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Low vagal tone in two rat models of psychopathology involving high or low corticosterone stress responses

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ABSTRACT

The two stress-responsive physiological systems, autonomic nervous system (ANS) and hypothalamus-pituitary-adrenal (HPA) axis exert complementary and interrelated actions in the organism. Individuals that suffer stress-related psychopathologies frequently present simultaneous alterations –i.e., either low or high- responsiveness– in both systems. However, there is scarce evidence establishing whether *a priori* alterations in these systems –i.e., independent of previous stress exposure– may predispose to the development of psychopathologies possibly due to the lack of animal models simultaneously involving aberrant HPA and SNS responses. In this study, we describe two animal models selectively bred according to their differential (either high, 'High', or low, 'Low') glucocorticoid responsiveness to stress, in comparison to a third line of rats that displays intermediate ('Inter') glucocorticoid responses. The two extreme lines may be considered distinct models of psychopathology; the High line representing a model of constitutive mood alterations while the Low line a model of vulnerability to develop stress-induced psychopathologies. We recorded the electrocardiogram in rats from the three lines and quantified heart rate variability and vagal tone indexes during rest and stress challenges. Rats from both High and Low lines displayed higher heart rate and lower basal vagal tone than the Inter group, both at resting and following stress exposure. Specific pharmacological manipulations probing the relative contribution of sympathetic and parasympathetic components on HR modulation confirmed a relative lower vagal tone in High and Low lines and discarded differences in the sympathetic regulation of heart rate between the lines. Therefore, the two genetically-selected High and Low glucocorticoid rat lines emerge as two valuable preclinical models of psychopathology involving two key risk factors for psychiatric and cardiovascular disorders, namely dysregulations in the HPA axis and cardiac vagal functioning.

1. Introduction

One of the current challenges in mental health research is the identification of risk factors and mechanisms related to individual vulnerability to develop psychopathologies (Weger and Sandi, 2018). Given the well-known impact of stress in triggering and exacerbating psychopathologies (de Kloet et al., 2016; McEwen et al., 2012), a great deal of research is concentrating on understanding the role of the major physiological stress systems [i.e., the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenocortical (HPA) axis] in this context (Carnevali et al., 2018; de Kloet et al., 2005; Pruessner et al., 1997; Sgoifo et al., 2015; Walker et al., 2018).

Importantly, the ANS and HPA axis exert complementary actions in the organism and their functions are closely interrelated (Ulrich-Lai and Herman, 2009). The cardiovascular system can increase corticosterone (CORT) production via sympathetic actions on the adrenal gland (Lowrance et al., 2016) and inhibit its production through the vagus nerve, the major outflow from the parasympathetic branch of the ANS (Thayer and Sternberg, 2006). Moreover, although much research is still needed, existing evidence indicates that several hormones from the activated HPA axis can modulate different aspects of cardiovascular functioning (Burford et al., 2017). There is strong evidence that aberrant -either low or high- glucocorticoid responsiveness to stress are related to the development of psychopathologies (Herman, 2013; Korte, 2001; Marques et al., 2009) and impaired cardiovascular functions (Doom and Gunnar, 2013; Peters and McEwen, 2015). A critical question is whether alterations in these systems can predispose to the development of psychopathologies or whether they are the consequence

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of stress-related disorders. However, the scarcity of animal models, simultaneously involving aberrant HPA stress responses and autonomic imbalance, precludes research addressed to interrogate the causal role of each of these systems in both their potential mutual dysregulation and in the pathophysiological development of stress-related disorders, as well as treatment development. Some animal studies have reported cardiovascular abnormalities and diminished vagal tone in animals showing either trauma-induced reduced basal CORT levels (Zoladz et al., 2012) or high stress-induced CORT levels (Cohen et al., 2003). However, to our knowledge, none of the existing models encompasses individual differences in the functioning of the HPA axis and ANS system simultaneously regardless of prior exposure to stress.

Our laboratory has recently performed selective breeding of rats according to their differential glucocorticoid responsiveness to repeated stress exposure during juvenility, resulting in three lines of high, intermediate or low responders (called 'High', 'Inter' and 'Low' lines, respectively) (Walker et al., 2017). Progeny of these lines, when tested at adulthood, shows the expected differences in CORT responsiveness to stress, with the High line showing higher and the Low line lower CORT levels than the control, Inter line (Walker et al., 2017). Importantly, each of these two lines of extreme CORT responsiveness represent a distinct model of psychopathology. The High line can be considered a model of constitutive mood alterations as they display increased anxiety-like, depression-like and aggressive behaviors (Walker et al., 2017; Walker and Sandi, 2018) that are not further altered by stress exposure at peripuberty (Walker and Sandi, 2018). On the contrary, the Low line can be considered a model of vulnerability to develop stress-induced psychopathologies, eventually related to post-traumatic stress disorder (PTSD). Indeed, although in the absence of life stress exposure rats from the Low line show emotional behaviors similar to controls (Walker et al., 2017; Walker and Sandi, 2018), these rats are particularly susceptible to trauma exposure, showing enhanced anxiety-like and aggressive behaviors following peripubertal exposure to fearful experiences (Walker and Sandi, 2018).

Affective disorders such as anxiety, depression and PTSD are highly comorbid with cardiovascular disorders (CVDs) (Cohen et al., 2015; Coughlin, 2011). Several studies have reported that individuals with psychiatric symptoms show cardiovascular complications, including autonomic nervous system imbalance and reduced heart rate variability (HRV) (Beauchaine and Thayer, 2015; Carnevali et al., 2017; Sgoifo et al., 2015). Similarly, CVDs seem to predispose individuals to develop psychiatric disorders (Rudisch and Nemeroff, 2003).

Individual differences in cardiac vagal outflow have been suggested to underlie differences in vulnerability and resilience to develop stress-related psychiatric disorders (Carnevali et al., 2018). Quantification of heart rate variability indexes vagal tone and it is used as a surrogate index of cardiac autonomic modulation. HRV assessment has become a clinical tool to determine susceptibility to develop both, CVDs (Billman et al., 2015) and psychological disorders (Flórez et al., 2017). Specifically, low vagally-mediated HRV has been associated with vulnerability to psychopathology (Beauchaine and Thayer, 2015). On the other hand, high levels of vagally-mediated HRV have been positively associated with overall health (Porges, 1995) and with resilience to stress (Carnevali et al., 2018).

Based on previous observations that a dysregulated (either high or low) CORT response to stress has been implicated in psychological states characterized by autonomic imbalance (Carnevali and Sgoifo, 2014; Danan et al., 2018; Zoladz and Diamond, 2013) we hypothesized that animals showing either low or high CORT responsiveness will exhibit decreased HRV and lower cardiac parasympathetic nervous system influence. To test these hypotheses, we performed a detailed characterization of the autonomic neural modulation of heart rate (HR) in the three CORT rat lines including (i) time- and frequency-domain analyses of HRV at rest and in response to social and restraint stress (ii) and pharmacological manipulations to reveal involvement of specific autonomic systems.

