1 **Biological Factors and Self-Perception of Stress Predict Human Freeze-Like**

2 **Responses in the Context of Self-Defence Training and Personal Experience**

3 **with Violence**

4 Peter Lenart,[1]*, Michal Vít[2]*, Klára Marečková[3], Jan Novák[2], Filip Zlámal[1], Michal Mikl[3], Zdenko

5 Reguli[2], Martin Bugala[2], Jitka Čihounková[2], Pavel Přecechtěl[2], Vojtěch Malčík[2], Tomáš

6 Vojtíšek[5,6], Jan Kučera[1], Jana Fialová Kučerová[4], Veronika Hajnová[1,7], Marie Tomandlová[8],

7 Radek Šíp[1,2,9], Lucie Ráčková[1], Markéta Grulichová [1,4], Josef Tomandl[8], Milan Brázdil[3], Julie

8 Bienertova-Vasku[1,4]

9 [1] Research Center for Toxic Compounds in the Environment (RECETOX), Faculty of Science,

10 Masaryk University, Kamenice 5, 62500 Brno, Czech Republic

11 [2] Faculty of Sports Studies, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic

12 [3] Central European Institute of Technology (CEITEC), Masaryk University, Kamenice 5, 62500

13 Brno, Czech Republic

14 [4] Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Kamenice

15 5, 62500 Brno, Czech Republic

16 [5] Department of Forensic Medicine, St. Anne's University Hospital, Tvrdého 562/2a, 602 00

17 Brno, Czech Republic

18 [6] Department of Forensic Medicine, Faculty of Medicine, Masaryk University, Tvrdého 562/2a,

19 602 00 Brno, Czech Republic

20 [7]Department of Mathematics and Statistics, Faculty of Science, Masaryk University, Kotlářská

21 2, 61137 Brno, Masaryk University

22 [8]Department of Biochemistry, Faculty of Medicine, Masaryk University, Kamenice 5, 62500

23 Brno, Czech Republic

24   [9] Department of Psychology, Faculty of Arts, Masaryk University, Arna Nováka 1, 60200, Brno,

25   Czech Republic

26   * authors contributed equally

27

Abstract

29   Many animals react to threatening stimuli such as a predator attacks by freezing. However,

30   little experimental research investigated freeze response in humans. Here, we have employed

31   practices commonly used in self-defence training to create two unique scenarios simulating

32   armed physical attacks. Sixty healthy men volunteers divided into three groups of twenty

33   (untrained, trained but unexperienced, trained and experienced) underwent these scenarios

34   accompanied by measurement of biochemical, physiological, and psychological markers of

35   stress. All participants also underwent an fMRI session during which they observed neutral

36   and negative images from the International Affective Picture System (IAPS). Our results show

37   that scenarios simulating physical attacks can induce a freeze-like response in men. In

38   addition, we demonstrate that while electrodermal activity (EDA), subjective stress

39   perception, and brain activity in fMRI predict freeze-like response in men, their effect on

40   freeze-like response is entirely dependent on the level of training and experience of a given

41   individual.

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Introduction

It is well established that animals react to danger by employing one of three main strategies: fight, flight, or freeze.[1–3] While processes aroused by the feeling of danger, fear, or anxiety are more complex in humans, they lead to very similar reactions.[4] From the human perspective, such reactions are relevant for a wide range of real-life situations and essential for various professions such as police officers, firefighters, etc., where dangerous high-stress situations are common. Of the three reactions to danger, freeze – sometimes conflated with tonic immobility[5] – stands out, since it is harmful in almost any imaginable situation a modern-day person might encounter. If, for example, a police officer is attacked by an armed criminal, or if the pilots of a commercial plane realize that the engines have stopped working and only have a limited time to react until the plane crashes, freezing is the worst possible reaction. Thus, in professional and personal self-defence training, it is especially important to reinforce the ability to switch between distinct defensive behaviors, such as freezing and active fight-or-flight responses.[6] However, while researchers have studied freeze response in animals for decades, studies of freeze response in humans are scarce. No published study to date has explored the human freeze response in reaction to a convincing simulation of a physical attack.[7,8]

Thus far, studies of humans have reported freeze-like behavior, assessed by postural control, skin conductance, and heart rate, in response to images of mutilation[9,10], images of angry faces[11], unpleasant images provided by the International Affective Picture System[12] or footage showing the aftermath of fatal car accidents.[13] Moreover, it was shown that humans might experience a freeze-like response operationalized as a feeling of immobility after breathing CO_2-enriched air.[5] Most interestingly, it was also demonstrated that the approaching motion

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69 of threatening stimuli (images of spiders or snakes) could induce a freeze-like response,

70 operationalized as a slowing of reaction times.[14] This observation seems to be more closely

71 connected to the origins of the concept of freeze than viewing images of angry faces.

72 Furthermore, an individual's vulnerability to freezing after being subject to such threatening

73 stimuli depends on their cognitive vulnerability to anxiety.[15] Studies where participants

74 observed images of an attacker aiming a firearm at them were presumably closest to real-life

75 conditions of interpersonal violence.[6,16] However, these simulations were still far from

76 convincing.

77 While the above-mentioned studies all contribute to research into human freeze response,

78 they all share the same limitations. The feeling of danger these studies induced was

79 relatively mild and thus hardly comparable with high-stress situations where freezing would

80 be expected to occur in real life, such as a physical attack. Therefore, in our study, 60 male

81 volunteers underwent two specially devised scenarios simulating armed physical attacks to

82 study human freeze response in a more realistic setting.

83 We hypothesized that freeze-like responses in humans may be affected by stress levels, and

84 we thus measured biochemical, physiological, and psychological markers of stress. We chose

85 cortisol and osteocalcin as biochemical stress markers since both are well-known stress-

86 related hormones[17–20] and because cortisol levels were previously associated with freezing in

87 rodents and primates.[21,22] As physiological stress markers we measured heart rate variability

88 (HRV) and Electrodermal activity (EDA).[23,24] Both have been utilized, with varying results, in

89 previous studies of human freeze-like response.[7,25] As psychological markers of stress we

90 recorded subjective ratings of physical and psychological stress reported by participants and

91 measured anxiety using the State-Trait Anxiety Inventory questionnaire which was previously

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92   connected to freeze-like response in humans.[26] In addition, we hypothesized that since parts

93   of the limbic system are involved in freeze response in animals,[7,27–31] its activation while

94   regulating the response to negative affective images presented during the fMRI task will

95   predict freeze-like response in scenarios simulating physical attacks.

96   **Methods**

97   **Study subjects**

98   We recruited 60 healthy men and divided them into three groups. The first group (A) consisted

99   of 20 healthy male controls with no previous experience with martial arts and combat sports

100   or self-defence training. The second group (B) included 20 students of the Special Education

101   of Security Bodies study program at the Faculty of Sports Studies at Masaryk University. These

102   individuals were specially educated to serve in first responder professions and well trained in

103   martial arts and combat sports as well as in personal self-defence. The third group (C)

104   consisted of 20 members of the Rapid response unit of the Police of the Czech Republic from

105   the South Moravian headquarters. This SWAT team is designed to intervene against

106   particularly dangerous and armed perpetrators. These participants have extensive

107   professional self-defence training (including hand-to-hand combat skills, arresting techniques,

108   and weapons training) as well as real-life experience with situations involving physical

109   violence.

110   Ethical approval for the study was obtained from the Research Ethics Committee of Masaryk

111   University under Ref. No.: EKV-2018-106-R1 on 3 June 2019 for project MUNI/A/1403/2018.

112   Written informed consent was obtained from all study participants. Participants could end the

113   scenario or leave the study altogether at any given time and were repeatedly made aware of

114   this fact. Special safety precautions were taken in scenarios with participants from group A.

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Study chronology

All participants visited the university three times. During their first visit they were introduced to the study and signed the informed consent form. They also provided a biological sample of blood and saliva, filled in the Trait part of the State-Trait Anxiety Inventory questionnaire (STAI-T), and underwent structural and functional magnetic resonance imaging (MRI).

