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Highlights

- As shown previously, MDD diagnosis went with a reduced respiratory sinus arrhythmia (RSA).
- Low RSA went with high values in anxiety-related indicators, and low extraversion.
- Low RSA went with high concentrations of several pro-inflammatory cytokines.
- Influencing vagal activity or pro-inflammatory factors may be of therapeutic value.

Associations between Heart Rate Variability, Peripheral Inflammatory Markers and Major Depressive Disorder

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Abstract

Background: Measures for the irregularity of the heartbeat, for example respiratory sinus arrhythmia, have been implicated as a measure for restorative functions of the vegetative nervous system. Methods: In the current observational study, we investigated 265 subjects, 70 of whom had a lifetime history of major depression, with a plethysmographic heartbeat monitor, blood sampling, as well as a range of psychiatric questionnaires. Results: Subjects with a history of MDE had significantly reduced respiratory sinus arrhythmia (RSA) as compared to never-depressed controls; in the whole sample, higher RSA went with lower anxiety/fear variables, especially in subscores related to cardiac symptoms as well as being afraid of dying. A reduced RSA was also associated with an increased concentration of cytokines (TNFa, IL1a, IL6, IFNg) and thyroid-stimulating hormone in the serum, pointing to a possible triangular relationship between immune system, vegetative nervous system, and emotional dysregulation. Limitations: We used a plethysmographic device for the measurement of heartbeat instead of an electrocardiogram, and had a single time point only. Conclusions: This data corroborate the idea that a disequilibrium of the vegetative nervous, especially if accompanied by a dysregulation system in immune function, can increase the risk for depression. Conversely, vagal stimulation and anti-inflammatory treatments may support the treatment with antidepressants.

keywords: depression, vagus, inflammation, heartbeat variability, respiratory sinus arrhythmia, anxiety

1. Introduction

Depression is a common illness that severely limits psychosocial functioning (Malhi and Mann, 2018); in 2008, WHO ranked major depression as the third cause of burden of disease worldwide (2004). Very often, depression is comorbid with anxiety (about 85% of patients with depression have significant anxiety, and 90% of patients with anxiety disorder have depression) (Tiller, 2013).

The search for biomarkers of depression has proven difficult (Kennis et al., 2020), partly because there may be subtypes that vary in these biomarkers (as exemplified by cortisol levels after challenge (Jurueña et al., 2018)).

One area where biomarkers have been investigated extensively is the autonomic nervous system and its impact on emotion regulation and dysregulation, for example in the model of neurovisceral integration (Thayer and Lane, 2000) and the polyvagal theory (Porges, 2001). One promising biomarker for vagal dysregulation is low heart rate variability (HRV) (Thayer and Lane, 2009). Indeed a large study using two different indicators for heart rate variability showed reduced heart rate variability in both current and remitted depressed as compared to non-depressed controls, which depended strongly on the use of psychoactive medication (Licht et al., 2008). In a meta-analysis on the impact of depression and antidepressant medication on HRV, depressed participants had lower HRV than non-depressed controls, and there was also a depression-severity effect in that more

severely depressed had a more strongly reduced HRV (Kemp et al., 2010). In the same meta-analysis, tricyclic medication reduced HRV, whereas SSRIs, mirtazapine and nefazodone did not (see also (Kidwell and Ellenbroek, 2018)).

Several markers of HRV have been implicated as biomarkers for psychopathology, using time or frequency domain analyses (Sgoifo et al., 2015), and indices such as the root mean sum of successive differences (RMSSD) (Jarczok et al., 2018; Koenig et al., 2018), standard deviation of normalized cardiac interbeat intervals (SDNN) (Karavidas et al., 2007; Licht et al., 2008), the high-frequency component of heart rate variability (HF-HRV) (Beauchaine and Thayer, 2015; Gruber et al., 2015; Koenig et al., 2016), or similarly, respiratory sinus arrhythmia (RSA) (Porges, 1995; Tonhajzerova et al., 2009). While these indicators are all facets of heart rate variability, it is possible that they tap slightly different aspects of autonomic involvement in emotion regulation.

For RSA, it has been implicated as a transdiagnostic biomarker for emotion dysregulation, because it has been found altered in many internalizing and externalizing psychiatric conditions, and it is supposed to reflect prefrontal activity, presumably of the same regions involved in emotion regulation (Beauchaine, 2015). In addition, because it can be quantified in a time frame of roughly 30 seconds, it can be used for biofeedback and experimental stress studies ('RSA reactivity'). Pharmacological blockade of cholinergic inputs reduces RSA (Coker et al., 1984), and can be induced by neck suction devices that influence the baroreceptor (Piepoli et al., 1997). Note that historically, RSA has been operationalised and measured in different ways (just as standard deviation of the RR-intervals, or as power in the HF band) (Beauchaine, 2015). RSA has been shown to increase significantly during propofol anaesthesia, and has even been proposed as an indicator to regulate automated propofol application in anaesthesia (Pomfrett et al., 1993).

