n=41). Standardized serum-based endocrinological assays and interview- and questionnaire-based psychiatric assessments were used. Smoking status was included as covariate for all analyses. **Results:** NSSI patients displayed altered HPT axis functioning as fT3/fT4 ratio values were blunted in comparison to HC. Negative correlations were further present between fT3, fT3/fT4 ratio and severity of BPD symptoms, depression scores and symptomatic distress. TSH correlated negatively with severity of BPD symptoms and symptomatic distress exclusively. Cortisol values differed neither significantly between experimental groups nor correlated significantly with clinical characteristics. **Conclusions:** Longitudinal examinations, assessing links between psychopathology and endocrinological alterations, are warranted to address potential clinical implications of thyroid markers in child and adolescent psychiatry.

Keywords: hypothalamic-pituitary-thyroid axis; adolescence; nonsuicidal self-injury; borderline personality disorder; depression

1. Introduction

Nonsuicidal self-injury (NSSI) is a serious and common phenomenon in adolescence. Consequently, NSSI has been introduced as a disorder warranting further research in the 5th version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[1]. Proposed criteria include intentional and self-inflicted damage to the surface of one's body without suicidal intent on five or more days within a year. Epidemiological research estimated that *single events of NSSI* occur in 17% of adolescent nonclinical samples while 5% even meet criteria for *NSSI disorder* (NSSID)[22]. NSSI is often accompanied by comorbid disorders such as borderline personality disorder (BPD) or major depressive disorder (MDD)[9]. Both disorders emerge commonly during adolescence and include severe emotion dysregulation. Existing evidence suggests that NSSI may serve as a coping strategy to diminish the intensity of negative emotions[13]. Taken together, research shows that NSSI, BPD and depression are common phenomena in adolescence, yet biological markers potentially underlying these psychopathologies have been investigated insufficiently.

To investigate somatic correlates of psychiatric disorders, there has been a growing interest in endocrinological markers, such as hormones of the hypothalamic-pituitary-adrenal (HPA) axis. A closely related axis, the hypothalamic-pituitary-thyroid (HPT) axis, has received far less interest, although it can be assumed that HPT axis functioning influences psychosocial health significantly. Here, thyrotropin-releasing hormone (TRH) stimulates secretion of thyroid-stimulating hormone (TSH) in the anterior pituitary gland, which prompts triiodothyronine (T3) and thyroxine (T4) secretion in the thyroid gland. T3 and T4 are available in free form (fT3; fT4) or attached to proteins. And importantly, thyroid hormones do not only regulate the metabolism, but also impinge upon the cardiovascular system, bone maintenance, pregnancy outcomes, child development, and mental health.

Given the absence of research investigating HPT axis hormones in NSSI, the present study aimed at investigating HPT axis functioning in adolescents engaging in NSSI compared with healthy controls. Based on prior research in BPD and MDD, NSSI patients were expected to show altered HPT axis hormones (i.e. elevated TSH and decreased fT3, fT4, fT3/fT4 ratio). Additionally, cortisol, the primary marker of the HPA axis, was included to reassess links between psychopathology and endocrinological functioning more generally. Lastly, we investigated whether comorbid psychopathological characteristics, i.e. severity of BPD and depression as well as symptomatic distress, would correspond to stronger blunting of HPT axis hormones or cortisol.

2. Methods

2.1. General Procedures and Participants

Data for the present analyses were collected drawing on a consecutive help-seeking cohort of adolescents (12-17 years) presenting at the outpatient clinic for adolescent risk-taking and self-harm behavior (*AtR!Sk*; Ambulanz für Risikoverhalten und Selbstschädigung) at the Clinic for Child and

Adolescent Psychiatry, University Hospital Heidelberg. Patients underwent a first-stage, structured psychiatric diagnostic assessment (ethical approval: ID S-449/2013) followed by the invitation to participate in a second appointment involving various neurobiological assessments (*AtR!Sk-Bio*; ethical approval: ID S-514/2015). NSSI patients were included when reporting acts of nonsuicidal self-injury on at least 5 days in the past 12 months, as defined by the DSM-5 criterion A[1]. Intention of self-injury as "nonsuicidal" was explicitly required to avoid a conceptual overlap between NSSI and suicide attempts. Patients showing acute psychotic symptoms or insufficient speech comprehension were excluded. HC were only included when history of NSSI, endorsement of psychiatric disorder and corresponding treatments could be excluded prior to participation in the study. Patients and their legal guardians signed written informed consent to participate in both assessments. Both studies were carried out in accordance with the declaration of Helsinki.

