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1 **A simulated avalanche search and rescue mission induces temporary physiological and**
2 **behavioural changes in military dogs.**

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23
24 **Running title: Physiological and behavioural changes in avalanche rescue dogs**

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27

28 **Abstract**

29 Saving human lives is of paramount importance in avalanche rescue missions. Avalanche
30 military dogs represent an invaluable resource in these operations. However, their
31 performance can be influenced by several environmental, social and transport challenges. If
32 too severe, these are likely to activate a range of responses to stress, which might put at risk
33 the dogs' welfare. The aim of this study was to assess the physiological and behavioural
34 responses of a group of military dogs to a Simulated Avalanche Search and Rescue mission
35 (SASR). Seventeen avalanche dogs from the Italian Military Force Guardia di Finanza (SAGF
36 dogs) were monitored during a simulated search for a buried operator in an artificial
37 avalanche area (SASR). Heart rate (HR), body temperature (RBT) and blood samples were
38 collected at rest the day before the trial (T0), immediately after helicopter transport at the
39 onset of the SASR (T1), after the discovery of the buried operator (T2) and 2 hours later (T3).
40 Heart rate (HR), rectal body temperature (RBT), cortisol, aspartate aminotransferase (AST),
41 creatine kinase (CK), non-esterified fatty acids (NEFA) and lactate dehydrogenase (LDH)
42 were measured. During the search mission the behaviour of each SAGF dog was measured by
43 focal animal sampling and qualitatively assessed by its handler and two observers. Inter-rater
44 agreement was evaluated. Snow and environmental variables were also measured.

45 All dogs successfully completed their search for the buried, simulated victim within 10
46 minutes. The SASR was shown to exert significant increases on RBT, NEFA and cortisol
47 ($P<0.001$), CK and HR ($P<0.01$), AST and LDH ($P<0.05$). These indicate the activation of a
48 response to stress probably induced by the addition of factors such as helicopter transport,
49 disembarking, and the search and rescue exercise. However, changes were moderate and
50 limited over time, progressively decreasing with complete recovery at T3 except for sera
51 cortisol that showed a slightly slower decline. More time walking within the search was
52 related to lower RBT, conversely to walking. Standing still with head up and exploring with
53 head-up were inversely related with HR. Agreement between handler and observers' opinions
54 on a dog's search mission ability was found only for motivation, signalling behaviour, signs

55 of stress and possessive reward playing. More time signalling was related to shorter search
56 time. In conclusion, despite extreme environmental and training conditions only temporary
57 physiological and behavioural changes were recorded in the avalanche dogs. Their excellent
58 performance in successful simulated SASR may be attributable to extensive training and good
59 dog-handler relationships. Simulated SASR did not seem to impair SAGF dogs' performance
60 or welfare.

61

62 **Keywords:**

63 AVALANCHE MISSION; DOG PHYSIOLOGY; DOG BEHAVIOUR; CORTISOL;

64 HEART RATE; SEARCH AND RESCUE DOGS

65

66 **Glossary**

67 GdF: Italian Military Force Guardia di Finanza

68 SAA: Simulated Avalanche Area

69 SAGF dogs: Avalanche Search and Rescue Military GdF dogs

70 SASR: Simulated Avalanche Search and Rescue mission

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82 1. Introduction

83 Dogs represent an irreplaceable resource in the case of avalanche disasters because of their
84 ability to pinpoint rapidly the location of victims buried beneath the snow [1]. Because of
85 their keen olfactory ability, dogs have been employed not only for locating disaster survivors,
86 lost persons or terrorists [2], but also cadavers and their resting places [3], drugs and
87 explosives [2][4] and wildlife [5]. The success of avalanche rescue missions depends upon a
88 wide range of factors, which can influence the dogs' ability to detect a human scent (i.e., air
89 temperature, direction of the wind, snow composition and burial depth). In addition, factors
90 such as training context [6], physical activity [7], environmental challenges [8] and quality of
91 the relationship between handler and dog have been shown to influence dogs' efficiency and
92 welfare [9]. The level of fitness is another potential factor of importance in avalanche rescue
93 dogs, because during a search they often need to cover large search areas in extremely harsh
94 climatic conditions. In the case of avalanche, the speed of rescue operations is of utmost
95 importance: a victim's chance of survival dramatically declines with time following snow
96 burial [10][11][12]. For this reason, rescue dog-handler units are mostly transported by
97 helicopter to avalanche rescue zones. Harsh climatic conditions, transport challenges, and
98 highly demanding physical activity involved in avalanche missions are likely to be stressful
99 for search and rescue dogs. These circumstances may be cause of delay in the discovery of
100 disperse victims and, over the time, might compromise dogs' welfare. Notwithstanding, so far
101 few studies have been conducted on search and rescue dogs [13][14][15].