Many studies highlight relationships between RSA/HF-HRV and various aspects of depression. One study showed that lower resting RSA in the first year of the study predicted depressive symptoms as assessed using the BDI in the second year of the study, even if controlling for state anxiety, body mass index, and medication use (Yaptangco et al., 2015). One paper involving two study samples showed that atypical RSA is an endophenotype of depression, in that atypical RSA patterns predicted current depressive episodes and remission status, were concordant across mothers and their juvenile offspring, and were more prevalent in siblings of depressed than in siblings of never-depressed (Yaroslavsky et al., 2014). One study found that RSA fluctuation was more stably found in depressed as compared to controls than baseline RSA, which did not survive correction for health or respiration (Rottenberg et al., 2007). A review found a lower fluctuation in heart rate and heart rate variability in MDD subjects than controls in well-established stress tasks (Schiweck et al., 2019). For cognition, one study found low RSA went with longer reaction times in a number-letter switching task (Hoffmann et al., 2017). One study reports a relationship between HF-HRV and anger in a stressful computer task, in that actual anger was uncorrelated with HF-HRV, but low HF-HRV went with a higher persistence of anger (Ellis et al., 2016). In addition, several papers have implicated RSA or HF-HRV in suicidality (e.g. (Tsypes et al., 2018)). In a study of RSA reactivity after stress, RSA reduction was observed both in MDD and non-depressed control subjects in a rumination condition, but for the depressed also in a cognitive distraction condition, which the authors interpret in terms of cognitive effort (LeMoult et al., 2016).

In addition to the direct relationship between heart rate variability measures and depression, there may also be bidirectional relationships of each with inflammation (Huston and Tracey, 2011). For example, in a paced respiration study in healthy human adults with immune system challenge with lipopolysaccharide, which induces depression-like behaviors in rodents, higher HRV (high frequency HRV) went with a lower production of IL-6 and TNFalpha (from now on TNFa), whereas the anti-inflammatory cytokine IL-10 was not affected (Marsland et al., 2007a). In patients with hypertension,

greater HRV (high-frequency power, baroreflex sensitivity, and RR interval) induced by heart rate biofeedback training went with lower high-sensitivity C-reactive protein concentration in the blood, a marker for inflammatory activity (Nolan et al., 2012). In human macrophage cultures, acetylcholine significantly attenuated the release of pro-inflammatory cytokines (TNF, IL-1 β , IL-6 and IL-18) upon endotoxin challenge; in rats, electrical stimulation of the vagus nerve reduced TNF production in the liver, attenuated TNF in the serum, and prevented the development of shock in endotoxemia (Borovikova et al., 2000). In depressed patients, high-sensitivity C-reactive protein was positively correlated with a lifetime history of suicidal ideation, which was negatively associated with total, low frequency and high frequency HRV (Chang et al., 2017).

In the present study, our main hypothesis was that human subjects with an actual or past depressive episode have a reduced RSA. Secondary research questions were: A) Does RSA explain more variability in internalizing psychiatric symptoms than other measures of heart rate variability, for example the standard deviation of the heart beat interval or root mean square of the successive differences (RMSSD)? B) Is RSA related to specific depressive and anxiety symptoms? and C) Do interindividual differences in RSA relate to other biomarkers in depression, particularly immune and metabolic markers that have been related to neurovisceral integration (e.g. TNF α , IL1 α , IL6, IFN γ and thyroid stimulating hormone TSH)?

2. Methods

The ethics committee of the Kanton Zurich approved for all procedures undertaken in the course of the study, and subjects gave written informed consent according to the declaration of Helsinki.

2.1 Subjects:

The sample consisted of a group of 296 young adults recruited from the general population via newspaper advertisements or university blackboard webpages. We included psychiatrically healthy or depressed adults between 18 and 40 years of age, right-handed, fulfilling the safety criteria for MRI; we did not include subjects with schizophrenia or subjects who had a first-degree relative with schizophrenia, subjects with drug abuse during the last year, with suicidal plans in the last eight weeks, or subjects with neurological or other medical disorders (such as asthma, diabetes, heart disease); women had to present a negative pregnancy test immediately before MR scans (requirement of the Ethics Commission). Of the 296 participants assessed in the MRI session, two were excluded for mild abnormalities in the structural MRI and one for extrasystoles; of the remaining 294 subjects, 28 were excluded for artefacts in the heartbeat signal, resulting in a dataset of 265 subjects, 70 of whom had a past or present MDE. Out of the 70 MDE subjects, only seven had taken medication in the past 90 days, in one case thyroid hormones, the other six antidepressants.