The manuscript reports on cross-sectional data from *AtR!Sk-Bio*, which was implemented in 2016, in combination with *AtR!Sk* clinical data. Recruitment for *AtR!Sk-Bio* took place within six weeks after the diagnostic assessment in *AtR!Sk*. HC were recruited via public advertisement and underwent an adapted form of the diagnostic assessment prior to being invited to *AtR!Sk-Bio*. For *AtR!Sk-Bio*, height (cm) and weight (kg) were measured after a structured assessment on fasting status, handedness, smoking status, present-day coffee consumption, menstrual status, contraception use, physical illnesses, and medication use. Subsequently, fasting blood samples were taken by qualified medical staff. HPT axis hormones and cortisol were examined using standardized serum-based blood draws. Preanalytical variation was minimized by performing venipuncture in a standardized manner around 0900 h. After blood collection, samples were immediately frozen in aliquots at -80 °C until analyzed. Participants received an allowance of 40€ for participation.

2.2. Measures

2.2.1. Psychological instruments. The diagnostic procedures have been described in detail by Kaess and colleagues[10]. Frequency and severity of NSSI and suicidality were examined separately using the *Self-Injurious Thoughts and Behavior Interview* (SITBI-G)[8]. BPD diagnoses were based on the *Structured Clinical Interview for DSM-IV Personality Disorders* (SKID-II)[7]. Current and lifetime Axis I disorders were assessed with the *M.I.N.I.-International Neuropsychiatric Interview for Children and Adolescents* (MINI-KID)[17]. Depressive symptoms were assessed based on self-reports using the *Depression Inventory for Children and Adolescents* (DIKJ)[20]. Symptomatic distress was examined based on the *Global Severity Index (GSI)* of the *Symptom-Checklist-90-Revised* (SCL-90-R)[4]. Adverse childhood experiences (ACEs) were examined using the *Childhood Experience of Care and Abuse Questionnaire* (CECA.Q)[19]. The self-report questionnaire was used to examine lack of parental care (i.e. neglect and antipathy), parental physical abuse, and sexual abuse from any adult before age 17. German versions were used for all questionnaires and interviews.

2.2.2. Endocrinological assays. Baseline thyroid function was evaluated based on TSH, fT3, fT4 and fT3/fT4 ratio. The reference range was 0.4 – 4.0 mU/l for TSH, 2.0 – 4.2 ng/l for fT3, and 8-18 ng/l for fT4. The intra-assay coefficient of variation (c.v.) was 2.41 – 2.48% for TSH, 2.35 – 3.08% for fT3, and 2.23 – 3.33% for fT4. The inter-assay c.v. was 2.05 – 5.31% for TSH and 2.33 – 4.00% for fT4. Baseline HPA axis functioning was examined using cortisol. The reference range was 56 – 200 ng/ml. The intra-assay c.v. was 2.9 – 4.2 %. The inter-assay c.v. was 4.4 – 6.0%. Fasting blood samples were thawed and analyzed by immunoassays (ADVIA Centaur® Assay). No prior thawing of the frozen plasma samples was performed. Blood analyses were conducted according to accredited routines at the Central Laboratory of the University Hospital Heidelberg.

2.3. Statistical Analyses

Adolescents reporting ≥ 5 acts of NSSI within the past year were included for the NSSI group, adolescents without history of NSSI were included for the HC group. Sociodemographic and clinical differences between groups were compared using *t*-tests for dimensional variables and χ^2-tests for categorical variables. Groups differed significantly with regard to smoking status ($p = .005$), which was therefore included as covariate to all subsequent analyses. Groups differed also with regard to school type ($p = .027$), however, there were no significant relationships with endocrinological parameters. As all variables other than smoking neither correlated with experimental groups nor biological markers, they were not included as covariates in the statistical analyses. Group differences on endocrinological parameters were analyzed using regression analyses. Associations between hormonal and clinical characteristics were analyzed using Pearson's correlations. Subsequently, semipartial correlations were run to determine the relationship between endocrinological markers and psychopathology whilst controlling for smoking status. Statistical analyses were performed using STATA (*Stata Statistical Software: Release 15*, 2017, StataCorp LP, College Station, TX, USA) with α set to .05.