During their second visit, all subjects participated in our low-anxiety scenario (LA) – simulating a physical attack – while wearing a set of sensors measuring physiological markers of stress (heart rate, galvanic skin response) and camera glasses. Several static cameras also recorded the scenario itself from different angles. Immediately before and after the scenario, participants provided blood and saliva samples, filled out the State part of the State-Trait Anxiety Inventory questionnaire (STAI-S), and reported a subjective rating of physical and psychological stress on a scale from 1 to 10.

During the third visit, all subjects participated in our high-anxiety scenario (HA) which simulated a physical attack. Again, several cameras recorded the scenario itself from different angles and each participant was equipped with camera glasses. All participants were equipped with the same set of sensors used during the low-anxiety scenario. They provided blood and saliva samples, filled out the STAI-S questionnaire, and reported a subjective rating of physical and psychological stress on a scale from 1 to 10.

Scenarios

Scenarios simulating a physical attack are common in personal self-defence training, police officer training, etc. However, such scenarios were, to the best of our knowledge, never before used to induce stress to study freeze response in humans. Therefore, both of the scenarios we employed were developed uniquely for this study. The intensity of the LA scenario was similar

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138   to scenarios commonly used for self-defence training. The HA scenario differed from classical

139   self-defence training in that it more prominently featured an element of surprise. In both

140   scenarios attackers modified the physical attack intensity according to the skill levels of

141   individual participants. In general, physical attacks were thus less intense in the case of

142   untrained individuals.

143   Physical attack intensity was also modified according to participant response. For example,

144   when participants froze, the attackers significantly decreased the speed and frequency of

145   active attacks and interacted more verbally, e.g., by encouraging participants to action by

146   phrases such as "Defend yourself!" or "Get out!". In case the participants did not begin to

147   actively defend themselves, the supervising researcher ended the scenario.

148   Prior to the beginning of the scenarios, all participants were informed that each scenario

149   would involve the staging of an attempted crime, including a verbal or physical attack by a

150   person equipped with a protective suit. The participants were instructed to react as they

151   would in real life, which means that reasonable physical contact with the attacker was

152   permitted. All participants were also repeatedly instructed that they could end the scenario

153   prematurely at any time by verbally stating "stop" or using any other words or sentences

154   indicating that they intended to stop the scenario. Participants from inexperienced group A

155   wore protective equipment (safety helmet) to minimize the risk of accidental injury. After the

156   scenario, all participants took part in a psychological debriefing. Immediately before and after

157   each of the scenarios and during the debriefing sessions, participants reported their subjective

158   feelings of physical and mental stress on a scale from 1 to 10.

159   **Low anxiety scenario**

160   In this scenario participants entered a gym used for martial arts and combat sports practice

161   and self-defence training, already expecting a potential unspecified verbal or/and physical

162   attacks. The synopsis of the low anxiety scenario was simple. Participants were instructed to

163   enter the venue, position themselves on an "X" marked on the floor, and observe the scene.

164   No specific task was set. Thirty seconds after the participant entered the scene, a mock

165   attacker dressed in a protective suit (High Gear Impact Reduction suit by Blauer Tactical

166   Systems Inc.) and armed with a fake rubber knife appeared on the opposite side of the gym.

167   For the first ten seconds, the attacker passively observed the participant. Then, the attacker

168   began to communicate with the participant. The communication escalated into a verbal

169   conflict and the attacker slowly began to approach the participant. The intensity of the verbal

170   attack increased and resulted in a simulated physical attack using the fake rubber knife. The

171   participants could react to the situation in any way (e.g., stop the scenario, escape, or resist

172   the attacker). After the scenario, all participants took part in a psychological debriefing

173   session.

174   **High anxiety scenario**

175   The high-anxiety scenario was more complex and task-specific. Participants were instructed

176   to walk through a complicated path divided into different rooms and corridors (total length of

177   approximately 500 meters). They were asked to enter a particular room, locate a piece of

178   paper with their code number, and bring it back to the starting location. However, these

179   instructions were only a distraction. In reality, the participant went down a flight of stairs to

180   the basement and entered a room specifically designed for the scenario (equipped with

181   protective equipment including carpets and mats). After a few steps, loud dog barking sounds

182   were played behind the participants, and, immediately afterward, a mock attacker appeared

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183   on the other side of the room. The attacker, dressed in a protective suit (High Gear Impact

184   Reduction suit by Blauer Tactical Systems Inc.), attacked each participant verbally and then

185   physically like in the low-anxiety scenario. The spatial conditions were the same as in the low

186   anxiety scenario (distance between the attacker and participant, room dimensions). However,

187   compared to the low-anxiety scenario, the attack simulation happened much faster, giving the

188   participants little time to plan their defence. In addition, the attacker used a special electric

189   plastic knife (Shocknife^{TM} SK-2 by Setcan Corporation) which imparts a very mild electric shock

190   upon contact and produces strong visual and audio effects while in use. By increasing the

191   intensity of the verbal attack, the attacker shortened the distance and started to simulate a

192   physical attack with a plastic knife. The participants could react freely to the situation in any

193   way (e. g. stop the scenario, escape, or resist the attacker). Participants from the

194   inexperienced group A wore protective equipment (safety helmets) to minimize the risk of

195   accidental injury. After the scenario, all participants took part in a psychological debriefing

196   session.

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198   **Psychological debriefing**

199   After both scenarios, participants were taken to a quiet room where they took part in a

200   psychological debriefing session. The interview was led by an experienced police psychologist

201   and the head of the Department of Gymnastics and Combatives, responsible for implementing

202   the scenarios.

203   The primary goal of the psychological debriefing session was to ensure that no mental or

204   physical harm was done to the participants during the course of the scenario and that they

205   returned to a resting mental state similar to that in which they came to the research facility.

206   The debriefing was conducted in the form of a semistructured interview, in which the

207   psychologist asked prepared questions and evaluated the participant's answers.

208   The secondary goal of the psychological debriefing session was to ascertain additional

209   information about the subjective perception of the course of the scenarios by the participants

210   (how they perceived the threat, how they evaluated their decision to solve the scenario, etc.).

211   The psychological debriefing session was recorded (audio) for documentation and further

212   analysis purposes. After confirming that all ethical and safety procedures were followed, the

213   participants were allowed to leave the research facility.

214   **Freeze-like response evaluation**

215   Participants' performance was assessed using videorecordings of the the scenarios by a panel

216   of six experts. Five of them were martial arts and combat sports and self-defence training

217   experts (see Supplementary Data 1 for qualifications) while the sixth was a forensic

218   pathologist with 22 years of experience, who estimated the likelihood of survival for each

219   participant in each scenario. The freeze-like response, operationalized as a passive reaction to

220   attack, was evaluated on a scale from 0 to 4, where 0 corresponded to a completely passive

221   reaction and 4 to a very active reaction. Precise criteria for the evaluation are listed in

222   Supplementary Data 1. The experts evaluated the video recordings simultaneously in one

223   room using the same video recordings. Importantly, expert evaluators sat behind different

224   computers and could not consult each other or share their scores between themselves while

225   scoring participants.

226   **MRI and fMRI data acquisition**

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227   MRI was acquired using a 3T Siemens Prisma scanner with a 64-channel head-neck coil. The

228   MRI protocol included T1 MPRAGE structural images and a mild visual stress fMRI task where

229   participants observed neutral and negative images from the International Affective Picture

230   System (IAPS) and were instructed to either observe or regulate their negative affect induced

231   by the stimuli (Supplementary Data 2). High-resolution T1 MPRAGE images were obtained

232   using 0.8 mm isotropic voxels covering the whole head. The fMRI acquisition details were as

233   follows: TR = 0.7 s; 3 echoes, TE = 16 ms, 38 ms, and 59 ms; voxel size 3 x 3 x 3 mm; whole-

234   brain coverage; MB factor 6; PAT factor 2; FA 47°, total of 2006 scans.