Among these 265 subjects, breathing (and consequently RSA) data were excluded in six subjects whose breathing signal did not show a sinusoidal cycling pattern, or artefacts in the heartbeat signal during five or more percent of the time. The relative number of depressed participants was comparable between the excluded subjects (12 out of 31) and included subjects (70 out of 265; $\chi^2=2.095$, $df=1$, $p=.148$). Further comparison between included and excluded subjects showed only a difference for body mass index (BMI; Students t-test $p=.016$, with a lower mean for the excluded than for the included subjects) and a nonsignificant trend for neuroticism (t-test $p=.067$, with a tendency towards higher values in excluded than in included subjects); no differences were

found for age, years of education, as well as depression measures (BDI, MADRS, HAMD) or further anxiety measures (BAI, STAI state; see 'procedure' below for the full name of the psychiatric indices).

2.2 Procedure:

Subjects filled out an online questionnaire before the study visit, namely ASI (Taylor and Cox, 1998), SKID (German version of SCID; see below), NeoFFI (McCrae and Costa, 1987), STAI trait version (Patterson et al., 1980)), and Aggression questionnaire (AQ) (Buss and Perry, 1992); during the study visit, more questionnaire data (STAI state, BDI (Beck et al., 1961), State-Trait-Anger-Expression Inventory (STAXI) (Spielberger et al., 1995), BAI (Beck et al., 1988), MADRS (Montgomery and Asberg, 1979), HAMD (Hamilton, 1960), PSS (Cohen et al., 1983), PANAS (Crawford and Henry, 2004), PTQ (Ehring et al., 2011)), Mind Wandering Questionnaire (MWQ) (Mrazek et al., 2013), and blood samples were collected. Subsequently, participants underwent an one hour MR scanning session during which heart and breathing activity were monitored; breathing and heart data were stored only during functional MRI sessions, and evaluated only for the longer resting-state fMRI session (looking at a fixation cross for 601 seconds). After the scanning session participants were tested with two economic games and an odd-ball reaction time task.

MDE (present and past) diagnoses were posed by a trained clinical psychologist based on the SKID (Wittchen, 1997), the German version of SCID (First, 1997). We were aware that MDE diagnoses would overlap greatly with other psychiatric diagnoses, especially lifetime presence of any anxiety disorder (specific phobia, social phobia, panic disorder, generalized anxiety disorder, posttraumatic stress disorder; in MDE subjects, 45 out of 70 fulfilled a diagnosis of an anxiety disorder, in non-MDE 20 out of 190; $\chi^2=78.85$, $df=1$, $p=.001$), for specific phobias (in MDE subjects, 22 out of 70 fulfilled a specific phobia, in non-MDE 14 out of 190; $\chi^2=24.82$, $df=1$, $p=.001$), but also for obsessive-compulsive disorder (in MDE subjects, 15 out of 70 fulfilled an OCD diagnosis, in non-MDE 1 out of 190; $\chi^2=38.70$, $df=1$, $p=.001$), MDE diagnosis overlapped with anxiety-related diagnoses.

2.3 Data evaluation:

Heartbeat and breathing measurements:

For the breathing and respiratory sinus arrhythmia calculations, the first 100 artefact-free inter-beat intervals collected during resting-state fMRI were used for each subject. The breathing signal was aligned to the heartbeat signal, and the R-peaks were timed and inter-peak intervals were calculated. Then offsets from the average inter-beat intervals were calculated. In the breathing signal, breathe-in intervals were separated from breathe-out signals, according to the relative maxima/ minima of the filling of the belt. For the maxima, if two peaks of equal height existed before the signal decreased by at least 250 units (maximum regulated to 3000 units), the second was taken as the cut-off point; if three peaks of equal height existed, the middle one was taken. Offsets from the average inter-beat-interval were summed for all peaks that ended in breathe-in intervals and for all peaks that ended in breathe-out intervals, and total RSA (in milliseconds) was defined as summed deviations from the mean in breathe-out periods minus summed deviations from the mean in breathe-in periods. In addition, relative RSA values (divided by the individual average RR-intervals) were calculated. Out of the 259 subjects with RSA measurements, only 7 had negative RSA values.