2. Material and methods

2.1. Animals

Experimental animals were male offspring from the eighth to eleventh generations of recently developed lines of differentially corticosterone reactive rats (Walker et al., 2017) developed in our animal facility (EPFL, Lausanne, Switzerland) as described below (Walker et al., 2017; as well *Protocol for selective breeding* below). Importantly, experimental rats used in this study were not exposed to the CAST procedure themselves; i.e., not exposed to stressors during juvenility. They were briefly handled on P28-30 and then left undisturbed, except for weekly cage changes, until they were submitted to experimental manipulations at adulthood (from P90 onwards). Male Wistar Han rats purchased from a commercial breeder (Charles River, L'Arbresles, France) acted as social stressors (during social instigation and social interaction protocols) and were used only once in each test.

Animals were maintained on a 12:12 h light-dark cycle (lights ON at 07:00 h) in a temperature (22 ± 1 °C) and humidity-controlled environment (55 ± 5% humidity). They had *ad-libitum* access to laboratory chow and water. All procedures were conducted in accordance with the Swiss National Institutional Guidelines on Animal Experimentation and were approved by the Swiss Cantonal Veterinary Office Committee for Animal Experimentation.

2.2. Protocol for selective breeding

The selected breeding of the rat lines used in this study started from Wistar Han rats obtained from a commercial breeder (Charles River, France) that were bred over generations in our animal facility, as previously described (Walker et al., 2017). Briefly, breeders were selected following a 'corticosterone-adaptation-stress-test' (CAST) protocol that involved exposure to different stressors over three consecutive days during the juvenile period (P28-P30; Supplementary Fig. 1A). The CAST is a truncated version (Tzanoulinou et al., 2014) of the peripubertal stress protocol developed in our laboratory (Márquez et al., 2013). Specifically, on P28, animals were exposed to an open arena during 5 min, followed by exposure to an elevated-platform (EP) for 25 min in a bright environment (> 300 lux). On P29, rats were placed in a new environment for 25 min where they were exposed to synthetic predator scent (trimethylthiazoline, TMT). Immediately afterwards, they were exposed to an EP (25 min). On P30, the same stressors as on P29 were applied but in a reverse order. Blood was sampled from tail incision immediately following exposure to stressors on P28 and P30, and following 30 min of recovery. Rats with extremely high (> 200 ng/ml) or extremely low (< 100 ng/ml) plasma CORT levels following stressor exposure on P30 are bred over generations, leading to the selected 'High' and the 'Low' breeding lines, respectively. A control line, 'Inter', consists of selectively bred animals with intermediate corticosterone values (> 100 ng/ml and < 200 ng/ml) following stressor exposure on P30. Supplementary Figs. 1B and 1C show the follow up of this selection procedure indicating CORT responses to P30 stressors on male and female rats from the three lines including generations F8-F11 whose offspring was used in the current study.

2.3. Corticosterone responsiveness to a novelty challenge

At P90, rats were exposed to a novelty challenge to measure plasma corticosterone reactivity, as previously described (Veenit et al., 2013). Immediately after 25 min exposure to a novel environment (a circular plastic container; Ø = 40 cm, height = 50 cm), blood samples were obtained by tail-nick and rats were returned to their homecage. Animals from the same homecage were simultaneously tested in adjacent containers. The containers were cleaned with a 5% ethanol solution and dried properly before placing the animals.

