

Modeling Brain Dynamics During Virtual Reality-Based Emergency Response Learning Under Stress

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Abstract

Background: Stress affects learning during training, and virtual reality (VR) based training systems that manipulate stress can improve retention and retrieval performance for firefighters. Brain imaging using functional Near Infrared Spectroscopy (fNIRS) can facilitate development of VR-based adaptive training systems that can continuously assess the trainee's states of learning and cognition.

Objective: The aim of this study was to model the neural dynamics associated with learning and retrieval under stress in a VR-based emergency response training exercise.

Methods: Forty firefighters underwent an emergency shutdown training in VR and were randomly assigned to either a control or a stress group. The stress group experienced stressors including smoke, fire, and explosions during the familiarization and training phase. Both groups underwent a stress memory retrieval and no-stress memory retrieval condition. Participant's performance scores, fNIRS-based neural activity, and functional connectivity between the prefrontal cortex (PFC) and motor regions were obtained for the training and retrieval phases.

Results: The performance scores indicate that the rate of learning was slower in the stress group compared to the control group, but both groups performed similarly during each retrieval condition. Compared to the control group, the stress group exhibited suppressed PFC activation. However, they showed stronger connectivity within the PFC regions during the training and between PFC and motor regions during the retrieval phases.

Discussion: While stress impaired performance during training, adoption of stress-adaptive neural strategies (i.e., stronger brain connectivity) were associated with comparable performance between the stress and the control groups during the retrieval phase.

Keywords: Training, firefighters, stress, fNIRS, neuroergonomics

Précis: Brain dynamics between the motor and frontal regions can effectively differentiate between the neural strategies of learning with and without stress. Integrating brain imaging in VR-based adaptive training systems can make them more effective in identifying states of stress and learning.

KEY POINTS

- Compared to the control group, the stress group exhibited poorer training performance, while retrieval performance was comparable between groups.
- Stress inhibits cognitive processing by suppressing DLPFC activation.
- Functional connectivity within the DLPFC and between DLPFC and the pre-motor regions is strengthened when learning under stress.
- fNIRS can prove to be an effective component of a VR-based adaptive training system for firefighter training.

INTRODUCTION

In safety-critical domains like emergency response, physical and mental stress burden workers strenuously (Benedek et al., 2007; Lentz et al., 2019). Emergency responders are more frequently exposed to various types of psychological stressors as compared to the general population, where in some cases, frequent exposure to traumatic events can lead to the development of post-traumatic stress disorder and other comorbidities (Regehr & LeBlanc, 2017). In 2012, 66% of the firefighters surveyed from United States reported to have occupational injuries and 56% reported having multiple injuries (Hong et al.,

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2012). This illustrates that firefighters are often in hostile environments exposing them to heightened risk and potential consequences associated with team and victim safety. Therefore, immersive firefighter trainings are needed that prepare them to effectively respond and perform in extremely hostile environment. Traditional firefighter training environments, however, are limited in exposing trainees to hazardous situations in interest of their safety (St Julien & Shaw, 2003).

Virtual Reality (VR) based systems have received substantial attention to enhance firefighter training experience (Bliss et al., 1997; Clifford et al., 2019; Engelbrecht et al., 2019; Narciso et al., 2020). VR-based trainings that use immersive headsets are able to simulate a wide range of cost-effective emergency scenarios, are highly immersive as compared to monitor-based virtual simulation trainings, and are significantly safer than live fire and emergency based exercises (Engelbrecht et al., 2019). Additionally, VR-based trainings also provide a unique opportunity for close monitoring of the trainee, offer simulation playback for constructive feedback, and make highly personalized and adaptive training systems possible. Adaptive training can be defined as a training module where the difficulty of the problem, stimulus, or the nature of task can be modified based on task performance or other desired measures (Kelley, 1969). Adaptive trainings that use performance, physiological data, and subjective feedback to assess the trainee's state are more effective than non-adaptive trainings (Jones et al., 2016; Peretz et al., 2011; Zahabi & Razak, 2020).

One physiological measure that provides an assessment of cognitive states and learning over time is functional brain activity. Parasuraman (2003) proposed neuroergonomics, a sub-discipline of human factors, which examines the neural bases of performance at work. Ambulatory functional brain imaging techniques, such as functional Near-Infrared Spectroscopy (fNIRS) and Electroencephalography, have been used in naturalistic or laboratory environments to assess cognitive states such as fatigue, workload, and stress (Ayaz et al., 2012; Borghetti et al., 2017; Newman et al., 2003), in addition to monitoring the learning process (Basso Moro et al., 2013).

Brain imaging has a comparative advantage over other continuously monitored physiological signals such as heart rate, dermal activity, or muscle activity, due to its increased spatial resolution provided through multiple sensor locations and region-specific responsibilities of the brain. Cognitive states can be examined based on numerous features of the neural signals (Newman et al., 2003). While region-based activation is traditionally used to identify cognitive states, network analysis techniques such as functional connectivity analysis can provide additional information about the coupling of different brain regions (Rogers et al., 2007). Functional connectivity measures the temporal correlation between signals of different regions of interest and represents the functional integration between specialized brain regions that may be spatially segregated (Friston, 1994).

Adaptive neurofeedback-based training systems can monitor behavior-based brain activity to assess the cognitive states and workload of the trainee for effective state adaptation (Dey et al., 2019) and can capture the neural changes associated with learning that can guide VR-based trainings to adjust attributes of the training task in real time for optimal performance (Afergan et al., 2014). Similar studies in the past have used neurofeedback to train and improve different cognitive states in VR. For example, Cho et al. (2004) used neurofeedback to capture and improve attention and impulsiveness in VR. Despite these advantages, very little effort has been made to integrate neuroergonomics into adaptive VR-based emergency response trainings, where stress manipulation for both task learning and retention plays a critical role in allowing firefighters to be better prepared for real-world emergencies.