As a specificity check for RSA relative to other measures of heart rate variability, we also calculated RMSSD, alpha1 and SD ratio (Tarvainen et al., 2014), based on the full 10-minute dataset.

Blood sampling and evaluation:

Venous blood sampling was done by a trained medical-technical assistant roughly 20min before the MR session started, in the afternoon (2.p.m., SD 1.1 hours). Blood samples were centrifuged 60 (plus/minus 15) minutes after collection; cortisol and DHEA were measured immediately after that, and aliquots were frozen and stored at -80°C for further analysis. Plasma cytokines/ chemokines (CRP, IFNg, IL1, IL6, TNFa) were measured using a multiplex assay using a commercially available human cytokine/ chemokine magnetic bead panel (MILLIPLEX MAP, HCYTMAG-60K-PX41) as per the manufacturer's instructions on Luminex-200 multiplex immunoassay system. Data were analyzed using Milliplex Analyst 5.1 software (EMD Millipore, Billerica, Massachusetts). Blood samples were assayed in duplicate; the Pearson correlation between the concentration values derived from each plate was .989 for CRP, .901 for TSH, .883 for IL1a, .988 for IL1b, .878 for IL6, and .855 for TNFa.

2.4 Statistics:

Statistical analyses were performed with IBM SPSS 24/ 27, the mediation analysis with R studio (see below). For the evaluation of our main hypothesis, the relationship between depression status and RSA, we used simple Anovas/ Student's t-tests; for the more exploratory analyses, we used Pearson correlations, partial correlations as well as AnCovas and chisquare tests, because the data scaling and approximately Gaussian distribution of the data allowed the use of these parametric tests. ICAs (with VARIMAX rotation) were used mainly to find out how many independent dimensions explained a set of measured variables. The main hypothesis could be answered with one t-test; for exploratory analyses involving many more variables, we did not correct for multiple comparisons, for two reasons: In the event of a significant main result, we wanted to explore possible candidate mechanisms for further studies; second, the psychiatric variables were highly correlated, so they can be considered as nuances of the same large concept ('internalizing psychiatric symptoms'). The results from these 'exploratory analyses' should therefore be considered with caution until they can be replicated with at least one independent sample. The mediation analysis was calculated with the sem package for RStudio (version 1.3.1093).

3. Results

3.1 Depression and RSA

Demographics and sample description of the subjects is given in Table 1. Subjects with lifetime MDD ('MDE subjects') had a highly significantly reduced respiratory sinus arrhythmia as compared to nondepressed controls ('non-MDE subjects', see Fig.1; Table 2a). As can be seen in Table 2a, this difference was relatively specific for RSA and could not be seen in other indicators of heart rate variability. We also conducted AnCovas controlling in turn for age, BMI, the average breathing intervals (which was longer in subjects than in non-MDD controls, see Tab.2a) or the standard deviation of the lengths of 100 inter-beat intervals, but the result remained significant (data not shown). In a subgroup analysis investigating differences in RSA between MDE subjects with and without a current major depressive episode, no significant difference in RSA was evident, but participants with current MDE showed lower variability in the interbeat interval (SD RR-interval and RMSSD; see Table 2b).

3.2 Correlates of psychiatric indices

To elucidate the specificity of this main finding, we correlated RSA with psychopathological features associated with MDE (which were often highly correlated with each other) and found negative associations between RSA and measures of anxiety (Anxiety Sensitivity Index (ASI), Beck's Anxiety Inventory (BAI), state version of the State Trait Anxiety Inventory (STAI state)), introversion and perseverative thinking (PTQ, especially the subindex 'mental capacity captured') (Table 3). We also looked for physiological correlates that could further elucidate the finding and found relationships of RSA with several pro-inflammatory proteins in the serum (IL1, IL6, IFNg, TNFa) as well as TSH (Table 4). TNFa was strongly correlated with TSH ($r=0.402$, $p=0.001$). Given the theoretically tight relationship between cytokines on the one hand and TSH on the other, we conducted partial correlation analyses to estimate which factor is more able to reduce the correlation of the other with RSA; for TSH and IL-6, they were almost identical in reducing the correlation of the effect size of the other with RSA, whereas for TSH and TNFa, TNFa was slightly stronger (reduction in the correlation TSH-RSA .080; TSH as control variable for TNFa-RSA just .061).