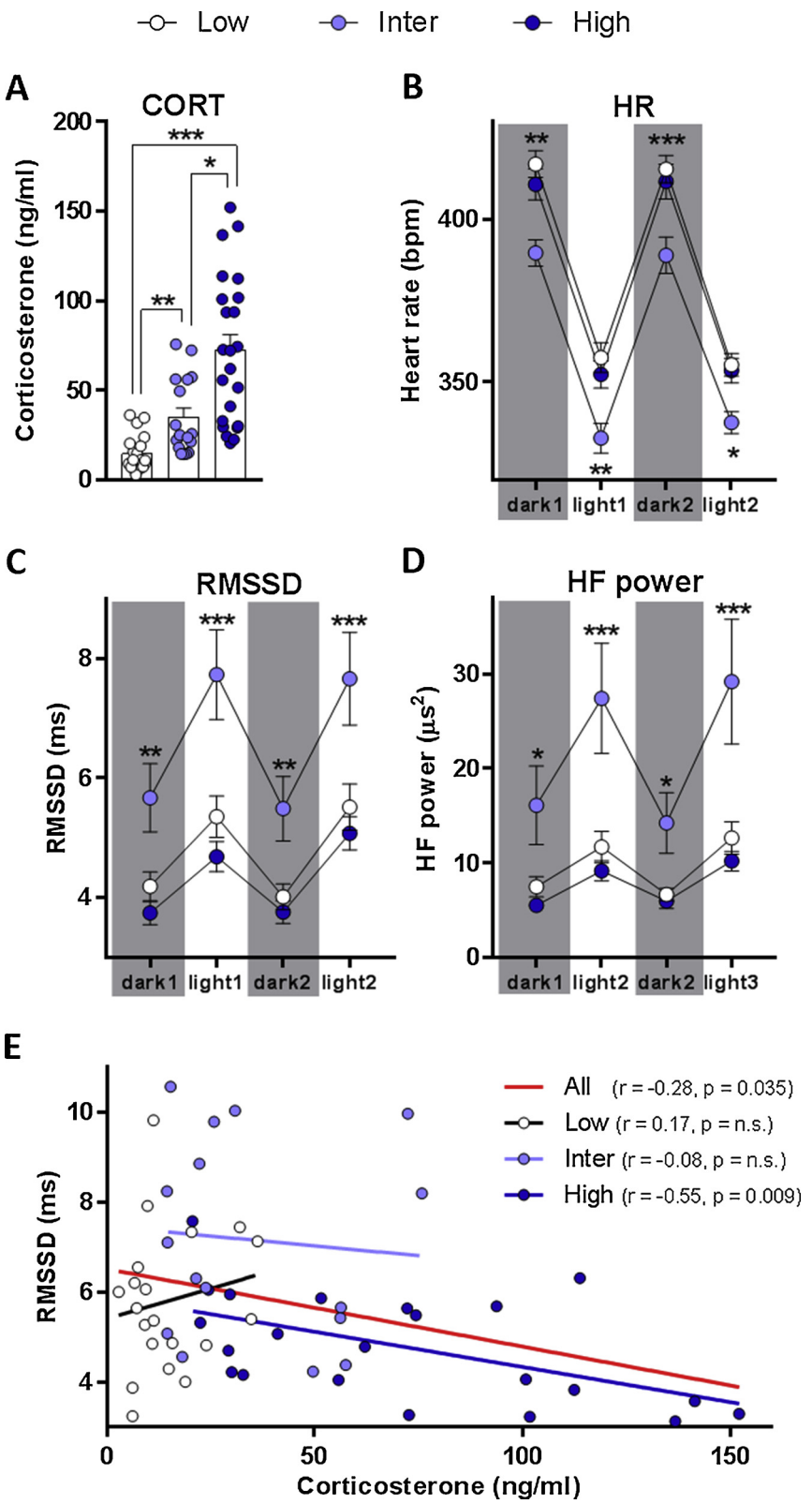

Fig. 1. Rats with high and low corticosterone responsiveness had higher resting heart rate and lower vagal tone. **A,** Corticosterone (CORT) responses to novelty exposure at adulthood differed in a line-dependent manner. Low (n = 20), Inter (n = 17) and High (n = 23). **B,** Heart rate (HR) averaged over two 12 h dark- and two 12 h light-phases was lower in the Inter line than in Low or High line rats. **C,** Vagal tone, indexed by the root-mean square differences of successive R-R intervals (RMSSD), was higher in Inter line rats than in Low or High lines. **D,** The high frequency (HF) power parameter was also higher in Inter line rats, indicating a higher vagal tone, than in Low or High lines. **E,** There was a negative linear correlation for the High line animals between corticosterone levels in response to novelty-stress and basal RMSSD. Pearson r is reported for correlations concerning Low, Inter and High lines; Spearman r is reported for the correlation concerning all subjects. Low (n = 20), Inter (n = 16) and High (n = 22). In Figures **B, C** and **D** : Low (n = 25), Inter (n = 17) and High (n = 27). In Figures **B, C and D:** Asterisks for each time point indicate statistical differences between Inter and Low/High lines. *p < 0.05, **p < 0.01, ***p < 0.001; n.s.: non-significant.

2.4. Corticosterone analysis

Blood samples were collected into heparin-coated capillary tubes (Sarsted, Switzerland), kept on ice until centrifugation (4 min, 4 °C and 9400 *g*), and stored at −20 °C. Plasma CORT levels were measured using a highly sensitive ELISA kit (ADI-900-097, Enzo Life Sciences, Switzerland). Blood plasma samples were diluted 20 times and the ELISA was performed according to manufacturer's instructions. Concentration values of CORT were calculated using a 4-parameter logistic fit (www.myassays.com). The Inter and intra-assay coefficients of variation were both below 10%.

2.5. Radiotelemetric transmitters for ECG recordings

Experiments with implanted radiotelemetric transmitters for recording electrocardiogram (ECG), core body temperature (T°) and locomotor activity (LOC), were performed as described below.

2.5.1. Surgery: radiotransmitter implantation

Following a procedure adapted from (Adeyemi et al., 2009; Sgoifo et al., 1996), rats were implanted with radiotelemetric transmitters (TA11CTA-F40, Data Sciences International (DSI), St. Paul, MN, USA). Briefly, rats were anaesthetized by inhalation of Isoflurane. Then, the transmitter body was inserted in the abdominal cavity and sutured to the abdominal wall for stability, one electrode was sutured to the xiphoid process and the other electrode was sutured between the sternomastoid and sternohyoid muscles (Adeyemi et al., 2009). After surgery, rats were individually housed and given 1 week to recover before baseline ECG recording.

2.5.2. Radiotelemetric data recording

The radiotelemetric transmitters allowed recording of electrocardiogram (1000 Hz), body temperature (256 Hz) and locomotor activity (expressed in counts/min, 256 Hz) signals, which were picked up by receivers (RPC-1, DSI) and recorded by a dedicated software (Dataquest ART-platinum, DSI). During baseline recordings, receivers were individually placed below rats' homecages. During behavioral testing, cages or apparatuses were placed on top of receivers in the experimental rooms.

2.5.3. Quantification of HRV

HR as well as time- and frequency-domain parameters of HRV were quantified using LabChart 8.0 software (AD Instruments, Sydney, Australia). ECG signals were visually inspected to ensure that all R-waves were properly detected. In the time-domain, we considered the Root Mean Square of Successive Differences between adjacent R-R intervals (RMSSD, ms), which quantifies short-term, high frequency variations of R-R intervals and therefore estimates the activity of the parasympathetic nervous system (Stein et al., 1994). For spectral (frequency-domain) analysis of HRV, the power spectrum was computed with a fast Fourier transform-based method. We considered the total power of the spectrum (ms^2), which reflects all the cyclic components responsible for variability, as well as the power of the low frequency (LF power: 0.2-0.75 Hz) and high frequency (HF power: 0.75–2.5 Hz) bands in absolute values (ms^2). The power of LF band is a non-specific index as it contains contributions of both the sympathetic and parasympathetic influences (Reyes del Paso et al., 2013). The power of HF band is due to the activity of the parasympathetic nervous system and includes respiration-linked oscillations of HR (Berntson et al., 1997).

2.6. Radiotelemetric experiments

2.6.1. Baseline recordings

After 1 week of recovery from surgery, baseline ECG, T° and LOC signals were recorded during two days for 4 min every hour and data were averaged over the 12 h light- and 12 h dark-phases for statistical analyses. One week later, cardiovascular parameters were recorded after rats' exposure to either social (social instigation and social interactions) or physical (restraint) challenges, as well as following specific pharmacological manipulations, as described below.

2.6.2. Social and physical stressors

Male rats offspring from the 8th generation of parental breeding lines were tested in three replication cohorts of 10 animals, each one including rats from the three lines. During testing and recording of an implanted cohort, rats from the remaining cohorts were left undisturbed in their homecage. All implanted rats were tested on four behavioral tests: light/dark-box, social instigation, resident-intruder (social interactions) and restraint stress (see below for experimental details). In each case, ECG was recorded during a baseline period of 30 min, during the test and during a recovery period of 30 min. The two social challenges were given during the beginning of the dark phase (between 19:00 and 22:00). Social instigation was performed for 30 min during which each rat was exposed to an unfamiliar conspecific male, separated by a clear plastic glass separator with small holes that allowed visual, olfactory and auditory interactions, while preventing physical interactions. The following day, a new unfamiliar conspecific was placed in the homecage of the experimental rat, allowing direct physical interactions for 30 min. Given the lack of differences between these two sets of data, results from both social tests (referred as *social stressors*) were averaged and combined. The physical stressors consisted on restraint stress performed during the light-phase, specifically between 09:00 and 12:00 am. Each animal was introduced for 15 min into a restrainer, made of mesh grid (approx. 8 x 25 cm), and returned to its homecage after the test. For social and physical stressors, HR and HRV parameters were analyzed for the immediate response to stress (named the 'peak' response, 5 min) and during the remaining stress exposure.

