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Distinct Effects of Early-Life Experience and Trait Aggression on Cardiovascular Reactivity and Recovery

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Abstract

We previously demonstrated independent effects of early-life experience (ELE) and trait aggression (TA) on resting heart rate (HR) and mean arterial pressure (MAP) in rats. The present study examined the effects of TA and ELE on stress-evoked cardiovascular reactivity and recovery. Pups born to Wistar-Kyoto dams were exposed to daily 180-min periods of maternal separation (MS) during the first two weeks of life, and aggression was assessed in adult offspring using the resident-intruder test. Radiotelemetry was then used to record stress-evoked HR and MAP responses in response to: strobe light, novel environment, intruder rat, or restraint. Maximal HR and MAP responses were quantified as indices of reactivity, and exponential decay curves were fitted to determine decay constants as a measure of recovery. Strobe light was the weakest stressor, evoking the lowest increases in MAP and HR, which were significantly greater in MS-exposed rats irrespective of TA. In contrast, reactivity to and recovery from exposure to a novel environment or an intruder were significantly influenced by TA, but not ELE. TA animals exhibited greater reactivity in both of these paradigms, with either decreased (novel environment) or increased (intruder) recovery. Restraint stress induced the largest changes in HR and MAP with the slowest recovery, and these responses were shaped by a significant ELE $\times$ TA interaction. These data indicate that cardiovascular reactivity and recovery are influenced by ELE, TA, or ELE $\times$ TA interaction depending on stressor aversiveness as well as its physical and psychological dimensions.

Keywords

rat; maternal separation; personality; heart rate; blood pressure

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1. Introduction

Early-life experience (ELE) can exert long-term consequences on health and can impact emergence of disease. Adverse early experiences have been shown to have deleterious influences on emotional, metabolic, and endocrine functioning throughout the lifespan across multiple species [1–5]. While there is a variety of ways to model different experiences during early development, a well-established model of differences in ELE in rodents is the maternal separation model. This approach involves daily separation of pups from their dam for different periods of time during the first two postnatal weeks. Previous studies using this approach demonstrated adverse changes in emotional behaviors [6–8] and cardiovascular functioning following prolonged separations [9–11], while brief separations appear to be protective [8, 12, 13].

Our previous work demonstrated that the effects of maternal separation depend on inherent stress-reactivity, with prolonged maternal separation having paradoxically protective behavioral and physiological effects in rats genetically predisposed to high stress susceptibility. Specifically, we utilized Wistar-Kyoto (WKY) rats that are highly sensitive to stress in terms of their emotional and physiological responses. These animals were initially bred from the parent Wistar strain as normotensive controls for the spontaneously hypertensive rats (SHRs) [14]. They were subsequently found to have pronounced differences in emotional behaviors, including increased anxiety- and depressive- like behavior, increased learned helplessness, decreased social interaction, and diminished exploration in a novel environment in comparison to other rat strains, including the parent Wistar strain [15–23]. WKY rats also exhibit altered physiological and neuroendocrine responses to stress. This includes increased circulating plasma corticosterone and adrenocorticotrophic hormone levels, which resemble abnormalities that are common in depressed patients, along with increased weights of the adrenal gland and the heart [18, 24–26]. WKY rats also display greater sensitivity to developing ulcers along with impaired gastric accommodation and visceral hypersensitivity, likely due to their exaggerated response to stress [27–29].

We previously found that exposing WKY pups to daily 180-min maternal separation (MS) from postnatal day (P)1 to P14 led to protective behavioral and cardiovascular adaptations in adulthood. Specifically, MS-exposed WKY rats manifested a more resilient adult behavioral profile, characterized by decreased anxiety- and depressive- like behaviors and increased social interaction [30]. In contrast, MS-exposed Wistar rats exhibited a more stress reactive behavioral profile, characterized by increased anxiety-like behavior and decreased social interaction in adulthood [30]. In addition to the protective behavioral alterations, MS exposure in WKY rats elicited adaptive cardiovascular changes. These included decreased resting heart (HR) and increased HR variability (HRV), both in the time domain and the frequency domain, throughout the lifespan [31]. Extensive clinical evidence demonstrates the protective effects of decreased resting HR and increased HRV in a variety of clinical settings [32–36].

In addition to ELE, cardiovascular health and disease is also strongly shaped by certain dimensions of personality. Specifically, aggression has been strongly linked to autonomic,

endocrine, and cardiovascular disturbances [37–40]. For example, highly aggressive rats manifest impaired cardiovagal regulation, propensity for cardiac arrhythmias, and increased sympathoadrenal activation [41–43]. Our previous work suggests that ELE in WKY rats does not influence expression of aggression. Exposing WKY male rats to MS during early development did not significantly influence whether the rats exhibited trait aggression (TA) [31]. Furthermore, WKY rats that exhibited TA manifested significantly higher resting levels of systolic, diastolic, and mean arterial pressures throughout the lifespan [31]. Interestingly, TA did not correlate with resting HR and HRV levels, while MS exposure did not correlate with blood pressure levels [31]. Taken together these findings suggest that ELE and TA are independent factors that determine baseline cardiovascular function, with ELE determining HR and TA contributing to blood pressure.

The current study sought to extend these observations by examining potential impact of MS exposure and TA on cardiovascular reactivity to and recovery from stress. Cardiovascular reactivity is defined as maximal HR and mean arterial pressure (MAP) responses elicited by a stressor [44]. Cardiovascular recovery refers to the return to pre-stress levels of these parameters following cessation of stressor exposure [44]. Numerous studies have linked cardiovascular reactivity and recovery to development of cardiovascular risk factors and associated morbidity and mortality [44–51]. Distinct personality characteristics also significantly impact cardiovascular reactivity, including depressive, anxious, socially isolating, and hostile traits [52–57]. TA correlates with increased resting MAP levels, while MS correlates with decreased HR and increased HRV [31]. Here we initially characterized cardiovascular response profiles to four distinct stressors: 1) strobe light; 2) novel environment; 3) exposure to a novel conspecific; and 4) restraint. We then examined the impact of TA and MS on shaping MAP and HR reactivity and recovery in response to these four stressors. Our data indicate that TA and MS significantly affect cardiovascular reactivity and recovery in a stress-dependent manner.

2. Methods

All animal handling and experimental procedures were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.1. Animals

Eight pairs of male and female Wistar-Kyoto rats were purchased from Charles River Laboratories (Kingston, NY). Upon arrival, rats were housed 2–3 per cage of the same sex and strain in a temperature-controlled animal housing facility with a 12/12 h light-dark cycle with the lights on at 6:00 a.m. Following a one-week acclimatization period, male and female pairs were mated to generate the pups for the experiment. From postnatal day(P)1 through P14, we initiated a separation procedure modeled after Plotsky and Meaney [12] and as previously described [58]. Newborn pups were either from their dam daily for 180 min (Maternal Separation: MS) or 15 min (Neonatal Handling: NH) respectively between 8:30 a.m. – 12:00 p.m. from P1–P14 as previously described. Entire litter was transferred to different room in a small cage, placed on a heating pad (~37°C) whereas each dam remained

in the home cage. All littermates remained in close contact throughout the separation period, and were returned to home cage after the conclusion of separation. After the final separation on P14, litters remained undisturbed until weaning on P21. For detailed procedure for maternal separation, please see [30].

At the time of weaning on P21, male pups were separated and group housed (3 per cage). Rats with the same ELE (NH or MS) were housed together and left undisturbed except for weekly weighing and standard cage changes until P60. Behavior testing was conducted from P60 - P70 to study anxiety-, depressive-like, and social behaviors. Methodology and results of these studies have been previously reported [30, 59]. Four weeks after the behavioral testing (~P101-P109), $n = 10$ MS and $n = 10$ NH rats were randomly chosen and instrumented with cardiovascular radiotelemetry probes (PA-C40, DSI International). At P263, NH and MS rats were exposed to an intruder rat (i.e. novel conspecific) to quantify their levels of aggressive behaviors as describe below. ELE did not impact emergence of TA, so that half of the animals in each group were classified as TA [31]. Experimental groups were as follows ($n = 5$ per group): MS-TA, MS -NA, NH-TA, NH-NA.

