# Multilevel Longitudinal Analysis of Shooting Performance as a Function of Stress and Cardiovascular Responses

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Abstract—Virtual reality (VR) systems are increasingly using physiology to improve human training. However, these systems do not account for the complex intra-individual variability in physiology and human performance across multiple timescales and psychophysiological demands. To fill this gap, we propose a theory of multilevel variability where tractable neurobiological mechanisms generate complex variability in performance over time and in response to heterogeneous sources. Based on this theory, we also present a study that examines changes in cardiovascular activity and performance during a stressful shooting task in VR. We examined physiology and performance at three important levels of analysis: task-to-task, block-to-block, session-to-session. Findings indicated joint patterns of physiology and performance that notably varied by the level of analysis. At the task level, higher task difficulty worsened performance but did not change cardiovascular activation. At the block level, there were nonlinear changes in performance and heart rate variability. At the session level, performance improved while blood pressure decreased and heart rate variability increased across days. Of all the physiological metrics, only heart rate variability was correlated with marksmanship performance. Findings are consistent with our multilevel theory and highlight the need for VR and other affective computing systems to assess physiology across multiple timescales.

Index Terms-Stress autonomic, nervous system, shooting performance

# **1** INTRODUCTION

## 1.1 Background

The last two decades have witnessed a surge of research on affective computing systems that aim to detect human emotion and stress based on physiological signals [1], [2], [3]. Such systems have been used in a variety of applications with the aim of inferring, with some specificity, the occurrence of emotional and/or cognitive states that in turn affect human user engagement, performance, and health [4], [5], [6], [7]. Especially important for operational domains such as the military, principles of affective computing can be implemented in virtual reality (VR) systems for the purpose of training human performance outcomes (e.g., shooting marksmanship) [8], [9], [10], [11]. In addition to simulating real-world stressors, VR may enhance training because it can be integrated into closed-loop systems that adapt to the user [12], [13]. Such systems aim to customize stimuli (e.g.,

Manuscript received 15 Feb. 2019; revised 3 May 2020; accepted 4 May 2020. Date of publication 20 May 2020; date of current version 3 Sept. 2021. (Corresponding author: Derek P. Spangler.) Recommended for acceptance by T. Percep. Digital Object Identifier no. 10.1109/TAFFC.2020.2995769 training aids) to changes in performance and psychological states (e.g., stress, workload) that are inferred from physiological responses [14], [15], [16], [17], [18], [19]. Cardiovascular (CV) responses in particular have shown promise as affordable and unobtrusive indicators of performance-relevant states within VR [20], [21], [22], [23], [24], [25].

VR systems that measure physiology, and affective computing systems more generally, are limited because they do not consider the multiple levels of variability that have been noted in prior psychophysiological theory [26], [27], [28]. Here, we define "levels" as the different timescales over which physiology and performance can change, in combination with the different sources (exogenous and endogenous factors) that drive such change. With respect to human performance in affective computing systems (e.g., VR systems using physiology), we outline three particularly important levels of variability: (1) Task-to-task: changes induced by exogenous tasks that typically last in the range of minutes. This level encompasses both changes between rest and a performance task as well as changes between different performance tasks, (2) Block-to-block: within-task changes that occur between chunks of similar trials. These changes can occur on a second-to-second and/or minute-to-minute basis (specifically, <0.1 Hz) and are driven by endogenous factors (e.g., intrinsic biological rhythms, thoughts), (3) Sessionto-session: much slower day-to-day changes across experimental sessions, where such changes are driven by both endogenous and exogenous factors.

Many existing systems and basic research in this area have used CV physiology to only estimate task-to-task changes in

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emotional and cognitive states (e.g., workload, stress) [23], [29], [30], [31], [32]. However, computing systems that model all three levels of variability simultaneously are likely to produce more precise estimates of state and performance. For example, also modeling block-to-block variability in physiology can prevent researchers from mistaking CV changes (e.g., heart rate increases) as being due to workload when such increases are due to intrinsic oscillations in blood pressure [33]. Also, since training often takes place across multiple days, computing systems that target training (e.g., VR-based training) could benefit from tracking slow day-to-day changes in physiology and then using this information to optimize training to daily levels of stress or fatigue [11], [34], [35].

We argue that affective computing systems—including VR training technologies-do not sufficiently exploit multilevel variability because basic research has not investigated such patterns of variability within the same analysis. The deeper root of the problem is likely that researchers in this domain lack a clear theoretical perspective that can generate specific hypotheses regarding multiple timescales and stressors. Addressing these gaps, the current paper proposes a theory of multilevel variability, where tractable neurobiological mechanisms link physiology and performance at multiple timescales and in response to heterogeneous stimuli. In support of this model, we also present a study that is the first to show unique patterns of physiology and performance at all three levels of variability (block, task, session) simultaneously. Importantly, the current paper presents and examines our theory with respect to VR. However, the theory can also be applied to any computing system that utilizes multi-timescale changes in physiology to infer performance-relevant states (e.g., emotion, cognition). We describe our theory below and highlight how prior work has failed to examine CV physiology and performance (and their relations) at all theoretically important levels.

#### 1.2 Multilevel Theory of Performance Variability

Synthesizing multiple theories in psychophysiology and neuroscience, we conceptualize the human as an assemblage of interacting yet non-redundant response systems at the cognitive, motor, emotional, and physiological levels (see Fig. 1) [36], [37], [38], [39], [40], [41]. Behavioral performance is determined by the interactions between these systems where limited energetic (e.g., metabolic, neural) resources are shared and exchanged across specialized neurobiological systems [42], [43]. That is, such interactions are regulated by an integrated neurobiological architecture composed of pathways in central, autonomic, and skeletal nervous systems as well as the endocrine system [44], [45]. Their flexible coordination permits diverse adjustments to cardiovascular physiology, cognition, and hence behavior on multiple timescales and in response to heterogeneous stimuli [36], [46]. Thus, depending on the stressor, energetic resources can be routed to the appropriate system(s) to yield adaptations to performance at the proper timescale. In this way, response systems and their neurobiological mechanisms exhibit dynamics that give rise to performance variability at all three levels outlined above (task, block, session) [47].

Our multilevel perspective posits two themes that are important for human performance in VR training. First, although multiple response systems influence performance

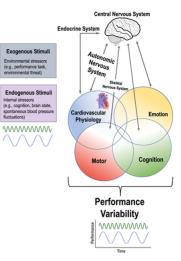


Fig. 1. Multilevel theory of performance variability. The circles represent different emergent, psychophysiological responses systems. The overlap between circles represent the interaction and/or exchange of limited energetic (neural, metabolic) resources between responses systems. The arrows represent bidirectional control of response system dynamics (i.e., variability) by varying yet integrated neural and endocrine systems. The differential functional specialization and frequency dynamics of these neural and endocrine systems allows for adjustments to the response systems in reaction to different stimuli (exogenous and endogenous stimuli; presented in boxes on the left) across a range of time-scales. The resulting dynamic interactions between response systems gives rise to complex, multi-timescale variability in human performance.

variability, their effects are often mediated through cognition (i.e., information-processing mechanisms that shape perception, thought, action) [48]. Attention is especially critical to performance and training and describes the selective processing of goal-relevant stimuli [49], [50]. Attention requires that the frontal lobes utilize energetic resources to suppress the processing of irrelevant information and to bias responses to task stimuli [51], [52]. Attention is also critical for effortful behavioral strategies that guide performance, such as the speed-accuracy tradeoff (i.e., slowing of response times to achieve better accuracy) [53]. Maintaining task attention and hence adaptive strategies is difficult because many stimuli (e.g., performance anxiety, environmental distractors) compete for neural resources during performance tasks [49].

Second, the above neurobiological architecture controls response systems with varying degrees of integration, such that a single pattern of CV physiology can reflect a number of cognitive and emotional processes [54]. The particular performance-related state that is indexed by a CV pattern depends on the: (i) timescale over which physiology changes, and (ii) stimuli that evoke such changes. For example, greater decreases in vagally mediated heart rate variability (vmHRV; a metric of parasympathetic regulation of heart rate) in response to a cognitive task might accompany better performance because these decreases track taskrelated augmentations in attention. In contrast, overall increases in vmHRV across days may reflect a lowering of tonic negative emotion and hence be associated with daily performance improvements [55], [56]. We now distinguish each level by its neurobiological and psychological processes and highlight relevant gaps in prior research.