102 Previous studies have measured physiological responses induced by a range of potentially
103 stressful situations in dogs, such as living in a shelter [16][17], agility work [18], training and
104 outdoor conditions [13][19]. Cortisol has been indicated as a major indicator of altered
105 physiological states that strongly correlates with stress [16][17]. It has been used to detect
106 poor dog welfare [16][20], especially when the stressor was an immediate challenge, because
107 it is known to facilitate energy release [21]. High levels of this hormone may indicate marked
108 stress during cold and/or heat stress, after physical exercise and performance in a competition

109 [13][19]. Variability of some physiological variables (i.e. heart rate and body temperature)
110 has also been considered as useful indicators for assessing the response to stress in the dog
111 [22][17]. Heart rate (HR) monitoring has been widely used as an accessible, quantifiable,
112 physiological measure underlying emotional responses in dogs [23][24]. Increased body
113 temperature, not linked to a state of disease, have also been used to as a measure of emotional
114 response to stress in other animals [25]. In addition, increases in blood concentrations of
115 creatine kinase (CK), aspartate aminotransferase (AST) and non-esterified fatty acids
116 (NEFA), as well as lactate dehydrogenase (LDH) have been used to evaluate muscle effort at
117 the end of a physical stress [26][14]. Behaviour can also differ in dogs when they are
118 challenged in different situations [27], being an expression of different coping strategies [28].
119 In order to assess behavioural changes both quantitative and qualitative methods can be used.
120 Quantitative methods of assessing behaviour are a way to measure objectively specific aspects
121 of the dogs' behaviour [29] and have been previously used to measure behavioural responses
122 in working dogs [8][20][30]. Modifications of the proportion of time spent in performing
123 specific behaviours during the search activity may indicate possible predictive behaviours
124 associated with enhanced mission success. However, to improve the success rate of search
125 and rescue missions, it is of utmost importance that dogs' handlers are able to judge
126 empirically the ultimate search performance of their dogs, including potential critical aspects,
127 which might cause a delay in the discovery of the missing victim. A qualitative method for
128 assessing search dog search and ability has been previously successfully exploited [31]. It
129 relayed upon agreement between trainers rating their dogs' behaviour while searching, and
130 results were comparable with those of a scientist expert on dog behaviour.

131 Notwithstanding a growing body of research examining stress in the dog, very few studies
132 have been focused on the impact of search and rescue training and work [14][32][33]. The
133 aim of this study was to analyse the physiological and behavioural responses of avalanche
134 military dogs to a simulated search and rescue mission, to investigate whether it might elicited
135 stress or influenced their performance. To the best of our knowledge, this is the first attempt

136 to measure the effect of a search and rescue mission on avalanche military dogs. We adopted
137 a multidisciplinary approach including the measurement of physiological and behavioural
138 changes in the dogs together with a qualitative assessment of the dogs' performance by means
139 of the trainers' subjective ratings at the end of the search missions. This group of avalanche
140 military dogs was homogeneous in terms of physical variables and search performance
141 ability, thus enhancing experimental reproducibility.

142

143 **2. Materials and Methods**

144 All experimental procedures in the present study were in agreement with the Ethical
145 Committee of Perugia University. This research complies with the laws of the Italian Ministry
146 of Health. There is a standing agreement between the Italian Military Force of Guardia di
147 Finanza (GdF) and the Department of Veterinary Medicine of Perugia for the ethical testing
148 and study of GdF working dogs.