2.2. Radiotelemetry Probe Implantation

Rats were surgically implanted with radiotelemetry probes during anesthesia with isoflurane (2.0–2.5%) in oxygen (induction 5% isoflurane in oxygen). Chronic indwelling catheters were implanted into abdominal aorta and glued with surgical glue (Vetbond), with body of device (PA-C40, Data Sciences International (DSI), St. Paul, MN) sutured into abdominal wall. Aseptic techniques were used throughout all the surgeries, and were treated with carprofen (5 mg/kg; subcutaneous) and buprenorphine (0.1 mg/kg; subcutaneous) injection before the surgical procedure. The rats were allowed to recover from anesthesia in a warm, clean cage with water provided in a petri-dish. Rats were single housed after the surgery, and were allowed to recover for a week before the first baseline recordings were made. Daily routine check (weight, visual inspection of sign of distress, food/water intake, and excretory function) was performed throughout the recovery period. Rats were treated with topical antibiotic cream (Triple antibiotic ointment) on the skin suture wound on daily basis while subcutaneous of buprenorphine (0.1 mg/kg) was injected when rats showed sign of distress and pain. Following the recovery, rats were housed in recording room equipped with DSI hardware and software (ART 4.3) for the rest of the experiment.

2.3. Data Acquisition and Analysis

Blood pressure and activity counts were acquired using Dataquest ART 4.3 software (Data Sciences International). Baseline levels of HR and blood pressure were evaluated by performing continuous 24-hour blood pressure recordings at specific time points during development: P130, P179, P193, P207, P221, and P277. Data from these baseline recordings have been described previously [31].

Blood pressure and HR responses to various acute stressors were acquired at the sampling rate of 500 Hz with 10-second averaged data. Data were averaged in 1-min bins, extracted offline, and used for further analyses. We were able to record from up to 8 rats simultaneously due to hardware limitations, thus the recording sessions were distributed

over a course of 3 days at the same time in the light-dark cycle. Data were acquired for 10 min before (baseline), 5–45 min during (depending on stressor), and up to 120 min after each stressor exposure. Rats were kept undisturbed without any human interference during the baseline recordings, i.e. prior to any stress exposure. Rats were exposed to following stressors in the order listed (developmental age indicated in parenthesis): 1) novel environment (P144 – 146), 2) restraint (P165 – 167), 3) strobe-light (P200 – 202), and 4) intruder (novel conspecific) (P263 – 265).

2.4. Novel Environment

At approximately 1:00 p.m. rats were transferred to a clean standard housing cage with new bedding. Similar cage changes were performed weekly as part of standard animal housing maintenance throughout the experiment. HR and blood pressure were recorded for 10 min prior to cage change, and for 120 min afterwards. On another occasion, this procedure was repeated at approximately 1:00 a.m.

2.5. Restraint

At approximately 1:00 p.m. rats were placed in clear Plexiglas restrainers (length: 23 cm, circumference: 22 cm) for a total of 45 minutes. Recordings were continued for another 120 minutes after the rats were released from the restrainers.

2.6. Strobe Light

Rats were exposed to stroboscopic lighting delivered as 10 flashes per second at 20 watts (E-105 Mini Strobe, Eliminator Lighting, Akron, OH). Total length of exposure was for a period of 5 minutes (from 10:00 – 10:05 p.m.). Activity, blood pressure, and HR were acquired before, during, and after the strobe-light exposure.

2.7. Resident-Intruder Test

The general procedure and quantification of behaviors during intruder encounter were adapted from previous studies utilizing the resident-intruder paradigm with modifications [60, 61] and has been described previously [31]. Testing was performed over the course of 3 days starting at P263 beginning. It was conducted in the early afternoon, when the resident was allowed to rest quietly in its homecage for 1–2 h before testing. Intruder rats were age-matched and socially housed (2–3 per cage) WKY male rats. At the beginning of the test phase, the intruder conspecific was placed into the resident homecage for 10 min. The encounter was recorded and digitized, and was subsequently scored by an experienced observer who was blinded to the treatment groups. Rats were closely observed to ensure that no physical injuries resulted from the encounter; despite aggressive displays by some of the rats, none of the rats needed to be separated because of potential for serious physical harm (e.g., bites, bleeding). Resident and intruder behavior was quantified in the social, nonsocial, and aggressive domains using published methodology and as previously described [31, 60]. The animals included in the current study were previously characterized in terms of their aggressive behavior [31]. One-half of the MS exposed rats ($n = 5$) and one-half of NH-exposed rats ($n = 5$) exhibited clinch attack behavior and were classified as exhibiting trait aggression (TA). The remaining animals from the MS ($n = 5$) and NH ($n = 5$) groups did not

exhibit clinch attack and were classified as non-aggressive (NA), indicating that ELE did not impact aggressive behavior ($\chi^2 = 0$, $P = 1$) [31]. MAP and HR were recorded for 10 min prior, 10 min during, and 2 hrs following intruder exposure.

2.8. Statistical Analyses

Data were analyzed using GraphPad Prism 6 (<http://www.graphpad.com/>). One-minute averages of HR and blood pressure were extracted and further analyzed. The average of 10 minutes prior to the start of stress exposure was regarded as baseline and used to calculate HR and MAP in response to various stressors as % change from baseline. Data were plotted and analyzed using curve fitting analysis using non-linear regression. One phase decay was used as the model to fit the HR and MAP recovery following stress exposure using the following formula: $Y = (Y_0 - \text{Plateau}) * \exp(-K * X)$, where Y is % change from baseline, Y_0 is maximal response, plateau is post-stress plateau, K is the decay constant, and X is time (Figs. 1 and 2). Maximal responses in HR and MAP were used as indices of cardiovascular reactivity. Decay constants calculated using curve fitting analyses of HR and MAP responses were used as indices of cardiovascular recovery. Potential differences in cardiovascular reactivity to and recovery from various stressors were analyzed using one-way ANOVA. Two-way ANOVA was used to analyze the impact of aggression and early-life experience on cardiovascular reactivity to and recovery from individual stressors. Sidak's multiple comparisons test was used post-hoc where appropriate. Significance was set at $p < 0.05$, and data are presented as mean $\pm$ SEM.

3. Results

3.1. Reactivity to and Recovery from Various Stressors

While all stressors utilized in the current study evoked rapid and reliable increases in HR and MAP, there were notable differences in the magnitude of these responses and in the pattern of recovery from each stressor. Among all the stressors, 5-min strobe light exposure during the dark phase appeared to induce the weakest responses in HR and MAP. In case of HR reactivity, maximal response was generally achieved within 3 min of stressor exposure and was $22.56 \pm 3.57\%$ in magnitude. During the recovery phase following cessation of strobe light, HR returned to baseline after ~15 min following stressor onset. Interestingly, HR continued to decrease and reached a stable plateau below the pre-test baseline of ~-7% (Fig. 1A). MAP response to strobe light showed a similar pattern, though the timing of maximal MAP response was delayed by 1–2 min with the peak around 4–5 min following stressor onset. Similarly, the magnitude of the maximal MAP response was smaller at $13.36 \pm 2.64\%$ (Fig. 2A).

Exposure to novel environment during the light phase induced a rapid increase in HR of $58.74 \pm 2.00\%$ in magnitude that reached its peak at ~4 min after stressor onset. HR then returned to baseline within ~25 min. (Fig. 1B). Maximal increase in MAP in the novel environment was $30.21 \pm 1.57\%$, which peaked at ~6 min following stressor onset. MAP then recovered within the next 20 min, reaching the pre-test baseline (Fig. 2B).

Ten-minute exposure to a novel intruder rat induced a rapid increase in HR that peaked within 5–6 min. and reached a maximal increase $79.82 \pm 4.26\%$ (Fig. 1C). HR then slowly recovered, and then reached a plateau after ~45 min of stressor onset. Interestingly, this plateau was ~10% above the pre-test baseline, and it was maintained for 2 hrs following cessation of intruder exposure (Fig. 1C). The MAP response to intruder followed a similar general pattern, though with smaller maximal response and a more sluggish recovery. Maximal increase in MAP was 48.89 ± 2.61 , which peaked at ~8–9 min after stressor onset (Fig. 2C). MAP then slowly decreased and reached a plateau at ~60 min following stressor exposure that was ~5% above the pre-test baseline (Fig. 3C).

Restraint induced a two-phase HR response, which corresponded to placing of the animal into the restrainer and the subsequent removal from the restrainer. During the initial phase, there was a nearly immediate increase of HR to ~45%, which then slowly diminished over the 45-min period of restraint period (Fig. 1D). Following removal of the restrainer there was a second phase characterized by a further increase of HR to $67.24 \pm 2.70\%$ above baseline, which peaked within ~4 min of removal of the restrainer (Fig. 1D). HR then gradually decreased, but remained elevated at ~20% above the pre-test baseline for the next 2 hrs (Fig. 1D). By contrast, MAP response to restraint was distinct from that of HR, and did not exhibit the two-phase response. It showed a nearly instantaneous peak increase to $44.55 \pm 3.45\%$ above baseline induced by placing the rat into the restrainer. MAP then gradually decreased over the next 2.5 hrs without reaching a plateau and maintaining a level above the pre-test baseline (Fig. 2D). In contrast to HR, restraint-induced MAP changes did not include the second phase, corresponding to the removal of the restrainer.