*Task-to-Task.* Initiating performance on a performance task requires shifting from a resting state to an active state of

attention to the environment. Here, neural resources must be routed from the default mode (task-off) to the dorsal attention (task-on) brain network, in order to support strategies required for optimal performance (e.g., speed-accuracy tradeoff) [57], [58]. Task-to-task changes also describe the impairing effects of negative emotional and stressful tasks (e.g., tasks with high difficulty or threat) on performance relative to neutral tasks. These effects are mediated by emotional stimuli hijacking resources away from prefrontal cortex regions that are critical for attention and behavioral strategies [59], [60]. Similar mechanisms are seemingly implicated in the worsening of performance during high versus low difficulty tasks. The increased effort during high difficulty conditions (which may inherently elicit negative emotion) consumes more limited energetic resources, which in turn worsens performance [43], [58], [61], [62].

Much research has examined task-to-task changes in CV physiology and their relations to concomitant performance. Task-to-task variability is often studied as change in CV activity between a baseline task and performance task (i.e., CV reactivity) [63]. Here, linear change in the CV metric (e.g., blood pressure) from baseline across the whole task (i.e., across all blocks) reflect physiological activity induced by exogenous stressors that require sustained effort [64], [65], [66], [67]. CV reactivity during performance tasks and other stressors is thought to be mediated by the autonomic nervous system (ANS) [68]. Parasympathetic withdrawal (measured as vmHRV decreases) and increases in sympathetic activity elicit CV activation, a pattern of reactivity marked by augmentations in heart rate and blood pressure. CV activation in turn mobilizes metabolic resources for task effort [69]. Consistently, greater decreases in vmHRV and higher CV activation from rest to stress have been linked to relatively better task performance [70], [71]. Task-to-task variability also encompasses differences in CV reactivity between performance tasks (e.g., mental arithmetic, speech preparation). Here, more difficult tasks evoke greater CV activation and more performance errors [72]. Such effects are likely due to difficult tasks eliciting greater effort and perhaps more negative emotion, both of which mobilize CV responses to cope with stressors [64], [73].

Block-to-Block. Even within the same task and in the absence of major environmental change, there are important changes in performance and physiology across trials. During a single task, the default mode exhibits endogenous <0.1 Hz (every 10s or slower) oscillations commensurate with changes between chunks of similar trials (i.e., block-to-block change) [74], [75]. Within such cyclical oscillations, some blocks of trials periodically exhibit heightened default mode activity, which is believed to drive the occurrence of: (1) spontaneous task-unrelated thoughts (often emotional in nature), (2) lapses in task attention and behavioral control (e.g., speed-accuracy tradeoff), and (3) hence decreases in performance on some blocks relative to others [76], [77], [78], [79], [80]. In sum, endogenous block-to-block oscillations in default mode activity yield concomitant block-toblock oscillations in performance.

Relative to the task level, the significance of block-related changes in CV physiology during performance is less clear. Some research, however, is beginning to show that endogenous activity in sensory/perceptual brain regions (e.g., default mode network) is modulated by intrinsic, cyclical oscillations in heart rate that are commensurate with block-toblock change (<0.1 Hz) [81]. Indeed, the notion that the endogenous attentional activity in the brain is modulated by the viscera implicates ANS afference from the vasculature to the cortex. Further linking the ANS to block-to-block performance, our group and others have begun to link greater vagal activity (measured with vmHRV) to fewer intermittent increases in response time (RT) [82], [83]. Here, high vagal activity may reflect increased outflow of top-down cortical mechanisms that regulate default mode activity [84]. Adding even more complexity to the issue, the aforementioned changes in CV activity and their default mode correlates perhaps overlap with the endogenous habituation of CV activation occurring later in a stressor (represented by curvilinear decreases in heart rate or blood pressure at the end of a task) [85], [86], [87]. Here, nonlinear decreases in CV activation across blocks may reflect the down-regulation of stressful offtask cognition that otherwise impairs performance [88]. Taken together, nonlinear block-to-block change (e.g., cyclical oscillations, nonlinear decreases) in CV activity may reflect a number of endogenous mechanisms beyond CV reactivity to the exogenous stressor (i.e., task-related variability that can be represented by linear change across blocks).

Session-to-Session. Relative to other levels, session changes more heavily implicate long-term neural adaptations underlying continuous learning and habituation, thereby making this level especially critical to track in VR training systems [89]. Prior research generally indicates that performance improves across days, likely due to practice/learning effects and the slow habituation of performance-harming stress responses. [90], [91], [92]. However, performance across sessions can also exhibit more diverse patterns of variability due to hormonal influences. That is, unlike the task and block levels, sessionrelated changes are slow enough to implicate the similarly protracted activity of the endocrine system [93]. Here, hormonal rhythms and their perturbations over days due to sleep and mood have complex influences on central and autonomic function [94]. Not surprisingly then, changes in daily emotion (i.e., mood) and sleep have been correlated with session-tosession changes in performance [95], [96], [97].

It is unclear whether session-related changes in performance can be tracked with CV physiology. This is because research has yet to identify the pattern and magnitude of day-to-day changes in CV physiology within healthy populations, let alone their relations to performance. Many prior studies examining longitudinal change in cardiovascular physiology are limited in that they have focused on two or three measurement occasions, often years apart, with the goal of examining consistency between time points [98], [99], [100], [101], [102]. The studies in question hence do not meaningfully clarify continuous (dayto-day) changes in CV physiology that are important for VR training applications that target daily use. Importantly, decreases in CV activation across days may in part reflect the slow habituation to performance-related stress, which could in turn increase performance across the sessions [103].

# 1.3 Current Study

Addressing gaps in prior research, our study is among the first to simultaneously investigate human performance and

CV physiology at all the levels of variability outlined above. In the current study, participants visited the laboratory on six separate days. During each daily session, participants completed the same stressful, VR-based shooting task. Here, participants were asked to maintain optimal marksmanship (i.e., shot accuracy) while under threat of enemy fire. Performance was measured as marksmanship as well as response time (RT) to shoot targets; RT metrics can clarify the strategies and cognitive functions driving changes in marksmanship. For example, under stress, participants might adopt a more impulsive strategy, thereby decreasing mean RT at the cost of accuracy (i.e., speed-accuracy tradeoff) [104]. Trial-to-trial RT variability, in contrast, may convey unique information about the stability and robustness of attention that underlies accuracy [105]. CV activity was simultaneously assessed as interbeat interval (IBI), heart rate variability (HRV), and blood pressure (BP). Such metrics are influenced by ANS dynamics and endocrine factors that give rise to performance variability [106], [107], [108].

Fig. 2 illustrates our experimental design in the light of our multilevel theory. The difficulty of shooting was manipulated to examine task-to-task variability. Here, performance variability represents the adaptation of behavior to the increased motor-perceptual demands and mental stress (e.g., anxiety) of high versus low difficulty tasks [63]. For CV physiology, we examined CV reactivity (changes in physiology between a preceding baseline and the shooting condition) and differences in reactivity between the shooting difficulty conditions. For block-to-block variability, we examined changes in shooting performance and physiology between chunks of trials (blocks) within a single difficulty condition. Importantly, blocks within a single condition did not notably differ with respect to exogenous demands. For session-to-session variability, we examined mean level changes in performance and physiology across the six sessions that were days apart.

We tested four hypotheses based on our multilevel theory, in order to inform how VR training systems should exploit variability in physiology and performance. For all hypotheses pertaining to marksmanship, we examined concomitant changes in RT metrics (mean RT and trial-to-trial RT variability) to clarify the underlying cognitive factors driving shifts in shooting performance.

Hypothesis 1- Task-to-Task. (1a) In line with difficult tasks requiring more energetic resources, marksmanship will worsen during the high difficulty relative to the low difficulty condition. (1b) Consistent with the VR shooting task requiring mental and physical effort, both the low and high difficulty conditions will elicit increases in CV activation from baseline across the blocks. By increases in CV activation, we mean linear increases in BP as well as linear decreases in IBI and HRV from baseline across the blocks. (1c) increases in CV activation will be stronger for the high versus low difficult shooting task, consistent with high difficulty eliciting greater stress and more effort. In the current findings, we validated predictions for performance. Although we confirmed CV activation across both shooting conditions, we falsified the hypothesis of CV reactivity differences between the conditions.

*Hypothesis 2- Block-to-Block.* (1a) Aligned with research documenting endogenous, within-task change in attentional

function, there will be systematic changes in marksmanship between blocks of each shooting condition. (1b) Over and above linear changes in CV metrics across blocks (i.e., taskrelated CV activation), CV activity will also exhibit nonlinear changes across blocks. These effects would likely represent the < 0.1 Hz oscillations and/or habituation-related changes in CV activity that are theorized to affect performance. The present findings validated the prediction for the performance metrics and for HRV.