235   **MRI and fMRI data processing**

236   All MRI data were processed in SPM12, build 6225 (Wellcome Trust Centre for Neuroimaging

237   at University College London, UK; http://www.fil.ion.ucl.ac.uk/spm/), running under Matlab

238   8.4. R2014b. The functional images were processed as follows: the time series were realigned

239   to the first volume to correct for head motion, transformed into standard stereotactic space

240   (MNI), and spatially smoothed with a Gaussian filter (FWHM = 5 mm). The T1-weighted

241   anatomical high-resolution data were registered to the mean functional image of each subject

242   and spatially normalized into MNI space. Task data were processed with a general linear model

243   as implemented in SPM12, modeling the three conditions and contrasting them. T-statistics

244   maps for individual contrasts were parceled with an AAL atlas. To decrease the number of

245   comparisons, we chose six brain regions from the limbic system (hippocampus,

246   parahippocampus, amygdala, thalamus, insula, cingulum) corresponding to 16 AAL defined

247   regions (6 AAL regions for cingulum 2 ALL for each of the remaining regions; the activity of

248   each brain region was obtained by averaging the responses over corresponding AALs, see

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249   Supplementary Data 3). Pearson's correlations between the activity of these six regions in

250   different contrasts and freeze-like scores in LA and HA scenarios were then calculated.

251   **Biomarkers**

252   Blood and saliva were collected at five time points (baseline and immediately before and after

253   each of the two scenarios). Saliva was kept on ice, centrifuged, and mixed with Protease

254   Inhibitor Cocktail (#P2714, MilliporeSigma). Plasma was extracted from EDTA-whole blood,

255   and both saliva and plasma samples were processed and stored at -80 °C within 90 minutes.

256   To assess salivary cortisol, thawed and centrifuged samples were run in duplicates using

257   Human Cortisol (Saliva) ELISA kit (#RCD005R, BioVendor LM, Czech Republic) according to the

258   manufacturer's instructions.

259   The quantitative measurement of undercarboxylated osteocalcin in plasma samples was

260   performed in duplicate using the Human ucOC ELISA Kit (#MBS700581, MyBioSource, CA, USA)

261   according to the manufacturer's instructions.

262   **HRV and EDA**

263   Physiological data of electrodermal activity (EDA) and heart rate variability (HRV) were

264   recorded during both scenarios. EDA signal was measured using the E4 Wristband device by

265   Empatica Inc. Company. The device was placed on each participant's right wrist. For EDA signal

266   acquisition, disposable pre-gelled ECG Ag/Cl electrodes were used. Electrodes placed on the

267   first phalanx of the index finger and middle finger were connected to an E4 Wristband by E4

268   EDA lead wires extension. Data was saved using the E4 Manager software and cloud.

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269 HRV signal was measured using Polar Vantage V with the heart rate sensor Polar H10 device

270 by Polar Electro Inc. The sensor was placed on each participant's chest. Data was saved using

271 the Polar Flow software and cloud.

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273 **Statistical analysis**

274 All descriptive statistics of continuous variables are represented by mean and standard

275 deviation. Intergroup comparisons (i.e. between performance groups A, B, and C) were

276 performed using parametric (ANOVA, linear model using generalized least squares – in the

277 case of unequal variances) or nonparametric tests (Kruskal-Wallis test). Normality was verified

278 using statistical tests of normality (Shapiro-Wilk, Anderson-Darling, Pearson tests) and

279 graphically (histogram, Q-Q plot). In case data did not follow normality, we log transformed the

280 data and verified the normality (of these transformed data) again. Dependence between

281 continuous variables was studied using pairwise Pearson's correlation coefficient.

282 The dynamic of cortisol and osteocalcin with regard to performance group membership was

283 studied with the linear model using generalized least squares (R package `nlme`)[32]. This type

284 of model facilitates the modelling of mean values of the response variable depending on

285 different kinds of independent variables and accounting for various types of correlations in

286 the data (time, spatial) and heteroscedasticity. In addition, *post hoc* tests were implemented

287 using multiple comparison tests with p-value adjustment using the Tukey method (R package

288 `emmeans`)[33].

289 Linear regression models were used to study the effect of BOLD activity (treated as

290 independent variable) on freeze like response (treated as dependent variable), separately for

291 LA and HA scenarios.

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292   Moreover, four group of variables (anthropometry, cortisol and osteocalcin levels, subjective

293   perception of stress and STAI levels) were incorporated as potential confounders.

294   Because of relatively high correlation levels between variables in each group first FA (in each

295   group) was conducted. Extracted factors were treated as independent variables in the models.

296   Statistical analysis was performed in statistical software R (version 3.5.1) using Rstudio

297   software (version 1.2.5019).[34] All results of statistical testing with p-values less than 0.05 were

298   considered significant.

299   **Results**

300   **Inter- and intra-group comparisons of freeze-like response**

301   As expected, participants in the untrained A group exhibited a more intense freeze-like

302   response in both scenarios. On the other hand, freeze-like response was least pronounced

303   among professionals in group C (Figure 1). However, more surprisingly, while the intensity of

304   freeze-like response in trained group B and professional group C participants increased in the

305   HA scenario compared to LA, untrained group A participants froze less in the more intense
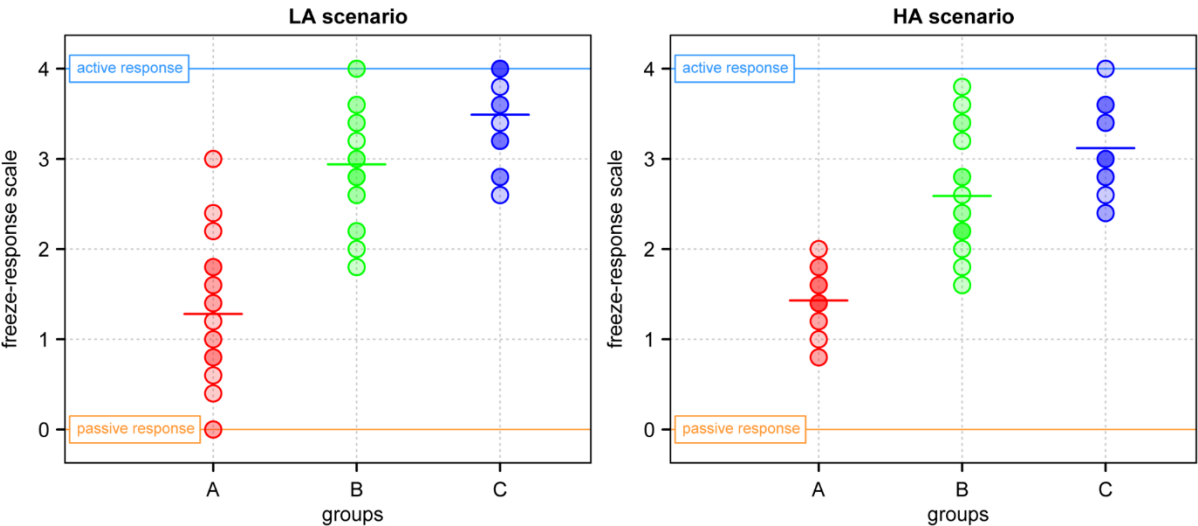
306   HA scenario.

Figure 1 Freeze-like response in low anxiety (LA) and high anxiety (HA) scenarios. A higher score reflects less intense freezing and conversely. Group A consisted of 20 healthy males without previous experience in martial arts, combat sports, or self-defence training. Group B included 20 males trained in martial arts, combat sports, and personal self-defence, but without real-life experience with violence. Group C consisted of 20 members of the Rapid Response Unit of the Police of the Czech Republic from the South Moravian headquarters. These males were both trained and had experience with real-life violence.

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314 The increase in average score in the HA scenario compared to the LA scenario in group 1 stems

315 from the fact that this was the only group where several participants scored 1 point or lower.

316 Interestingly, all participants who scored 1 or lower on the freeze-like response in the LA

317 scenario improved their score in HA, implying that participants who experienced the worst

318 freeze-like reaction also learned the most from this experience. On the other hand, only three

319 participants from trained group B and only two participants from professional group C

320 increased their freeze-like score in the HA scenario compared to the LA scenario.