One-way ANOVA of maximal HR responses revealed a significant effect of stressor type (Fig. 3A). Post-hoc analyses indicated that intruder exposure induced significantly greater responses than the other type of stressors, while strobe light induced significantly smaller responses (Fig 3A). One-way ANOVA of maximal MAP responses likewise revealed a significant of stressor type, with intruder and restraint exposure eliciting the greatest changes (Fig. 3B). Similar to HR, strobe light exposure elicited the smallest changes in MAP when compared to the other stressors (Fig. 3B).

Cardiovascular recovery was evaluated by quantifying recovery decay constants from one-phase decay curves fitted to the HR and MAP following peak responses in each animal. One-way ANOVA of the HR recovery decay constants revealed a significant effect of stressor, while post-hoc analyses revealed significant individual differences (Fig. 3C). HR recovery from exposure to the novel environment was associated with fastest recovery as indicated by its significantly greater decay constant ($0.11 \pm 0.0046 \text{ min}^{-1}$) as compared to other stressors (Fig. 3C). HR recovery from restraint was associated with the slowest recovery and the smallest decay constant ($0.022 \pm 0.0026 \text{ min}^{-1}$; Fig. 3C). HR recovery from intruder exposure ($0.074 \pm 0.0037 \text{ min}^{-1}$) and to strobe light ($0.070 \pm 0.011 \text{ min}^{-1}$) were associated with intermediate decay constants that were not significantly different from each other (Fig. 3C).

One-way ANOVA of the MAP recovery decay constants revealed a significant effect of stressor type (Fig. 3D). Post-hoc analyses also revealed significant group differences.

Similar to HR, restraint was associated with the slowest recovery and the smallest decay constant ($0.0083 \pm 0.00086 \text{ min}^{-1}$; Fig. 3D). Novel environment ($0.103 \pm 0.0068 \text{ min}^{-1}$) and strobe light ($0.111 \pm 0.0180 \text{ min}^{-1}$) exposures were associated with faster recovery and significantly greater decay constants as compared to the other groups (Fig. 3D). Intruder exposure yielded MAP decay constant of $0.069 \pm 0.0038 \text{ min}^{-1}$, which was significantly greater than restraint but smaller than strobe light and novel environment exposure (Fig. 3D).

3.2. Responses to Individual Stressors

3.2.1. Strobe Light—Cardiovascular reactivity to strobe light exposure was significantly impacted by TA, but not by ELE (i.e. maternal separation). Specifically, we detected significant effects ELE, so that MS-exposed rats manifested greater maximal HR responses as compared to their NH-exposed counterparts (Fig. 4A). Similar effects were observed on MAP responses, with significant effect of ELE, but not of TA or of TA $\times$ ELE interaction (Fig. 4B).

In terms of HR recovery, no significant effects of ELE ($F_{(1, 16)} = 2.25$, $p > 0.1$), TA ($F_{(1, 16)} = 0.20$, $p > 0.1$), or TA $\times$ ELE aggression interaction ($F_{(1, 16)} = 1.02$, $p > 0.1$) on the recovery decay constant were detected (data not shown). Similarly, MAP recovery decay constant was not significantly impacted by ELE ($F_{(1, 16)} = 0.55$, $p > 0.1$), TA ($F_{(1, 16)} = 0.087$, $p > 0.1$), TA $\times$ interaction ($F_{(1, 16)} = 3.70$, $p > 0.05$; data now shown).

3.2.2. Novel environment—During the light phase, HR recovery to novel environment exposure was significantly impacted by TA. HR decay constant was significantly smaller in TA rats, while no effects of ELE or of TA $\times$ ELE interaction were detected (Fig. 5).

During the dark phase, both cardiovascular recovery and reactivity were impacted by TA. There was a significant increase in MAP decay constant in TA rats, with no effects of ELE or of TA $\times$ ELE interaction (Fig. 6A). There was a trend toward similar effect of TA on HR decay constant (Fig. 6B). In terms of reactivity, maximal HR responses were significantly greater in TA rats, while no effects of ELE or of TA $\times$ ELE interaction were detected (Fig. 6C).

3.2.3. Intruder Stress—Similar to the exposure to a novel environment, cardiovascular responses to intruder stress were predominantly shaped by TA. These effects were confined to cardiovascular recovery, so that HR and MAP decay constants were greater in TA rats with no effects of ELE (Fig. 7 A, B). In contrast, cardiovascular reactivity did not differ with aggressive behavior or exposure to MS, so that no significant effects of TA (MAP: $F_{(1, 16)} = 0.0070$, $p > 0.1$; HR: $F_{(1, 16)} = 0.019$, $p > 0.1$), ELE (MAP: $F_{(1, 16)} = 1.28$, $p > 0.1$; HR: $F_{(1, 16)} = 0.16$, $p > 0.1$), or of TA $\times$ ELE interaction (MAP: $F_{(1, 16)} = 1.44$, $p > 0.1$; HR: $F_{(1, 16)} = 0.22$, $p > 0.1$) on maximal MAP or HR were detected (data now shown).

3.2.4. Restraint Stress—Exposure to restraint induced a complex two-phase HR response. The first phase corresponded to the 45-min period spent in the restrainer (Phase 1), while the second phase was associated with the 2-hour period following removal from the restrainer (Phase 2; see Fig. 1D). Therefore, HR reactivity and recovery associated with Phase 1 and Phase 2 were analyzed separately. In contrast, MAP response to restraint was

characterized by a one-phase response, which included a rapid increase at the onset of restraint followed by a gradual decay (Fig. 2D). Therefore, quantification of maximal MAP responses and recovery was performed using the same approach as for the other stressors.

While cardiovascular reactivity to restraint was not impacted by either TA or ELE, or by their interaction, significant TA $\times$ ELE interaction associated with recovery was detected. Specifically, decay constant associated with HR recovery during Phase 2 was significantly impacted by TA $\times$ ELE interaction (but not by either factor alone; Fig. 8A). Interestingly, HR recovery during Phase 1 (i.e. during restraint), quantified as slope, was not significantly impacted by TA ($F_{(1, 16)} = 3.49$, $p > 0.05$), ELE ($F_{(1, 16)} = 0.035$, $p > 0.5$), or by TA $\times$ ELE interaction ($F_{(1, 16)} = 2.28$, $p > 0.1$; data not shown). Similar to HR recovery during Phase 2, MAP recovery decay constant was significantly influenced by TA $\times$ ELE interaction, but not by TA or ELE alone (Fig. 8B).

In terms of HR reactivity, no effects of TA (Phase 1: $F_{(1, 16)} = 0.28$, $p > 0.5$; Phase 2: $F_{(1, 16)} = 0.010$, $p > 0.5$), ELE (Phase 1: $F_{(1, 16)} = 1.36$, $p > 0.1$; Phase 2: $F_{(1, 16)} = 0.23$, $p > 0.5$), or TA $\times$ ELE interaction (Phase 1: $F_{(1, 16)} = 0.40$, $p > 0.5$; Phase 2: $F_{(1, 16)} = 1.84$, $p > 0.1$) on maximal HR responses were observed (data not shown). Similarly, for MAP reactivity no effects of TA ($F_{(1, 16)} = 0.84$, $p > 0.1$), ELE ($F_{(1, 16)} = 1.74$, $p > 0.1$), or TA $\times$ ELE interaction ($F_{(1, 16)} = 0.032$, $p > 0.5$) on maximal MAP were detected (data not shown).

4. Discussion

4.1. Overview

We previously showed that TA and ELE independently influence resting levels of HR and MAP [31]. Those data indicated that ELE determines resting HR and HRV throughout the lifespan. Specifically, MS-exposed WKY rats showed lower resting HR and increased HRV in the time and frequency domain throughout adulthood [31]. In contrast to ELE, TA did not impact resting levels of HR and HRV, but showed significant effects on resting blood pressure. Accordingly, TA animals manifested significantly higher levels of diastolic, systolic, and MAP during the light and the dark phases of the 24-hr cycle throughout adulthood [31]. Data presented in the current paper extend these earlier observations to demonstrate that these two factors – ELE and TA – also shape reactivity to and recovery from stress. Specific effects of TA and ELE on cardiovascular reactivity and recovery depend on characteristics of a particular stressor. Stressor intensity and its physical, social, and psychological components interact to determine specific influences of TA and ELE on shaping cardiovascular reactivity and recovery.

4.2. Patterns of Cardiovascular Reactivity and Recovery

Our initial observations demonstrated that that exposure to strobe light, novel environment, intruder, and restraint all elicit robust increases in HR and MAP. These changes occur quickly and reliably following the onset of each stressor. HR and MAP recovery from strobe light, novel environment, and intruder exposure is characterized by a one phase exponential decay. In contrast, restraint induces a two-phase HR response characterized by a rapid

increase after the animal is placed in the restrainer. This is followed by a further increase following removal of animal from the restrainer. In contrast to HR, restraint induces a one-phase response in MAP. It is characterized by rapid increase in MAP after placement of animal in the restrainer, which then gradually recovers to baseline over the next 2.5 hrs.