149

150 ***2.1. Experimental conditions***

151 This study is part of a broader collaborative project among the Italian Military Force of GdF,
152 the Department of Veterinary Medicine (Perugia University), the Agenzia Regionale per la
153 Prevenzione e Protezione Ambientale del Veneto (ARPAV) and the Istituto per la Dinamica
154 dei Processi Ambientali del Consiglio Nazionale delle Ricerche (IDPA-CNR-Venice
155 University) aimed at identifying limiting factors affecting the success of avalanche search and
156 rescue missions. The study was carried out during a 2nd retraining course (February 2012) for
157 the Avalanche Search and Rescue Military Dog-Handler Units (SAGF Units) at the GdF
158 Alpine School of Passo Rolle (Trento, Italy). During the course the SAGF Units were trained
159 using two sessions of a Simulated Avalanche Search and Rescue mission (SASR) carried out
160 on different days, with half the group tested on one day, the other half on the next. The
161 avalanche victims were played by military personnel, safely buried beneath the snow prior to
162 the arrival of the SAGF, with the burial site then masked to match the remaining search area.

163 A search area of 1 hectare was established at an altitude of 2170 m and prepared to resemble
164 an avalanche fall environment (Simulated Avalanche Area: SAA). The snow was moved and
165 compressed by a snow-cat. Three identical pits (1.5m deep, 50 x 100cm wide) were dug,
166 carefully avoiding contaminating these areas with human scent. In each of the trials the
167 'victim' was entirely buried in snow to a depth of 1m in one of the three pits, chosen at
168 random, the snow further compressed by the snow-cat following initial burial. For safety
169 reasons a hole for air turnover was left in the pits, but not open to the air, and 'victims' were
170 limited to a maximum of 60 minutes burial.

171 During each SASR, a team of experts from the ARPAV continuously monitored
172 meteorological conditions. They collected data through a small Oregon Scientific portable
173 station (Oregon Scientific Wireless Weather Station, Tualatin, OR, USA) installed at a
174 campsite nearby the SAA. During the experimental period, the environmental air
175 temperatures ranged from -8.5 to -10.4°C with 28% humidity and a relative wind-chill of -
176 29°C.

177

178 **2.2. Subjects**

179 The study used 17 Avalanche Search and Rescue Military GdF Dogs (SAGF dogs); fifteen
180 male and two female, aged from 1 to 8 years, and belonging to various breeds (eight German
181 shepherd, six Belgian Shepherd Malinois, three Border Collies) (Table 1). All SAGF dogs
182 were physically (i.e. X-ray negative for hip dysplasia and found in good health from a
183 veterinarian) and behaviourally tested (i.e. absence of behavioural pathologies identified by a
184 veterinary behaviour consultant) to certify their suitability for training work. The SAGF dogs
185 came from different areas of Italy and lived with their handlers all year round. All of them
186 arrived at Passo Rolle a week before the beginning of the study, to allow time for adaptation
187 to the new environment. During both the adaptation and the experimental period the SAGF
188 dogs were individually kennelled in indoor pens (2.9 x 2.1 x 2.3m) at a station beside the GdF
189 Alpine School at Passo Rolle and fed with a commercial, dry dog food. All SAGF dogs had

190 been operational in a search and rescue working dog-handler unit for a minimum of 1 year.

191 All dogs had been previously trained to be transport by helicopter. Their handlers had at least

192 2 years of working experience with search and rescue dogs at the start of the experiment.

193

194 ***2.3. Experimental design***

195 Each SAGF dog was individually tasked with a full SASR. Baseline samples (T0) were

196 collected from the SAGF dogs outside their living pen, the day before SASR, between 08.00

197 and 09.00 h. The SASR protocol consisted of: in turn, and one at a time starting from 09.00 h,

198 each SAGF Unit, equipped with pulley harness, was loaded into a helicopter and transported

199 for approximately 8 minutes to the area above the SAA. Here, the SAGF dog's handler

200 lowered himself and the dog, safely fastened to his harness, from the hovering helicopter to

201 the ground. Upon arrival, the SAGF Unit moved to the study sampling station, where the GdF

202 veterinarian subjected the dog to the first HR, rectal body temperature (RBT) and blood

203 sample collection (T1) (Fig. 1). Then, the SAGF Unit began the SASR. During the SASR the

204 handler could vocally encourage the dog, moving with it on the SAA at a variable distance.