*Hypothesis 3- Session-to-Session.* Marksmanship will improve and average levels of CV activation will diminish with increasing sessions. This hypothesis is consistent with practice effects and stress habituation (respectively) taking place over days. We confirmed the hypothesis only for marksmanship, blood pressure, and HRV.

Hypothesis 4- Associations Between CV Physiology and Performance. Intra-individual changes in marksmanship performance will be related to concomitant changes in CV physiology. Here, we explored the level at which these relations occurred (block-to-block, task-to-task, session-to-session). We predicted that these intra-individual associations between physiology and behavior could be detected even without modeling task variables as covariates. Such findings would lend support to CV physiology as a robust estimator of performance in real-world VR training systems where detailed information about the environment may not be available. This hypothesis was partially confirmed such that HRV metrics at the task and block levels were related to marksmanship.

#### 1.4 Participants

Participants were seventeen students (7 women, 10 men) from a university in the Mid-Atlantic region of the United States. All participants were volunteers recruited through flyers and word-of-mouth on campus, and they provided informed consent in accord with the university's Institutional Review Board. The sample had a mean age of 26.18 years (SD = 3.70) and was predominantly Asian (76 percent Asian, 12 percent White, 12 percent Other). Participants were not excluded based on illness, disease, smoking, drug/ medication usage, or history of video gameplay (i.e., factor that could affect behavior in our VR game). We chose not to exclude participants on these factors in order to retain the real-world noise inherent to actual VR trainings, and to therefore increase the odds that present findings would generalize to real-world contexts. However, participants were still screened on the aforementioned characteristics. No participants reported cardiovascular or skeletomuscular conditions. Furthermore, no participants indicated using drugs or medications that notably influence cardiovascular function. Only one participant reported smoking (1-2 times per month), and one participant identified themselves as an active video game player. All results were identical (p < .05) when excluding these two participants.

Data were collected during six sessions that were on separate days. The spacing of sessions was in the order of magnitude of days; the average time between sessions was 4.23 days ( $\sigma = 4.75$ ). The time-of-day for sessions was not systematically controlled. The average hour of the session start time was 12:30PM ( $\sigma = 2.3$  hours). Importantly,

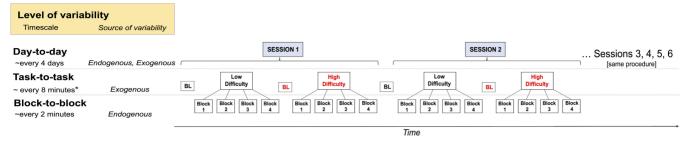


Fig. 2. Multilevel variability in the current study. Here, we link study elements to the conceptual aspects of our Multilevel Theory. At the Block level, we examined changes between 90-trial blocks within a single shooting difficulty condition. The blocks did not appreciably differ in environmental demands; thus, block-to-block variability reflected endogenously driven changes in physiology and behavior. At the Task level, we examined variability that is driven by environmental or exogenous change. This involved investigating change in physiology from the baseline (BL) period across the whole difficulty condition (i.e., cardiovascular reactivity), which encompasses all four blocks of a given difficulty condition. The task level also involved changes between the low and high difficulty conditions with respect to: (1) the degree of baseline-to-condition CV reactivity, and (2) shooting performance. The Session level examined average changes in physiology and performance across the six sessions. Session-related changes reflected slower, tonic changes in neurophysiological function in response to diverse environmental and internal stimuli. Additional notes: Timescales are approximate since the spacing of sessions and block durations were variable. \*When examining baseline-to-condition differences in physiology, the task level examined changes between a one-minute baseline and an eight-minute task condition.

supplemental analyses suggest that time-of-day could not account for any of the session-related effects presented in the paper (results not presented).

Data for some sessions had to be excluded due to missing data caused by hardware failure and excessive artifact in the physiological signals. Specifically, most participants completed five sessions. The mean number (i.e., count) of sessions completed was 4.29 (SD = 1.31). Here, we indicate the number of subjects who completed each session: Session 1 = 9, Session 2 = 13, Session 3 = 16, Session 4 = 12, Session 5 = 9, Session 6 = 14. Although not ideal, missing data is inherent to longitudinal designs like our own, especially when physiological signals are recorded over multiple days [109]. The issue of unbalanced data was addressed in the statistical analysis (described below).

## 1.5 General Procedures

The present data were extracted from a larger ongoing study that aims to examine the effects of neurofeedback training (between-participants factor) on shooting performance. Since our focus is on multilevel patterns of intraindividual variability, we collapsed across training conditions and focus on the VR shooting data. To examine the potential effects of training on VR data, neurofeedback training was added as a between-subject predictor (dummy code, 0= control, 1=experimental) to the multilevel models. Entry of the training variable did not significantly improve model fit in any case (results are presented in Supplementary Materials, which can be found on the Computer Society Digital Library at http://doi.ieeecomputersociety.org/ 10.1109/TAFFC.2020.2995769). Thus, in accord with multilevel modeling guidelines, the training predictor was dropped from the models [110]. These findings suggest unlikely effects of training on physiological response and performance, and they validate our approach of collapsing across training conditions. See the Supplemental Materials, available online, for a description of the neurofeedback training.

Participants visited the lab for seven sessions that were days apart. On the first day, participants were familiarized with the VR and physiological recording systems. They also

performed a thresholding shooting session that was used to titrate the difficulty conditions to individual participants (see below for more detail). On subsequent days, participants completed Session 1-6 such that they completed the same VR shooting task every session, while CV physiology was continuously recorded. The procedure of each session was largely identical. Specifically, participants completed two difficulty conditions of the VR shooting task whose order was randomly counterbalanced across participants and sessions. Before each difficulty condition, there was a one-minute resting baseline while participants wore the VR headset. Here, participants were asked to calmly attend to a fixed dot at a central location of the screen while remaining quiet and as still as possible. The low and high difficulty conditions were each comprised of four blocks. Each block contained 90 shooting trials (i.e., targets). This arrangement yielded eight blocks (720 trials) per session. Before every block, there were participant-determined break periods, which ended when participants pressed the space bar. Participants were asked to remain as still and quiet as possible during the break periods. Shooting blocks then commenced after a 3s, visually presented numerical countdown. The average duration of break periods across both difficulty conditions was 9.03s ( $\sigma$  = 20.53s). The low and high difficulty conditions had mean break durations of 8.29 ( $\sigma$  = 13.34) and 9.77 ( $\sigma$  = 25.76), respectively. Importantly, the break period durations were uncorrelated with performance and CV metrics (p>.05, results not presented).

After completing the entire shooting session, participants were detached from the VR headset and physiological equipment, and the experimental session ended. The next session was scheduled by research assistants in person or via email or phone; participants returned to complete the same procedures described above. This was done until participants completed all six sessions.

## 1.6 Physiological Recording

Electrocardiography (ECG) was continuously recorded at a modified lead II configuration using disposable spot electrodes on the thorax. ECG was collected during each session using an auxiliary channel of a commercially-available



Fig. 3. On the left, we show participant outfitted with the physiological and VR recording system. On the right, we show a screenshot from the VR shooting task.

Biosemi ActiveTwo system (http://www.biosemi.com/ products.htm). Beat-by-beat BP was simultaneously recorded using a commercially available Finapres Medical Systems Portapres system (http://www.finapres.com). Here, two finger cuffs were placed on the non-dominant hand to acquire values of both systolic and diastolic BP for each heartbeat. Other physiological signals were recorded but not analyzed in the current paper (see Supplemental Materials, available online). A picture of the physiological and VR recording suite is displayed in Fig. 3. All physiological, behavioral, and VR game event data were synchronized using Lab Streaming Layer (LSL) [111] and stored for offline analysis.