Our data also revealed notable differences in cardiovascular recovery patterns in response to these stressors. Interestingly, strobe light led to decreases in HR and MAP within 10 min of cessation of the stressor to ~5–7.5% below baseline levels. This new baseline for HR and MAP persisted for the duration of recording (~40 min.). In contrast, exposure to intruder or restraint led to persistently elevated MAP and HR levels for up to 2 h following cessation of each stressor. Following intruder exposure, MAP and HR reached a new baseline at ~10% above pre-stress levels. While following restraint, both HR and MAP remained elevated ~20–50% and were gradually decaying during the subsequent 2-hr period. These observations suggest that specific environmental stimuli can lead to lasting cardiovascular changes. This notion is consistent with the observation that an acute bout of exercise hypotension can lead to long-lasting (i.e. minutes to hours) decreases in resting MAP [62]. This post-exercise hypotension is mediated in part by inhibitory synaptic mechanisms in the brainstem [63]. It would be interesting to determine if some of the same systems mediate lasting changes in HR and MAP following exposure to strobe light, intruder, or restraint.

Novel environment exposure led to a rapid increase in HR and MAP, both of which returned to baseline within 20 min and maintained stable pre-stress levels afterwards. It is feasible that these differences in cardiovascular recovery may be due to habituation. Exposure to novel environment was achieved by placing each animal into a new cage with clean bedding. All animals experienced this on a weekly basis as part of routine maintenance of the rat colony. Indeed, a previous study demonstrated attenuation in peak MAP response and improved recovery in response to cage change in rats exposed to two previous weekly cage changes [64]. That study, which was performed in Sprague-Dawley rats, demonstrated that MAP recovered to pre-stress levels over >100 min following cage change. In the current study we observed full recovery of HR and MAP within 20 min of novel environment exposure [64]. This difference may be due to habituation, as the animals in the current study likely experienced over a dozen weekly cage changes. Alternatively, this effect may be due to strain differences as previous studies have documented significant differences in cardiovascular reactivity and recovery in WKY rats [65].

In contrast, intruder exposure and restraint were novel psychological stimuli, which the animals never experienced before. It seems feasible that this novelty aspect is what contributed to HR and MAP not recovering to pre-stress levels following both restraint and intruder exposure. On the other hand, the observed effects of strobe light exposure in producing a modest hypotensive and bradycardic response may be due to the unique aspects of this stressor. Strobe light was a relatively weak stressor as it caused the smallest hypertensive and tachycardic responses among the stressors examined (see Fig. 3A,B). It was the only stressor not to involve a social or a psychological component. It also did not involve the presence of an experimenter, since the strobe light was set to turn on automatically for a pre-determined period of time during the dark cycle. These unique

features of strobe light most likely contribute to these differences in recovery as compared to the other stressors.

4.3. Quantitative Analyses

We further characterized the magnitude of tachycardic and hypertensive changes induced by each stressor along with their recovery. This analysis is consistent with our qualitative observations in that strobe light appears to be the weakest stressor among the ones examined. It is associated with the lowest maximal HR and MAP responses (Fig. 3A, B), along with the largest HR decay constant and intermediate MAP decay constant (Fig. 3C, D). In contrast, restraint produces the largest maximal MAP responses (Fig. 3B), 2nd largest HR responses (Fig. 3A), and much lower HR (Fig. 3C) and MAP (Fig. 3D) decay constants. These findings indicate that responses to restraint reach a higher peak and persist longer than the other stressors. In contrast, intruder exposure leads to similar maximal hypertensive (Fig. 3B) and tachycardic (Fig. 3A) responses restraint. But intruder exposure is associated with significantly greater HR (Fig. 3C) and MAP (Fig. 3D) decay constants, indicating more robust cardiovascular recovery as compared to restraint. Finally, novel environment leads to maximal HR (Fig. 3A) and MAP (Fig. 3B) responses similar to intruder exposure. But it is also associated with significantly greater HR (Fig. 3C) and MAP (Fig. 3D) decay constants, indicating a more robust recovery, suggesting that it is a weaker stimulus. Based on these observations, we propose the following ranking of stressor aversiveness based on combined cardiovascular reactivity and recovery: restraint > intruder > novel environment > strobe light. We are not aware of any previous studies in rodents utilizing this approach to classify stressor aversiveness in terms of effects on cardiovascular function. This ranking of stressors is in line with previous observations ranking stressor aversiveness based on corticosterone secretion. Accordingly, restraint stress is associated with significantly greater corticosterone levels when compared to novel environment exposure [66]. Previous work in avian species revealed diminished HR reactivity and enhanced HR recovery in response to strobe light as compared to other stressors [67], which is in line with our current observations.

4.4 Effects of ELE and TA

Increased cardiovascular reactivity has been linked with adverse cardiovascular morbidity and mortality [44–51]. However, predictive effects of cardiovascular reactivity are stressor- and disease- specific. While decreased cardiovascular reactivity is thought to confer diminished risk of adverse cardiovascular outcomes, it is also associated with clinical depression [53, 54, 57, 68]. Furthermore, blunted cardiovascular reactivity is also associated with Type D personality profile [52], which is characterized by prominent negative affectivity and social withdrawal [69]. Interestingly, Type D personality itself has been linked to greater cardiovascular morbidity in multiple clinical populations [70]. These findings extend the traditional view that heightened cardiovascular reactivity portends adverse cardiovascular outcomes [71]. They suggest that blunting of cardiovascular reactivity may also be a marker of adverse emotional health (i.e. depression and/or negative affectivity).

Our findings of diminished cardiovascular reactivity in NH rats to strobe light are consistent with that notion (see Fig. 4). We previously demonstrated that when compared to MS WKY

rats, NH WKY rats demonstrate higher levels of depressive- and anxiety- like behaviors, along with diminished prosocial behaviors [30]. These earlier observations suggest that NH WKY rats recapitulate key clinical features of depression and Type D temperament. Our current observations extend these earlier findings and suggest that blunted cardiovascular reactivity to strobe light may be a marker of negative affectivity in rodents.

We have previously demonstrated that MS in WKY rats leads to protective cardiovascular changes, namely a decrease in resting HR and an increase in HR variability [31]. Our finding that HR decay constant in response to intruder exposure is significantly greater in MS animals (see Fig. 7A) is consistent with protective effects of MS in WKY rats. Prior work has demonstrated protective effects of enhanced HR recovery, presumably via enhanced activation of cardiac parasympathetic nerves, in clinical populations [47, 49, 72, 73].

Previous work in humans has established increased risk of heart disease in hostile and aggressive subjects [74–76] along with increased cardiovascular reactivity in such individuals [55, 56]. Similar studies have also demonstrated a correlation between increased cardiovascular reactivity and resting blood pressure in humans [77, 78]. We previously demonstrated that TA in WKY rats correlates with increased resting levels of MAP throughout the 24-hr cycle [31]. Based on these previous observations, one would expect that TA rats would demonstrate increased cardiovascular reactivity and/or impaired recovery. Indeed, these animals show increased HR reactivity in response to a novel environment during the dark phase (see Fig. 6C). This increase in HR reactivity may be related to greater cardiac sympathetic drive, as we have previously demonstrated increased norepinephrine content in the hearts of TA rats [31].

TA animals also manifest impaired HR recovery to a novel environment during the light phase (see Fig. 5). However, these animals show improved MAP (see Fig. 6A) and HR (Fig. 6B) recovery in response to a novel environment during the dark phase. These conflicting observations may be due to day-night differences, as circadian cues significantly impact cardiovascular function [79–81]. TA animals also show enhanced cardiovascular recovery to intruder (Fig. 7), which may relate to stressor controllability. TA animals manifest an active coping response that invariably leads to submissive behavior by the intruder [31]. Extensive evidence implicates stressor controllability as being a significant determinant of cardiovascular and endocrine responses to stress [82, 83].