205 When the dog signalled the location of the 'victim', the handler rewarded the dog by playing

206 with it for a maximum of 3 min. Then, the SAGF Unit returned to the sampling station and

207 the dog was submitted to the second HR, RBT and blood collection (T2). A maximum of 10

208 min of SASR time was allowed for each SAGF Unit to locate the 'victim'. Considering the 3-

209 min reward time and the 1–2 min required to move to the sampling point, the T1 to T2

210 sampling interval ranged from 6 up to 15 min. Following T2 sampling, the SAGF handler

211 walked and skied alongside his dog to return him back to his living pen and to let him rest.

212 There, just outside the SAGF dog pen, following 2 hours from T2, the third HR, RBT and

213 blood collection was carried out (T3) (Fig. 1).

214

215 ***2.4. Physiological data***

216 The procedure to collect HR, RBT and blood at each sampling time (T0, T1, T2 and T3) from
217 the SAGF dogs is described in more detail: Each handler asked his SAGF dog to stand and
218 stay still for 1 min, allowing the GdF veterinarian to measure HR and RBT whilst the handler
219 gently manipulated and distracted the dog. HR, in beats per minute, was measured by
220 counting the number of heart beats within a 30 second time window and multiplying it by
221 two. A stethoscope was used (Classic II S.E., 3M™ Littmann®). RBT, in °C, was measured
222 with a digital thermometer (MB TERMO7126500, Reckitt Benckiser SpA, Milano, Italia)
223 gently inserted in the rectum. Following RBT measurement, the handler asked the dog to sit
224 and then gently held it while the veterinarian collected the blood sample (approximately 1mL)
225 from the radial vein using a syringe. Blood collected was rapidly transferred to a glass tube,
226 then centrifuged at 3800 rpm and sera stored at -20°C until analysis. HR, RBT and blood
227 sampling time ranged from 2 to 3 min. During this period, the SAGF dogs did not display any
228 behavioural signs of stress or anxiety but remained calm and attentive.

229 Sera were analysed for cortisol, CK, AST, LDH and NEFA. Serum cortisol concentrations
230 were assayed in duplicate with the commercially available ¹²⁵I Cortisol Radioimmunoassay
231 RIA kit (Beckman Coulter®), following the standard procedure. The measurement range was
232 from 10 to 2000 nM. The intra-assay and inter-assay coefficients of variation (CV) were 5.8%
233 and 9.2% respectively. The antibody used in the immunoassay is highly specific for cortisol,
234 with extremely low cross reactivities reported in human samples against other naturally
235 occurring steroids (Aldosterone, Corticosterone, Cortisone, 11-Desoxycortisol, Progesterone,
236 etc.). All samples were processed within a single assay. The concentrations of CK, AST, LDH
237 and NEFA were measured using the automatic bio-analyser AU 400 Olympus®. The
238 analytical methods and reagents (CK, AST and LDH: Beckman Coulter®; NEFA: Randox®)
239 and measure units (CK, AST and LDH: U/L; NEFA: µmol/L) were those designed for this
240 instrument.

241

242 **2.5. Behavioural observations**

243 An operator located on a nearby slope giving good overall view, filmed the SAGF dogs
244 during the SASR period (from T1 to T2) using a Digital Video Camera Recorder (DCR-
245 SR58, Sony®). For the behavioural analysis, a range of predefined behavioural categories was
246 used [20], modified to the experimental conditions (Table 2). Behaviour variables were
247 mutually exclusive. Behaviour categories were analysed in terms of duration or frequency of
248 occurrence, in relation to the patterns seen in the dog. Behaviours of relatively long duration
249 were considered states and measured as total duration (digging, run, walk, explore head down,
250 explore head up, still head down, still head up - Table 2). Behaviours of relatively short
251 duration were considered events and measured as frequency of occurrence (contact, digging,
252 intensive digging, play, sign of stress - Table 2). We defined as signs of stress a miscellaneous
253 of behaviours (panting, barking, paw-lifting, snout and lip-licking, circling, body shaking,
254 yawning) that have been associated to stress in previous studies [34][35][36][17]. Duration
255 was measured by continuous focal animal sampling, frequency as total number of occurrence
256 of the behaviour within the SASR period. A single operator recorded behavioural data from
257 all the videos after the event. Intra-observer reliability exceeded 90% for all behavioural
258 categories.