3. Results

3.1. Sociodemographic and Clinical Characteristics

The study sample comprised $n = 117$ NSSI patients and $n = 41$ HC (see **Supplement 1** for in- and exclusion criteria and sociodemographic characteristics). NSSI patients smoked more frequently in the past month ($p = .005$) and attended lower school types than HC ($p = .027$). Groups did not differ on age, body weight, body height, body mass index (BMI), average physical activity per week, estradiol levels, menstrual status, hormonal contraceptive use, medical condition within the past three months, alcohol consumption, or illicit drug use (all $p \geq .117$). Comorbid diagnoses, clinical characteristics, as well as frequency of ACEs are shown in **Supplement 2**. Approximately one third of the NSSI group ($n = 34$; 29%) met at least five BPD criteria in the clinical interview; diagnostic criteria for depression were met by $n = 72$ (62%). Besides, NSSI patients reported a significant higher frequency of ACEs in

general ($p < .001$) and also scored higher on several subscales of the CECA.Q, i.e. parental antipathy ($p < .001$), parental neglect ($p < .001$), and sexual abuse from any adult before age 17 ($p < .001$). Traumascores, which were calculated based on frequency and severity of ACEs, were positively correlated with number of BPD criteria ($p = .013$), depression scores ($p = .024$) and suicide attempts in the past 12 months ($p = .017$). Traumascores were neither correlated with GSIs ($p = .081$) nor with frequency of nonsuicidal self-injury in the past 12 months ($p = .382$).

3.2. Hormonal Levels

As shown in **Table 1**, groups differed significantly with regard to fT3/fT4 ratio. Ratio values were lower in NSSI patients (M=0.30, SD=0.05) than in HC (M=0.32, SD=0.05). For fT3, the regression model was significant (p=.004), yet groups did not differ significantly ($t_{(156)}$=-1.78, p=0.77).

Table 1

Group differences on thyroid markers and cortisol

Biomarker	NSSI	HC	Comparison			
	Mean ± SD	Mean ± SD	*F*	*p*	adj. R^2	*ES*
TSH (mU/l)	2.16 ± 1.05	2.31 ± 1.18	0.59	.557	< -.01	0.06
fT3 (ng/l)	3.38 ± 0.35	3.53 ± 0.42	5.81	.004	.06	0.57
fT4 (ng/l)	11.52 ± 1.50	11.07 ± 1.21	2.69	.071	.02	0.39
fT3/fT4 ratio	0.30 ± 0.05	0.32 ± 0.05	5.46	.005	.06	0.56
Cortisol (ng/ml)	161.55 ± 66.22	175.73 ± 71.45	2.19	.116	.02	0.01

Note. Sample sizes for all TSH, fT3, fT4, and fT3/fT4 ratio were $n = 117$ for NSSI patients and $n = 41$ for HC. Degrees of freedom (*df*) were (2, 141) for these endocrinological markers. Sample sizes for cortisol were $n = 110$ for NSSI patients and $n = 39$ for HC. Degrees of freedom (*df*) were (2, 132) for cortisol. Nicotine use in the past month (yes/no) was included as covariate. Due to reasons of space, statistics for these covariates are not shown but available on request. TSH = thyroid-stimulating hormone; fT3 = free triiodothyronine; fT4 = free thyroxine; fT3/fT4 ratio = ratio between free triiodothyronine and free thyroxine.

3.3. Associations between Clinical Characteristics and Hormone Levels

As shown in **Table 2**, BPD severity correlated negatively with TSH ($p = .027$), fT3 ($p = .009$), fT3/fT4 ratio ($p = .009$), and smoking status ($p < .001$). As shown in **Table 3**, semipartial correlations for BPD severity were significant for TSH ($p = .013$) and fT3/fT4 ratio ($p = .009$). Depression severity correlated negatively with fT3 ($p = .008$), fT3/fT4 ratio ($p = .003$), and smoking status ($p = .034$).