A neuroergonomics perspective allows for further understanding of the mechanistic brain behavior associated with movement and learning under certain environmental or cognitive conditions. Voluntary motor movements are organized and executed by the motor areas of the brain. While, the primary motor cortex (M1) is directly responsible for sending neural drive to the muscle, the premotor (PM) and supplementary motor areas (SMA) are responsible for motor planning and movement in response to sensory

information (Roland, 1984; Tanji, 1994). SMA and PM activation increases for more complex motor tasks (Gerloff et al., 1997). The functional connections between the prefrontal cortex (PFC) and the premotor areas can also indicate learning of motor skills and adaptation to stress. A study assessing the bimanual skills of surgeons under temporal pressure found that the signatures of neural adaptation to stress over years of training were characterized by more efficient functional organization between the PFC and the motor regions (Deligianni et al., 2020). Vice et al., 2011 used the same approach to investigate the fidelity requirements of a virtual training environment to real-world military tasks for future virtual training platforms for military trainings. Performance and neural data indicated that there was an effect of fidelity conditions on learning, where the virtual environment was associated with better performance and higher cognitive processing. The study also found that neurophysiological data was better at identifying response to stimulus than performance. Similar investigations into the ability of VR environment to simulate real-world environments relevant to firefighter training and the neural signatures of learning in these environments are also needed.

Stress plays a major role in emergency response training; thus, it is important to understand how stress impacts the learning process in order to ensure effective performance. Stress is associated with decreased activity in the PFC but strengthened connectivity between the PFC and motor areas for motor tasks (Deligianni et al., 2020; Qin et al., 2009). Studies on learning in classroom settings have found that successful memory retrieval under a stressed state is more probable if encoding of information also occurs in a similar stressed state (Joëls et al., 2006; Vogel & Schwabe, 2016). This effect is due to the nature of memory consolidation under stress, where any information unrelated to the task at hand is suppressed and memory formation of the task related details of the event are strengthened. Similarly, Hordacre et al. (2016) found motor learning is also facilitated under stress. Therefore, it is important to simulate stress during firefighter training, not only to prepare them for emergency situations but to also ensure that memory retrieval is possible in such situations. Therefore, any

neuroergonomics-based adaptable system needs to be efficient at recognizing the states of stress and how the process of learning is affected by it. Understanding how stress affects the process of learning will provide insight into when and what is needed in training regimens for better retention and performance in the field.

The present study aimed to model the neural dynamics associated with learning and retrieval under stress in a VR-based emergency response training exercise. The VR environment was manipulated to induce stress during the visuospatial sequence learning phase and participant firefighters' ability to retrieve the learned sequence was assessed under stress and in no-stress conditions. Along with performance during the training and retrieval exercises, functional hemodynamic responses from the stress-motor circuitry brain regions (i.e., dorsolateral PFC, SMA, and PM) were monitored using fNIRS. We hypothesized that learning under stress would be detrimental to memory retrieval in non-stressful condition and facilitative to retrieval in stressful condition. Based on results reported in Amsten (2009); Saleh et al. (2021), we also hypothesized that activity in the PFC would decrease, but functional connectivity between the PFC and the premotor areas would be strengthened under stress. Additionally, we aimed to identify fNIRS-based neural signatures of learning under stress. We hypothesized that neural activation and functional connectivity of the prefrontal and premotor regions of the brain would change as the firefighters learned the sequence and it would be different for learning with and without stress. The results from this study may facilitate development of personalized algorithms to accelerate and strengthen emergency response training in VR environments.

METHODS

Experiment Variables

In order to test the hypotheses mentioned above, we designed a study to capture sequence learning and associated hemodynamic activity of the brain in the VR environment. The independent and dependent measures are outlined below and further described in the remainder of the methods section.

Independent Measures

To study the effect of stress on learning, a between-subject design was employed, where participants were randomly assigned to a stress group and a control group. The stress group learned a sequence in a stressful VR environment, whereas the control group learned the sequence in a non-stressful environment. We were also interested to see the changes in the dependent measures based on different states of learning. The two analyzed phases of learning were the training phase, where the participants memorized and practiced the sequence with feedback, and the retrieval phase, where the participants had to retrieve the sequence from memory without any feedback or help. Finally, to study whether retrieval is facilitated if it occurs in the state of stress, both the stress and control groups retrieved the sequence from memory in stressful and non-stressful environment.

Dependent Measures

Task performance was measured during the training phase, and during the retrieval phase in the stressful and the non-stressful environments. The State-Trait Anxiety Inventory (STAI) scores were reported by firefighters after relaxing for 3 minutes, after being familiarized with the valve sequence, and after completing the training phase. Activation of the prefrontal and pre-motor areas of the brain were calculated for training and retrieval phases. Finally, the temporal correlation between the brain regions was calculated for training and retrieval phases.

Participants

Forty firefighters were recruited from the local firefighting community in Bryan, TX, to participate in the study. Of the recruited firefighters, four participants were not able to complete the study due to VR sickness, and two participants performed a different study protocol that was not used for further analysis. The participants were randomly assigned to either a control group or a stress group. All participants were healthy males of at least 18 years of age who spoke English fluently. We were unable to recruit women

participants due to a lack of diversity in the local firefighter community; women make up a very small percentage of the firefighting community (Hulett et al., 2008). Table 1 provides demographics of the included participant pool. This research complied with the American Psychological Association Code of Ethics and was approved by the Institutional Review Board at Texas A&M University (IRB2019-0943DCR). All participants provided their informed consent in writing before data collection.

Experiment Task

The experimental task employed in this study was a pipe maintenance task performed in a VR environment set in a chemical power plant that holds hazardous and combustible elements. The task chosen was to simulate a shutdown maintenance scenario, where firefighters need to perform a shutdown procedure to close valves in a particular order to cut the supply of hazardous materials during a fire (Shi et al., 2020). The purpose of the task was to simulate a real-world scenario where firefighters are required to learn a shutdown sequence of valve closure in situations like safety-critical gas leaks, fires, or explosions in manufacturing or power plants. The task was based on the instruction manual of Alfa Laval plate heat exchangers to simulate a real-world scenario (AlfaLaval, 2016). The task was aimed to assess the firefighters' spatial sequence learning capabilities. The participants were to turn the valves in an 8-step sequence, given in Figure 1, using a hand-held Controller. The tasks were either visually guided or unguided. In the visually guided trials, arrows highlighting the correct valve appeared sequentially as the participants selected each valve. In the unguided trials, the participants had to turn the valves sequentially without any aid.

VR-Based Stressors

The stressful environment was simulated via sudden explosions, alarm sounds, and smoke as these cues induce stress and represent a naturalistic environment for a firefighter on the job (Hall et al., 2016; Proulx, 1993). The second and third trials in the familiarization phase, and the

Table 1. Demographics of Participants. All Values are Given as Mean ± SD.