259

260 **2.6. Trainers' behavioural assessment**

261 At the end of the SASR, each SAGF handler, together with an additional two external
262 observers (military experts on training avalanche search and rescue dogs), all independently,
263 qualitatively assessed the search performance of the dog. For this subjective assessment, all
264 three trainers rated the SAGF dogs for 14 different attributes considered to be important in
265 search and rescue military dogs (Table 3). For clarity, we defined: *possessive reward playing*,
266 when the dog was playing with the reward but was reluctant to return it to the handler;
267 *collaborative reward playing*, when the dog was playing to leave and take again the reward
268 with the handler; *sign of stress* when the dog showed at least one of the stress-related
269 behaviours described in Table 2. We differentiate the attributes *marking* and *signalling*

270 according to the behaviour of the dog: we defined as *marking* when the dog was digging for
271 less than 5 seconds and not barking to explore a point, *signalling* when the dog was digging
272 for longer than 5 seconds and was barking to indicate the location of the ‘disperse victim’.
273 These 14 attributes were chosen taking into account the general opinion and suggestions
274 given by all SAGF military trainers combined with characteristics highlighted by previous
275 research [37][30]. Similarly to the behavioural assessment approach suggested by Rooney et
276 al., [31], the trainers rated each of nine characteristics of the dog as either: 1, extremely low; 2,
277 low; 3, intermediate; 4, high; or 5, extremely high, whereas for the remaining five
278 characteristics they provided a Yes/No answer (Table 3)..

279

280 **2.7. Statistical analysis**

281 A simple polynomial was fitted to obtain an overall summary across all dogs of the response
282 over time of each of the physiological variables measured. The polynomials were fitted using
283 the statistics package MLwiN [38], which allowed the repeated measurements on each dog to
284 be properly modelled. A polynomial of increasing order was tested in the regression model of
285 each variable and retained when the highest order was of $P \leq 0.05$. Inter-rater agreement was
286 evaluated by means of Cohen’s Kappa using the statistics package Stata v12 (StataCorp LP).
287 All behaviour scores were assessed between each possible pair of assessors.

288 The proportion of time spent carrying out of the eight different behaviours was calculated
289 from the total search time (including the last 10 seconds of digging). The effect of the
290 proportion of the time spent carrying out each of the eight behaviours were tested, using
291 multiple regression, for their effects on each of temperature, total heart rate and total cortisol
292 at time T2. Temperature and heart rate and cortisol at T1 were included in each of the three
293 regressions, respectively, to adjust to T1 as a baseline.

294

295 **3. Results**

296 *Physiological responses*

297 All SAGF dogs successfully completed the SASR within 10 minutes (Fig. 2). SASR
298 significantly affected all measured variables (Fig. 3). The overall line fitted for RBT data
299 showed an increase from T0 ($38.51\text{ }^{\circ}\text{C} \pm 0.09$) (mean \pm SD) to T1 ($39.13\text{ }^{\circ}\text{C} \pm 0.12$)
300 remaining approximately constant at T2 ($39.17\text{ }^{\circ}\text{C} \pm 0.15$), then falling back to initial values
301 at T3 ($38.59\text{ }^{\circ}\text{C} \pm 0.15$) ($P = 0.000$).

302 A trend ($P = 0.007$) was observed for the overall line fitted for HR data, that showed a steady,
303 linear decline from T0 ($116.20\text{ bpm} \pm 5.03$) to T3 ($97.65\text{ bpm} \pm 5.54$). However, inspection of
304 the responses for individual dogs showed that for some subjects the HR rose from T0 to reach
305 a peak at T1 ($110.02\text{ bpm} \pm 5.54$), then declining to T2 ($103.84\text{ bpm} \pm 3.91$).