2.6.3. Pharmacological autonomic treatments

Male rats offspring from the 11th generation of parental breeding lines were used. The study was designed to assess the relative contribution of sympathetic and parasympathetic components on HR modulation by, respectively, testing the effects of pharmacological administration of beta-adrenoceptor and muscarinic receptor antagonists in consecutive days. Rats received a subcutaneous (SC) saline injection as a control manipulation. Sympathetic blockade was induced by injecting atenolol (2 mg/kg, SC; Sigma, St Louis, MO, USA), a β-1 adrenergic receptor antagonist. The muscarinic receptor antagonist, methylscopolamine bromide (0.05 mg/kg, SC; Sigma, St Louis, MO, USA) was injected in order to block the vagal component of HR. Drug doses were selected based on previous studies (Carnevali et al., 2014, 2013). In all experiments, ECG recordings were started 30 min prior to injection to obtain data during the habituation period and continue being recorded until 50 min post-injection.

2.7. Statistics

Data distribution were checked for normality and outliers removed using the robust regression and outlier removal (ROUT) method (Motulsky and Brown, 2006). Two-way ANOVA with repeated measures with *line* as a between-subject factor (three levels: High, Inter and Low lines) and with *time* as a within-subject factor (number of levels depend on individual cases) was applied when data followed a repeated measures design. All other analyses involved one-way ANOVA with *line* as a between-subject factor. *Post hoc* analyses were performed with a Fischer's test for multiple comparisons. When data comparisons involved groups displaying differences in variance (e.g., CORT data), additional analyses of CORT results were performed by applying a non-parametric Kruskal-Wallis test with uncorrected Dunns' multiple comparison tests, reported in the main Results section, and a logarithmic transformation [Log(CORT)], reported in Supplementary Information. Linear regressions were applied in order to examine potential correlations between CORT and RMSSD values. Specifically, Pearson r was computed when data were normally distributed (this was the case for data from each of the lines separately) and non-parametric Spearman r was computed when data did not follow a Gaussian distribution (this was the case when data from all subjects was considered together). The area-under-the-curve (AUC) was calculated as HR and HRV changes with respect to baseline values. Data are presented as mean ± standard error of the mean (SEM). Statistics and graphs were performed using the GraphPad Prism software (version 7). Statistical significance was set for $p < 0.05$.

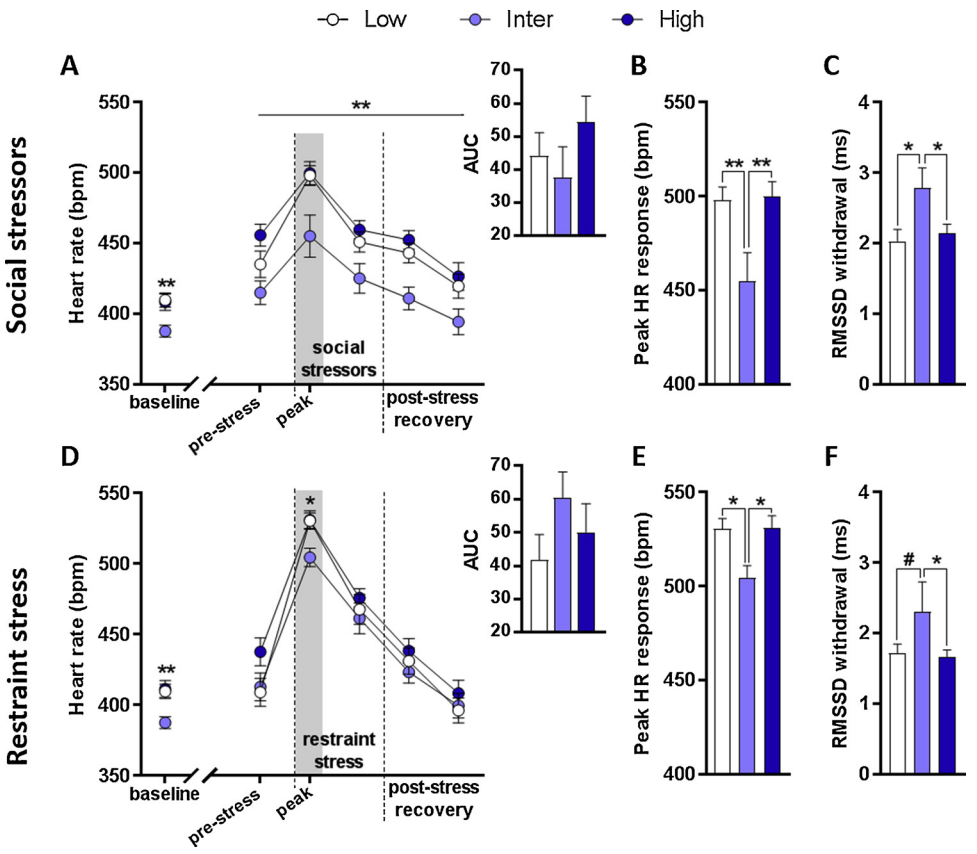

Fig. 2. Low and High line rats had higher heart rate response during social stressors but not during restraint stress. **A,** During social stressors, Low and High line rats had higher heart rate (HR) than Inter line animals. There was no difference in the area under the curve (AUC) between the electrocardiogram (ECG) recorded during social stressors and the baseline recording (**A, right panel**). **B,** The peak HR response during social stressors was higher in the Low and High lines compared to Inter line rats. **C,** The root-mean square differences of successive R-R intervals (RMSSD) during peak social stressor responses was lower in the Low and High lines compared to Inter line rats. The 'RMSSD withdrawal' illustrates the vagal withdrawal following stress during the early response to stress (5 min following stress onset). **D,** During restraint stress there were no differences in HR between the three lines and there was no difference in the AUC response (**D, right panel**). **E,** The peak HR response to restraint did not differ between the lines. **F,** There was no difference in RMSSD peak value in response to restraint stress. In Figures **A and D,** the baseline time point represent the average of the dark-phases during baseline recording. In Figures **A, B and C**: Low (n = 11), Inter (n = 5) and High (n = 10). In Figures **D, E and F**: Low (n = 11), Inter (n = 6) and High (n = 11). Asterisks represent statistical differences. $^{\#}$ $p < 0.1$, *$p < 0.05$, **$p < 0.01$.

3. Results

3.1. Corticosterone responsiveness at adulthood

First, we verified that rats from the different lines differed in their CORT responses to stress at adulthood (Fig. 1A). Indeed, plasma CORT levels in response to novelty (i.e., 25 min bucket exposure) were significantly different between the lines (Kruskal-Wallis, $H_3 = 31.71$, $p < 0.001$). As expected, High line rats displayed higher CORT response than animals from the Low (Dunn's $p < 0.001$) and Inter lines (Dunn's $p = 0.011$). In addition, rats from the Inter line had significantly higher CORT response to novelty than Low line rats (Dunn's $p = 0.006$). In addition, these significant differences were further supported when ANOVA was performed on Log(CORT) transformed data (see Supplementary Fig. 2A).