321 When merged together, all three groups exhibited a strong significant correlation between

322 freeze-like response in LA and HA scenarios (r = 0.80, p < 0.001). However, this was likely

323 caused mostly by the intergroup differences in freeze-like response. There was no significant

324 correlation between freeze-like response in LA and HA scenarios in groups A and B and a weak

325 correlation in group C (r = 0.40, p = 0.077; r = 0.21, p = 0.374; r = 0.60, p = 0.005; respectively).

326 Interestingly, while the intensity of the freeze-like response did not affect the likelihood of

327 survival in the LA scenario (p = 0.738), it significantly affected survival in the HA scenario (p =

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328   0.014). Unsurprisingly, the likelihood of survival was also modified by group membership (p

329   < 0.001) in both scenarios.

330   **State-Trait Anxiety Inventory questionnaire**

331   STAI questionnaire scores significantly differed between groups at all time points (Table 1).

332   Surprisingly, baseline STAI-S values (time points 2 and 4) were highest in group B, suggesting

333   that group B experienced the highest anticipatory stress levels.

Questionare (time point)	Groups (average score ± SD)			p-value	transformation	test
	A	B	C			
STAI-T (1)	36.45 ± 8.13	34.75 ± 7.12	27.55 ± 4.20	<0.001	ln	ANOVA
STAI-S (2)	32.84 ± 6.66	39.40 ± 9.25	30.35 ± 5.00	0.001	ln	ANOVA
STAI-S (3)	43.55 ± 8.88	42.75 ± 9.14	32.05 ± 7.10	<0.001	id	ANOVA
STAI-S (4)	36.45 ± 8.90	37.70 ± 10.12	30.80 ± 5.22	0.005	id	MEM
STAI-S (5)	40.90 ± 9.62	40.26 ± 12.37	32.65 ± 5.88	0.015	id	ANOVA

334   **Table 1. Average group scores from the State-Trait Anxiety Inventory questionnaire.** 1 – before MRI. 2 – before
335   LA scenario. 3 – after LA scenario. 4 – before HA scenario. 5 – after HA scenario. Group A consisted of 20 healthy
336   males without previous experience in martial arts, combat sports, or self-defence training. Group B included 20
337   males trained in martial arts, combat sports, and personal self-defence, but without real-life experience with
338   violence. Finally, group C consisted of 20 members of the Rapid Response Unit of the Police of the Czech Republic
339   from the South Moravian headquarters. These males were both trained and had experience with real-life
340   violence.

341   After adjustment for multiple comparisons, professionals from group C had significantly lower STAI-T

342   scores than either groups A or B (p = <0.001; p = 0.001). No significant difference between groups A

343   and B was established (p = 0.725). Group C also had significantly lower STAI-S score than group B at

344   every time point (p = 0.001; p = <0.001; p = 0.030; p = 0.042 for time points 2 to 5). Group C also had a

345   significantly lower STAI-S score than untrained group A after both scenarios (p = <0.001 and p = 0.024

346   for LA and HA scenarios). However, STAI-S scores for groups C and A measured before the scenarios

347   were not significantly different (p = 0.521 and 0.052). STAI-S scores measured before the LA scenario

348   differed significantly between the A and B groups (p = 0.030), but at every other time point, the STAI-

349   S scores of groups A and B were virtually the same (p = 0.952; p = 0.910; p = 0.977 for time points 3 to

350   5).

351   Across all groups, STAI-T scores measured before MRI sessions showed a weak but significant

352   correlation with STAI-S scores across all time points (Supplementary Data 4). Across individual groups,

353   the professional group C showed the highest correlation between STAI-T and STAI-S scores (r= 0.50, p

354   = 0.025; r= 0.66, p = 0.002; r= 0.58, p = 0.008; r= 0.55, p = 0.012; for time points 2 to 5) (Supplementary

355   Data 4). Trained group B also showed a consistent correlation between STAI-T and STAI-S scores across

356   all time points (Supplementary Data 4). However, the untrained group A exhibited a very different

357   trend. Not only were the correlations between STAI-T and STAI-S significant only after each of the

358   scenarios (time points 3 and 5), but the correlation between STAI-T and STAI-S was negative (r= -0.53,

359   p = 0.016; r= -0.47, p = 0.034; for time points 3 and 5) (Supplementary Data 4). These results thus

360   suggest that STAI-T scores have a very different meaning in differently trained and experienced

361   individuals.

362   While STAI-S scores were affected by the LA and HA scenarios and significant correlations between

363   STAI-T and STAI-S scores were established, no significant correlation between STAI-T or STAI-S scores

364   and freeze-like response was observed in HA scenarios. Accordingly, no significant correlation was

365   found for groups B and C in the LA scenario. However, STAI-S measured before the LA scenario showed

366   a significant negative correlation with freeze-like response in untrained group A (r= -0.56, p = 0.014).

367   **Cortisol and osteocalcin levels**

368   In this study, cortisol and osteocalcin were measured for two related reasons. First, cortisol

369   and osteocalcin were measured immediately before and after each scenario to assess if the

370   scenarios were as stressful as intended for all groups despite differences in training and

371   experience with real-life violence. In this regard, our results show that both LA and in

372   particular the HA scenario were stressful even for the most trained and experienced

373   participants from group C (Figure 2). Furthermore, it seems that osteocalcin is a more reliable

374   marker of stress of the two, as, unlike in the case of cortisol, its baseline levels remained

375 relatively stable during all three baseline samplings (before MRI, before LA scenario, and

376 before HA scenario). In addition, osteocalcin levels were consistent with the STAI

377 questionnaire data showing that group B exhibited the highest anticipatory stress: their

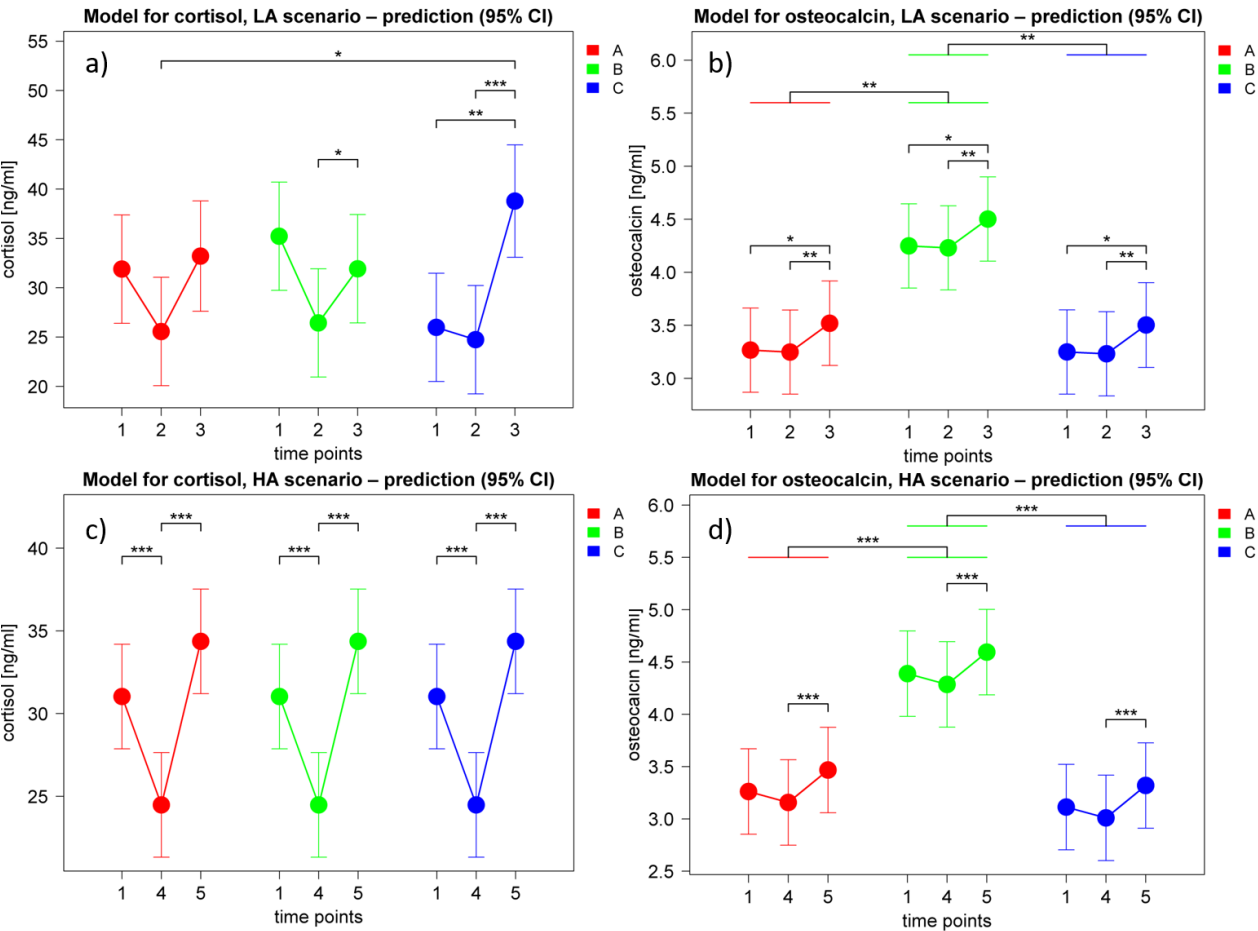378 osteocalcin levels were consistently higher than groups A and C.