306 The overall line fitted for sera cortisol concentrations increased throughout the period from
307 T0 ($16.43\text{ ng/mL} \pm 2.17$) to T1 ($32.50\text{ ng/mL} \pm 5.91$), and to T2 ($38.78\text{ ng/mL} \pm 6.89$),
308 afterwards slightly declining to T3 ($35.27\text{ ng/mL} \pm 5.29$) ($P = 0.0003$).

309 The overall line fitted for CK data, showed an increase in T1 ($181.13\text{ U/L} \pm 86.89$) and T2
310 ($200.71\text{ U/L} \pm 30.06$) compared T0 ($122.34\text{ U/L} \pm 10.10$) and decreased a T3 ($181.09\text{ U/L} \pm$
311 18.86) ($P = 0.004$).

312 A linear, progressive and incremental response from T0 ($31.45\text{ U/L} \pm 1.04$), T1 ($37.34\text{ U/L} \pm$
313 9.78) and T2 ($43.22\text{ U/L} \pm 9.37$) to T3 ($49.10\text{ U/L} \pm 7.49$) was recorded for AST ($P = 0.016$).

314 The overall line fitted for LDH data, similarly to CK, showed the greatest increase in activity
315 from T0 ($129.39\text{ U/L} \pm 15.62$) to T1 ($196.79\text{ U/L} \pm 29.62$) and T2 ($210.02\text{ U/L} \pm 28.51$) and
316 decreasing at T3 ($169.06\text{ U/L} \pm 7.00$) ($P = 0.024$).

317 The overall line fitted for NEFA data mirrored the response time course of CK and LDH,
318 increasing from T0 ($509.22\text{ }^{\mu}\text{mol/L} \pm 97.34$) to T1 ($994.15\text{ }^{\mu}\text{mol/L} \pm 141.03$) and reaching a
319 peak to T2 ($1128.90\text{ }^{\mu}\text{mol/L} \pm 201.69$), then declining to T3 ($913.58\text{ }^{\mu}\text{mol/L} \pm 143.00$)
320 ($P = 0.001$).

321

322 ***Behaviour – Quantitative assessment***

323 The duration (including the last 10 seconds of digging) of the search for the individual SAGF
324 dogs is shown in Fig. 2. All dogs successfully found the buried ‘victim’ within 600 seconds,
325 many of them (8 out of 17) completing their search within 100 to 200 seconds. Search time
326 ranged from a minimum of 127 seconds to a maximum of 564 seconds. The duration of time
327 spent performing different behaviours, as well as the number of occurrences during the search
328 is reported in Figures 4. On average, SAGF dogs spent most time exploring (37% with the
329 head down and 12% with the head up), 13% of the time running, 11% digging, 5% walking,
330 and staying still (6% with the head down and 3% with the head up). For 13% of the time the
331 behaviour of the SAGF dogs was not visible. No significant relationships were found for
332 duration or frequency of analysed behaviours in relation to search time (to discovery).
333 There was a significant effect of the proportion of time spent walking and a trend for an effect
334 of the proportion of time spent running on RBT T2 (adjusted for RBT at T1). The higher the
335 proportion of walking, the lower the RBT ($b = -7.622$, $P = 0.020$), and higher proportion of
336 running, the higher the RBT ($b = 2.099$, $P = 0.064$).
337 There was a significant effect of the proportion of time spent standing still with head up and
338 the proportion of time spent exploring with head-up on overall HR at T1 and T2. The higher
339 the proportion of time spent standing still with head up within the search the lower the HR (b
340 $= -481.7$, $P = 0.006$). The higher the proportion of time exploring with the head-up the lower
341 the HR ($b = -161.5$, $P \leq 0.004$).
342 There was no association between the proportion of time spent in any of the recorded
343 behaviours and plasma cortisol levels.

344

345 ***Behaviour - Qualitative assessment***

346 In the qualitative behavioural assessments, we recorded a significant agreement between
347 assessors only for the conclusive motivation, signalling behaviour, signs of stress and
348 possessive reward playing. In Table 4 are reported these paired agreements, identifying the
349 assessor pair and the particular behaviour, and showing the value for Kappa with its

350 significance (conclusive motivation, signalling, sign of stress, possessive reward playing).
351 The analysis for variables indicative of decreased search time found only one variable, signal
352 average, to be significantly associated ($P = 0.009$). The variable ‘signal average’ was coded
353 from 1 to 5, with 5 indicating greater signalling. The analysis showed that for every integer
354 increase in the scale the search time was reduced by a mean of 150.6 (se = 57.70) seconds.