Semipartial correlations for depression severity were significant for fT3 (p = .019) and smoking status (p = .020), as well as fT3/fT4 ratio (p = .003). Symptomatic distress correlated negatively with TSH (p = .014), fT3 (p = .017) and fT3/fT4 ratio (p = .003). Semipartial correlations for symptom distress were significant for TSH (p = .022), fT3 (p = .026) and smoking status (p = .010), as well as fT3/fT4 ratio (p = .002).

Table 2

Partial correlations between endocrinological markers and clinical characteristics for the full study sample

Biomarker	No. BPD criteria (SCID-II) *n* = 158		Depression score (DIKJ) *n* = 144		Global Severity Index (SCL-90-R) *n* = 145	
	r	*p*	*r*	*p*	*r*	*p*
TSH (mU/l)	-0.176	.027*	-0.097	.250	-0.203	.014*
fT3 (ng/l)	-0.206	.009**	-0.221	.008**	-0.198	.017*
fT4 (ng/l)	0.072	.367	0.123	.143	0.139	.096
fT3/fT4 ratio	-0.206	.009**	-0.249	.003**	-0.246	.003**
Cortisol (ng/ml)	-0.020	.809	-0.115	.183	-0.087	.313
Smoking (yes/no)	0.401	< .001***	0.186	.034*	0.114	.192

Note. SCID-II = Structured Clinical Interview for DSM-IV Personality Disorders; DIKJ = Depression Inventory for Children and Adolescents. SCL-90-R = Symptom-Checklist-90-Revised. Cortisol samples were available for $n = 149$ participants for correlation analyses with BPD criteria, for $n = 135$ participants for correlation analyses with depression scores, and for $n = 136$ participants for correlation analyses with global severity indices. Details related to smoking status were available for $n = 144$ participants for correlation analyses with BPD criteria, for $n = 131$ participants for correlation analyses with depression scores, and for $n = 132$ participants for correlation analyses with global severity indices. * $p < .05$, ** $p < .01$, *** $p < .001$.

Table 3

Semipartial correlations between endocrinological markers and clinical characteristics for the full study sample

Biomarker	No. BPD criteria (SCID-II)				Depression score (DIKJ)				Global Severity Index (SCL-90-R)			
	n = 158				*n* = 144				*n* = 145			
	Clinical		Smoking		Clinical		Smoking		Clinical		Smoking	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
TSH (mU/l)	-0.208	.013*	0.134	.105	-0.095	.285	0.030	.735	-0.200	.022*	0.040	.645
fT3 (ng/l)	-0.153	.061	-0.154	.059	-0.200	.019*	-0.199	.020*	-0.188	.026*	-0.218	.010*
fT4 (ng/l)	0.119	.158	-0.084	.317	0.149	0.090	-0.060	.496	0.165	.060	-0.054	.534
fT3/fT4 ratio	-0.215	.009**	-0.032	.692	-0.261	.003**	-0.085	.314	-0.265	.002**	-0.103	.222
Cortisol (ng/ml)	-0.091	.290	0.172	.046*	-0.125	.167	0.148	.105	-0.080	.378	.134	.140

Note. SCID-II = Structured Clinical Interview for DSM-IV Personality Disorders; DIKJ = Depression Inventory for Children and Adolescents. SCL-90-R = Symptom-Checklist-90-Revised. Details related to smoking status were available for *n* =144 participants for correlation analyses with BPD criteria, for *n* = 131 participants for correlation analyses with depression scores, and for *n* = 132 participants for correlation analyses with global severity indices. * $p < .05$, ** $p < .01$, *** $p < .001$.