## 1.7 Shooting Task

The VR shooting task was a first-person marksmanship task performed in a head-mounted display using a commercially available HTC Vive (https://www.vive.com/us/product/ vive-virtual-reality-system/). Our particular simulation was inspired by similar VR studies on marksmanship [10], [11], but the particular design was novel and scripted by our research team in the Unity game environment (https:// www.unity.com). The simulation mimicked a nine-station shooting range in a daytime, desert environment. The nine stations were sandbags arranged at three distinct vertical distances from the shooter and at three distinct horizontal positions, as shown in Fig. 3. Targets could appear at any one of the nine stations, and the specific locations of the targets were randomized across trials. Targets could be either enemies or friends, and the order of target types was randomized across trials. Enemy targets were colored red and held rifles that were raised toward the participant. Enemy targets were programmed to shoot the player right before disappearing, unless the participant successfully hit the target first. That is, targets disappeared after successfully being hit by the player. Friendly targets were blue and held their rifles down vertically by the sides of their bodies. Friendly targets never shot at the player. Using a virtual handgun and simulated laser sight, participants were instructed to shoot the enemy targets but refrain from shooting the friendly targets. As mentioned above, each shooting block consisted of 90 trials (i.e., targets), yielding 360 targets per difficulty condition and 720 per session. For each difficulty condition, 90 percent of targets were enemies and 10 percent were friends (i.e., 324 enemies, 36 friends).

As aforementioned, a thresholding session was performed for each individual before the six experimental sessions [112]. Prior to the thresholding procedure, each participant became familiar with experimental setup and completed practice trials until they were comfortable with the task. The goal of the thresholding session was to individualize the level of difficulty for both conditions. Low difficulty was defined as the target exposure time (TET) that produced 90 percent shot accuracy on enemy targets, and high difficulty was defined as the TET that produced 50 percent shot accuracy on enemy targets. In the low difficulty condition, TETs were randomly selected from a Gaussian distribution with a mean of the TET corresponding to 90 percent ( $\sigma$ = .18s). In the high difficulty condition, the TETs were randomly selected from a similar distribution with the 50 percent accuracy TET ( $\sigma$ = .18s). The inter-target interval (ITI) was preserved across difficulty levels, in that it was randomly selected from Gaussian distribution with a  $\mu$  of 1.5s and  $\sigma$  of 0.5s.

The durations of the blocks and difficulty conditions were variable depending on how quickly participants shot at the targets. Collapsing across conditions, the average duration of a single block and task condition was 131.09s ( $\sigma$ = 9.20s) and 512.88s ( $\sigma$ = 66.69s), respectively. For low stress, the average duration of a single block and condition was 143.48s ( $\sigma$ = 12.97s) and 562.53s ( $\sigma$ = 80.24s), respectively. For, the average duration of a single block and condition was 118.70s ( $\sigma$ = 6.69s) and 463.23s ( $\sigma$ = 56.30s), respectively.

#### 1.8 Measures

All performance and physiological measures were computed for each block (1–4) within the difficulty conditions (low and high) of the VR shooting task. In the case of physiological metrics, measures were additionally computed for the two baseline periods. For every measure, this approach yielded multiple scores for each difficulty condition and session within a participant. In the case of performance, each subject would ideally have eight scores per session (four scores for each difficulty condition) and ten scores for each physiological metric (inclusive of baseline periods). However, some participants had less scores due to missing data caused by hardware failure and excessive noise in the physiological data. Taking this into consideration, participants had an average of 7.56 ( $\sigma$  = .88) scores for performance and an average of 9.47 ( $\sigma$  = 1.06) scores for physiological metrics.

## 1.8.1 Behavioral Measures

Shooting Performance. Our primary metric of shooting performance was marksmanship M, defined as the proportion of enemy targets correctly hit: M = H/T, where, H is the number of enemy targets hit and T is total number enemy targets per block.

Based on prior work, response time (RT; milliseconds) may elucidate the cognitive functions and strategies (e.g., attention, speed-accuracy tradeoff) underlying marksmanship. As such, we measured shot response time (RT) as the delay in milliseconds between target onset and the depression of the trigger on the controller. RTs for each target were averaged for each block to compute mean RT. As an additional metric of performance, we indexed RT variability with the coefficient of variation (CV; unit-free):  $\sigma(RT)/\mu(RT)$ . CV adjusts RT variability for the mean RT because prior research has shown that statistical relations involving  $\sigma$  of RT can be confounded by  $\mu$  [113].

## 1.8.2 CV Physiology

ECG signals were pre-processed in Python using the Biosppy toolbox [114]. Once the ECG signal was cleaned, a Pan-

Tompkins algorithm was used to detect R-spikes [115]. Interbeat Intervals (IBI) were computed as the time between consecutive R-spikes in milliseconds. IBIs were classified as abnormal if they were less than 300 ms or greater than 2000 ms, or if the IBI was more than 30 percent different than the preceding interval [116]. These abnormal IBIs were removed from the ECG record and interpolated with a cubic spline function [117]. Across all participants, this procedure affected less than 2 percent of IBIs in the ECG records. Corrected IBIs were then averaged per each block.

*Heart rate variability (HRV)* was computed with two wellestablished time-domain metrics reflecting autonomic nervous system influence on cardiac chronotropy [118]. First, SDNN (standard deviation of normal IBIs) was computed as the overall SD of corrected IBIs across the time series within each block. SDNN is believed to reflect a mixture of sympathetic and parasympathetic (or vagal) influences on cardiac chronotropy. Second, we computed the root mean square of successive differences (RMSSD) in the corrected IBI time of each block. RMSSD is an established metric of vagally mediated HRV, thus reflecting cardiac vagal influence [119]. Both HRV metrics were transformed with a natural logarithm to normalize their distributions for statistical analysis. These transformed metrics are denoted as ln (SDNN) and ln(RMSSD) in the Results.

*Blood pressure (BP)* was computed from the Portapres pressure signal in Python using a standard peak and trough detection algorithm. The pressure (millimeters of mercury; mmHg) values for each neighboring peak and trough in a cardiac cycle served to index beat-to-beat values of systolic (SBP) and diastolic blood pressure (DBP), respectively. These scores were then averaged to derive pressure values for each block.

## 1.9 Statistical Analysis

Hypotheses were tested with multilevel modeling, which is effective in disambiguating inter- and intra-individual variability [110]. Each model included a random intercept of participant in order to account for inter-individual variability, including inter-individual differences in initial performance and physiology scores due to prior VR experience [120]. This approach therefore allowed for a precise test of intra-individual associations involving physiology and performance. Multilevel modeling is also appropriate for the current data, because, unlike many other regression methods, they are robust to unbalanced data resulting from missing observations [121].

To test Hypotheses 1-3, different models (with the same predictors) were used so that each performance and physiological metric was a dependent measure in its own model. See Supplemental Materials, available online, for the specific structure of these models. At the individual level (Level-1), we entered the effects of Block (continuous predictor; 1-4), Task Difficulty (dummy variable; Low= 1, High=2) and Session (continuous predictor; 1–6). For the models predicting physiology, Block was coded 0–4, to accommodate baseline values (Block=0) and analysis of physiological reactivity from rest.

In order to test the intra-individual associations between physiology and performance (Hypothesis 4), two-level random intercept models were conducted with the same general form as above. To examine block-to-block associations, each block-level physiological variable was entered as a predictor of performance as the dependent measure. Models were conducted for each performance metric separately in order to avoid issues with multicollinearity. Difficulty condition, block, and session codes were not entered as predictors so that we could be blind to condition. The same approach was taken for task-level relations except that block scores were averaged for each physiological metric, and then corresponding baseline scores were subtracted from the task mean (to index reactivity). For session-level relations, the same procedure was carried out, except that we examined session means for each metric. That is, in a separate model for each physiological variable, we predicted session-level performance with session-level CV activity.

Models were built in accord with guidelines of Kreft and de Leuuw [110], which are detailed in the Supplemental Materials, available online. For all models, continuous variables were group-mean centered, and significant interactions were probed with simple slope analysis [122]. In order to bolster statistical conclusions, standard errors and confidence intervals were bootstrapped (5,000 resamples) [123]. Model fits were compared with likelihood ratio tests. We report unstandardized beta coefficients (*B*) in order to facilitate the interpretation of each effect's practical significance. All effects were tested with two-tailed 95 percent confidence intervals (bootstrapped) and p-values.

## 2 RESULTS

## 2.1 Hypothesis 1: Task-to-Task Changes

# 2.1.1 Hypothesis 1a: Shooting Performance Will be Worse During the High Difficulty Relative to the Low Difficulty Condition

Speaking to the efficacy of the shooting difficulty manipulation, there was a significant fixed effect of Difficulty on marksmanship (*B* = -.30, *SE* = .007, 95 % *CI* [-.32, -.29], *p* < .05). That is, participants hit 30 percent less enemy targets during the high relative to the low difficulty condition. Fig. 4 displays each performance metric and their mean differences between difficulty conditions. The decline in marksmanship was accompanied by an unsurprising 71ms decrease in RT during high relative to low difficulty, as indicated by the significant fixed effect of Difficulty on mean RT (B = -70.79, SE = 2.80, 95 % *CI* [-76.21, -65.27], *p* < .05). Also paralleling the decrease in marksmanship, the high difficulty condition was associated with an increase in the coefficient of variation, a metric reflecting RT variability (Fixed effect of Difficulty: B =.56, SE = .07, 95% CI [.42, .70], p < .05). Taken together, when the target exposure times were faster (i.e., the task was more difficult), marksmanship worsened and RTs became both shorter and more unstable.