355

356 **4. Discussion**

357 The aim of this study was to characterise the physiological and behavioural responses of
358 military avalanche dogs to a search and rescue work. A deeper knowledge of their responses
359 is important because these animals play a pivotal role in rescue missions, making the
360 difference between the survival or death of a victim. The SASR induced significant increases
361 on RBT and HR, cortisol, CK, AST, LDH and NEFA. However, only AST values were
362 higher than the upper limit reported for healthy dogs at rest [39]. Overall, physiological
363 changes progressively decreased to have completely recovered within two hours following a
364 successful rescue (T3), with the exception of sera cortisol levels that showed a slower decline
365 and also AST that was still increasing. For the baseline values (T0), most dogs had blood
366 values, RBT and HR within published reference ranges (70-120 bpm, 37.9 - 39.9°C) [39].
367 Some individuals showed a peak of HR soon after the descent from the helicopter (T1)
368 indicating a fast activation of the sympathetic-adrenal-medullary axis as a component of the
369 stress response. The range of HR and RBT increases were similar to those observed in healthy
370 Labrador Retrievers 5 minutes post-exercise during training and field trial competition [40].
371 Heart rate rises could reflect that the SASR stimulated an “emotional tachycardia” as shown
372 in other species [25], often also associated with body temperatures increases. These changes
373 might be an adaptive response to the SASR, but also a consequence of the dogs’ increased
374 locomotor activity. Similar changes were observed in military dogs during a three-day
375 terrestrial search operation in response to physical and mental challenge [41] and after 20
376 minutes of physical activity on a treadmill in explosive detection dogs [7].

377 In agreement with Bergeron et al., [42], the elevated heart rate values at baseline sampling
378 (T0, 116.20 bpm) might indicate the presence of a high level of arousal in the SAGF dogs
379 possibly elicited by the novelty of blood sampling procedure. SAGF dogs did show overt
380 signs of excitement when the operator and the handlers entered the area outside their living
381 pen. This elevated HR baseline values might also reflect the excitement of the anticipation of
382 the search, an emotional response to learning known as “Eureka effect” [43], where dogs
383 showed an anticipatory response when experiencing a learning process after recognition of the
384 assigned operational task. It is probable that the SAGF dogs were experiencing a similar
385 excitement. They were daily trained, and showed always signs of excitement when their
386 handlers in the morning let them out of their living pen to work. They were accustomed to this
387 practice, thereby this could have represented a cue of anticipation of the search work.

388 Cortisol baseline values detected in our study were similar to those reported in other studies
389 on working dogs [20]. The SASR induced an increase of the adrenocortical response in the
390 SAGF dogs soon after helicopter transport (T1) reaching peak values after the SASR (T2).
391 However, this increase of plasma cortisol levels was very moderate, values remaining always
392 within the normal range compared to other studies in dogs [8][44]. Cortisol increase showed a
393 tendency to progressively decline over the time, even though not until a complete recovery
394 after 2 hours from the end of the SASR (T3). Haverbeke et al., [8] registered a temporary
395 increment of cortisol levels in a group of military dogs submitted to consecutive training
396 sessions, but this was temporary and declined back to baseline after challenge as a sign that
397 dogs might have a reduced welfare, but were not chronically stressed.

398 Similar trends in the cortisol response, comparable to those of our study, had been observed
399 following intensive work in search and rescue dogs [41], in search and rescue dogs subjected
400 to physical exertion and working in adverse weather conditions (cold and heat stress) and
401 after exercise [13], in dogs after playing [45] and short-term challenges [27], as well as in
402 explosive detection dogs as a response to an emotional reactivity test [46].