4. Discussion

The current study examined HPT axis hormones in adolescents with NSSI compared to healthy controls. Above all, findings suggest that altered fT3/fT4 might be a biological correlate of NSSI in adolescence, which in turn might indicate disrupted conversion from T4 to T3 in NSSI patients. Abnormal conversion from T4 to T3 may lead to fatigue, depression, and difficulty concentrating, which suggests that these symptoms should be taken seriously during physical examinations of NSSI patients. In this context, one may further question whether a higher frequency of NSSI coincides with stronger endocrinological alterations. Yet, our results suggest no such association and exploratory analyses assessing links between frequency of NSSI and thyroid markers failed to reach statistical significance. However, as we investigated a clinical help-seeking sample presenting with severe and repetitive NSSI, findings might not generalize to population- or college-based samples frequently studied in the field. Put differently, as a higher frequency of NSSI is associated with greater psychopathological distress, our findings might not generalize to occasional NSSI, which has not readily been captured in the present sample. However, and given the heterogeneity of NSSI frequency in the present sample, it seems rather unlikely that the inclusion of patients with low to mild NSSI frequency would have challenged these findings. Additionally, it should be mentioned that most endocrine markers were in the normal physiological range in the current study. Yet, HPT axis markers have relatively fixed *individual* setpoints, which tend to be stable over lifetime[16]. As our analyses showed that NSSI patients reported more frequent and severe adverse childhood experiences and as these were further associated with BPD severity and suicide attempts in the past year, it might be particularly important to examine long-term relationships between psychopathology and HPT axis alterations to investigate whether such early experiences lead to changes in individual HPT axis setpoints. And, as setpoints can be affected by factors such as inflammation and lack of sleep, a normalization of HPT axis functioning resulting from psychosocial stabilization, for instance due to psychotherapy, needs to be investigated in future studies. Taken together, the current findings are in line with empirical evidence, however, antecedents leading to altered HPT functioning in adolescent NSSI patients need to be examined in greater detail.

Second, negative associations could be demonstrated for focal clinical characteristics and HPT markers. Here, BPD severity predicted blunting of TSH, fT3 and fT3/fT4 ratio, while depression severity predicted blunting of TSH and fT3/fT4 ratio. Symptomatic distress predicted blunting of TSH, fT3 and fT3/fT4 ratio. As suggested by Duval and colleagues[6], such blunted thyroid levels may either originate from a downregulation of TRH receptors of the pituitary thyrotrophs secondary to a prolonged increase in hypothalamic TRH stimulation or from previously increased thyroid hormone levels and subsequent negative feedback of the HPT axis, which in turn could be associated with more pronounced psychopathology. Since we rely on single assessments, we can only speculate on the underlying cause. In any case, the associations signified in the current study may be linked with emotion dysregulation – commonly underlying NSSI – as thyroid receptors are localized on limbic structures acting on mood regulation[3]. Besides, it has previously been speculated that serotonin (5-hydroxytryptamine; 5-HT) –

which likely plays a crucial role with regard to depressive symptoms and BPD[15] – may act peripherally on the thyroid gland and could thereby decrease 5'-deiodinase activity[21]. In this context, it has been hypothesized that patients with mood disorders are particularly sensitive to changes in thyroid status, even when peripheral thyroid hormone assays in the normal range[14]. This could suggest that even minor deviations of thyroid hormones may parallel markers for psychopathology. To further investigate this hypothesis, it may be worthwhile to investigate if pharmalogical treatment of 5-HT receptors comes along with an increase of thyroid hormones and clinical characteristics.

The finding that most endocrinological markers were not directly associated with NSSI behavior but rather with general psychopathology (such as symptomatic distress) further suggests that HPT axis dysfunction may present a non-specific mechanism promoting the development and maintenance of NSSI via general psychopathological distress. Recently, we proposed a temporal framework[11], within which neurobiological factors associated with NSSI should be distinguished as (1) *distal biological traits* (e.g. biological predisposition or vulnerability for NSSI), (2) *proximal biological traits* (e.g. biological processes underlying NSSI that are of moderate stability) and *biological states* directly preceding or following NSSI. As such, and based on the present findings, HPT axis dysfunction can be considered a distal biological trait, not necessarily linked to NSSI, but to functional abnormalities related to the predisposition of the behavior. This hypothesis is further supported by the fact that HPT levels may not change in the short term or transient depending on the current frequency of NSSI. Further longitudinal research is needed, addressing the longitudinal course of HPT axis function in association with psychopathology in those developing or terminating the behavior. And, while findings were consistent with earlier research [12,18], we were the first to show that such associations are present at an early developmental stage. However, future studies should examine separate clinical groups to examine the specificity of thyroid hormones, psychopathology, and emotion dysregulation. Here, future studies may also investigate if a higher frequency of NSSI coincides with more pronounced psychopathology to eventually investigate core mechanisms related to altered HPT axis functioning.