# 2.1.2 Hypothesis 1b: Both Difficulty Conditions Will Elicit Increases in CV Activation From Baseline Across Blocks

In the model examining IBI, there was a significant linear effect of Block (B = -6.73, SE = 1.38, 95% *CI* [-9.39, -4.02], p < .05), which indicates decreases in IBI from baseline across the shooting blocks. This pattern is depicted in Fig. 5

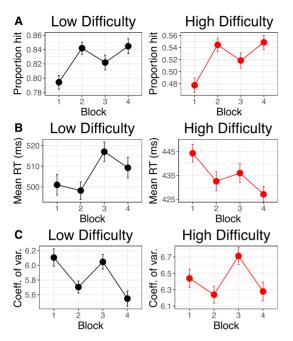


Fig. 4. Shooting performance metrics by block and difficulty condition (averaged across sessions). Within-participant means and standard errors are depicted to purely reflect intra-individual patterns apart from inter-individual variability [124]. Proportion hit (i.e., marksmanship) refers to the total number of enemy targets hit out of total number enemies per block. Mean RT (in milliseconds) refers to the  $\mu$  of response times per block. Coeff. of var. (unit-free) refers to  $\sigma$  of response time adjusted by  $\mu$  of response time per block and is a metric of trial-to-trial response time variability. The black lines depict the low difficulty condition, and the red lines depict the high difficulty condition.

and indicates that this IBI decrease was similar between difficulty conditions. Consistently, the Block X Difficulty interactions was not significant (B = -1.20, SE = 2.71, 95% CI [-6.46, 4.11], p > .05).

When examining  $\ln(\text{SDNN})$ , there was a significant quadratic (B = .04, SE = .006, 95 % CI [.03, .05], p < .05) and a small cubic (B = -.01, SE = .006, 95 % CI [-.02, -.0009], p <.05) effect for Block. Importantly, there was a significant interaction between the linear term of Block and Difficulty (B = -.03, SE = .01, 95% CI [-.06, -.004], p < .05). Simple slopes revealed that there was no significant linear change in  $\ln(\text{SDNN})$  for the low difficulty condition (B = -.005, SE =.02, 95% CI [-.05, .04], p > .05). During the high difficulty condition, there was a decrease in  $\ln(\text{SDNN})$  but the magnitude of the slope was not different from zero (B = -.04, SE =.02, 95% CI [-.08, .007], p > .05). Given that the simple slopes were not significant, we do not further interpret the Block X Difficulty interaction. In sum, reactivity in  $\ln(\text{SDNN})$  can be characterized as a curvilinear decrease from baseline across the blocks (reflecting quadratic effect), such that the decrease is comparable between conditions (see Fig. 5B). This pattern of reactivity is somewhat consistent with our prediction of a decline in HRV in response to the shooting task; however, it was surprising that this was a nonlinear rather than a linear decline.

We next to turn to changes in  $\ln(\text{RMSSD})$ . As indicated by the significant linear effect (B = -.03, SE = .008, 95% CI [-.05, -.02], p < .05), it can be said that  $\ln(\text{RMSSD})$  linearly decreased across the shooting blocks. The magnitude of the decrease was not different between conditions, as seen in

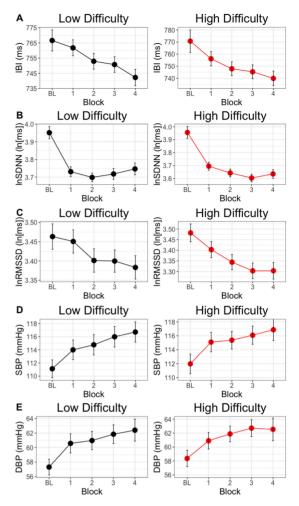


Fig. 5. Cardiovascular metrics by block and difficulty condition (averaged across sessions). Within-participant means and standard errors are depicted to purely reflect intra-individual patterns apart from inter-individual variability [124]. IBI refers to interbeat interval (in milliseconds). LnSDNN refers to the natural logarithm (In) of the standard deviation of normalized interbeat intervals (in natural logarithm of milliseconds). LnRMSSD refers to the natural logarithm (In) of the root mean square of successive differences in interbeat intervals (in natural logarithm of milliseconds). SBP refers to systolic blood pressure in millimeters of mercury. The black lines depict the low difficulty condition, and the red lines depict the high difficulty condition.

Fig. 5C and based on the non-significant Block X Difficulty interaction (B = -.02, SE = .02, 95% CI [-.05, .007], p > .05).

In the model predicting systolic blood pressure (SBP), there was a significant linear effect of Block (B = 1.22, SE =.31, 95% CI [.61, 1.82], p < .05). As can be seen in Fig. 5D, SBP increased across the shooting blocks equally for both difficulty conditions. Supporting the latter point, there was no significant Block X Difficulty interaction (B = -.18, SE =.63, 95% *CI* [-1.43, 1.03], *p* > .05). The pattern of change in diastolic blood pressure (DBP) was the same as that of SBP, such that there was only a linear effect of Block (B = 1.10, SE = .28,95% CI [.56, 1.64], p < .05). As above, the Block X Difficulty interaction (B = -.08, SE = .54, 95% CI [-.19, .95], p > .05) was not significant and was therefore dropped from the model. See Fig. 5E for the decrease in DBP across both shooting conditions. The nonlinear effects in the above models have bearing on block-to-block effects, which are discussed later.

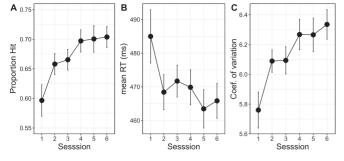


Fig. 6. Mean performance by session. Here, we present session-level means that were computed by averaging metrics across all blocks, difficulty conditions, and participants for each session. Within-participant means and standard errors are depicted to purely reflect intra-individual patterns apart from inter-individual variability [124]. Proportion hit (i.e., marksmanship) refers to the total number of enemy targets hit out of total number enemies per block. Mean RT (in milliseconds) refers to the  $\mu$  of response times per block. Coeff. of var. (unit-free) refers to  $\sigma$  of trial-to-trial response time variability.

## 2.1.3 Hypothesis 1c: CV Activation Will be Stronger for the High versus Low Difficult Shooting Task

As reported above, there was no strong evidence that the pattern of block-related changes in CV metrics differed between the difficulty conditions. This pattern can be seen in Figs. 5A, 5B, 5C, 5D, and 5E. Although the interaction between Block and Difficulty was significant in the model testing ln(SDNN), the simple effects of Block on ln(SDNN) at low and high difficulty separately were not significant. Thus, they are not interpreted further.

## 2.2 Hypothesis 2: Block-to-Block Changes

# 2.2.1 Hypothesis 2a: There Will be Changes in Performance Metrics Between the Shooting Blocks

We tested the magnitude of performance shifts between the shooting blocks with the fixed linear and polynomial effects of Block. For marksmanship (i.e., proportion of enemy targets hit), there was a significant linear effect of Block (B = -.02, SE = .01, 95% *CI* [-.04, -.0008], p < .05), which indicates an overall increase in marksmanship across the blocks (Fig. 4A). We also detected significant quadratic (B = -.009, SE = .003, 95% *CI* [-.02, -.002], p < .05) and cubic (B = .02, SE = .003, 95% *CI* [-.02, -.002], p < .05) effects of Block. Based on these findings, there are significant block-to-block shifts in marksmanship during both difficulty conditions. Confirming the latter point, none of the interactions between the Block and Difficulty were significant (results not presented for clarity).