	Control (n = 17)	Stress (n = 17)
Age (years)	31.71 ± 4.01	29.76 ± 4.25
Service (years)	7.29 ± 4.1	6.51 ± 3.95
Height (m)	1.83 ± 0.08	1.78 ± 0.06
Weight (Kg)	98.51 ± 14.91	94.83 ± 20.4
BMI (Kg/m ²)	29.2 ± 3.11	29.66 ± 5.09
Sex	17 men, 0 women	17 men, 0 women

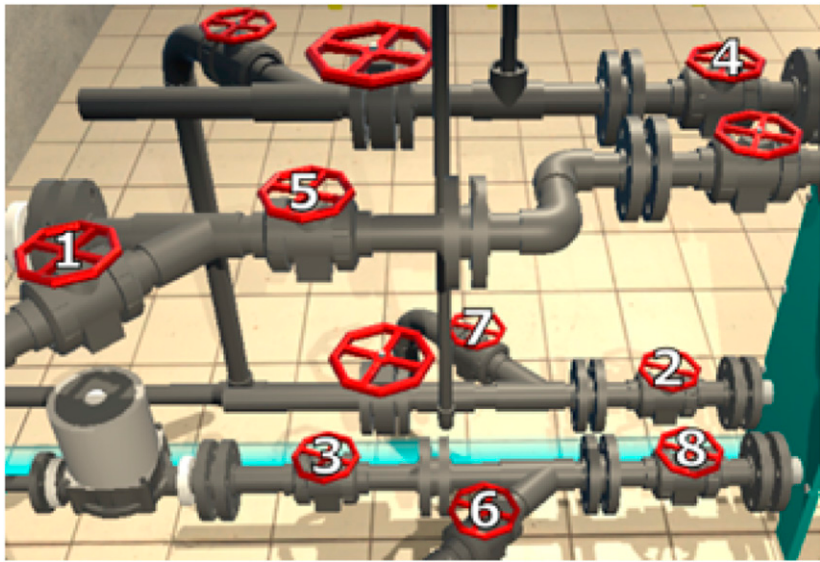


Figure 1. The valve sequence for the pipe maintenance task.

second, third, fifth, sixth, and seventh trials in training phase included the stressors to keep their occurrence unpredictable to prevent the participant from becoming accustomed to the stressor.

Procedure

Following informed consent, participants filled a background questionnaire about their previous experience with virtual reality and completed the State-Trait Anxiety Inventory (STAI) (Spielberger, 2010). STAI has two scales, form Y1, which measures the “state” of anxiety of a person in a particular situation, and form Y2, which measures their “trait” or disposition for anxiety. Participants were then

familiarized with subjective assessment scales of the NASA Task Load Index (NASA-TLX; Hart, 2006). The results of STAI scores are reported here; however, detailed analysis of NASA TLX is discussed elsewhere. Participants were then instrumented with the fNIRS head-cap (NIRSport 2, NIRx Medical Technologies, New York, NY, USA) and the virtual reality headset (HTC Vive Head Mounted Display, USA). The signal quality of the fNIRS probes were tested before beginning the experiment using the fNIRS acquisition software (Aurora fNIRS, NIRx Medical Technologies, New York, NY, USA) and monitored throughout the study.

Figure 2 illustrates the timeline of the experiment, which lasted for approximately 45 minutes. *Baseline:* Participants sat quietly with their