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380 **Figure 2 Change in cortisol and osteocalcin levels in LA and HA scenarios.** Cortisol and osteocalcin were
381 measured at five time points. 1 – before MRI. 2 – before LA scenario. 3 – after LA scenario. 4 – before HA scenario.
382 5 – after HA scenario. Group A consisted of 20 healthy males without previous experience in martial arts, combat
383 sports, or self-defence training. Group B included 20 males trained in martial arts, combat sports, and personal
384 self-defence, but without real-life experience with violence. Finally, group C consisted of 20 members of the
385 Rapid Response Unit of the Police of the Czech Republic from the South Moravian headquarters. These males
386 were both trained and had experience with real-life violence
387
388 While cortisol and osteocalcin levels confirmed what the STAI-S scores indicated, i.e. that both

389 LA and HA scenarios were stressful, and osteocalcin levels even affirmed the higher basal

390 stress levels of participants from group B, no correlation between STAI-S scores and cortisol

391 and osteocalcin levels (Supplementary Data 4).

18

392   The second reason for measuring cortisol and osteocalcin was to assess whether their levels

393   predict subsequent freeze-like responses in LA and HA scenarios. In short, they do not. Adding

394   freeze-like response score, BMI, or age to regression models predicting osteocalcin or cortisol

395   levels did not significantly improve the model over models predicting osteocalcin or cortisol

396   levels from a time point and group affiliation (Supplementary Data 4).

397   **Subjective stress perception**

398   In both scenarios, subjectively perceived physical and mental stress significantly rose after the

399   scenario and then significantly decreased before the start of the debriefing session for all three

400   groups (in all cases $p < 0.001$; Figure 3). Furthermore, in all comparisons, self-perceived

401   physical and mental stress levels after the debriefing were no longer significantly different

402   from self-perceived physical and mental stress levels before the scenario (Figure 3). This

403   observation constitutes further evidence suggesting that our scenarios were stressful as

404   intended for all groups despite their different levels of training and experience with real-life

405   violence. In addition, these findings also show that the stress induced by the scenarios was

406   transient and short-term as intended and that participants calmed down to pre-scenario levels

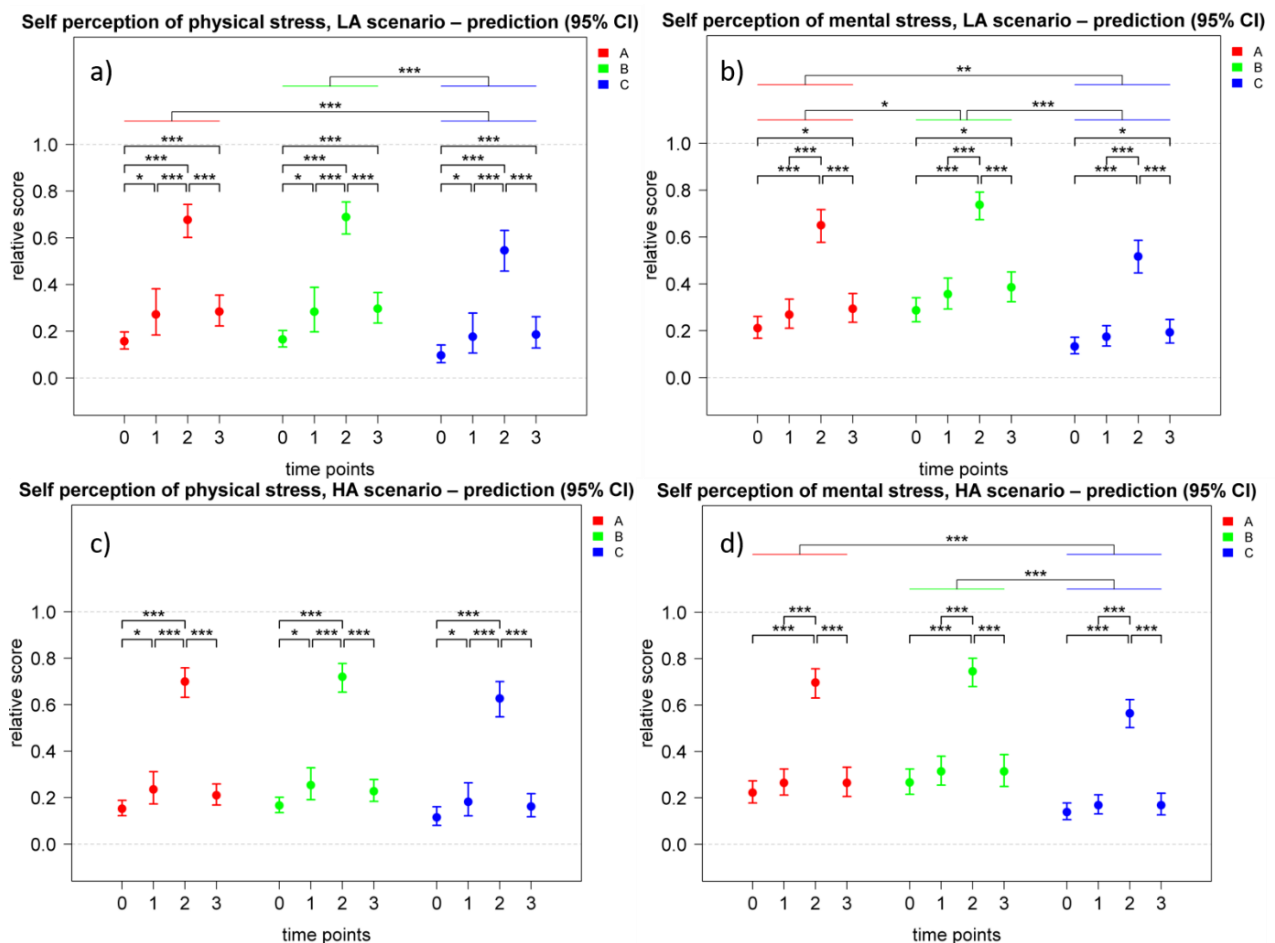407   even before the start of the debriefing session.

Figure 3 Development of self-perceived physical and mental stress in LA and HA scenarios. Participants were asked to rate their personal, physical, and mental stress levels at four time points. 0 – before MRI (day without scenario). 1 – before scenario. 2 – after scenario. 3 – immediately before debriefing. The subjective rating of physical and mental stress levels were linearly transformed to scale 0-1. Group A consisted of 20 healthy males without previous experience in martial arts, combat sports, or self-defence training. Group B included 20 males trained in martial arts, combat sports, and personal self-defence, but without real-life experience with violence. Finally, group C consisted of 20 members of the Rapid Response Unit of the Police of the Czech Republic from the South Moravian headquarters. These males were both trained and had experience with real-life violence.