4.5. Limitations and Perspectives

In the current study we focused on examining the impact of ELE and TA on cardiovascular reactivity and recovery. Prior studies have frequently analyzed these variables by examining area under the curve (AUC) values in response to various stressors [64, 65, 84]. However, using AUC to analyze our data would be misleading for several reasons. Firstly, HR and MAP responses recovered in different ways depending on a stressor, including: 1) return to baseline pre-test levels (i.e. novel environment), 2) remaining above pre-test baseline (i.e. intruder and restraint), or 3) decrease to below pre-test baseline levels (i.e. strobe light). Furthermore, length of time of our recordings was not uniform across stressors. Therefore, AUC measurements would be skewed by differences in the length of recordings of the different stressors and the non-uniform shape of responses. Also, AUC measurements

capture both reactivity (i.e. maximal response) and recovery (i.e. return to baseline). We were interested in measuring these variables separately as both independently contribute to cardiovascular health and disease. The difficulty in adequately quantifying cardiovascular recovery has been noted by others [85]. One of the challenges is attempting to quantify an inherently dynamic process using static variables, which reduces accuracy and reliability. Indeed, previous authors have argued that curve-fitting analyses may yield more accurate and reliable measures of cardiovascular recovery in response to multiple stressors [85–87]. Previous studies have also established that first-order kinetics may be used to accurately quantify HR recovery from exercise in humans [49, 87, 88]. Visual inspection of HR and MAP curves elicited by stressors in our study revealed that first-order kinetics may be an appropriate model of our data. Goodness of fit analyses for curve fitting in our study were consistent with this notion.

Stressors used in the present study were quite different, including the nature of stressors as well as their duration. This is likely what contributed to the varying intensity of these stressors as indicated by the reactivity and recovery data presented in Fig. 3. It is certainly possible that these responses would be altered by increasing or decreasing the time that each stressor was presented. It would be interesting to see whether these differences would persist if all of the stressors were presented for the same length of time.

Another consideration is that differences in experimental paradigms may significantly impact observed differences in cardiovascular responses. In the current study we chose to use a novel cage as a model of a novel environment as others have done in the past [89]. However, other investigators have used an entirely different apparatus, such as the open field [90]. It is important to keep methodological details, including physical layout and dimensions of behavioral equipment, in mind when interpreting data from current study.

Studies in human populations have utilized a variety of stressors to understand the role of cardiovascular recovery and reactivity in health and disease. Such stressors included: voluntary exercise, handgrip, cold water immersion test, along with public speaking and arithmetic tasks [45, 48, 53, 55–57, 77, 85, 91–96]. Most of these tasks are not readily adaptable to rodent studies. However, it is possible to model various physical and psychological dimensions of stress, as we attempted to do in the current study. Unfortunately, this approach is inherently limiting, given much greater complexity of human behavior as compared rodents. Future work will be required to determine if cardiovascular reactivity and recovery differences that we observed are translatable to relevant clinical populations.

One of the limitations of the current study is that it focused entirely on male rats. It would be interesting to expand this work in the future to study females as well. This is especially important in light of significant differences between men and women in their cardiovascular health and disease [97, 98], and in their stress-elicited physiological responses [99, 100].

Although much of the work on early-life stress focuses on its adverse consequences, emerging evidence suggests a more nuanced view. Match-mismatch and stress inoculation theories have been proposed to explain protective effects of stress [101–106]. It is thought

that early-life adversity triggers adaptive plasticity, to confer resilience to subsequent stressors [107, 108]. This plasticity may be initiated by limited resources during early development, such as low maternal care and limited nutrition [101]. This phenomenon is likely mediated by brief alterations in HPA axis by these early-life stressors, and may be more pronounced in stress-susceptible individuals [106]. Our work in the stress-susceptible WKY rats is consistent with this notion, as we have demonstrated protective behavioral and physiological effects of MS in these animals [30, 31]. These effects appear to be mediated by epigenetic modifications, including increased whole genome methylation [109].

Extensive literature has linked different dimensions of personality with cardiovascular risk factors. However, our understanding of biological mechanisms that underlie these associations is limited. The animal model presented in the current study can be useful to dissect the complex interplay between brain and body. It may be utilized to decipher neuromolecular mechanisms that drive the relationship between personality and cardiovascular health and disease.

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References:

- [1]. Suomi SJ Early stress and adult emotional reactivity in rhesus monkeys. *Ciba Found Symp* 1991,156:171–83; discussion 83–8. [PubMed: 1855411]
- [2]. Daniels WM, Pietersen CY, Carstens ME, Stein DJ Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to a subsequent stressor. *Metab Brain Dis* 2004,19:3–14. [PubMed: 15214501]
- [3]. Talge NM, Neal C, Glover V, Early Stress, T. R., Prevention Science Network, F., Neonatal Experience on, C., et al. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry* 2007,48:245–61. [PubMed: 17355398]
- [4]. Rensel MA, Wilcoxon TE, Schoech SJ The influence of nest attendance and provisioning on nestling stress physiology in the Florida scrub-jay. *Horm Behav* 2010,57:162–8. [PubMed: 19879277]
- [5]. Thompson AL Developmental origins of obesity: early feeding environments, infant growth, and the intestinal microbiome. *Am J Hum Biol* 2012,24:350–60. [PubMed: 22378322]
- [6]. Lippmann M, Bress A, Nemeroff CB, Plotsky PM, Monteggia LM Long-term behavioural and molecular alterations associated with maternal separation in rats. *Eur J Neurosci* 2007,25:3091–8. [PubMed: 17561822]
- [7]. Gardner KL, Thiruvikraman KV, Lightman SL, Plotsky PM, Lowry CA Early life experience alters behavior during social defeat: focus on serotonergic systems. *Neuroscience* 2005,136:181–91. [PubMed: 16182451]
- [8]. Huot RL, Plotsky PM, Lenox RH, McNamara RK Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. *Brain Res* 2002,950:52–63. [PubMed: 12231228]
- [9]. Loria AS, Brands MW, Pollock DM, Pollock JS Early life stress sensitizes the renal and systemic sympathetic system in rats. *Am J Physiol Renal Physiol* 2013,305:F390–5. [PubMed: 23678041]
- [10]. Loria AS, Kang KT, Pollock DM, Pollock JS Early life stress enhances angiotensin II-mediated vasoconstriction by reduced endothelial nitric oxide buffering capacity. *Hypertension* 2011,58:619–26. [PubMed: 21876076]

- [11]. Loria AS, Pollock DM, Pollock JS Early life stress sensitizes rats to angiotensin II-induced hypertension and vascular inflammation in adult life. *Hypertension* 2010;55:494–9. [PubMed: 20026758]
- [12]. Plotsky PM, Meaney MJ Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Brain Res Mol Brain Res* 1993;18:195–200. [PubMed: 8497182]
- [13]. Francis DD, Diorio J, Plotsky PM, Meaney MJ Environmental enrichment reverses the effects of maternal separation on stress reactivity. *J Neurosci* 2002;22:7840–3. [PubMed: 12223535]
- [14]. Louis WJ, Howes LG Genealogy of the spontaneously hypertensive rat and Wistar-Kyoto rat strains: implications for studies of inherited hypertension. *J Cardiovasc Pharmacol* 1990;16 Suppl 7:S1–5.
- [15]. Nam H, Clinton SM, Jackson NL, Kerman IA Learned helplessness and social avoidance in the Wistar-Kyoto rat. *Front Behav Neurosci* 2014;8:109. [PubMed: 24744709]
- [16]. Pare WP, Kluczynski J Developmental factors modify stress ulcer incidence in a stress-susceptible rat strain. *J Physiol Paris* 1997;91:105–11. [PubMed: 9403783]
- [17]. Lopez-Rubalcava C, Lucki I Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. *Neuropsychopharmacology* 2000;22:191–9. [PubMed: 10649831]
- [18]. Rittenhouse PA, Lopez-Rubalcava C, Stanwood GD, Lucki I Amplified behavioral and endocrine responses to forced swim stress in the Wistar-Kyoto rat. *Psychoneuroendocrinology* 2002;27:303–18. [PubMed: 11818168]
- [19]. Tejani-Butt S, Kluczynski J, Pare WP Strain-dependent modification of behavior following antidepressant treatment. *Prog Neuropsychopharmacol Biol Psychiatry* 2003;27:7–14. [PubMed: 12551720]
- [20]. Pare WP Stress ulcer susceptibility and depression in Wistar Kyoto (WKY) rats. *Physiol Behav* 1989;46:993–8. [PubMed: 2634265]
- [21]. Pare WP, Redei E Depressive behavior and stress ulcer in Wistar Kyoto rats. *J Physiol Paris* 1993;87:229–38. [PubMed: 8136789]
- [22]. Armario A, Gavalda A, Marti J Comparison of the behavioural and endocrine response to forced swimming stress in five inbred strains of rats. *Psychoneuroendocrinology* 1995;20:879–90. [PubMed: 8834094]
- [23]. Marti J, Armario A Forced swimming behavior is not related to the corticosterone levels achieved in the test: a study with four inbred rat strains. *Physiol Behav* 1996;59:369–73. [PubMed: 8838618]
- [24]. Solberg LC, Olson SL, Turek FW, Redei E Altered hormone levels and circadian rhythm of activity in the WKY rat, a putative animal model of depression. *Am J Physiol Regul Integr Comp Physiol* 2001;281:R786–94. [PubMed: 11506993]
- [25]. Pardon MC, Gould GG, Garcia A, Phillips L, Cook MC, Miller SA, et al. Stress reactivity of the brain noradrenergic system in three rat strains differing in their neuroendocrine and behavioral responses to stress: implications for susceptibility to stress-related neuropsychiatric disorders. *Neuroscience* 2002;115:229–42. [PubMed: 12401336]
- [26]. De La Garza R 2nd, Mahoney JJ 3rd. A distinct neurochemical profile in WKY rats at baseline and in response to acute stress: implications for animal models of anxiety and depression. *Brain Res* 2004;1021:209–18. [PubMed: 15342269]
- [27]. Gunter WD, Shepard JD, Foreman RD, Myers DA, Greenwood-Van Meerveld B Evidence for visceral hypersensitivity in high-anxiety rats. *Physiol Behav* 2000;69:379–82. [PubMed: 10869605]
- [28]. Nielsen MA, Bayati A, Mattsson H Wistar Kyoto rats have impaired gastric accommodation compared to Sprague Dawley rats due to increased gastric vagal cholinergic tone. *Scand J Gastroenterol* 2006;41:773–81. [PubMed: 16785189]
- [29]. Pare WP Stress ulcer and open-field behavior of spontaneously hypertensive, normotensive, and Wistar rats. *Pavlov J Biol Sci.* 1989;24:54–7. [PubMed: 2726299]
- [30]. Rana S, Pugh PC, Jackson N, Clinton SM, Kerman IA Inborn stress reactivity shapes adult behavioral consequences of early-life maternal separation stress. *Neurosci Lett* 2015;584:146–50. [PubMed: 25451726]