3.3 Reliability and specificity checks

We tested the reliability of the RSA measurements in a random selection of $n=30$ subjects, in which we used 100 RR-cycles in the middle of the 10min-sequence (versus at the beginning); the Pearson correlation between the two RSA measurements in these 30 subjects was $r=0.835$, $p=.001$.

Taking out the 7 medicated subjects did not change the result pattern (data not shown).

To evaluate the specificity of HRV abnormalities with MDD, we compared MDD with anxiety disorders. For specific phobias, there were a reasonable number (14) of cases without depression for comparison. In a 2x2 Anova with depression diagnosis and specific phobia (both lifetime) as independent factors, MDD was significant ($p=0.016$, $\eta^2=0.023$), whereas specific phobias were not ($p=0.278$, $\eta^2=0.005$).

In an item-wise analysis, we used an ICA to order all subitems of STAI state, BAI and ASI and extracted five factors ('not being joyful'; 'cardiac symptoms'; 'trembling and being afraid of death'; 'afraid of losing mind' and 'short breath or nausea in public') of which two ('trembling and being afraid of dying' and 'cardiac symptoms') were significantly correlated with RSA.

Among the subitems of the ASI, low RSA was correlated with being afraid of catastrophic processes inside the body (including the heart), rather than being afraid of socially painful situations (Table 5).

In an explorative analysis, we also tested the relationship between emotion dysregulation and RSA in 4 selected items of the SCID, 4 selected items of STAI trait, STAXI and the subscores of the aggression questionnaire and found significant group differences in subjects. For the SCID, item 36 'constantly worrying' was highly significant (not constantly worrying subjects mean RSA 1135(SEM51, $n=183$), constantly worrying subjects mean RSA 834(72, 76); item 28 'often in a bad mood, quarreling' was also significant ($p=.039$), whereas items 45 ('not forgetting other's lapses') and 46 ('unforgiving') were not. Pearson correlation with the STAXI total score was significant ($r=-.170$, $p=.006$, $n=259$), and the subscore correlating highest was hostility ($r=-.124$, $p=.046$, $n=259$). For the four selected STAI trait items, all were correlated with RSA, in the order of the effect size item 11 ('I'm inclined to take things hard', $r=-.190$, $p=.002$, $n=259$), 18 ('I take disappointments so keenly that I cannot put them out of my mind', $r=-.182$, $p=.003$, $n=259$), 8 ('I feel that difficulties are piling up so that I can not overcome them', $r=-.163$, $p=.009$, $n=259$), and 20 ('I get in a state of tension or turmoil as I think over my recent concerns and interests', $r=-.152$, $p=.014$, $n=259$).

3.4 Testing whether Cytokines Mediate the RSA-Psychiatric Outcome Association

We conducted a mediation analysis in R. Introducing cytokine concentrations in the serum reduced the betas for the direct paths from RSA to ASI, STAI trait and BDI (from -0.19 to -0.15/-0.14 in the case of ASI as outcome variable, from -0.20 to -0.13/-0.11 in the case of STAI, from -0.14 to -0.11/-0.12 trait for IL6/ TNFa). None of the indirect effects (via cytokines) did not turn out to be significant ($p > .2$), and indirect effects were always numerically smaller than direct effects (RSA-psychiatric index).

On reviewer's request, we compared the effect sizes of the Pearson correlations between RSA and cytokine concentrations in the serum and found that numerically, the effect sizes were always larger in the MDE subjects than in the non-MDE subjects (in IL1a, the correlation reached significance in MDE, but not in non-MDE).

4. Discussion

The main finding of the present study is that MDE was associated with reduced respiratory sinus arrhythmia (Fig. 1, Tab.2a). Of note, this association was independent of the presence of an acute major depressive episode at the time of the scan. However, acute depression was related to reduction of other measures of heart rate variability (Tab. 2b). Earlier studies already showed a link between depression and reduced heart rate variability, and that antidepressants can influence heart rate variability (Kemp et al., 2010). Our study corroborates the view that reduced heart rate variability can be found in subjects with at least one lifetime depressive episode; with our sample of overwhelmingly unmedicated subjects (except $n=7$, and excluding the medicated subjects did not have any influence on the pattern of results). However, given the small number of medicated subjects we are not able to assess whether medication influences heart rate variability.

In an exploratory analysis of the relationships between psychiatric indices and RSA, RSA was associated with a wide range of mood and anxiety symptoms (Tab.3). Our item-wise analyses pointed to a relatively specific dominance of heart symptoms (also in contrast to breathing symptoms) and being afraid of dying in subjects with low RSA. As a specificity check, we compared the RSA of non-MDE and MDE cases with or without specific phobias and found a significant result only for MDE, but not for specific phobia status, providing further evidence for a relatively specific link between MDE and RSA (see also (Licht et al., 2008)). Similarly, we did not find a difference between subjects with traumatic memories (39%) and subjects without traumatic memories in RSA (data not shown).