As a freeze-like response predictors, the self-perception of physical and mental stress was of

limited usefulness. In all groups merged together, no significant correlation with freeze-like

response was established and only physical stress after the LA and mental stress after the HA

scenario showed a weak but significant negative correlation with freeze-like response scores

in LA scenarios (r = -0.30, p = 0.019; r = -0.28, p = 0.032). In the untrained and inexperienced

group A, no significant correlation was observed between stress perception and freeze-like

response in the LA scenario. In the HA scenario, group A showed a significant correlation with

both mental and physical subjective stress before the scenario and freeze-like score (r = 0.45,

426   p = 0.049; r = 0.53, p = 0.017, for physical and mental stress, respectively), meaning that the

427   more stressed the inexperienced participants felt, the less they were freezing in the HA

428   scenario. In the trained group B, the perception of physical and mental stress negatively

429   correlated with the freeze-like response score in the LA scenario (r = -0.51, p = 0.021; r= -0.56,

430   p = 0.011; respectively); no significant correlation was established for the HA scenario. No

431   significant correlation was observed between freeze-like response and the subjective

432   perception of stress in either scenario for the trained and experienced group C.

Electrodermal activity

434   In addition to the subjective stress perception and biochemical markers of stress levels, we

435   also measured heart-rate variability (HRV) and electrodermal activity (EDA) as physiological

436   markers of stress[23,24]. Unfortunately, HRV data were full of artifacts and thus unsuitable for

437   further analysis (Supplementary Data 5), likely due to the dynamic and contact nature of the

438   employed scenarios. Fortunately, EDA data fared much better, with 47 LA and 49 HA records

439   available for analysis.

440   Results of marginal models show that in the LA scenario (Supplementary Data 6) all studied

441   EDA-related variables were significantly affected by predetermined time points in the

442   scenarios, thus suggesting that the skin conductivity of participants reacted to events in

443   scenarios. In addition, almost every variable also had a different time development in different

444   groups (group : timepoint), showing that, as expected, the skin conductivity of trained and

445   untrained individuals differs. Results of marginal models in the HA scenario (Supplementary

446   Data 7) show that all studied EDA related variables except the response latency of the first

447   significant SCR wrw (CDA.Latency) were significantly affected by predetermined time points

448   in the scenarios, suggesting that the skin conductivity of participants reacted to events in

449   scenarios. In addition, mean tonic activity (CDA.tonic) significantly differed between groups.

450   Furthermore, the area (i.e. time integral) of the phasic driver wrw (CDA.ISCR) and the sum of

451   SCR-amplitudes of significant SCR wrw (reconvolved with corresponding phasic driver peaks)

452   (CDA.AmpSum) had different time development in different groups (group: timepoint).

453   Interestingly, EDA correlations with freeze-like response differed between groups as well as

454   between scenarios. In the LA scenario, CDA.tonic upon verbal contact with the attacker

455   negatively correlated with freeze-like response score in group A (r = -0.53, p = 0.034). In

456   addition, CDA.tonic in group A also showed non-significant but suggestive negative

457   correlations upon scenario start (-0.46, p = 0.074) and visual contact with the attacker (-0.49,

458   p = 0.054). On the other hand, groups B and C did not indicate a correlation between CDA.tonic

459   and freeze-like response. Still, instead, both showed negative correlations between non-

460   specific responses in EDA (CDA.nSCR) and freeze-like response score. However, the timing of

461   these correlations was different. In group B, CDA.nSCR negatively correlated with the freeze-

462   like score upon visual contact with the attacker (r = -0.53, p = 0.034), while the negative

463   correlation between CDA.nSCR became significant only after verbal contact (r = -0.56, p =

464   0.028).

465   No significant correlations were observed between EDA and freeze-like responses in any of

466   the groups in the HA scenario. This finding may have resulted from the faster pace of the

467   scenario as all significant correlations during the LA scenario were in time points

468   corresponding to visual and verbal contact with the attacker and both of these parts happened

469   much faster in the HA scenario (see descriptive statistics in Supplementary Data 6 and 7).

470   However, a non-significant but suggestive pattern of correlation with CDC.tonic and freeze-

471   like response scores was present in group B across all parts of the scenario, i.e. after the start

22

472   of the measurement (r = -0.50, p = 0.096), at the start of the scenario (r = -0.49, p = 0.104), on

473   visual contact (r = -0.49, p = 0.104), on verbal contact (r = -0.49, p = 0.104), on physical contact

474   (r = -0.56, p = 0.061), and at the end of the scenario (r = -0.57, p = 0.055).

475   **Brain activity during the fMRI task correlates with freeze-like response**

476   Changes in blood oxygenation level-dependent (BOLD) signal in all contrasts during the fMRI

477   task where participants viewed neutral or negative IAPS images were associated with freeze-

478   like response with corelaction distinctly different between groups an almost non-existent in

479   merged sample. Since the contrast regulate > negative reflects the ability to regulate response

480   to negative affective stimuli, we report it as the most relevant predictor of one's ability not to

481   freeze in threatening situations below, and provide results for the remaining contrasts in the

482   Supplementary Data 3.

483   In untrained participants from group A, BOLD signal in all analyzed anatomical parts of the

484   brain (hippocampus, parahippocampus, amygdala, thalamus, insula, cingulum) during the

485   regulate > negative contrast negatively correlated with the subsequent score on the freeze-

486   like response scale in the HA scenario (Figure 4). In other words, Higher BOLD response in the

487   limbic system was associated with lower freeze-like score and thus more freezing behavior.

488   However, there were no correlations between change in BOLD signals and a freeze-like

489   response in the LA scenario. The brain region with the strongest association with freeze-like

490   response was cingulum (r = -0.66 p = 0.002), suggesting that higher cingulum activation in

491   untrained men results in more freezing.

492   The situation was dramatically different in trained group B. There were no significant

493   correlations of BOLD signal in regulate > negative contrast with a freeze-like response in the

494   HA or LA scenario (Figure 4).

23

495   The situation was again different in the trained and experienced group C, where BOLD activity

496   in regulate > negative contrast in all analyzed brain regions correlated significantly with a

497   higher freeze-like score (less freezing) in the HA scenario (Figure 4). From the analyzed brain

498   regions, the BOLD signal in the amygdala showed the strongest correlation with the freeze-

499   like response in the HA scenario (r = 0.73 with p <0.001). Notably, professional group C was

500   the only one to show a significant correlation between BOLD signal and freeze-like response

501   in the LA scenario. Specifically, increased thalamic activity showed a moderately strong (r =