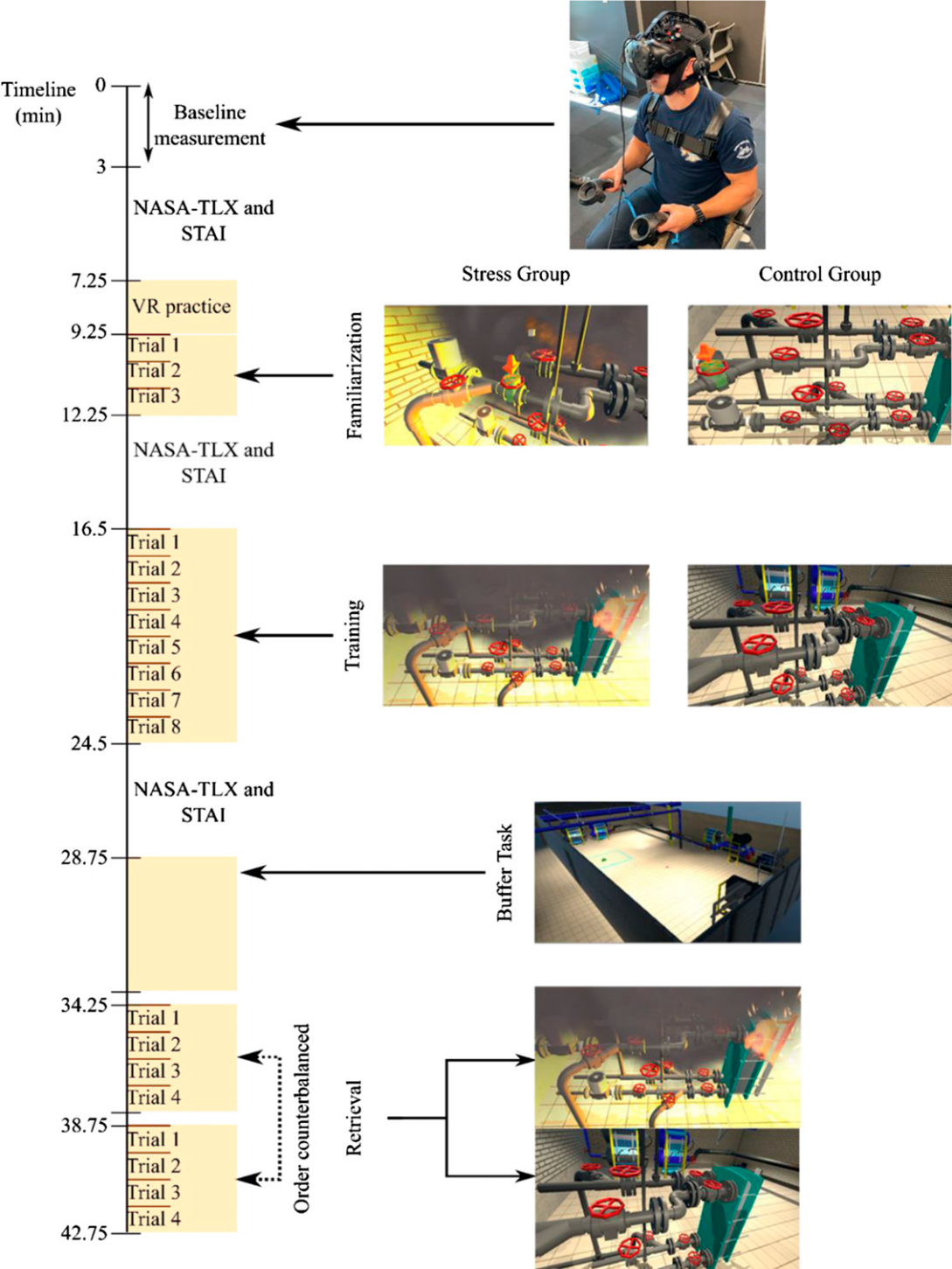


Figure 2. Experiment protocol for the study. Note that the timeline is to scale and the sections in yellow signify the participants being in the VR environment. Participants were randomly assigned to the control and stress groups. The tasks performed in the familiarization phase were guided and the tasks in the training and retrieval phase were

unguided. If a participant made an error in one of the training trials, the next trial was guided. The stress group performed two of the familiarization trials and five of the training trials under stress in random order.

eyes closed for 3 minutes for measurement of their baseline brain activity. *VR practice*: Participants entered the VR environment with the shutdown maintenance scenario and were demonstrated how to turn valves in a sequence based on visual cues. The purpose of this phase was to acquaint the participants with the hand-held controller to close valves. *Familiarization*: Participants performed three visually guided trials of the task, where the sequence of the valves was the one given in [Figure 1](#). The start times for consecutive trials were 60 s apart. *Training*: Participants completed eight trials. Each trial required participants to complete the same pipe maintenance task as fast and accurately as they could without visual guidance. In the event that a participant made a mistake (i.e., incorrectly played out the sequence) in any training trial, the trial was terminated, and the subsequent trial presented was visually guided. The start times for consecutive trials were 60 s apart. After training trials, participants were again asked the subjective assessment questionnaires. *Buffer*: During the buffer task, participants were in a VR environment for 5 minutes and were instructed to move around freely without any specific goal. The aim of the buffer task was to provide a break between the training and retrieval phases to provide adequate time for memory consolidation ([Nielson & Powless, 2007](#); [Tse et al., 2007](#)). *Retrieval*: Participants were asked to perform the pipe maintenance task without visual guidance as quickly and as accurately as they could, and they were not given any feedback on their performance. Participants underwent two sets of four retrieval trials, where one set was in the stressful environment and the other in no-stress environment. The order of the stress and no-stress retrieval trials was counterbalanced between individuals.

Task Performance and STAI

The performance score was calculated as the number of correct valves turned in sequence before a wrong valve was selected for the unguided

trials of the training phase and all trials for the retrieval phase. Speed was not considered since the trials where participants made an error were terminated immediately. The medians of the performance scores were calculated for training early (first four training trials), training late (last four training trials), stress retrieval and no-stress retrieval phases. The STAI scores were calculated for the trait anxiety scores collected in the beginning of the experiment, and state anxiety scores collected after baseline and training period. The percent change in STAI state scores from baseline to training were calculated for each participant.

Neural Activity

Functional Near-Infrared Spectroscopy (fNIRS) was employed to measure brain activations and connectivity strengths. fNIRS is a brain imaging technique that measures hemoglobin dynamics to determine cortical activations through a variety of different features. The cortical locations are defined following the 10–20 international systems using a sixteen-probe design with 21 measurable channels that covered the regions of the brain involved in motor learning, such as the supplementary motor area, and premotor area ([Halsband & Lange, 2006](#)) and working memory function, such as the dorsolateral prefrontal cortex ([Figure 3](#); [Levy & Goldman-Rakic, 2000](#)). The 21 channels were allocated into the six regions of interest (ROIs) according to their Brodmann locations and functionalities, three in Brodmann area 9: Dorsolateral PFC (Left/Mid/Right DLPFC), and three in area 6/8: the Premotor (Left/Right PM) and Supplementary Motor Area (SMA) ([Brodmann Area Function Atlas, 2010, January](#); [Feng, 2020, October 29](#)).

Signal processing was completed using algorithms built into MATLAB based processing software ([Homer2, 2021](#)). The first corrective algorithm was for motion artifacts ([Figure 3](#)). Each data channel was reviewed for sharp fluctuations within a time range of 0.5 s using a SD threshold of 50 and an amplitude threshold of five optical units. Any segment that met the criteria

was marked as a motion artifact and removed along with 0.75 s before and after the segment (Molavi & Dumont, 2012). Each participant had an average of 14.5 flagged segments. While trials were not removed based on the number of artifacts, specific channels were removed based on recommendations from Homer2. No participant had more than four bad channels, and each ROI had at least two channels averaged together for all participants. The flagged and removed segments were then interpolated using a spline function (Jahani et al., 2018). Following, a bandpass filter was applied that kept the data's frequencies between 0.010 Hz and 0.50 Hz (Zhu et al., 2020). The next major correction was to convert the optical density to concentrations to measure oxygenated, deoxygenated, and total hemoglobin measures. Of these, oxygenated hemoglobin measure was chosen for further analysis since it is the most sensitive to changes in cerebral blood flow and motor related tasks (Hoshi et al., 2001). The partial pathlength factors for each wavelength were kept at six as the absorption change was assumed to be uniform over the tissue in the prefrontal cortex.

Following signal processing, the baseline measurement was calculated by averaging oxygenated hemoglobin (HbO) over the last 2 minutes of the baselining period. Every trial within each of the phases (familiarization, training, buffer, and retrieval phases) was optimized to find the peak activation for each channel individually. Once the peak was identified, the 2 s segment around the peak was averaged followed by subtraction of the baseline value to obtain peak ΔHbO (Zhu et al., 2020). The resulting values were then averaged by region of interest. All phases with repeated measurements were averaged together resulting in six ΔHbO values (one for each region) per participant for each phase. Specifically, within the training phase, some of the trials were visually guided and were thus not included in the analysis.