For mean RT, there was a significant linear (B = 23.55, SE = 5.72, 95% *CI* [12.39, 34.74], p < .05) and cubic (B = -6.21, SE = 2.01, 95% *CI* [-10.15, -2.32], p < .05) effect of Block. In addition, there was a significant Block X Difficulty interaction (B = -7.60, SE = 2.60, 95% *CI* [-12.83, -2.56], p < .05). As indicated by simple slope analysis and by Fig. 4B, RT decreased across blocks for the low difficulty condition (B = 15.95, SE = 4.43, 95% *CI* [7.23, 24.80], p < .05) but did not significantly change across blocks during the high difficulty condition (B = 8.36, SE = 4.44, 95% *CI* [-.54, 16.85], p > .05). Although block-related changes in mean RT could be characterized as cubic for both difficulty conditions, low difficulty was associated

with a stronger linear increase in mean RT. Fig. 4 suggests that marksmanship positively correlated with mean RT at the block level. This is supported by a separate random intercept model testing mean RT as a predictor of proportion of hits (B = .002, SE = .0001, 95% CI [.0017, .0022], p < .05).

When examining the coefficient of variation, there was a linear effect of Block (B = .44, SE = .10, 95% *CI* [.24, .64], p < .05) that was qualified by a significant cubic term (B = -.25, SE = .05, 95% *CI* [-.34, -.16], p < .05). As seen in Fig. 4C, there were notable block-to-block changes in the coefficient of variation for both difficulty conditions. Consistent with our interpretation, there were no interactions between Block and Difficulty in the prediction of the coefficient of variation (results not presented).

# 2.2.2 Hypothesis 2b: Over and Above Linear Changes (Representing Task-Related CV Activation), CV Metrics Will Exhibit Nonlinear Changes Across Shooting Blocks

All effects of Block are reported under Hypothesis 1b. In this section, we highlight statistically significant curvilinear effects of Block because (unlike linear Block effects) the curvilinear effects better speak to endogenously driven block-to-block variability. Only ln(SDNN) exhibited a statistically significant curvilinear term. As depicted in Fig. 5B), there were significant quadratic (B = .04, SE = .006, 95% CI [.03, .05], p < .05) and cubic (B = -.01, SE = .006, 95% CI [-.02, -.0009], p < .05) effects of Block. All other CV metrics exhibited strictly linear change across blocks (see above).

## 2.3 Hypothesis 3: Session-to-Session Changes

## 2.3.1 Hypothesis 3a: Performance Will Improve Across Sessions

As hypothesized, there was a linear change in marksmanship across sessions (fixed effect of Session: B = .01, SE =.005, 95% CI [.002, .02], p < .05). Improved prediction of marksmanship was afforded by the addition of both quadratic (B = -.007, SE = .002, 95% CI [-.01, -.004], p < .05) and cubic (*B* = .002, *SE* = .001, 95% *CI* [.0001, .004], p < .05) terms of Session, although these effects were small. Despite significant polynomial effects, the change in marksmanship can best be characterized as a linear increase across sessions, since the effect size of the linear term is notably larger than that of the polynomial terms. See Fig. 6A. To further clarify the main effects of session, we also plotted the session-level averages by session number in Fig. 6. Fig. 6 suggests that marksmanship negatively correlated with mean RT across sessions. This is supported by a separate random intercept model that tested mean RT as a predictor of proportion of hits (B = -.001, SE = .0003, 95% CI [-.002, -.0007], p < .05).

In the model predicting mean RT, there was a significant linear effect of Session (B = -3.49, SE = .90, 95% *CI* [-5.30, -1.78], p < .05). This result indicates that, as predicted, mean RT declined across sessions. The linear effect was qualified by a quadratic term of Session (B = 2.18, SE = .66, 95% *CI* [.92, 3.49], p < .05). Suggested by visual inspection (Fig. 6B), much of the decline in RT occurred from session 1 to session 2, with this relationship flattening across the rest of the sessions.

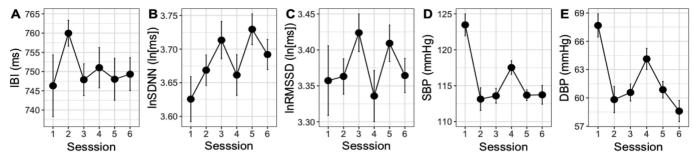


Fig. 7. Mean cardiovascular metrics by session. Here, we present session-level means that were computed by averaging metrics across all blocks, difficulty conditions, and participants for each session. Within-participant means and standard errors are depicted to purely reflect intra-individual patterns apart from inter-individual variability [124]. IBI refers to interbeat interval (in milliseconds). LnSDNN refers to the natural logarithm (In) of the standard deviation of normalized interbeat intervals (in natural logarithm of milliseconds). LnRMSSD refers to the natural logarithm (In) of the root mean square of successive differences in interbeat intervals (in natural logarithm of milliseconds). SBP refers to systolic blood pressure in millimeters of mercury. DBP refers to diastolic blood pressure in millimeters of mercury.

For CV, there was a significant linear (B = .12, SE = .02, 95% *CI* [.07, .16], p < .05) and quadratic (B = -.03, SE = .02, 95% *CI* [-.07, -.001], p < .05) effect of Session. Similar to the findings for marksmanship, the change in CV across sessions can best be characterized as a linear increase. This inference is based on the notably larger effect size of the linear versus the cubic term and based on visual inspection of Fig. 6C.

# 2.3.2 Hypothesis 3b: CV Activation Will Decrease Across Sessions

In the model testing IBI, there was a significant quadratic term of Session (B = 1.97, SE = .88, 95% *CI* [.21, 3.67], p < .05). In Fig. 7A, it is apparent that the change in IBI across session could be characterized as notably quadratic.

Session-related changes in ln(SDNN) could be characterized as a strictly linear increase, as indicated by the fixed effect of Session (B = .02, SE = .006, 95% CI [.005, .03], p <.05). Although the pattern of change for ln(SDNN) is graphically similar to that of ln(RMSSD) (Figs. 7B and 7C), sessionrelated changes in ln(RMSSD) could be characterized as linear (B = .04, SE = .02, 95% CI [.004, .07], p < .05), quadratic (B = .01, SE = .005, 95% CI [.005, .02], p < .05), and cubic (B = -.008, SE = .003, 95% CI [-.01, .-.002], p < .05). The patterns of session-related change for systolic (SBP) and diastolic (DBP) blood pressure were highly similar to one another (Figs. 7D and 7E). Specifically, there was a significant negative linear effect of Session on both SBP (B = -1.22, SE = .28, 95% CI [-1.74, -.68], p < .05 and DBP (B = -1.23, SE = .24,95% CI [-1.72, -.77], p < .05). However, both linear effects were qualified by significant quadratic terms (SBP: B = .92, SE = .20, 95% CI [.53, 1.32], p < .05; DBP: B = .59, SE= .18,95% CI [.23,.94], p < .05).

## 2.4 Hypothesis 4: Direct Intra-Individual Associations Between Physiology and Performance

## 2.4.1 Task-to-Task

Ln(RMSSD) reactivity scores were significantly related to marksmanship (B = .81, SE = .31, 95% *CI* [.21, 1.39], p < .05), such that task conditions with weaker decreases in ln (RMSSD) from baseline to task had relatively higher marksmanship. Ln(RMSSD) reactivity was also significantly related to mean RT (B = 200.16, SE = 92.68, 95% *CI* [19.70, 378.90], p < .05). Here, task conditions with weaker

decreases in ln(RMSSD) had relatively higher mean RT. Other task-to-task relations between physiology and performance were not statistically significant and appear in the Supplemental Materials, available online.

## 2.4.2 Block-to-Block

There was a significant relation between IBI and mean RT (B = .17, SE = .05, 95% CI [.07, .26], p < .05), suggesting that block-to-block increases in IBI predicted concomitant increases in mean RT. Similarly, ln(SDNN) was related to marksmanship (B = .09, SE = .03, 95% CI [.02, .15], p < .05), mean RT (B = 43.42, SE = 8.72, 95% CI [26.12, 60.37], p < .05), and the coefficient of variation (B = -.63, SE = .16, 95% CI [-.95, -.32], p < .05). Here, increases in  $\ln(\text{SDNN})$ predicted concomitant augmentations in accuracy and mean RT, but declines in RT variability. The pattern of significant correlations were the same for  $\ln(RMSSD)$ . That is, increases in ln(RMSSD) also predicted increases in both marksmanship (B = .06, SE = .03, 95% CI [.004, .17], p <.05) and mean RT (B = 33.18, SE = 7.67, 95% CI [17.83, 48.71], p < .05), as well as declines in the coefficient of variation (B = -.57, SE = .14, 95% CI [-.85, -.30], p < .05). Other effects were not statistically significant (see Supplemental Materials, available online)

## 2.4.3 Session-to-Session

Here, we examined session-level mean scores of each performance metric as a function of session-level mean scores of each physiological metric. None of the associations were statistically significant; they are hence presented in the Supplemental Materials, available online.