- [31]. Rana S, Pugh PC, Katz E, Stringfellow SA, Lin CP, Wyss JM, et al. Independent effects of early-life experience and trait aggression on cardiovascular function. *Am J Physiol Regul Integr Comp Physiol* 2016;311:R272–86. [PubMed: 27280432]
- [32]. Ponikowski P, Anker SD, Chua TP, Szelemiej R, Piepoli M, Adamopoulos S, et al. Depressed heart rate variability as an independent predictor of death in chronic congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 1997;79:1645–50. [PubMed: 9202356]
- [33]. Tsuji H, Larson MG, Venditti FJ Jr., Manders ES, Evans JC, Feldman CL, et al. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* 1996;94:2850–5. [PubMed: 8941112]
- [34]. Kristal-Boneh E, Silber H, Harari G, Froom P The association of resting heart rate with cardiovascular, cancer and all-cause mortality. Eight year follow-up of 3527 male Israeli employees (the CORDIS Study). *Eur Heart J* 2000;21:116–24. [PubMed: 10637085]
- [35]. Jensen MT, Suadicani P, Hein HO, Gyntelberg F Elevated resting heart rate, physical fitness and all-cause mortality: a 16-year follow-up in the Copenhagen Male Study. *Heart* 2013;99:882–7. [PubMed: 23595657]
- [36]. Opdahl A, Ambale Venkatesh B, Fernandes VRS, Wu CO, Nasir K, Choi EY, et al. Resting heart rate as predictor for left ventricular dysfunction and heart failure: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2014;63:1182–9. [PubMed: 24412444]
- [37]. Natarajan D, Caramaschi D Animal violence demystified. *Front Behav Neurosci* 2010;4:9. [PubMed: 20407576]
- [38]. Caramaschi D, de Boer SF, Koolhaas JM Is hyper-aggressiveness associated with physiological hypoarousal? A comparative study on mouse lines selected for high and low aggressiveness. *Physiol Behav* 2008;95:591–8. [PubMed: 18817794]
- [39]. Veenema AH, Meijer OC, de Kloet ER, Koolhaas JM Genetic selection for coping style predicts stressor susceptibility. *J Neuroendocrinol* 2003;15:256–67. [PubMed: 12588514]
- [40]. Caramaschi D, de Boer SF, Koolhaas JM Differential role of the 5-HT1A receptor in aggressive and non-aggressive mice: an across-strain comparison. *Physiol Behav* 2007;90:590–601. [PubMed: 17229445]
- [41]. Carnevali L, Trombini M, Porta A, Montano N, de Boer SF, Sgoifo A Vagal withdrawal and susceptibility to cardiac arrhythmias in rats with high trait aggressiveness. *PLoS One* 2013;8:e68316. [PubMed: 23861886]
- [42]. Sgoifo A, Costoli T, Meerlo P, Buwalda B, Pico'-Alfonso MA, De Boer S, et al. Individual differences in cardiovascular response to social challenge. *Neurosci Biobehav Rev* 2005;29:59–66. [PubMed: 15652255]
- [43]. Sgoifo A, De Boer SF, Buwalda B, Korte-Bouws G, Tuma J, Bohus B, et al. Vulnerability to arrhythmias during social stress in rats with different sympathovagal balance. *Am J Physiol* 1998;275:H460–6. [PubMed: 9683433]
- [44]. Linden W, Earle TL, Gerin W, Christenfeld N Physiological stress reactivity and recovery: conceptual siblings separated at birth? *J Psychosom Res* 1997;42:117–35. [PubMed: 9076640]
- [45]. Panaite V, Salomon K, Jin A, Rottenberg J Cardiovascular recovery from psychological and physiological challenge and risk for adverse cardiovascular outcomes and all-cause mortality. *Psychosom Med* 2015;77:215–26. [PubMed: 25829236]
- [46]. Heponiemi T, Elovainio M, Pulkki L, Puttonen S, Raitakari O, Keltikangas-Jarvinen L Cardiac autonomic reactivity and recovery in predicting carotid atherosclerosis: the cardiovascular risk in young Finns study. *Health Psychol* 2007;26:13–21. [PubMed: 17209693]
- [47]. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS Heart-rate recovery immediately after exercise as a predictor of mortality. *N Engl J Med* 1999;341:1351–7. [PubMed: 10536127]
- [48]. Moseley JV, Linden W Predicting blood pressure and heart rate change with cardiovascular reactivity and recovery: results from 3-year and 10-year follow up. *Psychosom Med* 2006;68:833–43. [PubMed: 17132835]
- [49]. Imai K, Sato H, Hori M, Kusuoka H, Ozaki H, Yokoyama H, et al. Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J Am Coll Cardiol* 1994;24:1529–35. [PubMed: 7930286]

- [50]. Steptoe A, Marmot M Impaired cardiovascular recovery following stress predicts 3-year increases in blood pressure. *J Hypertens* 2005;23:529–36. [PubMed: 15716693]
- [51]. Lovallo WR Can exaggerated stress reactivity and prolonged recovery predict negative health outcomes? The case of cardiovascular disease. *Psychosom Med* 2015;77:212–4. [PubMed: 25829241]
- [52]. Kupper N, Denollet J, Widdershoven J, Kop WJ Type D personality is associated with low cardiovascular reactivity to acute mental stress in heart failure patients. *Int J Psychophysiol* 2013;90:44–9. [PubMed: 23361112]
- [53]. Schwerdtfeger AR, Gerteis AK Is the blunted blood pressure reactivity in dysphoric individuals related to attenuated behavioral approach? *Int J Psychophysiol* 2013;90:58–65. [PubMed: 23395561]
- [54]. Schwerdtfeger A, Rosenkaimer AK Depressive symptoms and attenuated physiological reactivity to laboratory stressors. *Biol Psychol* 2011;87:430–8. [PubMed: 21679744]
- [55]. Fichera LV, Andreassi JL Stress and personality as factors in women's cardiovascular reactivity. *Int J Psychophysiol* 1998;28:143–55. [PubMed: 9545652]
- [56]. Fichera LV, Andreassi JL Cardiovascular reactivity during public speaking as a function of personality variables. *Int J Psychophysiol* 2000;37:267–73. [PubMed: 10858572]
- [57]. Salomon K, Bylsma LM, White KE, Panaite V, Rottenberg J Is blunted cardiovascular reactivity in depression mood-state dependent? A comparison of major depressive disorder remitted depression and healthy controls. *Int J Psychophysiol* 2013;90:50–7. [PubMed: 23756147]
- [58]. Clinton SM, Watson SJ, Akil H High novelty-seeking rats are resilient to negative physiological effects of the early life stress. *Stress* 2014;17:97–107. [PubMed: 24090131]
- [59]. Rana S, Nam H, Glover ME, Akil H, Watson SJ, Clinton SM, et al. Protective effects of chronic mild stress during adolescence in the low-novelty responder rat. *Stress* 2016;19:133–8. [PubMed: 26473581]
- [60]. Koolhaas JM, Coppens CM, de Boer SF, Buwalda B, Meerlo P, Timmermans PJ The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *J Vis Exp* 2013:e4367. [PubMed: 23852258]
- [61]. Malkesman O, Maayan R, Weizman A, Weller A Aggressive behavior and HPA axis hormones after social isolation in adult rats of two different genetic animal models for depression. *Behav Brain Res* 2006;175:408–14. [PubMed: 17069898]
- [62]. MacDonald JR Potential causes, mechanisms, and implications of post exercise hypotension. *J Hum Hypertens* 2002;16:225–36. [PubMed: 11967715]
- [63]. Chen CY, Bonham AC Postexercise hypotension: central mechanisms. *Exerc Sport Sci Rev* 2010;38:122–7. [PubMed: 20577060]
- [64]. Duke JL, Zammit TG, Lawson DM The effects of routine cage-changing on cardiovascular and behavioral parameters in male Sprague-Dawley rats. *Contemp Top Lab Anim Sci* 2001;40:17–20.
- [65]. McDougall SJ, Paull JR, Widdop RE, Lawrence AJ Restraint stress : differential cardiovascular responses in Wistar-Kyoto and spontaneously hypertensive rats. *Hypertension* 2000;35:126–9. [PubMed: 10642286]
- [66]. Kabbaj M, Devine DP, Savage VR, Akil H Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. *J Neurosci* 2000;20:6983–8. [PubMed: 10995843]
- [67]. Nephew BC, Kahn SA, Romero LM Heart rate and behavior are regulated independently of corticosterone following diverse acute stressors. *Gen Comp Endocrinol* 2003;133:173–80. [PubMed: 12928007]
- [68]. Phillips AC Blunted cardiovascular reactivity relates to depression, obesity, and self-reported health. *Biol Psychol* 2011;86:106–13. [PubMed: 20347924]
- [69]. Mols F, Denollet J Type D personality in the general population: a systematic review of health status, mechanisms of disease, and work-related problems. *Health Qual Life Outcomes* 2010;8:9. [PubMed: 20096129]
- [70]. Kupper N, Denollet J Type D personality as a prognostic factor in heart disease: assessment and mediating mechanisms. *J Pers Assess* 2007;89:265–76. [PubMed: 18001227]