3.2. Resting heart rate and vagal tone

Basal HR was averaged separately for the 12 h light- and 12 h dark-phases (Fig. 1B). There was a main effect of day period on HR ($F_{3,138} = 164.9$, $p < 0.001$) with higher HR during the active, dark-phase. There was a line effect ($F_{2,66} = 7.49$, $p = 0.002$) with Inter line rats having lower basal HR than both Low ($t_{66} = 5.1$, $p = 0.002$) and High lines ($t_{66} = 4.96$, $p = 0.003$). There was no interaction between light/dark-phases and lines ($p = 0.32$).

RMSSD and HF values were used as surrogate measures of vagal modulation during the light- and dark-phases (Fig. 1C & 1D). First, RMSSD values (Fig. 1C) fluctuated greatly according to the light/dark-phases ($F_{3,198} = 72.64$, $p < 0.001$) with higher RMSSD during the non-active light-phases. Moreover, there was a highly significant line effect on RMSSD ($F_{2,66} = 10.94$, $p < 0.001$) with higher RMSSD values in Inter line rats than Low ($t_{66} = 5.1$, $p = 0.002$) and High line animals ($t_{66} = 6.44$, $p < 0.0001$). There was no difference between Low and High line rats in RMSSD values during baseline recordings ($t_{66} = 1.4$, $p = 0.586$). Similarly, HF power values (Fig. 1D) were higher during light-phases ($F_{3,198} = 42.59$, $p < 0.001$). There was also a line effect on HF values ($F_{2,66} = 10.1$, $p < 0.001$) with Inter line rats having higher HF values than Low ($t_{66} = 5.17$, $p = 0.0015$) and High line animals ($t_{66} = 6.07$, $p < 0.001$). There was no difference between the Low and High lines in HF values during baseline recording ($t_{66} = 0.919$, $p = 0.793$). Baseline results of the different HRV parameters are summarized in Supplementary Table 1.

Then, we assessed whether plasma CORT levels in response to the bucket test correlated with resting vagal tone. As illustrated in Fig. 1E, when considering the three line independently, the High line exhibited a negative correlation between CORT and RMSSD values ($r = 0.545$, $p = 0.009$), whereas the Low and Inter lines did not show correlations ($p = 0.48$ and $p = 0.75$ respectively). When data from all animals were analyzed together, there was a negative correlation between the CORT levels and resting RMSSD values ($r = -0.28$, $p = 0.035$; Spearman r was computed here as these data from all subjects is not normally distributed). In addition, similar findings were obtained when the correlation was performed on data corrected for normality using logarithmic transformation (see Supplementary Fig. 2B).

3.3. Cardiac autonomic responses to stress

In order to verify the maintenance of behavioral differences between the lines in anxiety tests as previously described (Walker et al., 2017), we tested rats in an elevated plus maze prior to surgery and in a light/dark box test after recovery from surgery (see Supplementary Material). As expected, during both time points (before and after surgery), rats from the High line displayed higher anxiety-like behaviors than rats from the Low and Inter lines (Supplementary Fig. 3A and B).

HR and HRV responses to social and restraint challenges are reported in Fig. 2 as well as in Supplementary Tables 2 and 3. Fig. 2 shows the baseline values recorded during the active dark-phases, the pre-stress values after moving the cages in the experimental room, the peak