Figure 3 illustrates the analysis of fNIRS signals performed to obtain functional connectivity maps (steps 3–6) to identify the magnitude of the coupling of regions that may be functionally working together. Within a phase, each trial was segmented into active sections, where the firefighters performed the pipe maintenance task, removing any downtime between trials

(Step 3). These segments were concatenated and detrended using a first order linear model for each channel (Step 4). Channels within the same region of interest were then averaged together (Step 5). Pearson correlations were calculated by comparing the resulting curves between two regions of interest effectively over the entire active phase (Step 6). Pearson correlations were transformed into a Fisher z-score, and any scores below a threshold of 0.4 were reduced to zero to reduce the likelihood of a false positive connection (Rhee & Mehta, 2018).

Statistical Analysis

To test the influence of group (control vs. stress) and training phase (training early vs. training late) on performance (calculated as the number of correct valves turned in sequence before a wrong valve was selected), ordinal logistic regression was performed with performance as the dependent variable, and group, phase, and their interaction as predictor variables. Similarly, ordinal logistic regression was also performed with the performance of retrieval phase as the dependent variable and with group (control vs. stress), condition (stress retrieval vs. no-stress retrieval), and their interaction as predictor variables. In both analyses, the coefficients of the regression models were tested against the null hypothesis at significance level $p < .05$. Additionally, to test if both stress and control group went through a similar number of training trials, the Mann–Whitney U test at significance level $p < .05$ was performed on the number of visually guided trials performed in the training phase. To evaluate group differences in anxiety scores, independent t -tests were performed on the trait scores and on the percent change from baseline in state scores during the training period at significance level $p < .05$.

The Anderson–Darling test with threshold $\alpha = 0.05$ was performed on the peak activation of each ROI to check the goodness of fit of the data to assume normality. Once normality was confirmed, separate phase (training early vs. training late) $\times$ group (stress vs. control) and condition (stress retrieval vs. no-stress retrieval) $\times$ group (stress vs. control) ANOVAs were performed on peak ΔHbO from each ROI. To capture significant

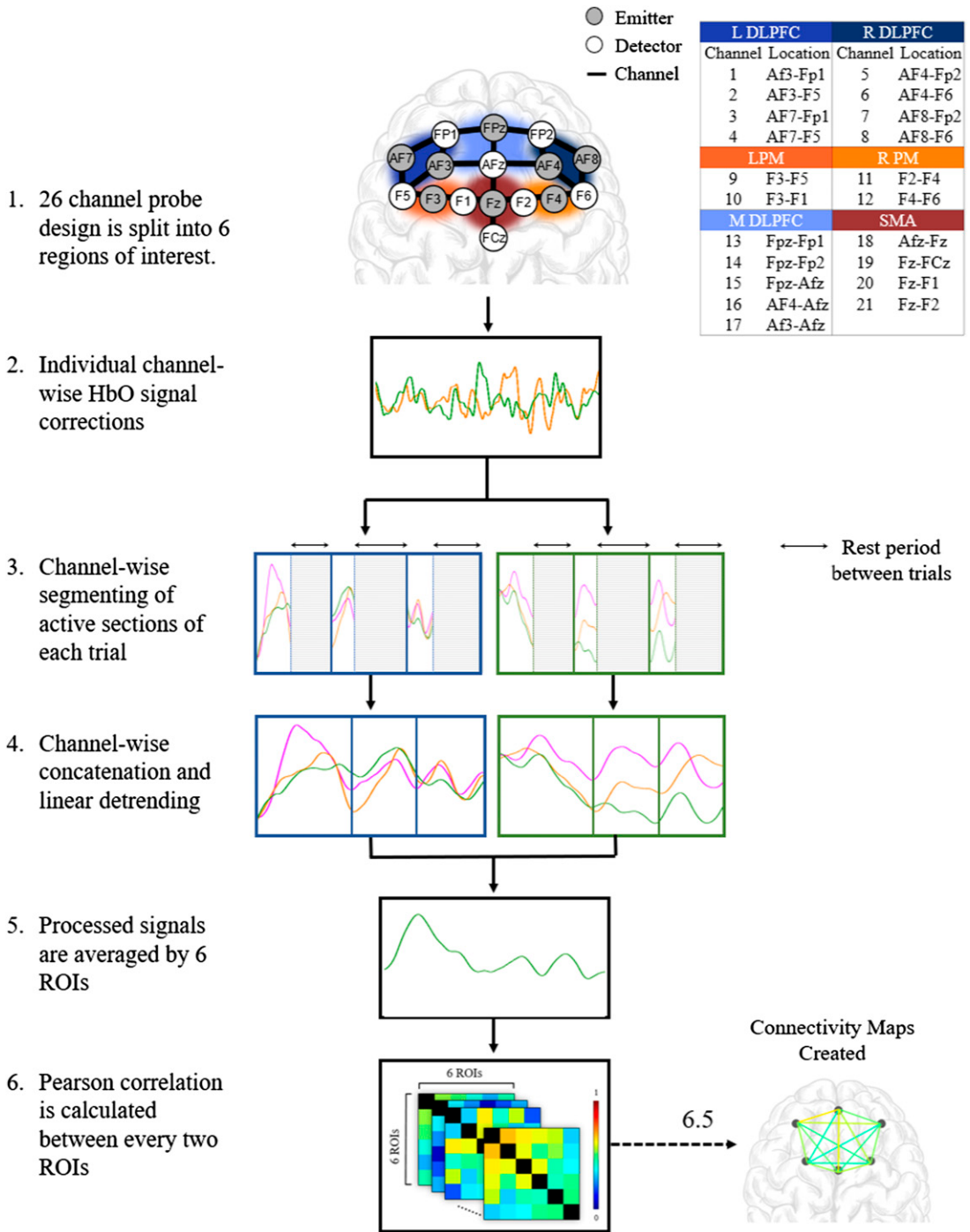


Figure 3. Probe design and overview of fNIRS processing and analysis approach. The fNIRS channels were split into six regions of interest (ROIs) and signal corrections were applied. The peak Δ HbO values were obtained for each channel then averaged to an ROI, where trials were averaged over phases. The corrected signal was further analyzed via steps 3–6 to obtain functional connectivity maps.