502   0.45) and significant (p = 0.045) correlation with freeze-like response in the LA scenario.
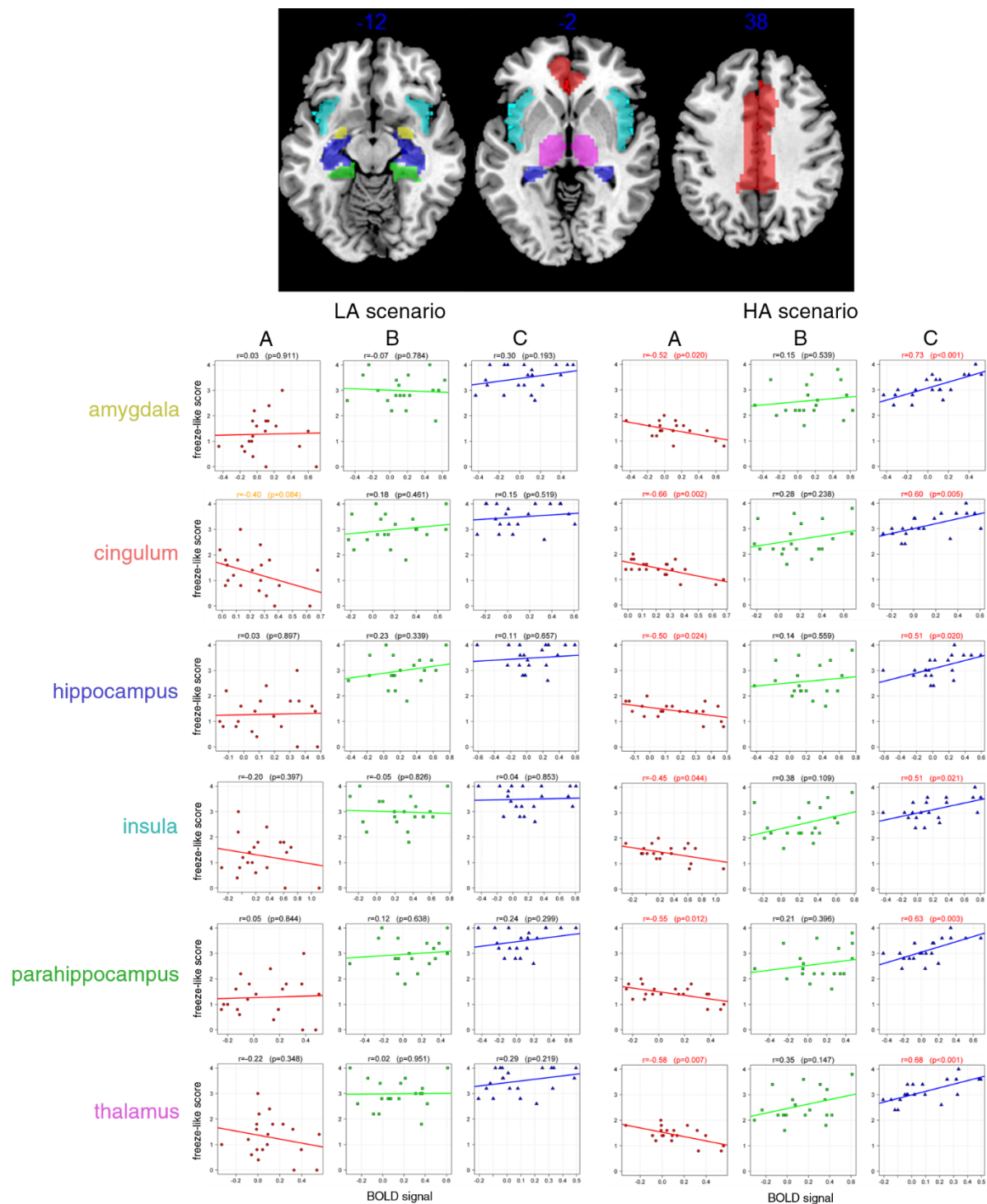
Figure 4 Brain activity correlates with freeze-like response in training and experience-dependent manner. Top part shows the AAL-based regions of interest on axial slices at z=-12, z=-2 and z=38. The graphs show Pearson's correlations between the BOLD signal in the analyzed brain regions and subsequent freeze-like response in either LA or HA scenario. Significant correlations are highlighted in red and trends are highlighted in orange. Group A consisted of 20 healthy males without previous experience in martial arts, combat sports, or self-defence training. Group B included 20 males trained in martial arts, combat sports, and personal self-defence, but without real-life experience with violence. Finally, group C consisted of 20 members of the Rapid Response Unit of the Police of the Czech Republic from the South Moravian headquarters. These males were both trained and had experience with real-life violence.

513   When the BOLD activity was analysed for all groups combined, there was only one significant

514   (p = 0.041) but very weak correlation (r = 0.27) between the BOLD signal in the insula during

515   neutral-negative contrast and freeze-like response.

516   **Complex model**

517   To assess the relative importance of the factors affecting the freeze-like response, we have

518   decided to construct series of linear regression models. Because most of the measured

519   variables were correlated, we have first used factor analysis (with varimax rotation) on four

520   groups of analyzed variables (State-Trait Anxiety Inventory scores, cortisol and osteocalcin

521   levels, subjective stress perception, BOLD activity in limbic system in regulate > negative

522   contrast). The resulting factors were used in combination with anthropometric data (age,

523   height, weight) to create linear regression models predicting freeze-like response in LA and

524   HA scenarios (Supplementary data 8).

525   In the LA scenario, only results from State-Trait Anxiety Inventory questionnaires and age of

526   participants significantly improved the regression model compared to its basic version

527   containing just information about the group participants belonged to. Therefore, the freeze-

528   like response in the LA scenario can be predicted from the subject's group, score from the

529   State-Trait Inventory questionnaires, and age (Table 2). (effects for Stai-Trait and age are

530   specific for each group - due to significant interaction between these variables and group)

	variable	estimate	95% CI	p
	group			
	A		(ref)	
	B	-0.99	(-4.44 ; 2.47)	**0.569**
	C	-1.05	(-3.78 ; 1.68)	**0.444**
	age:A	-0.09	(-0.16 ; -0.02)	**0.015**
slopes	age:B	0.02	(-0.10 ; 0.15)	**0.699**
	age:C	0.04	(-0.03 ; 0.11)	**0.234**

	stai2:A	-0.60	(-0.97 ; -0.24)	**0.002**
	stai2:B	-0.02	(-0.22 ; 0.18)	**0.846**
	stai2:C	0.29	(-0.09 ; 0.67)	**0.136**

531 **Table 2. Best linear regression model for the LA scenario.** Effects for Stai-Trait (Factor stai2) and age are specific for each
532 group - due to significant interaction between these variables and group

533   In the HA scenario, neither results from State-Trait Anxiety Inventory questionnaires or age of

534   the participants significantly improved the basic model. However, the model was significantly

535   improved by including information about the levels of cortisol and osteocalcin and

536   information about the BOLD activity in the limbic system in regulate > negative contrast.

537   Therefore, freeze-like response in the HA scenario can be predicted from the individual's

538   group, levels of cortisol and osteocalcin, and limbic system activity during the task in fMRI

539   (Table 3).

	variable	estimate	95% CI	p
	group			
	A		(ref)	
	B	1.18	(0.90 ; 1.46)	**<0.001**
	C	1.54	(1.26 ; 1.83)	**<0.001**
	ko.HA1:A	0.10	(-0.10 ; 0.30)	**0.331**
	ko.HA1:B	-0.20	(-0.38 ; -0.02)	**0.030**
	ko.HA1:C	-0.12	(-0.36 ; 0.13)	**0.341**
	ko.HA2:A	0.13	(-0.06 ; 0.32)	**0.182**
	ko.HA2:B	0.33	(0.13 ; 0.52)	**0.001**
	ko.HA2:C	0.09	(-0.05 ; 0.23)	**0.221**
slopes	ko.HA3:A	0.05	(-0.13 ; 0.22)	**0.595**
	ko.HA3:B	0.27	(0.12 ; 0.43)	**0.001**
	ko.HA3:C	-0.15	(-0.35 ; 0.06)	**0.155**
	AAL1:A	-0.18	(-0.37 ; 0.02)	**0.072**
	AAL1:B	0.15	(-0.02 ; 0.32)	**0.091**
	AAL1:C	0.22	(0.07 ; 0.37)	**0.006**

540 **Table 3. Best linear regression model for the HA scenario.** Effects for cortisol and osteocalcin levels (factors ko.HA1-3) and
541 limbic system activity (Factor AAL1) are specific for each group - due to significant interaction between these variables and
542 group

543

544 **Discussion**

27

545   This is the first study to show that scenarios simulating armed physical attacks can elicit a

546   freeze-like response in humans. The intensity and tendency to freeze are profoundly affected

547   by personal training and experience. Interestingly, our HA scenario, which featured an

548   element of surprise, elicited a freeze response even in highly trained and experienced

549   professionals who performed without any signs of freezing in our more conventional LA

550   scenario. We also show that feelings of anxiety assessed by STAI-S and cortisol and osteocalcin

551   levels are at best of limited use in predicting freeze-like response in male subjects. On the

552   other hand, we show that the subjective perception of stress and EDA correlates with freeze-

553   like response. Nevertheless, these correlations are entirely dependent on the level of training

554   and experience as well as on scenario intensity. Furthermore, our results suggest that BOLD

555   signal levels in several brain regions while subjects observed neutral and negative images from

556   the IAPS in fMRI predict freeze-like responses in subsequent scenarios. However, we also show

557   that with respect to freezing tendency, the activity of these regions means very different

558   things in untrained males, trained males, and males with both training and real-life experience

559   with situations involving physical violence.