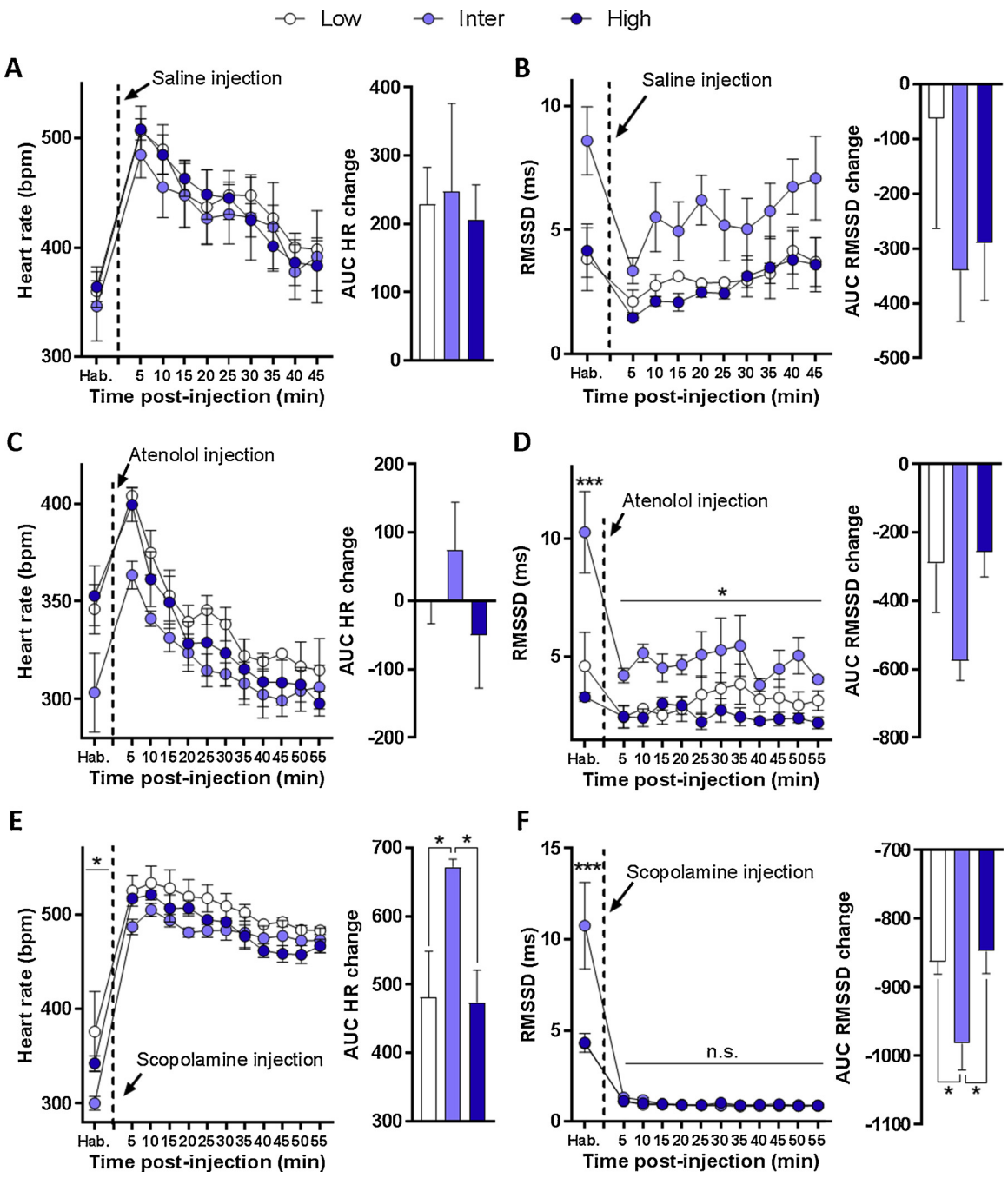

Fig. 3. Low and High line rats had a lower vagal modulation of heart rate in response to pharmacological manipulations. **A, B,** Saline injection was performed as control manipulation. There was no difference in heart rate (HR) increase (**A, left**) and no difference in relative changes in HR (**A, right**) in response to saline injection. Low and High lines had lower root-mean square differences of successive R-R intervals (RMSSD) throughout recording compared to Inter line rats (**B, left**). There were no significant differences in area under the curve (AUC) of RMSSD values (**B, right**). **C, D,** Injection of atenolol blocked sympathetic influence on HR (**C, left**). There was no line effect on HR (**C, left**) and there was no difference in AUC of HR changes (**C, right**). There was a stable difference in RMSSD (**D, left**) with Low and High lines having lower RMSSD values throughout ECG recording compared to Inter line rats and there was no difference in AUC of RMSSD changes (**D, right**). **E, F,** Scopolamine injection blocked the parasympathetic influence on HR. There was no difference in HR after scopolamine injection between the three lines (**E, left**), but there was a relative lower increase in HR for Low and High lines compared to Inter line rats (**E, right**). Scopolamine decreased RMSSD values in the three lines (**F, left**) but Low and High lines had lower decrease of RMSSD than Inter line rats (**F, right**). Asterisks represent statistical differences between the Inter line and the Low /High lines. *p < 0.05and ***p < 0.001; n.s.: non-significant. N = 3/line.

and late responses to the stressors as well as the post-stress recovery period (two blocks of 15 min each). Supplementary Tables 2 and 3 present HR, HRV, T° and LOC parameters during habituation, stressors and recovery phases. Data from HF power and RMSSD were highly correlated thus only RMSSD values were used as surrogate measures of vagal modulation, as depicted in Fig. 2.

Analyses of HR and HRV changes during social stressors are represented in Fig. 2A, B and C. There was a line effect on HR ($F_{2,24} = 5.84$, $p = 0.009$), but no interaction between time-bins and lines ($F_{8,96} = 1.1$, $p = 0.37$). The line effect (Fig. 2A) involved a lower HR throughout the experimental phases in Inter line rats compared to Low ($t_{24} = 2.59$, $p = 0.02$) and High lines ($t_{24} = 3.37$, $p = 0.003$). There was no difference in HR values between Low and High lines ($t_{24} = 0.97$, $p = 0.34$). AUC values did not differ between the three

lines (Fig. 2A, inner panel: $F_{2,24} = 1.01$, $p = 0.38$). In response to the introduction of an unfamiliar rat in the homecage (Fig. 2B), there was a line effect on stress-induced peak HR ($F_{2,24} = 5.96$, $p = 0.008$). Inter line rats had a lower peak HR than Low ($t_{24} = 3.09$, $p = 0.005$) and High line rats ($t_{245} = 3.16$, $p = 0.004$). The RMSSD values during social stressors did not differ between the lines (data not shown). Analysis of the peak response to stress (Fig. 2C) illustrated a line effect ($F_{2,24} = 3.98$, $p = 0.032$), with Inter line rats having a significantly higher RMSSD than Low ($t_{24} = 2.73$, $p = 0.012$) and High line rats ($t_{24} = 2.29$, $p = 0.031$).

During restraint stress (Fig. 2D), there was no line effect on HR values ($F_{2,25} = 2.11$, $p = 0.14$), and no interaction between time-bins and lines ($F_{8,100} = 1.17$, $p = 0.31$). AUC values did not differ between the three lines (Fig. 2D, inner panel: $F_{2,25} = 1.1$, $p = 0.348$). For the peak HR reached following restraint (Fig. 2E), there was a line effect ($F_{2,25} = 4.37$, $p = 0.024$). Inter line rats reached a lower peak HR than Low ($t_{25} = 2.66$, $p = 0.014$) and High lines ($t_{25} = 2.71$, $p = 0.012$) in response to restraint, while there was no difference between Low and High lines ($t_{25} = 0.06$, $p = 0.96$). There was no line effect on RMSSD values during restraint stress (data not shown), however, analysis of the peak response to restraint stress (Fig. 2F) indicated a statistical tendency for a difference in RMSSD values between the lines ($F_{2,25} = 2.66$, $p = 0.089$). While there was no difference between Low and High lines ($t_{25} = 0.19$, $p = 0.85$), Inter line rats had higher RMSSD than Low ($t_{25} = 2.01$, $p = 0.056$) and High lines ($t_{25} = 2.17$, $p = 0.04$).

In addition, we verified other behavioral and metabolic parameters. The three lines did not differ in total locomotion during stressor application (Supplementary Tables 2 and 3). The T° of the animals differed only during the habituation period before restraint stress (Supplementary Table 3) with Inter line rats having higher T° than High line rats. However, there were no differences in T° during behavioral testing or recovery periods following stressors.

3.4. Pharmacological autonomic manipulations

Since data from RMSSD and HF power parameters were positively correlated, only RMSSD results are illustrated to evaluate vagal modulation of HR (Fig. 3). As a control manipulation, rats were injected with a saline solution (Figs. 3A and 3B), which caused a significant increase in HR ($F_{9,54} = 17.96$, $p < 0.001$) and decrease in RMSSD ($F_{9,54} = 6.66$, $p < 0.001$) immediately following the injection. There was no line effect ($F_{2,6} = 0.20$, $p = 0.822$) on HR response to saline injection (Fig. 3A, left panel). This was confirmed by the AUC values for HR (Fig. 3A, right panel) ($F_{2,6} = 0.056$, $p = 0.94$). There was a significant line effect ($F_{2,6} = 5.19$, $p = 0.049$) on RMSSD changes in response to saline injection (Fig. 3B). Inter line animals had higher RMSSD values throughout the entire recording (Fig. 3B, left panel) than Low ($t_6 = 2.64$, $p = 0.039$) and High lines ($t_6 = 2.92$, $p = 0.027$). There was no group difference in RMSSD AUC values (Fig. 3B, right panel) ($F_{2,6} = 1.1$, $p = 0.391$).