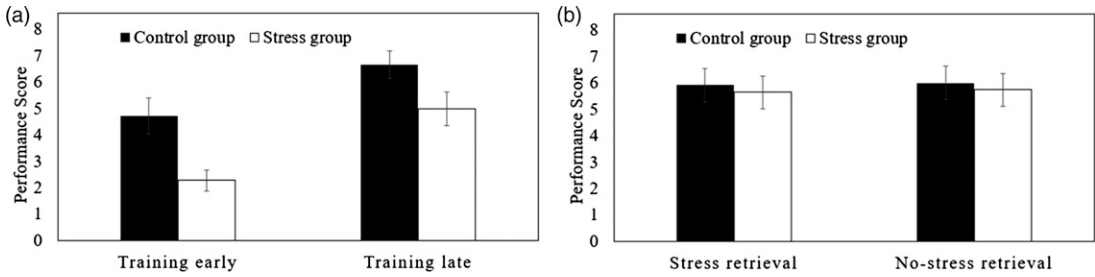


Figure 4. The median performance scores in the control and stress groups for the (a) training and (b) retrieval phases. Error bars represent SE.

connectivity differences between the groups, independent *t*-tests were performed on the Fisher *z*-scores of ROIs between the stress and control groups for the training, stress retrieval, and no-stress retrieval phases. The level of significance for all analyses was set at $p < .05$. Where required, post hoc comparisons were performed using simple effects tests with a predetermined alpha of 0.05. All analyses were conducted using SPSS 26 (IBM SPSS Statistics, NY, USA).

RESULTS

Performance and Anxiety Scores

Training. The median performance scores for training early and training late phases are illustrated in Figure 4a. Group had a significant relationship with performance (coefficient = 2.268 ± 0.742 , $p = .002$), where performance of the stress group was lower than that of the control group. Phase also significantly predicted performance (coefficient = 1.493 ± 0.700 , $p = .033$), where performance was significantly higher in training late phase as compared to training early phase. There was no significant relationship of the phase $\times$ group interaction with performance ($p = .278$). On average, the stress group underwent significantly ($p = .005$) greater number of visually guided trials (3.19 ± 0.81) than the control group (2.00 ± 1.37).

There was no statistical difference between the trait anxiety scores of the control and stress groups recorded before the experiment ($p = .884$). The state anxiety scores for the control group increased by $8.4\% \pm 24.9\%$ from the baseline to training period and increased by $16.9\% \pm 26.9\%$ for the stress group. The independent sample *t*-tests

revealed no significant difference between the scores of the control and stress groups ($p = .383$).

Retrieval. The median performance scores for stress and no-stress retrieval are illustrated in Figure 4b. There were no significant relationships between any of the predictor variables with performance (all $p > .886$).

Brain Activation

Training. Table 2 lists all significant main and interaction effects of phase and group across each ROI. The stress group exhibited lower brain activation than the control group in the MDLPFC, RDLPFC, and LDLPFC, illustrated in Figure 5 (top panel). Activation in the LPM and the RPM increased from the early to late training phase. Finally, a phase $\times$ group interaction effect was also observed in the SMA. Post hoc analysis revealed that activation in the SMA increased from the early to the late training phase for the stress group, but SMA activation remained comparable over time in the control group (Figure 5, bottom panel).

Retrieval. The stress group exhibited lower brain activation than the control group in the MDLPFC, LDLPFC and the RDLPFC, as shown in Figure 5 (top panel). There was a main effect of condition, where activation in the MDLPFC was higher during stress retrieval than no-stress retrieval. The effect of group $\times$ condition interaction was not significant for any other ROIs (all p 's $> .503$).

Functional Connectivity

Training. Figure 6 illustrates the functional connectivity magnitudes between different brain

Table 2. *F*-statistics, *p*-values, and Effect Sizes (η_p^2) for Significant Main and Interaction Effects for ANOVAs in the Training and Retrieval.

			MDLPFC	LDLPFC	RDLPFC	SMA	LPM	RPM
Training	Group	<i>F</i> (1,31)	6.827	6.923	6.643			
		<i>p</i> -value	.014	.013	.015			
		η_p^2	0.18	0.183	0.18			
	Phase	<i>F</i> (1,31)					6.643	7.466
		<i>p</i> -value					.015	.01
		η_p^2					0.18	0.194
Retrieval	Group	<i>F</i> (1,31)	7.236	9.499	4.861			
		<i>p</i> -value	.011	.004	.035			
		η_p^2	0.189	0.235	0.136			
	Condition	<i>F</i> (1,31)				4.899		
		<i>p</i> -value				.034		
		η_p^2				0.136		
	Group × Phase	<i>F</i> (1,31)						
		<i>p</i> -value						
		η_p^2						

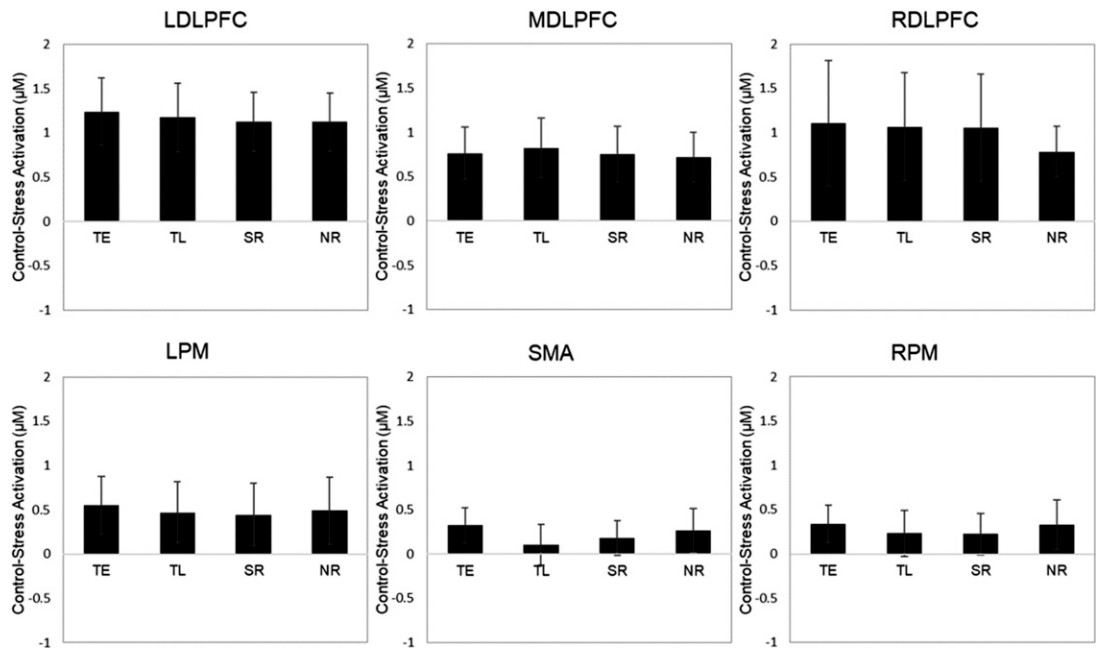


Figure 5. Difference in activation of control group and stress group for the six ROIs for training early (TE), training late (TL), stress retrieval (SR), and no-stress retrieval (NR) across the PFC and motor regions. Error bars represent SE.