## 3 DISCUSSION

This is among the first studies to examine joint patterns of intra-individual variability in CV physiology and human performance, both within and across multiple daily study sessions. The present findings largely confirm the heuristic value of our multilevel model of performance, where the task, block, and session levels galvanize unique neurobiological mechanisms and performance-relevant states. Consistently, we found that the joint patterns of CV physiology and performance (as well as their direct correlations) differed depending on the level of analysis. At the task level, we found large decreases in both marksmanship and mean RT-as well as increases in RT variability-from the low to high difficulty condition. The performance changes were not accompanied by changes in CV physiology between difficulty conditions. Rather, there was a similarly robust pattern of CV activation from baseline to task for both shooting conditions. At the block level, there were cyclical, intermittent increases in marksmanship across blocks that were accompanied by increases in mean RT and decreases in RT variability. Unlike the task level, these performance changes were paralleled by block-related changes in physiology, specifically by nonlinear decreases in cardiac autonomic regulation (i.e., SDNN) across blocks. At the session level, a very different pattern of performance emerged where there were increases in marksmanship, decreases in mean RT, and increases in RT variability across days. At the same time, SDNN linearly increased across sessions and BP metrics showed large attenuation between the first two sessions; other CV metrics showed unanticipated patterns of change across sessions. Lastly, of all the CV metrics and levels of analysis, only changes in HRV at the task and block levels were directly correlated with simultaneous changes in marksmanship.

Taken together, the findings imply that CV metrics show diverse patterns of intra-individual change depending on the timescale of change and whether such change is driven by exogenous versus endogenous stimuli. We next describe how performance and physiological variability reflect different psychophysiological mechanisms at each level of analysis. We then discuss their implications for future affective computing systems (e.g., VR training systems).

#### 3.1 Hypothesis 1: Task-to-Task

We increased task difficulty by shortening the target exposure time. In line with hypotheses, the high difficulty condition was associated with relatively worse marksmanship. This effect was accompanied by shorter mean RT during high relative to low difficulty, which is likely a consequence of the shorter target exposures. Importantly, trial-to-trial RT variability was also increased during high difficulty, suggesting increased demands on the effortful control of attention [105], [125]. Together, these findings suggest that the high difficulty condition required more effort, thus more strongly taxing energetic and perhaps attentional resources [43]. These effects are also consistent with a speed-accuracy tradeoff where participants had to shoot faster at the briefer targets during high difficulty, thus leading to worse marksmanship [104].

Despite condition differences in performance, it is likely that *both* difficulty conditions elicited substantial shifts in underlying brain state compared to baseline, such that energetic resources were mobilized for on-task attention [57], [64]. Indeed, this notion was supported by the statistically significant linear increases in CV activation (increases in BP and decreases in IBI from baseline across blocks) for both difficulty conditions. These changes were hypothesized and are generally consistent with a well-established pattern of increased CV activation in response to stress. Such activation putatively facilitates mental effort and the activation of metabolic resources for motor action [63], [64], [108]. In line with others, the above patterns of CV activation were in part mediated by the ANS, specifically by cardiac vagal (parasympathetic) withdrawal [68], [126]. The latter inference is supported by the linear decreases in RMSSD from baseline across the entire task condition (i.e., across all blocks). The pattern was detected for both difficulty conditions. A sympathetic involvement in shortening IBI and increasing vasoconstriction (partly indexed by BP) is also likely responsible for the observed physiological response [127]. However, pure sympathetic metrics cannot be inferred from the present metrics. It should be noted that SDNN (broadband index of HRV) showed curvilinear rather than linear decreases in response to the shooting conditions. The differential pattern between HRV metrics may reflect the additional contributions of sympathetic nervous system and lower frequency vagal influences to SDNN [128].

Contrary to predictions, performance impairment during the high versus low difficulty conditions was not accompanied by a robust increase in CV reactivity. This finding is contrary to previous studies that report greater CV activation in response to higher task difficulty and load [106], [107]. One reason for the null finding could be that the high difficulty condition was not sufficiently stressful to elicit robust cardiovascular activation compared to the low difficulty condition. Indeed, others have noted that cardiac indices such as HRV are only sensitive to very large changes in load [129]. In addition, there are notable individual differences in reactivity to stress documented in the literature, suggesting that task-related differences in CV activation may be more prominent for some participants [130]. However, we were statistically underpowered at the participant-level to examine such effects. Future studies should investigate this issue more closely.

## 3.2 Hypothesis 2: Block-to-Block

We also examined variability between chunks of trials on the same task in order to probe endogenously driven fluctuations in performance and their CV concomitants. Confirming our predictions, there was significant block-to-block oscillations in shooting marksmanship as well as RT metrics. These oscillations were reflected as cubic changes in marksmanship, mean RT, and trial-to-trial RT variability. Since blocks were nearly identical in terms of external demands (i.e., the timing and nature of stimuli did not notably change between blocks), it is likely that the oscillations in performance here (<0.1Hz) reflect endogenous oscillations in attention that have been noted in prior work [74].

Importantly, the block-to-block changes in the present study may partially represent a speed-accuracy tradeoff where participants were less cautious responders on some blocks. [104]. Importantly, block-related decreases in accuracy may also be owed to a less controlled mode of responding, which is consistent with the fact that RT variability increased on blocks when accuracy lowered [43], [78], [105], [131], [132], [133]. That is, high levels of RT variability are believed to reflect a relative lack of control of attention whereby energetic resources are perhaps not appropriately utilized to maintain focus and suppress endogenous distractors (task-unrelated thought) [105], [132], [134]. Our inference is consistent with the literature where dorsal attention networks implicated in attentional control are anticorrelated with block-to-block oscillations in default mode activity [57], [74]. In our findings, blocks with worse marksmanship but high RT variability feasibly reflect the phase of this oscillation when default mode activity is on and attentional control is off. Attentional control being off in turn yields a relatively impulsive response style (i.e., fast RTs and worse marksmanship).

The cubic changes in performance above were accompanied by nonlinear changes in SDNN across blocks; here, SDNN increased after an initial decrease from baseline. Consistent with prior studies, this curvilinear decrease likely reflected an endogenous habituation response to the external task [66], [85]. Our inference is supported by the lack of a plausible exogenous mechanisms that would account for cubic shifts in physiology between nearly identical blocks. We also detected linear increases in CV activation and decreases in vagal influence (i.e., RMSSD) across the blocks. As discussed above, however, the linear changes CV reactivity were probably exogenously evoked by the shooting task.

We should note that our attempt to linearly decompose exogenous and endogenous influences in the CV response is heuristic. Exogenous and endogenous factors may interact in at least two ways within the present study. First, nonlinear responses reflecting habituation necessarily require some prolonged external stimulus. Second, in some cases, continued linear change in CV activity after the initial stress reaction may be affected by thoughts (e.g., worrisome thoughts) about the stressor [135], [136]. Despite such caveats, it is still likely—based on the literature—that linear and curvilinear CV responses across block more strongly reflect exogenous and endogenous factors, respectively. Future work should refine methods to more precisely decompose these influence on physiological variability.

#### 3.3 Hypothesis 3: Session-to-Session

As predicted, marksmanship improved across days. In parallel, there were decreases in mean RT and augmentations in RT variability across the sessions. These changes were largely linear but also had curvilinear components. Improved marksmanship at later sessions is consistent with a basic learning effect, where participants became more skilled at the task with increased practice [90], [91]. Importantly, RT decreased across the sessions as marksmanship improved, which is a very different pattern than what was observed at the task level and, to some extent, the block level (i.e., mean RT increased with improved marksmanship). Also unlike the task and block levels, session-to-session increases in marksmanship were paralleled with increases rather than decreases in RT variability. Taking these results together, improved marksmanship at the task and block levels appeared to reflect effective cognitive control over impulsive responding. In contrast, marksmanship improvements across sessions (accompanied by mean RT decreases and RT variability increases) appear to reflect the development of a more efficient mode of responding [137]. That is, the increased trial-to-trial RT variability at later sessions is consistent with a reduction in the top-down control of attention, where a more bottom-up or automatic mode of cognitive processing accompanies superior performance on well-learned tasks. [138].