- [71]. Treiber FA, Kamarck T, Schneiderman N, Sheffield D, Kapuku G, Taylor T Cardiovascular reactivity and development of preclinical and clinical disease states. *Psychosom Med* 2003,65:46–62. [PubMed: 12554815]
- [72]. Lauer MS Heart rate recovery: what now? *J Intern Med*. 2011,270:597–9. [PubMed: 21910769]
- [73]. Goldberger JJ, Le FK, Lahiri M, Kannankeril PJ, Ng J, Kadish AH Assessment of parasympathetic reactivation after exercise. *Am J Physiol Heart Circ Physiol* 2006,290:H2446–52. [PubMed: 16415073]
- [74]. Friedman M, Rosenman RH Association of specific overt behavior pattern with blood and cardiovascular findings; blood cholesterol level, blood clotting time, incidence of arcus senilis, and clinical coronary artery disease. *J Am Med Assoc* 1959,169:1286–96. [PubMed: 13630753]
- [75]. Friedman M, Rosenman RH Type A Behavior Pattern: its association with coronary heart disease. *Ann Clin Res* 1971,3:300–12. [PubMed: 5156890]
- [76]. Rosenman RH, Brand RJ, Jenkins D, Friedman M, Straus R, Wurm M Coronary heart disease in Western Collaborative Group Study. Final follow-up experience of 8 1/2 years. *JAMA* 1975,233:872–7. [PubMed: 1173896]
- [77]. Matthews KA, Woodall KL, Allen MT Cardiovascular reactivity to stress predicts future blood pressure status. *Hypertension* 1993,22:479–85. [PubMed: 8406652]
- [78]. Manuck SB, Kasprovicz AL, Muldoon MF Behaviorally-Evoked Cardiovascular Reactivity and Hypertension: Conceptual Issues and Potential Associations. *Ann Behav Med* 1990,12:17–29.
- [79]. Bonnemeyer H, Richardt G, Potratz J, Wiegand UK, Brandes A, Kluge N, et al. Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability. *J Cardiovasc Electrophysiol* 2003,14:791–9. [PubMed: 12890036]
- [80]. Wang D, Ruan W, Chen Z, Peng Y, Li W Shift work and risk of cardiovascular disease morbidity and mortality: A dose-response meta-analysis of cohort studies. *Eur J Prev Cardiol* 2018,25:1293–302. [PubMed: 29929393]
- [81]. Muller JE Circadian variation in cardiovascular events. *Am J Hypertens* 1999,12:35S–42S. [PubMed: 10090293]
- [82]. Peters ML, Godaert GL, Ballieux RE, van Vliet M, Willemsen JJ, Sweep FC, et al. Cardiovascular and endocrine responses to experimental stress: effects of mental effort and controllability. *Psychoneuroendocrinology* 1998,23:1–17. [PubMed: 9618748]
- [83]. Maier SF, Ryan SM, Barksdale CM, Kalin NH Stressor controllability and the pituitary-adrenal system. *Behav Neurosci* 1986,100:669–74. [PubMed: 3022767]
- [84]. Sedova L, Berube J, Gaudet D, Dumont M, Tremblay J, Hamet P, et al. Diet-induced obesity delays cardiovascular recovery from stress in spontaneously hypertensive rats. *Obes Res* 2004,12:1951–8. [PubMed: 15687396]
- [85]. Christenfeld N, Glynn LM, Gerin W On the reliable assessment of cardiovascular recovery: an application of curve-fitting techniques. *Psychophysiology* 2000,37:543–50. [PubMed: 10934913]
- [86]. Llabre MM, Spitzer S, Siegel S, Saab PG, Schneiderman N Applying latent growth curve modeling to the investigation of individual differences in cardiovascular recovery from stress. *Psychosom Med* 2004,66:29–41. [PubMed: 14747635]
- [87]. Pierpont GL, Stolpman DR, Gornick CC Heart rate recovery post-exercise as an index of parasympathetic activity. *J Auton Nerv Syst* 2000,80:169–74. [PubMed: 10785283]
- [88]. Pierpont GL, Voth EJ Assessing autonomic function by analysis of heart rate recovery from exercise in healthy subjects. *Am J Cardiol* 2004,94:64–8. [PubMed: 15219511]
- [89]. Blanchard MM, Mendelsohn D, Stamp JA The HR/LR model: Further evidence as an animal model of sensation seeking. *Neurosci Biobehav Rev* 2009,33:1145–54. [PubMed: 19497336]
- [90]. van den Buuse M, Van Acker SA, Flutters M, De Kloet ER Blood pressure, heart rate, and behavioral responses to psychological “novelty” stress in freely moving rats. *Psychophysiology* 2001,38:490–9. [PubMed: 11352138]
- [91]. Andreassi JL The psychophysiology of cardiovascular reactivity. *Int J Psychophysiol* 1997,25:7–11. [PubMed: 9067795]

- [92]. Brosschot JF, Thayer JF Anger inhibition, cardiovascular recovery, and vagal function: a model of the link between hostility and cardiovascular disease. *Ann Behav Med* 1998;20:326–32. [PubMed: 10234427]
- [93]. Chida Y, Steptoe A Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: a meta-analysis of prospective evidence. *Hypertension* 2010;55:1026–32. [PubMed: 20194301]
- [94]. Cohen S, Hamrick N Stable individual differences in physiological response to stressors: implications for stress-elicited changes in immune related health. *Brain Behav Immun* 2003;17:407–14.
- [95]. Lovallo WR Do low levels of stress reactivity signal poor states of health? *Biol Psychol* 2011;86:121–8. [PubMed: 20079397]
- [96]. Sherwood A, Davis MR, Dolan CA, Light KC Cardiovascular reactivity assessment: effects of choice of difficulty on laboratory task responses. *Int J Psychophysiol* 1992;12:87–94. [PubMed: 1740406]
- [97]. Traustadottir T, Bosch PR, Matt KS Gender differences in cardiovascular and hypothalamic-pituitary-adrenal axis responses to psychological stress in healthy older adult men and women. *Stress* 2003;6:133–40. [PubMed: 12775333]
- [98]. Huikuri HV, Pikkujamsa SM, Airaksinen KE, Ikaheimo MJ, Rantala AO, Kauma H, et al. Sex-related differences in autonomic modulation of heart rate in middle-aged subjects. *Circulation* 1996;94:122–5. [PubMed: 8674168]
- [99]. Kajantie E, Phillips DI The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology* 2006;31:151–78. [PubMed: 16139959]
- [100]. Stoney CM, Davis MC, Matthews KA Sex differences in physiological responses to stress and in coronary heart disease: a causal link? *Psychophysiology* 1987;24:127–31. [PubMed: 3602263]
- [101]. Kentner AC, Cryan JF, Brummelte S Resilience priming: Translational models for understanding resiliency and adaptation to early life adversity. *Dev Psychobiol* 2018.
- [102]. Santarelli S, Lesuis SL, Wang XD, Wagner KV, Hartmann J, Labermaier C, et al. Evidence supporting the match/mismatch hypothesis of psychiatric disorders. *Eur Neuropsychopharmacol* 2014.
- [103]. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, et al. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 1998;351:173–7. [PubMed: 9449872]
- [104]. Parker KJ, Buckmaster CL, Justus KR, Schatzberg AF, Lyons DM Mild early life stress enhances prefrontal-dependent response inhibition in monkeys. *Biol Psychiatry* 2005;57:848–55. [PubMed: 15820705]
- [105]. Parker KJ, Buckmaster CL, Schatzberg AF, Lyons DM Prospective investigation of stress inoculation in young monkeys. *Arch Gen Psychiatry* 2004;61:933–41. [PubMed: 15351772]
- [106]. Parker KJ, Buckmaster CL, Sundlass K, Schatzberg AF, Lyons DM Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. *Proc Natl Acad Sci U S A* 2006;103:3000–5. [PubMed: 16473950]
- [107]. Schmidt MV Animal models for depression and the mismatch hypothesis of disease. *Psychoneuroendocrinology* 2011;36:330–8. [PubMed: 20674180]
- [108]. Gluckman PD, Hanson MA, Beedle AS Early life events and their consequences for later disease: a life history and evolutionary perspective. *Am J Hum Biol* 2007;19:1–19. [PubMed: 17160980]
- [109]. McCoy CR, Rana S, Stringfellow SA, Day JJ, Wyss JM, Clinton SM, et al. Neonatal maternal separation stress elicits lasting DNA methylation changes in the hippocampus of stress-reactive Wistar Kyoto rats. *Eur J Neurosci* 2016;44:2829–45. [PubMed: 27643783]