Maximal HR response to atenolol was significantly lower than the HR response to saline injection in the three lines (Fig. 3C, $p < 0.001$). There was no line effect ($F_{2,6} = 2.76$, $p = 0.142$) on HR values during the overall recording and no interaction between time-bins and lines ($F_{22,66} = 0.86$, $p = 0.639$). The relative changes in HR, illustrated by the AUC (Fig. 3C, right panel) did not differ between lines ($F_{2,6} = 1.01$, $p = 0.417$). The RMSSD response to atenolol (Fig. 3D, left panel) showed an important decline following injection for the three lines ($F_{11,66} = 7.03$, $p < 0.001$). There was a line effect on RMSSD values ($F_{2,6} = 8.81$, $p = 0.016$) as well as an interaction between time-bins and lines ($F_{22,66} = 2.5$, $p = 0.002$). During habituation, Inter line rats exhibited higher RMSSD values than Low ($t_6 = 5.9$, $p = 0.001$) and High lines animals ($t_6 = 7.25$, $p < 0.001$). In addition, during the entire post-injection period Inter line rats maintained a higher RMSSD than Low ($t_6 = 2.85$, $p = 0.029$) and High lines rats ($t_6 = 3.89$, $p = 0.008$). The relative difference in RMSSD, illustrated in Fig. 3D (right panel) by the AUC, did not show group difference in vagal response after sympathetic blockade ($F_{2,6} = 3.16$, $p = 0.115$).

The HR and HRV responses to vagal blockade by scopolamine injection are shown in Figs. 3E and 3 F. Scopolamine injection (Figure 4E, left panel) provoked a marked increase in absolute values of HR ($F_{11,66} = 83$, $p < 0.001$) in the three lines, without group differences ($F_{2,6} = 2.45$, $p = 0.166$). There was a tendency for an interaction between time-bins and lines ($F_{22,66} = 1.61$, $p = 0.072$). Inter line rats had a higher increase in HR ($F_{2,6} = 5.56$, $p = 0.043$) as shown by higher AUC values (Fig. 3E, right panel) in comparison to Low ($t_6 = 2.83$, $p = 0.03$) and High lines ($t_6 = 2.94$, $p = 0.026$). When the parasympathetic influences on HR was blocked there was a remarkable decrease in RMSSD in the three lines ($F_{11,66} = 44.2$, $p < 0.001$) which lasted for the entire recording session (Fig. 3F, left panel). There was a line effect on RMSSD values ($F_{2,6} = 10.9$, $p = 0.01$) and an interaction between time-bins and lines ($F_{22,66} = 6.65$, $p < 0.001$). The three lines had different RMSSD values during habituation ($F_{2,6} = 7.01$, $p = 0.027$) with Low and High lines having lower RMSSD values than Inter line rats ($t_{72} = 11.1$, $p < 0.001$), but the three lines had similar RMSSD values post-injection ($t_{72} = 0.42$, $p > 0.67$). Additionally, the AUC of the RMSSD changes (Fig. 3F, right panel) differed between the lines ($F_{2,6} = 5.72$, $p = 0.041$) and Inter line rats had a greater vagal decrease than Low ($t_6 = 2.73$, $p = 0.034$) and High line animals ($t_6 = 3.09$, $p = 0.021$).

In order to assess HR changes following pharmacological treatments (i.e., free from potential injection-induced SNS activation), HR values were also analyzed 45 min after injections (see Supplementary Figure 4). By 45 min after saline injection, HR was similar to baseline HR (Supplementary Figures 4A and 4B). Furthermore, after atenolol and scopolamine injections, HR (Supplementary Figures 4C and 4E, respectively) and HR AUC (Supplementary Figures 4D and 4 F, respectively) did not differ between the three lines.

4. Discussion

Here, we have characterized cardiac autonomic regulation in two rat models of psychopathology involving constitutive differences in their glucocorticoid responsiveness (either high or low) to stressor exposure. The rat lines used here were selected over several generations for their constitutive difference in the adaptation of the corticosterone response to juvenile stress (Walker et al., 2017; Walker and Sandi, 2018). In addition, we report here that when tested at adulthood, the three lines were shown to display differential corticosterone responsiveness to stressors, as previously reported (Walker et al., 2017). We report that rats from both High and Low lines have higher resting HR and lower basal vagal tone than the control -Inter line- group. Similar differences in these two parameters were also observed following responses to stressors. Specific pharmacological challenges probing the relative contribution of sympathetic and parasympathetic components on HR modulation indicated a relative lower vagal (i.e., parasympathetic) modulation in the two extreme rat lines as compared to the Inter line. However, there was no evidence for line-related differences in the sympathetic regulation of HR.

Specifically, we measured HR and HRV parameters from ECG recordings performed both, at baseline (i.e., resting state) and following exposure to different stressors. Rats from the High and Low lines displayed higher HR and lower vagal-mediated HRV (lower RMSSD and HF indexes) than rats from the Inter line at both baseline and in response to stressors. Importantly, we did not observe locomotor or other behavioral differences during these tests (locomotor activity reported in Supplementary Tables). Therefore, these data indicates line-related differences in basal and maximal cardiac capacities (with High and Low lines displaying lower parasympathetic activity leading to higher HR), but no differences in vagal withdrawal in response to stress. In support of this view, pharmacological blockade of cardiac vagal influences with scopolamine led to a smaller increase in HR in High and Low lines than

in Inter line animals. Moreover, scopolamine treatment induced a larger decrease in RMSSD in Inter line animals than in the two other lines, particularly due to the lower baseline values in High and Low lines as compared to the Inter line. These results represent a reliable pharmacological confirmation of a lower relative contribution of the vagal control over resting HR in High and Low lines compared to Inter line animals. Importantly, following saline control injection and sympathetic blockade by atenolol, there were no significant differences in HR changes and Inter line rats constantly exhibited higher RMSSD values. These data further reinforces the view that the three lines do not differ in the sympathetic modulation of HR and that higher resting HR in High and Low lines can be ascribed to their lower parasympathetic influence on cardiac peacemaker activity than in the Inter line.

A limitation in our study is that SNS is typically activated by animals' handling and general procedures involved in the pharmacological injections. Consequently, there might be an overlap between ANS activation induced by the injection per se and ANS-induced changes by the specific drugs. Importantly, in our study, we verified that 45 min after saline injection, HR was similar to baseline HR (Supplementary Figures 4A and 4B). For atenolol and scopolamine injections, we focused our analyses of ECG recordings on the 45–60 min time-window following injection (Supplementary Figures 4C-F). After atenolol injection, we found no difference in HR values between the three lines (Supplementary Figure 4C), and no statistical difference in the AUC of HR changes (Supplementary Figure 4D) supporting the view that sympathetic blockade did not affect cardiovascular responses differently in the different lines. Although we did not find significant differences in HR changes following atenolol injections, we cannot exclude that the Low and High lines might have shown a more important SNS blockade compared to the Inter line at higher drug doses. Further experiments focusing on SNS manipulations of the lines may provide a more extensive characterization of this potential differentiation between the lines. Analysis of ECG recordings following scopolamine manipulation and injection-induced SNS activation allowed us to determine that without differences in vagal influence on the heart, HR did not differ between the three lines (Supplementary Figures 4E and 4 F). Indeed, the observed difference in HR is mainly due to differences in vagal activity already observed during resting state. SNS activation by injections did not affect specifically a rat line.