According to a review paper (Berk et al., 2013), depression is associated with a chronic, low-grade inflammatory response and activation of cell-mediated immunity, as well as activation of the compensatory anti-inflammatory reflex system. This can be seen in the blood increases of the cytokines IL-6, TNF, and CRP (Dowlati et al., 2010), prostaglandine E2 (Nishino et al., 1989). Inflammation seems to increase serotonin turnover (Godbout et al., 2008; Janssen et al., 2010). A possible pathophysiological process is that cytokines from the innate immune system reach the brain by crossing the blood-brain barrier, activating microglia, which can disrupt the plasticity and development of nerve cells (Weber et al., 2017). Note that two possible PET tracers for neuroinflammatory processes are being tested (Berdyeva et al., 2019; Setiawan et al., 2015). Conversely, parasympathetic and cholinergic influences seem to inhibit inflammation in depression (Borovikova et al., 2000; Halaris, 2013). At least some antidepressants seem to normalize cytokines

in the blood (Janssen et al., 2010; Kubera et al., 2001), and anti-inflammatory agents have been tried to treat depression (Husain et al., 2017; Kohler et al., 2014; Muller et al., 2006). Interestingly, ECT seems to have a pro-inflammatory effect (Beurel et al., 2020).

In our study, lower RSA correlated with increased inflammation-related cytokines (most strongly TNF α , but also IL-6, IL1 α and IFN γ ; not with CRP). Relationships between heart rate variability and cytokines have been reported earlier in patients with a broad range of diseases such as those with heart failure (Malave et al., 2003; Nikolic et al., 2013), obesity and diabetes (Parish et al., 2016; Wegeberg et al., 2020), obstructive sleep apnea (Xie et al., 2020), infants with hypoxic ischemic encephalopathy (Al-Shargabi et al., 2017; Yasova Barbeau et al., 2019), in traumatic brain injury (Deepika et al., 2018), to name just a few, and in larger samples of healthy subjects (Cooper et al., 2015; Lampert et al., 2008; von Kanel et al., 2008). Note that all papers cited demonstrated a low-HRV-associated-with-high-cytokines relationship, although not always using the same measures for HRV/cytokines, but according to a recent meta-analysis, SDNN and HF-HRV should show the largest effect sizes (Williams et al., 2019). An experimental study in rats showed a decrease in heart rate and temperature going with a peak in TNF α , followed by a longer reaction with reduced HRV and increased levels of other cytokines, including IL-6, after receiving a bacterial endotoxin (Fairchild et al., 2009). Human studies with low-dose endotoxin also found increases in TNF α and IL-6 and reductions in HRV (Herlitz et al., 2015; Koenen et al., 2021; Marsland et al., 2007b). Interestingly, studies in arthritic patients showed that an implantable vagus nerve stimulator was able to reduce TNF and IL-6 (Koopman et al., 2017), and that patients with low HRV profited less from anti-TNF α therapy (Holman and Ng, 2008). In addition, at least one paper on a genetic polymorphism in humans demonstrates that a common proinflammatory polymorphism in the IL-6 promoter reduces heart rate variability in subjects older than 50 years, along with a higher sensitivity to air-pollution on HRV, which the authors interpret in terms of an influence of inflammation on HRV (Adam et al., 2014). Note that this discussion is also relevant for the reported relationship between depression and coronary heart disease (Halaris, 2013). Theoretical and review papers highlight the influence of the cholinergic anti-inflammatory pathway, based on the observation that macrophages can be deactivated by cholinergic inputs from the vagus nerve (Borovikova et al., 2000; Huston and Tracey, 2011; Pavlov and Tracey, 2005; Tracey, 2002), but could also noradrenergic influences (Fitzgerald, 2020).

Another factor strongly correlating with RSA was TSH in the serum (note that this was strongly correlated with cytokine concentration, especially TNF α). This finding was not based on a hypothesis and needs replication in future studies.

So far, few studies collected data about depressed mood, immune activation and measures of heart rate variability in the same subjects; as a notable exception, one study in older subjects found higher CRP and IL-6 as well as lower heart rate variability (SDNN, SDANN, RMSSD) in depressed than in nondepressed subjects, along with higher cardiovascular risk factors (higher cholesterol and reduced flow-mediated dilation) (Pizzi et al., 2008). Beyond replicating these relationships in a single sample, our study also indicates that RSA, despite the critique for its age dependency in the literature, may be a more specific trait marker for the diathesis of internalizing psychiatric disorders than other measures of heart rate variability, whereas other measures (e.g. standard deviation of the RR-intervals) may be better indicators for acute depressive episodes.