Besides, several limitations of the current study need to be acknowledged. First, generalizability with regard to male participants is limited. However, females develop thyroid diseases more frequently[2] and receive NSSI and BPD diagnoses more often[23], which may point to a high ecological validity of our sample. Second, analyses were based on singular endocrinological assessments using blood draws, which necessitates repeated assessments in future studies to confirm the current findings. This seems especially important with regard to cortisol assessments, as prior research has shown that cortisol changes dynamically depending on current stress responsivity, which is why repeated and dynamic measurements may reflect subjective stress levels more adequately[5]. Third, and notwithstanding that NSSID has been added to the DSM-5 as an independent disorder requiring further research[1], the validity of the diagnosis has only been examined empirically as of recently[24] and a recent study by our group indicated that NSSID as a stand-alone diagnosis is rare in help-seeking adolescents[9]. As a matter of fact, NSSID is frequently accompanied by several comorbid disorders and marked functional impairments.

In order to investigate the validity of NSSI as a stand-alone diagnostic entity, biological studies have the potential to enrich clinical descriptions, and can make an important additive contribution to existing studies on the nosology of NSSI as an independent disorder. Our results seem in line with an increasing body of evidence that NSSI may rather serve as a transdiagnostic symptom than a disorder of its own given that many of its biological correlates may be driven by underlying psychopathology. Replication studies using thyroid markers as well as related endocrinological markers in larger samples may have sufficient power to facilitate both classification and differentiation of this clinical picture. A strength of the current study lies in its comprehensive link between biological and psychiatric data within a unique patient population. Moreover, the young age of the sample corresponds with little chronification and marginal interference of psychotropic drugs, which complements research on adult patient groups.

In summary, this study highlights potentially altered thyroid functioning in female adolescents with NSSI as well as associations between thyroid functioning and specific clinical characteristics in NSSI. Nonetheless, research based on long-term and dynamic endocrinological assessments is needed to confirm its etiological and diagnostic value. Once the clinical utility of thyroid markers can be demonstrated, parallel effects of altered hormones and psychiatric symptoms present a promising avenue for further research. Eventually, such research may facilitate efficient treatment options for adolescents engaging in NSSI.

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Statement of interest

None to declare.

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Specification of own contribution to data collection and evaluation

Manuscript 1

P. van der Venne wrote the manuscript and contributed essentially to the statistical analyses in collaboration with J. Koenig and M. Kaess. He was critically involved in the project coordination, participant recruitment and data collection. He revised the manuscript for important intellectual content in collaboration with all co-authors.

Manuscript 2

P. van der Venne wrote the manuscript and performed the statistical analyses in collaboration with J. Koenig and M. Kaess. He was critically involved in the project coordination, participant recruitment and data collection. He revised the manuscript for important intellectual content in collaboration with all co-authors.

Manuscript 3

P. van der Venne was critically involved in the project coordination, participant recruitment and data collection. He revised the manuscript for important intellectual content and approved the final version of the manuscript in collaboration with all co-authors.

Manuscript 4

P. van der Venne was critically involved in the project coordination, participant recruitment and data collection. He revised the manuscript for important intellectual content and approved the final version of the manuscript in collaboration with all co-authors.

Additional topic-related Manuscripts

The following additional publications were produced during the doctoral project. While not explicitly addressed in this work, they are closely related thematically and shall therefore be listed here.

Kindler, J., Koenig, J., Lerch, S., **van der Venne**, P., Resch, F., & Kaess, M. (2022). Increased immunological markers in female adolescents with non-suicidal self-injury. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2022.08.125

Cavelti, M., Rinnewitz, L., Walter, M., **van der Venne**, P., Parzer, P., … & Kaess, M. (2022) Psychobiological Correlates of Aggression in Female Adolescents with Borderline Personality Disorder. *Psychopathology, 55*(1), 37-48. https://doi.org/10.1159/000520228

Mielke, E. L., Koenig, J., Herpertz, S., Steinmann, S., Neukel, C., … & Kaess, M. (submitted) Dimensional association between plasma oxytocin and borderline personality disorder symptom severity. *Progress in neuro-psychopharmacology and biological psychiatry*.

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Declaration in accordance to § 8 (1) c) and d) of the doctoral degree regulation of the Faculty

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