Highlights:

- Among different stressors, strobe light is least aversive
- Maternally-separated rats have enhanced cardiovascular reactivity to strobe light
- Trait aggressive rats have enhanced recovery to novel environment in dark phase
- Trait aggressive rats have enhanced recovery following novel conspecific exposure
- Trait aggression and maternal separation interact to shape responses to restraint

Figure 1.

Heart rate responses to strobe light (A), novel environment (B), intruder (C), and restraint (D). Values were averaged in one-minute intervals and expressed as percent change from baseline recorded over a 10-min. period prior to stress; data are shown as mean $\pm$ SEM. Exponential decay curves were fit to evaluate cardiovascular recovery from peak response to each stressor. Goodness of fit (r values) for curve fits: 0.41 (A), 0.72 (B), 0.64 (C), and 0.56 (D). Black bars indicate onset and duration of stress (A, C, D); timing of cage change is shown by arrow (B). $N = 20$.

Figure 2.

Mean arterial pressure responses to strobe light (A), novel environment (B), intruder (C), and restraint (D). Values were averaged in one-minute intervals and expressed as percent change from baseline recorded over 10-min. period prior to stress; data are shown as mean $\pm$ SEM. Exponential decay curves were fit to evaluate cardiovascular recovery from peak response to each stressor. Goodness of fit (r values) for curve fits: 0.37 (A), 0.55 (B), 0.60 (C), and 0.74 (D). Black bars indicate onset and duration of stress (A, C, D); timing of cage change is shown by arrow (B). $N = 20$.

Figure 3.

Effect of stressor type on cardiovascular reactivity and recovery. Cardiovascular reactivity was quantified as maximal heart rate and mean arterial pressure responses to stress expressed as percent change from baseline. Cardiovascular recovery was quantified as the decay constant calculated from the one-phase decay curve fits. Data are expressed as percent change from baseline. One-way ANOVA revealed significant effect of stressor type for maximal heart rate ($F_{(3, 76)} = 57.12$, $p < 0.0001$; A) and mean arterial pressure ($F_{(3, 76)} = 36.57$, $p < 0.0001$; B) responses. Similarly, there was a significant effect of stressor type on the recovery from stress as evidenced by group differences in the recovery decay constant for heart rate ($F_{(3, 76)} = 30.17$, $p < 0.0001$; C) and in the recovery decay constant for mean arterial pressure ($F_{(3, 76)} = 22.53$, $p < 0.0001$; D). Significant group differences are indicated by letters above each bar ($p < 0.05$ for a vs. b, a vs. c, and b vs. c); $n = 20$. Data for novel environment stress were collected during the light phase of the 24-hr cycle.

Figure 4.

Cardiovascular reactivity to strobe light exposure. Maximal heart rate (HR) responses (A) were significantly impacted by early-life experience (ELE; $F_{(1, 16)} = 4.70$, $p < 0.05$), but not by trait aggression (TA; $F_{(1, 16)} = 0.46$, $p > 0.1$), or by TA $\times$ ELE interaction ($F_{(1, 16)} = 1.65$, $p > 0.1$). Similarly, mean arterial pressure (MAP) responses (B) were significantly impacted by ELE ($F_{(1, 16)} = 6.69$, $p < 0.05$), but not by TA ($F_{(1, 16)} = 0.88$, $p > 0.1$), or by TA $\times$ ELE interaction ($F_{(1, 16)} = 0.37$, $p > 0.1$). * - $p < 0.05$. Abbreviations: MS – maternal separation, NA – non-aggressive, NH – neonatal handling, and TA – trait aggressive.

Figure 5.

Cardiovascular recovery in the novel environment during the light phase. HR recovery decay constant in the novel environment was significantly impacted by TA ($F_{(1, 16)} = 6.72$, $p < 0.05$), but not by ELE ($F_{(1, 16)} = 0.19$, $p > 0.1$), or by TA $\times$ ELE interaction ($F_{(1, 16)} = 0.14$, $p > 0.1$). * - $p < 0.05$. Abbreviations: MS – maternal separation, NA – non-aggressive, NH – neonatal handling, and TA – trait aggressive.

Figure 6.

Cardiovascular recovery and reactivity in the novel environment during the dark phase. MAP recovery decay constant was significantly affected by TA ($F_{(1, 16)} = 5.82$, $p < 0.05$; A); it was not affected by ELE ($F_{(1, 16)} = 2.09$, $p > 0.1$), or by TA $\times$ ELE interaction ($F_{(1, 16)} = 0.04$, $p > 0.1$). There was a trend toward significance in the impact of TA ($F_{(1, 16)} = 4.16$, $p < 0.06$) on HR recovery decay constant (B). No effects of ELE ($F_{(1, 16)} = 2.09$, $p > 0.1$), or of TA $\times$ ELE interaction ($F_{(1, 16)} = 0.015$, $p > 0.1$) on HR recovery were detected. Maximal HR responses were significantly affected by trait aggression (TA; $F_{(1, 16)} = 6.40$, $p < 0.05$;

C), but not by early-life experience (ELE; $F_{(1, 16)} = 1.41, p > 0.1$), or by $TA \times ELE$ interaction ($F_{(1, 16)} = 0.43, p > 0.1$). * - $p < 0.05$. Abbreviations: HR – heart rate, MS – maternal separation, NA – non-aggressive, NH – neonatal handling, and TA – trait aggressive.

Figure 7.

Cardiovascular recovery following intruder exposure. Heart rate decay constant (A) was significantly affected by TA ($F_{(1, 16)} = 12.17$, $p < 0.01$; A) and by ELE ($F_{(1, 16)} = 4.58$, $p < 0.05$; A), but not by TA $\times$ ELE interaction ($F_{(1, 16)} = 0.067$, $p > 0.1$). MAP decay was significantly impacted by TA ($F_{(1, 16)} = 13.0$, $p < 0.01$; B), but not by ELE ($F_{(1, 16)} = 1.11$, $p > 0.1$), or by TA $\times$ ELE interaction ($F_{(1, 16)} = 0.061$, $p > 0.1$). * - $p < 0.05$. Abbreviations: MS – maternal separation, NA – non-aggressive, NH – neonatal handling, and TA – trait aggressive.

Figure 8.

Cardiovascular recovery following restraint. Decay constant associated with HR recovery during phase 2 was significantly impacted by TA $\times$ ELE interaction ($F_{(1, 16)} = 10.47$, $p < 0.01$; A). No main effects of TA ($F_{(1, 16)} = 0.0004$, $p > 0.5$) or of ELE ($F_{(1, 16)} = 0.53$, $p > 0.1$) on HR decay constant were detected. Similarly, for the MAP recovery decay constant a significant TA $\times$ ELE interaction was observed ($F_{(1, 16)} = 13.07$, $p < 0.01$; B), while no significant main effects of TA ($F_{(1, 16)} = 0.76$, $p > 0.1$) or of ELE ($F_{(1, 16)} = 0.024$, $p > 0.5$) were detected.