As a next step, it would be important to collect longitudinal data to investigate potential intraindividual variability in RSA, whether it reflects changes in psychiatric symptoms, and whether they precede or follow changes in internalizing psychiatric symptomology. The relationship between depression and inflammation seems to have trait-like aspects, as exemplified by genetic studies

involving cytokines and monocyte chemoattractant protein-1 (Barnes et al., 2017), but also state-like aspects, as demonstrated in a psycho-social stress study (Fagundes et al., 2013).

Our data are consistent with earlier theories that a dysregulated vegetative nervous system, especially if combined with chronic activation of the immune system, may increase the risk for depressive episodes. As discussed in the literature, this may apply especially to a subgroup of depressed participants (Lopresti et al., 2014; Lotrich, 2015); specifically those with a poor response to antidepressant medication (Lanquillon et al., 2000), reversed vegetative symptoms (Hickman et al., 2014), or the suicidal and non-melancholic patients (Beurel et al., 2020; Chang et al., 2017; Dunjic-Kostic et al., 2013; Woelfer et al., 2019), patients with childhood adversity (Miller and Cole, 2012) or with cumulated episodes (Copeland et al., 2012). An increased risk may not only be seen for depression, but also for bipolar disorder, schizophrenia (Goldsmith et al., 2016) or PTSD (Gill et al., 2008), but note that another source claims specificity for depression (Baune et al., 2012). While the direction of cause and effect is not fully clear, vagal stimulation may be able to improve the depressive symptoms of long-term depressed (Rush et al., 2005), and anti-inflammatory medication can support the treatment with antidepressants (Beurel et al., 2020; Kohler et al., 2014; Muller et al., 2006), but there are no large studies in non-comorbid depression yet (Bullmore, 2018) although clinical trials are on the way (Sakamoto et al., 2021).

5. Strengths and Limitations

We did not use ECG to measure the heartbeat, but a plethysmographic device as used in MRI scanners for cardiac triggering or to control for heartbeat artefacts. We evaluated only a single dataset roughly in the middle of a one-hour MR session (we collected a second one during task fMRI, but did not evaluate it since it may have been influenced by task demands), so we were not able to check day-to-day reliability and could not ensure if the measured RSA values constitute rather a state or trait variables. The setting may have been relaxing for a subset of the subjects but more arousing for other (presumably fearful) subjects; this could have increased situation-related differences between subjects, but also reduced trait-like differences between subjects. However, our sample was quite large, and we collected enough questionnaire data to search for psychological correlates of reduced RSA/ heart rate variability.

6. Conclusion

Our data, although being purely observational, corroborate the idea that a disequilibrium of the vegetative nervous system, especially if accompanied by a dysregulation in immune function, can increase the risk for depression. Conversely, vagal stimulation and anti-inflammatory treatments may support the treatment with antidepressants, at least in some MDD patients.

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Authors Contribution

Andreas Buchmann: Conceptualization, Analysis, Writing. Christopher Ritter: Investigation, Review and Editing. Sabrina Theresia Müller: Investigation. Melanie Haynes: Investigation. Carmen Ghisleni:

Investigation. Ruth Tuura: Conceptualization, Supervision, Review and Editing. Gregor Hasler: Conceptualization, Supervision, Writing, Review and Editing. The funding sources were not involved in in study design, collection, analysis or interpretation of the data.

All authors have seen and approved the final version of the manuscript being reviewed. The article is our original work, has not received prior publication and is not under consideration for publication elsewhere.

Conflict of Interest

none of the authors declares conflicts of interest

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Fig. 1

Table 1a: Demographics, for whole sample and non-MDE/ MDE subjects
p-values according to Students t-tests a), Mann-Whitney U-tests b) resp. Chisquare tests c)

	all	non-MDE	MDE	p
n (%)	265	195 (73.6)	70 (26.4)	
female sex (%)	166(62.6)	118(60.5)	48(68.6)	.146 c
age [y] (M, (SD))	24.7(4.6)	24.1(4.0)	26.4(5.6)	.001 a
education [y] (M, (SD))	13.9(2.8)	14.0(2.7)	13.7(3.1)	.391 a

self-estimated social status (M, (SD))	5.79(1.60)	6.09(1.38)	5.06(1.85)	.001 b
parents' income, categorized (M, (SD))	2.67(2.33)	2.67(2.36)	2.65(2.25)	.964 b
BMI (M, SD)	22.3(3.0)	22.2(2.6)	22.7(3.8)	.228 a