Hypotheses pertaining to decreases in average CV activation across sessions were not universally supported across metrics. BP robustly decreased across sessions, although this decrease was predominantly mediated by heavy decreases from session 1 to session 2, with relatively constant levels of BP for the remainder of the sessions. This finding is consistent with the rapid nature of physiological habituation to mental stress and/or negative emotion documented in the literature [139]. SDNN appeared to show a progressive increase across the sessions, perhaps due to habituation-related augmentations in low-frequency vagal regulation [128]. Unlike RMSSD, SDNN reflects low-frequency oscillations in IBI that have been attributed to autonomically mediated baroreflex function, which serves to regulate BP [140]. Such putative increases in baroreflex function (vis-a-vis increases in SDNN) could support the down-regulation of BP over sessions [141]. Further research is needed to directly test this possibility.

It should be noted that session-to-session changes in CV physiology pertained to mean physiology across all task blocks and baselines. Differences in reactivity between sessions were not statistically significant. Thus, habituation effects appear to affect more tonic (i.e., mean) levels of cardiovascular activation across baseline and performance, such that participants might have felt more acclimated to the experimental setting at the second session [100], [103]. Such lowered levels of stress and/or negative emotion may have in turn led to less competition for neural resources during the task, thereby accounting for the linear improvement in performance over sessions [59].

Interestingly, the patterns of change in the other physiological metrics (IBI, RMSSD, SDNN) were more complex, in that there were nonlinear trajectories of change across sessions. Such complex patterns suggest involvement of endocrine mechanisms. Unlike task and block-related shifts in CV responses, day-to-day shifts are slow enough to implicate hormonal chemical signaling. Relative to the neural control of the ANS, the chemical messaging of the endocrine systems must affect organs via the bloodstream, thus making endocrine control of CV activity much slower and more diffuse than ANS modulation [93]. As such, with day-to-day fluctuations in physiology, there is potential for many interactions between varied organ systems and autonomic feedback mechanisms, thereby resulting in potentially complex patterns of change [142], [143]. Such endocrine-mediated changes likely encompass important exogenous (e.g., daily temperature, stressful life events) factors and endogenous oscillations in biological function (uterine cycle) that were not directly measured in this study [144]. Taken together, the session effects in the present study are at odds with prior longitudinal studies that emphasize the test-retest reliability of cardiovascular measures [98], [99]. Unlike these prior studies, we showed that cardiovascular measures may show significant variability across daily testing sessions.

## 3.4 Hypothesis 4: Direct Associations Between CV Physiology and Performance

The fact that performance and CV physiology both exhibited statistically significant changes across blocks and sessions implies that there should be direct associations between performance and CV measures at these levels. However, when directly testing relations between CV and performance metrics, only a few statistically significant effects emerged at the task and block levels. At the task level, the task condition with less vagal withdrawal (weaker RMSSD decreases from rest to the task condition) had relatively better marksmanship and longer mean RT. In other words, increased vagal outflow during the task was associated with better performance, which is consistent with other reports [145]. A similar pattern of relationships were detected at the block level where blocks with increased HRV (both SDNN and RMSSD) had increased marksmanship and RT as well as decreased RT variability. Although SDNN is affected by sympathetic activity, it shows relations with performance that strongly resemble analogous associations involving RMSSD (pure vagal metric). This suggests that the aforementioned SDNN and RMSSD effects are reflective of vagal activity.

The relations between HRV and performance metrics at both the task and block are consistent with theorized links between intra-individual increases in vagal activity and augmentations in PFC activity to support the context-appropriate, top-down control of behavior, cognition, and emotion [84]. Consistently, higher levels of vagal activity (i.e., vagally mediated HRV) have been linked to the superior control over both exogenous and endogenous emotions and cognitive processes (e.g., worrisome thoughts) that would otherwise impair performance [84], [146]. Through similar mechanisms, increases in vagal activity (higher HRV) on some blocks may have supported an adaptive increase in RT to improve marksmanship (i.e., speed-accuracy tradeoff favoring accuracy) on the same blocks. Blocks with higher HRV could seemingly reflect the onphase of the dorsal attention network (and concomitant offphase for default mode) of the < 0.1 Hz oscillation described above. Some prior work has certainly tied the neural hubs of these networks to HRV, but additional research is required to directly test the latter inferences [147], [148]. At the task level, relatively greater vagal outflow (less vagal withdrawal) likely reflects similar top-down control mechanisms underlying adaptive control of behavior, thereby leading to a cautious strategy that promoted higher marksmanship [84], [106].

Positive block-to-block associations between HRV and mean RT were paralleled by an unsurprising positive association between IBI and mean RT. The latter effect appears to index a vagally mediated lengthening of IBI that supports a cautious strategy. Indeed, vagal slowing of the heart, manifested as longer IBIs, is a well-documented and incredibly important for inhibiting motor action in accord with task goals [149]. IBI findings at the task level may implicate similar motor inhibitory processes, as they were in the same direction but not statistically significant.

The findings above underscore the importance of measuring HRV to estimate shifts in performance during a stressful, real-world task. Our findings here thus extend on a rich body of applied work using HRV to track performance within operational settings [129], [150]. More specifically, we add to this work by showing that, even under a constant level of difficulty, within-task (block-to-block) shifts in HRV can help index endogenous shifts in performance.

In sum, the complexity of the present response patterns are consistent with the similarly complex interactions between autonomic, skeletal, brain, and endocrine systems that support adaptive behavior. In light of the present results and our theoretical model, it is unlikely that a given CV metric such as HRV indexes the same psychological construct (e.g., workload) at all levels of variability (task, block, session). The latter notion is important for researchers who use CV physiology to estimate specific performance-relevant states in VR training and similar computing applications. That is, simply adding a heart rate measurement to index stress of fatigue across training sessions may be a more nuanced affair than some researchers believe.

## 3.5 Limitations and Future Directions

The present study has a number of limitations. First, there were missing session data that may have affected the statistical conclusions presented here. We attempted to alleviate this issue with multilevel modeling which is less sensitive to unbalanced data compared to traditional regression approaches. Second, we did not control for a number of variables such as sleep, stressful life events, or uterine cycle phase that may have driven the observed patterns of intraindividual variability. Although future research should examine these explanatory factors more closely, the goal of the present study was to depict the rich variability in behavior and physiology that are driven by diverse stimuli. We thought this initial wide scope was necessary to depict the amount of variability that researchers in affective computing spaces could eventually leverage. This is in contrast to artificially constraining such variability through stringent experimental and statistical controls. That said, future work should more systematically examine how different endogenous and exogenous stimuli interact to affect CV response across both short and long timescales. Third, all results come from a sample that also completed a neurofeedback training, which may limit the generalizability of our results to other samples. However, we believe that this is unlikely since many of the current patterns of performance and CV responding were consistent with prior work and with predictions. Fourth, we did not ask participants about prior experience with VR, which could have affected the results. Yet, our sample size was too low to statistically test whether observed intra-individual effects differed by individual attributes such as VR history, gender, age. Future research should examine if these individual differences impact patterns of multilevel variability in physiology and performance within VR environments. Fifth, the nature of the VR shooting task was fairly low in stress such that the present results may not generalize to more emotional tasks or VR shooting tasks with more a realistic scenario. Additional work is needed to replicate the present results in different VR tasks and affective computing systems. Sixth, it should also be noted that the direct relations between CV physiology and performance are low in effect size magnitude, calling into question their practical significance. Seventh, we did not have formal recovery periods in the present study, thus preventing us from establishing whether CV activity returned to baseline before additional blocks and tasks were administered. Furthermore, CV recovery might also reveal neurobiological dynamics relevant to stress, adaptation, and performance which are putatively distinct from the

reactivity and habituation effects emphasized in the current paper [151], [152]. Future work should apply our multilevel theory in diverse experimental contexts beyond VR and alongside data-driven methods such as machine learning. Such work would refine our theoretical model based on complex physiology-behavior associations that are hard to detect solely with hypothetico-deductive approaches.

#### 3.6 Implications and Conclusion

Taken together, the present findings suggest that intra-individual changes in performance are accompanied by dynamic and, at times uncorrelated, intra-individual changes in CV activity. These findings highlight the need for VR training systems and perhaps other computing paradigms to measure physiology and performance across multiple timescales. We hope, more broadly, that the present paper can provide a roadmap for affective computing researchers to approach CV measures beyond simplistic mappings between a single CV metric (e.g., heart rate) and single psychological construct (e.g., load). We provided a multilevel model that researchers can use to generate much more complicated hypotheses and neurophysiologically tractable inferences pertaining to how CV measures relate to human performance. Since our model synthesizes perspectives across disparate fields in neuroscience, psychology, and psychophysiology, its use to motivate research in affective computing could broaden the impact of such work. It could also help build a richer and more interconnected base of research that informs how physiology can be utilized in computing applications and other applied domains.

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