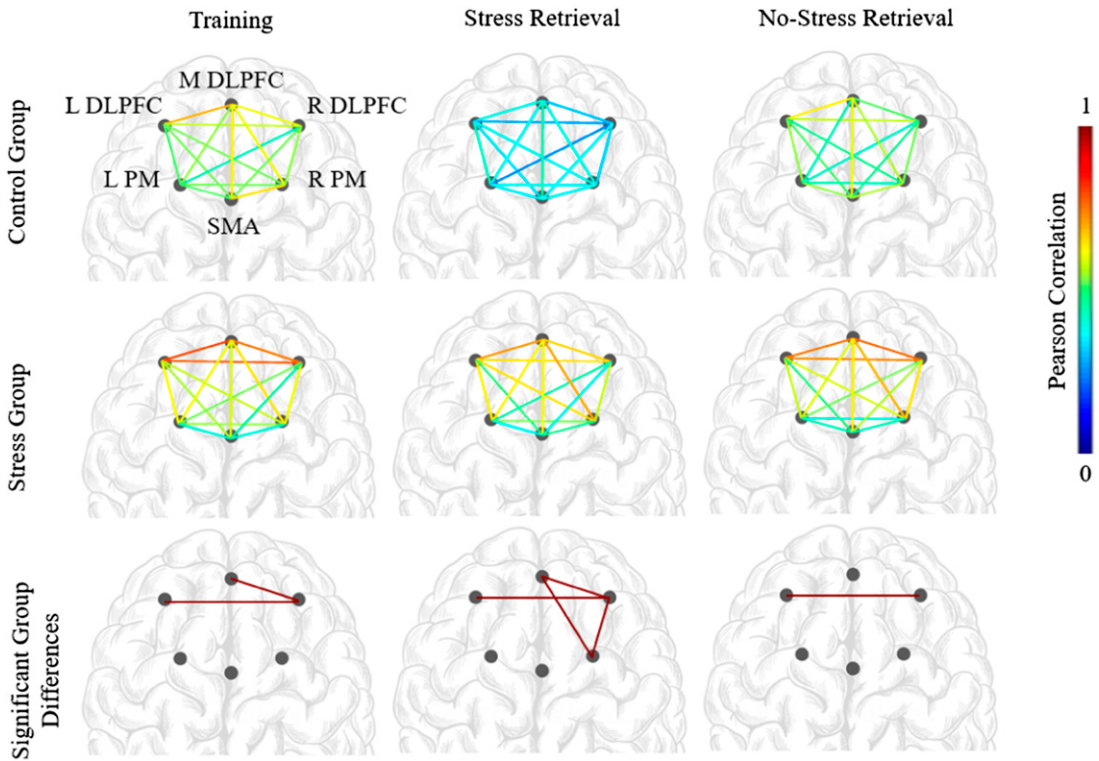


Figure 6. Functional connectivity maps of the control (top row) and stress (middle row) group during training, stress retrieval, and no-stress retrieval phases, averaged by participant. No negatively correlated connections were found when averaged across group, thus connectivity is plotted from no correlation to highly positively correlated based on the color bar map indicated in the figure. Significant increases in connectivity in the stress group, compared to the control group, are highlighted in the bottom panel.

regions. The stress group exhibited significantly stronger functional connectivity than the control group between LDLPFC-RDLPFC ($p = .0136$), and MDLPFC-RDLPFC ($p = .0378$). No other connectivity patterns were found significant between groups (all p 's $> .1467$).

Retrieval. The stress group exhibited significantly greater strength in functional connectivity than the control group between MDLPFC-RDLPFC ($p = .0195$), and a marginally greater strength in functional connectivity between LDLPFC-RDLPFC ($p = .0596$), MDLPFC-RPM ($p = .0554$), and RDLPFC-RPM ($p = .0576$) during the stress retrieval phase. Apart from a significant group difference in connectivity between MDLPFC-RDLPFC ($p = .0002$), and marginal group difference in connectivity between LDLPFC-RDLPFC ($p = .0537$) where the stress

group exhibited stronger connectivity in no stress retrieval, no other connectivity patterns were found significant between groups during the no-stress retrieval phase (all p 's $> .1927$).

DISCUSSION

This study investigated the neural activity associated with sequence learning and memory retrieval under stress for VR-based firefighter training. Important findings of this investigation can be summarized as follows: (1) while the rate of learning was different between the stress group and control group, retrieval performance was similar between groups; (2) the stress group performed a higher number of visually guided trials as compared to the control group, and (3) stress-adaptive neural strategies, such as increased

activation in the SMA and stronger functional connectivity within the frontal region and between the frontal and pre-motor regions may contribute to the comparable retrieval performance between groups.

Performance scores of both the stress group and control group improved significantly from the early to late training phases. The experimental task entailed participants to close valves, distributed spatially, in a specific 8-sequence order, requiring them to incorporate both motor and cognitive resources to perform the task. As such, the performance improvements were accompanied by increases in neural activation in the pre-motor areas for both groups and activation in the SMA for the stress group. Indeed, the pre-motor and supplementary motor areas are involved in motor planning and execution (Picard & Strick, 2003; Tanji, 1994). These areas are also recruited for working memory maintenance during cognitive tasks (Marvel et al., 2019) and to account for increases in cognitive demands (Küper et al., 2016).

During the early stage of training, the stress group's performance scores were poorer than those of the control group. It is likely that early performance by the stress group was poorer than that by the control group due to the distracting nature of the types of stressors employed, such as explosions, fire, smoke, and alarms that burden auditory and visual cognitive resources. The stress group experienced suppressed activity in the left and medial DLPFC in comparison to the control group. Previous studies have also reported suppression of working memory-related activation in the DLPFC in the presence of stress (Mehta, 2016; Qin et al., 2009; Shortz et al., 2015). Qin et al. (2009) attributed this phenomenon to the sensitivity of the PFC to the neurochemical changes caused by the increased sympathetic activity and decreased parasympathetic activity under stress. These neural findings also provide support that the VR-based environmental stressors were effective in manipulating neurophysiological responses in the stress group.