Table 1b: Descriptives, for whole sample and non-MDE/ MDE subjects
p-values according to Students t-tests

	all	non-MDE	MDE	p
n (%)	265	195 (73.6)	70 (26.4)	
BDI (M, SD)	4.76(7.00)	2.11(3.09)	12.38(9.13)	.001
neuroticism (M, SD)	19.0(8.8)	15.8(6.5)	27.7(8.3)	.001
TSH (M, (SD))	207(113)	210(108)	196(133)	.547
IL-6 (M, (SD))	37.6(28.5)	37.2(27.3)	39.3(33.5)	.714
TNFa (M, (SD))	51.8(16.4)	51.6(13.7)	52.7(24.4)	.743
cortisol in peripheral blood, afternoon [nmol/l] (M, SD)	317(157)	314(118)	319(177)	.917

Tab. 2a: measures of heart rate variability in subjects with no past or present major depressive episode ('no MDE') or with past or present major depressive episode ('MDE')

	non-MDE	MDE	eta-squared	p
n	195	70		
average respiration interval (ms) [M(SEM)]	3640(50)	3860(90)	.019	.028
heartbeat variability (SD) [M(SEM)]	72.1(2.2)	69.1(4.1)	.000	.502
mean RR-interval [M(SEM)]	967(10)	953(22)	.002	.518

SD RR-interval [M(SEM)]	54.2(2.1)	50.1(3.1)	.004	.30 9
RMSSD [M(SEM)]	62.9(2.9)	58.2(4.3)	.003	.38 8
alpha1 [M(SEM)]	.859(.018)	.864(.034)	.009	.88 1
SD ratio (high-freq/low-freq) [M(SEM)]	1.51(.03)	1.58(.07)	.004	.28 7
RSA [M(SEM)]	1130(50)	816(75)	.041	.00 1

Tab. 2b: measures of heart rate variability in subjects with past (but not actual) major depressive episode ('past MDE') versus subjects with an actual major depressive episode ('actual MDE')

	past MDE	actual MDE	eta-squared	p
n	54	16		
average respiration interval (ms) [M(SEM)]	3870(110)	3810(180)	.001	.7 70
heartbeat variability (SD) [M(SEM)]	72.6(5.0)	57.7(5.4)	.035	.1 23
mean RR-interval [M(SEM)]	944(22)	942(45)	.001	.9 80
SD RR-interval [M(SEM)]	54.1(3.8)	36.9(3.3)	.079	.0 20
RMSSD [M(SEM)]	61.6(5.1)	42.2(4.9)	.056	.0 51
alpha1 [M(SEM)]	.866(.040)	.893(.060)	.002	.7 37
SD ratio (high-freq/low-freq) [M(SEM)]	1.59(.08)	1.53(.12)	.003	.6 75
RSA [M(SEM)]	881(87)	599(143)	.037	.1 14

Tab.3: Pearson correlations of respiratory sinus arrhythmia (RSA) with age and various psychiatric scores

	all	males	females
age	-.245	n.s.	-.308
anxiety sensitivity (ASI)	-.191	-.279	-.166
neuroticism NeoFFI	-.152	n.s.	-.201
extraversion NeoFFI	.181	.268	n.s.
MADRS depression score	-.139	n.s.	n.s.
HAMD depression score	-.137	(-.212)	n.s.
state anxiety (STAI state)	-.204	-.239	-.187
BDI depression score	-.138	(-.181)	n.s.
BAI anxiety score	-.222	-.240	-.245
PTQ sum score	-.149	-.298	n.s.
PTQ mental capacity captured score	-.222	-.361	n.s.

p<.05; **bold**: p<.01; (in brackets: .05<p<.10)

Tab.4: Pearson correlations between RSA and hormones/ cytokines in the full sample and the male/ female subsamples

	all	male	female
TSH concentration serum [pg/ml]	-.219	-.293	n.s.

IFNg concentration serum [pg/ml]	-.167	-.423	n.s.
IL1a concentration serum [pg/ml]	-.189	(-.276)	n.s.
IL6 concentration serum [pg/ml]	-.226	-.305	n.s.
TNFa concentration serum [pg/ml]	-.238	-.416	n.s.

p<.05; **bold**: p<.01; (in brackets: .05<p<.10)