This set of data is consistent with previous studies showing impaired vagal tone and reduced HRV in rats displaying anxiety-like (Carnevali et al., 2014), depressive-like behaviors (Wood et al., 2012), and aggressive behaviors (Carnevali et al., 2013). Anxiety and depression disorders are associated with reduced HRV in humans (Chalmers et al., 2014; Kemp et al., 2012), and depression severity correlates inversely with HRV (Sgoifo et al., 2015). Therefore, our data supports the view that the High line might be a relevant model to investigate the contribution of autonomic dysfunction and heightened HPA axis responsiveness to constitutive anxiety- and depression-like psychopathologies.

Conversely, the blunted corticosterone stress responsiveness, high HR and low levels of resting cardiac vagal modulation are reminiscent of similar alterations frequently reported in patients with post-traumatic stress disorder (PTSD). In addition to frequent reports of low cortisol levels (Cohen et al., 2006; Danan et al., 2018; Zoladz and Diamond, 2013), PTSD patients typically show greater baseline HR and blood pressure (Zoladz and Diamond, 2013) and decreased HRV (Park et al., 2017; Shah et al., 2013; Zoladz and Diamond, 2013). Similarly, some animal models of PTSD involve cardiovascular abnormalities, including diminished vagal tone (Cohen et al., 2003). Importantly, rats from our genetically-selected Low CORT line do not show behavioral alterations in tests for emotional responses (Walker et al., 2017), but develop increased anxiety- and depression-like behaviors, as well as abnormal aggressive behaviors following exposure to traumatic experiences earlier in life (Walker et al., 2017; Walker and Sandi, 2018). Note that high levels of aggressive and violent behavior have been reported in trauma-exposed PTSD patients (MacManus et al., 2015). Therefore, our data supports the view that reduced vagal modulation might enhance vulnerability to traumatic experiences and, thus, an autonomic imbalance may contribute to PTSD vulnerability. This view is in line with evidence from studies in US soldiers (Minassian et al., 2015; Pyne et al., 2016) and subjects with recent traumatic injuries (Shaikh al arab et al., 2012) indicating that lower HRV at baseline predicts post-deployment PTSD symptoms.

Reactivity of the HPA axis and ANS has been previously linked to coping strategies; notably, a higher sympathetic reactivity was related to active coping, while high HPA axis reactivity with passive coping (Koolhaas et al., 2010). Here, differences in corticosterone stress responses and/or resting autonomic activity between the three rat lines do not seem to be specifically related to clear-cut differences in coping style. We have previously shown that, as compared to the Inter line, the High line displays enhanced passive coping in the forced-swim test (less swimming), while enhanced active coping in the resident intruder test (more aggression) (Walker et al., 2017). Accordingly, in these rat lines the relationship by coping styles and HPA axis and ANS reactivity may be context specific (social-or non-social) and requires further investigation. This possibility aligns with the view that individual vulnerability can depend on context-specific coping and physiological phenotypes (Koolhaas et al., 2017).

Vagal tone is an indicator of cardiac health, representing behavioral and physiological flexibility of an organism, as well as its ability to adapt in response to stress (Porges, 1995). Low vagal tone is a marker of reduced behavioral, physiological and stress response flexibility (Porges, 1995). Lower HRV at rest has been related to prefrontal cortex hypo-activation and impaired performance in cognitive control (Gillie and Thayer, 2014) and cognitive flexibility tasks (Colzato et al., 2018). Consistently, lower HRV at baseline has been reported to be associated with more cognitive control deficits, including the degree of intrusive memories experienced following exposure to stressful experiences (Rombold-Bruehl et al., 2017). Thus, lower baseline HRV before a trauma has been suggested to be a potential vulnerability factor for the development of intrusive memories (Rombold-Bruehl et al., 2017) and dysfunctional emotional attention (Park et al., 2013), all dysfunctions that can have implications for various affective disorders, including depression, anxiety disorders and PTSD. Furthermore, moderate vagal tone at rest was shown to predict greater prosociality in children, representing a physiological preparedness or tendency to engage socially (Miller et al., 2017).

The HPA axis and the ANS are highly complementary and can exert mutual modulatory influences (Burford et al., 2017; Lowrance et al., 2016; Thayer and Sternberg, 2006). The vagus nerve is frequently assumed to play an inhibitory role in the regulation of the HPA axis (Thayer and Sternberg, 2006). Recently, a larger decrease in HRV during the anticipation of a stressful task was reported to predict higher stress task-induced cortisol increase (Pulopulos et al., 2018), supporting the existence of an adapted homeostatic regulation involving the coupling of low vagal tone to high cortisol levels. Although similar mechanisms may account for the physiological deviations displayed by the High line, different regulatory mechanisms should account for the mismatch involving low vagal tone and low CORT levels in the Low line. Given the cardiovascular impact of vasopressin (Ulrich-Lai and Herman, 2009), the high vasopressin levels displayed in the hypothalamic paraventricular nucleus by rats from the Low line (Walker et al., 2017) might be a promising starting candidate for future investigations.

Interestingly, preliminary evidence supports the effectiveness for transcutaneous vagal nerve stimulation to improve vagal tone and modulate autonomic responses to stress in patients with PTSD and mild traumatic brain injury (Lamb et al., 2017). Similarly, HRV biofeedback in stress relaxation training given pre-deployment successfully increased HRV and, hence reduced arousal, during a post-training combat simulation (Lewis et al., 2015). Future studies addressed to investigate the impact of modulating vagal tone on the impact of traumatic experiences in the rat lines described here can help elucidating the causal

involvement of autonomic imbalance in the development of PTSD-like symptoms.

Therefore, our results support the view that the genetically-selected High and Low CORT lines constitute two valuable models linking two key risk factors for psychiatric and cardiovascular disorders, namely dysregulations in the HPA axis and cardiac vagal functioning. Therefore, these two lines emerge as two attractive preclinical models of psychopathologies such as depression and vulnerability to trauma-induced PTSD.

Disclosure of interests

The authors report no conflicts of interest.

Contributions

D.H. and C.S. conceived and designed the study and wrote the manuscript. D.H. performed experiments, analyzed data and wrote the first version of the manuscript. S.G., L.C. and A.S. helped designing pharmacological experiments, interpreting data and contributed to manuscript drafting. J.G. provided technical support. C.S. obtained funding and supervised the study. All authors have approved the final version of the manuscript.

Data statement

Access to the dataset in this study can be obtained by following a formal request procedure to the corresponding author.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.11.003.

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