In the retrieval phase, the stress group's performance was comparable to the control group. One possibility to explain this outcome is to compare the differences in the nature of learning that the control and stress group experienced

during the training phase. Because the stress group made more mistakes, they received more visually guided training trials than the control group. This means that, while control group learned the sequence primarily by repetition, the stress group learned the sequence via visual guidance. However, while visual guidance has shown to reduce cognitive load during training and facilitate translation of cognition to action (Carroll & Bandura, 1987), it has also shown to be less effective for learning (Yuviler-Gavish et al., 2011). Thus, the differential learning experiences (repetition vs. visual guidance) do not completely explain how both groups exhibited comparable retrieval performances despite different learning rates during the training phase.

Brain dynamics during the retrieval phase may potentially explain comparable performances between the control and stress groups. Despite the stress group having suppressed activation in the DLPFC regions during training, they exhibited stronger connectivity within the DLPFC during training and retrieval as well as between the DLPFC and the pre-motor areas during retrieval, when compared to the control group. While neural activation indicates increase or decrease in activity in a region of the brain, functional connectivity is an indicator of functional integrity between brain regions and tells us if the brain regions work together (Friston, 1994). These findings implicate a stress-adaptive neural strategy, in that while activation within the DLPFC region was lower under stress, the different DLPFC subregions developed stronger positive connections. Kohn et al. (2017) found that under stress, better performance was associated with stronger connectivity within the Executive Control Network (ECN), which includes the DLPFC and pre-motor regions. The reasoning given behind this phenomenon was that while stress is related to lower executive functioning to facilitate reduction in processing of irrelevant information, accuracy of task is achieved by an increased recruitment of a widespread network of brain regions associated with the ECN. The ECN is responsible for planning, decision making, and error detection and works with other attentional networks to facilitate perceptual processing (Callejas et al., 2005). Therefore, an increase in functional connectivity

between the DLPFC and the pre-motor regions, observed during the retrieval phase in the stress group, could be an indication of a strategy to improve ECN performance hindered by suppression of the PFC by expanding the recruitment to the pre-motor regions by the PFC.

In contrast to our hypothesis, there was no statistical difference between performances in either retrieval phases (stress or no stress) across both groups (Callejas et al., 2005). This can be explained by the fact that since the stress group exhibited poorer performance in the beginning of the training trials, they were provided with a greater number of opportunities to learn the sequence via the visually guided trials (Carroll & Bandura, 1987). Additionally, the buffer period provided to both groups may have allowed for memory consolidation that facilitated learning (Tse et al., 2007). As discussed before, the stress group exhibited strengthened functional connectivity as compared to the control group between the prefrontal regions and the pre-motor regions during stress retrieval. This strengthened functional connectivity within the DLPFC was also seen in the no-stress retrieval phases in the stress group. The altered functional connectivity during retrieval phase indicates the difference in neural strategies for memory retrieval between individuals who learned under stress and those who learned under no-stress conditions. Therefore, these activation and functional connectivity patterns can act as markers of learning under stress for adaptive training systems for firefighters. Identification of the neural strategies of learning can determine how well simulated stress was manipulated during emergency trainings. Future work is also needed to identify which of these different neural strategies of learning will prove to be more effective in memory retrieval in field conditions.

As with many studies, the limitations need to be addressed. This study was limited to men, who may adopt different learning strategies under stress than women. Future work that compares the neural process of learning under stress between sexes is warranted. Within the fNIRS design, only the frontal and premotor regions of the brain were monitored. Future studies should expand by monitoring visuomotor regions to systematically understand the effect of stress on

attention and to capture the neural strategies involved in regulating selective attention in response to stress. This study evaluated firefighter performance for visuospatial sequence learning under stress and provided reinforcement via visually guided trials when a mistake was made. However, due to initial poor performance of the stress group, both groups had different learning experiences. While the neural data may partially help explain the comparable retrieval performances between the two groups, future work should use a more standardized protocol that ensures consistent learning experiences for both groups. The results of the analyses of activation and functional connectivity were not corrected for multiple comparisons in this study. This is because the power of the statistical analysis was small to yield significant results after Bonferroni or FDR corrections. Limitations to the power of analysis arise from the fact that there were multiple regions monitored (6 regions and therefore, 15 connections). We hope that the results of this study contribute to localizing the areas of interest for functional connectivity analysis, so that future studies can use fewer regions of interest and minimize errors due to multiple comparisons.

The anxiety scores also were not statistically significantly different between conditions, perhaps due very high variability in self-reported scores. Future studies should recruit a larger participant pool to increase the power of their analyses, but could also include a measure of participants' cortisol levels before and after stress is applied to determine successful stress induction. It should be noted that there were significant differences between performance scores of the stress and control groups, where the stress group performed poorly in the early stages of the training phase, and as a result, underwent more guided trials than the control group. The stress group also exhibited stunted PFC activity, which is a common indicator of stress (Arnsten, 2009; Mehta, 2016). However, the STAI, which is a common subjective measure for stress and anxiety, was found comparable between the stress and control group. Previous evaluations of this questionnaire have indicated that the STAI scores can produce inconsistent results and misfit responses (Tenenbaum et al., 1985). The present study found that the STAI scores did not

differentiate between the states of stress produced by the different VR environments, which were otherwise captured by both performance and brain hemodynamic data. The stressors that were used to simulate an emergency were provided to the firefighters continuously and consistently during a trial. More sudden and randomly distributed stressors, such as sudden explosion sounds and structural collapse, can provide a more realistic setting for emergency training and stronger stress responses. Finally, brain dynamics differ under virtual versus physical realities (Vice et al., 2011), and as such comparative studies that examine the commonalities and deviation of learning under stress in a range of virtual, to mixed, to physical realities will be beneficial.

CONCLUSION

In this study, we identified the differences in neural strategies, activation and connectivity patterns, involved in learning under stress versus without stress. Firefighters who experienced the stressors during learning compensated with PFC suppression under stress and with strengthened functional connectivity between the PFC and pre-motor regions. These strategies can serve as potential learning markers during training to alter or enhance skill acquisition in VR training systems, which have typically relied on downstream performance outcomes that are lagging indicators of learning. Integrating brain dynamics using ambulatory neuroergonomic techniques, such as fNIRS, within VR-based trainings can facilitate expertise development with adaptive models that account for both trainee states of stress and associated learning and consolidation strategies.

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