560   Our results show that while highly trained and experienced professionals perform perfectly in

561   normal training routines (LA scenario), they may still freeze in scenarios featuring an element

562   of surprise (HA scenario). This observation is in accordance with previous findings that the

563   shooting accuracy of trained police officers changes from 90% in training to only 50% during

564   real life-threatening situations.[35,36] However, beyond merely demonstrating that even trained

565   individuals experience freeze-like response, our results also show the tremendous

566   effectiveness of training in preventing freeze response. In our study, training was by far the

567   most important single factor affecting the likelihood and intensity of freezing. Even a highly

568   trained professional sometimes experienced a freeze, but untrained individuals experienced

569   it more often and more intensely. This also suggests that it may be possible to develop training

570   strategies further reducing the likelihood and intensity of freeze response, e.g. by

571   incorporating an element of surprise into training routines.

572   Our results also show that freezing, particularly in untrained individuals, may be so intense as

573   to prevent any meaningful defence. Individuals with a score of 1 or lower on the freeze-like

574   response scale reacted at best by taking a small step back and extending their hands slightly

575   to an attack, which in real life would prove deadly in real life. This suggests that in some cases

576   the absence of evidence of defensive behaviour in victims of violent crimes may result from

577   freezing.

578   While the role of stress hormones in freeze response seems logical and the associations

579   between freezing and basal and stress-induced cortisol levels were previously reported in

580   primates and rodents,[21,22] we found no connection between basal or stress-induced levels of

581   cortisol or osteocalcin and freeze-like response. A possible interpretation might be that

582   cortisol and osteocalcin levels in humans may correspond to the intensity of the reaction

583   rather than to its type (fight, flight, freeze), which occurs depending on context. Our results

584   agree with such interpretations as they show that persons experiencing the same perceived

585   threat and showing the same overall response in terms of cortisol and osteocalcin levels react

586   very differently based on previous training and experience.

587   An unexpected element in our study was the increased stress levels before scenarios in trained

588   group B judged by the STAI-S scores and osteocalcin levels. This might be explained by the fact

589   that, unlike participants from group A and C, participants from group B were enrolled in a

590   study program taught by some of the researchers involved in the study. Therefore, they may

591   have felt more pressured to perform well in the scenarios.

29

592   The neuroimaging part of this study showed that the activation of the same brain areas, while

593   viewing the same IAPS pictures, has an entirely different connection to freeze response based

594   on training and experience levels. While the activation of all studied parts of the limbic system

595   (hippocampus, parahippocampus, amygdala, thalamus, insula, cingulum) in the regulate >

596   negative contrast correlated strongly with more freezing in the untrained group A, the

597   activation of the same parts of the brain in the trained and experienced group C correlated

598   with less freezing. Interestingly, there were no correlations between brain activity in fMRI and

599   freezing in group B – which had training but lacked real-world experience with violence. This

600   may be due to the fact that the group B, is in terms of experience somewhere in the middle

601   of groups A and C which show opposite trends. Additionaly group B also had the highest

602   heterogeneity in freeze-like respone in the HA scenario (figure 1). There are undoubtedly

603   many ways to interpret our findings that indicate that the activity of the same brain regions

604   has a different effect based on an individual's level of experience and training. Neverthless,

605   given that all analysed brain regions are connected to reaction to threatening stimuli[37–43] one

606   plausible interpretation is that the activation of the limbic system while viewing images from

607   IAPS reflects a higher tendency to recognize threats. Thus, in untrained individuals the

608   activation of the limbic system correlates with a higher tendency to freeze in the face of

609   physical violence as these untrained individuals do not have the resources to react differently.

610   However, the higher propensity for limbic system activation leads to less freezing in trained

611   individuals as the stronger recognition of the threat strengthens the behavioral patterns

612   learned for such situations.

613   At first sight, it may seem that our results showing the tremendous importance of self-defense

614   training in determining an individual's propensity to freeze are in conflict with results of animal

615   studies showing that freezing is not subject to habituation[7]. However, this is a false

616 comparison as training in self-defense is very different from habituation. Habituation, is a

617 decreased responsiveness to a stimulus with repeated presentation.[44] Self-defense training is

618 a process of active learning how to respond better when someone attacks you accompanied

619 by training to increase physical fitness. Therefore, parallel to self-defense training in animal

620 models is not a mouse that is confronted with a cat multiple times. The parallel of self-defense

621 training in mice would be a mouse that learns how to effectively defend against a cat and

622 significantly improves its strength and stamina in order to be able to do so in practice.

623 The main limitation of this study is that it was conducted solely on males; it is thus difficult to

624 assess how well its findings might be applicable to females. Initially, we intended to continue

625 the study by recruiting the same number and groups of female participants for altered

626 scenarios (including female attackers). However, the ongoing COVID-19 pandemic delayed

627 and complicated the recruitment to the point where we instead decided to publish the male

628 half of the study. Nevertheless, even though we cannot be sure whether the frequency of

629 freezing in a threatening situation differs between males and females, it seems unlikely that

630 the frequency would be lower in females given that up to 70% of female rape victims report a

631 significant feeling of immobility during sexual attacks.[45]

632 Overall, we have shown that training and experience are crucial in the context of human

633 freeze-like response. Focusing entirely on biochemical, biological, or psychological factors or

634 brain activity without taking training and experience into account can lead to a loss of most

635 relevant information, making the results uninformative. Therefore, future research into

636 human fight, flight, and freeze response should always consider the role of training and

637 experience. In addition, by showing that freezing is common in untrained men and can also be

638 induced in professionals by simple scenarios simulating verbal and physical attacks, our study

31

639   suggests that it is reasonable to expect an absence of active defence in a significant percentage

640   of victims of all types of crimes. This finding has a potentially far-reaching implication in

641   various fields ranging from forensic medicine to policy-making.

642

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Author contributions

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759 P.L. identified the research question, helped with the study design, wrote the first draft of the

760 manuscript, and interpreted the results. M.V. designed and supervised the scenarios,

761 including EDA and HRV measurements and methodology of freeze-like response evaluation,

762 and recruited study participants. K.M. designed the MRI protocol, contributed to data analysis.

763 J.N. helped with design scenarios, including EDA and HRV measurements, and methodology

764 of freeze-like response evaluation, and recruited study participants. F.Z. analyzed the data.

765 M.M. processed fMRI data, including statistical parametric mapping-based analysis and

766 exported fMRI features for complex statistics. Z.R. lead the debriefing. M.B., J.Č., P.P., V.M.,

767 and Z.R., evaluated the freeze-like response from the video recordings. T.V. assessed survival

768 scores using video recordings. J.T., J.K., J.F.K., M.T., performed the measurement of

769 osteocalcin/cortisol. V.H. helped with data management and analysed HRV data, R.Š., L.R.,

770 M.G., contributed by data acquisition. M.B. provided feedback on MRI studies and critically

771 revised the manuscript. J. B.V. supervised the workflow of the project and controlled and co-

772 designed its critical steps.. All authors co-wrote the manuscript. P. L. and M.V. contributed to

773 the manuscript in equal measure.

Competing interests

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775 The authors declare no competing interests.

Data availability

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777 Anonymized data are either presented with the manuscript, or present in supplementary data

778 further information is available upon reasonable request. Unfortunately, to protect the

779 identity of the study participants, we are unable to share most video recordings. Illustrative

780   recordings of the study scenarios were provided to the reviewers upon obtaining a written

781   agreement from the affected participants.