403 Plasma CK, AST and LDH activities are measured to assess the degree of metabolism and
404 muscular effort. We recorded a significant increase of CK activity during the experimental
405 period compared with baseline values. Knob and Seidl, [47] observed that the normal range of
406 CK enzyme activity was higher in dogs than humans, and considered a normal reference CK
407 range up to 84 U/L. That the peak of CK activity occurred immediately after the helicopter
408 trip may indicate that loading and transport induced an intense muscular stress. Since CK is
409 the first enzyme to appear in the blood as a result of damage of muscle cells and just as
410 quickly it tends to disappear [48], this temporary rise in CK activity could indicate the
411 presence of an acute state of muscle exertion in the SAGF dogs. Also Schneider et al., [41]
412 observed that the CK activity was the most obvious change among the measured
413 haematological variables after physical stress. SASR also induced a progressive increase of
414 AST activity over the time, but this was very moderate, confirming that the physical effort in
415 the SAGF dogs was not excessive enough to cause substantial muscle damage. Previous
416 studies showed that only elevated increases of AST activity after exercise were associated
417 with damage to the muscle cell membranes [49][50]. Conversely, similar to CK activities,
418 LDH rose after helicopter transport also implying an intense muscular effort. However, LDH
419 activity tended to return to baseline values after the period of rest (T3), suggesting a fast
420 recovery in the muscle metabolism. Similarly, Rovira et al., [14] found that exercise induced
421 elevation in plasma CK, AST, LDH activity and cortisol concentrations, that returned to
422 resting values after 30 minutes of a passive recuperation in search and rescue-trained dogs.
423 Plasma NEFA levels rose moderately above baseline values soon after the helicopter trip
424 (T1), and the SASR (T2), and then progressively declined at rest (T3), suggesting fat
425 mobilization to produce energy. High plasma NEFA concentrations after exercise play an
426 important role in the regulation of the oxidative metabolism by promoting muscle glycogen
427 resynthesis by increasing glucose availability and inhibiting muscle glycolysis through the
428 glucose-fatty acid cycle [51]. Large elevation of plasma NEFA levels post-exercise has been
429 previously reported in dogs after a 4 hour field search [15].

430 Overall, we recorded only moderate and temporary changes in the physiological parameters
431 suggesting that search and rescue activities involved in the SASR elicited only a short-lasting
432 stress response in the avalanche SAGF dogs. This will have been evoked by the additive
433 factors within the SASR (helicopter transport, winching to the ground, physical effort during
434 the search and rescue work). The overall response remained within the physiological limits
435 and was also limited over the time. This was quite remarkable when considering the extreme,
436 harsh environmental and working conditions (environmental air temperatures ranged from -
437 8.5 to -10.4°C with 28% humidity and a wind-chill effect of -29°C) to which the SAGF dogs
438 were exposed and indicate good adaptation to these conditions by the SAGF dogs. In this
439 study they demonstrated excellent search performances, probably due to a good physical
440 training program that allowed them to tackle the search task without showing particular signs
441 of stress or fatigue. In a previous study we found that SAGF dogs were able to recover within
442 2 hours following the exercise stress represented by the SASR, as shown by the return to pre-
443 SASR values of the expression of metabolism and oxidative stress-related genes [33]. This
444 appears to confirm that the physical training these military dogs undergo prepared them well
445 for ASR, enhancing their performance and capacity to potentially repeated high-intensity
446 effort. This finding is highly desirable from the standpoint of the operational use of these
447 animals, as if dogs do not show early signs of fatigue after searching for the first missing
448 person, they could be used in succession for the discovery of other buried victims, to
449 successfully complete other searches during the same avalanche rescue mission. Haverbeke et
450 al., [8] found that military working dogs had a good adaptation capacity to an additional
451 challenge, thereby might have a less diminished welfare than supposed. Rooney et al., [52]
452 found that working dogs that showed high levels of physiological stress were also those that
453 tended to perform poorly during training.

454 The analysis of the videos made during the avalanche simulated search missions gave further
455 insights. As regards the behavioural, quantitative assessment of the performance of the SAGF
456 dogs during the SASR, it was found that duration or frequency of analysed behaviours did not

457 differ in relation to time of search. Therefore, it did not highlight any predictive behaviour
458 associated with a shorter, successful search. This could be due to the lack of variability of the
459 search time amongst all the dogs: a maximum search time of only 564 seconds. As would be
460 expected, exploring (both with the head down and up) and running were recorded as the most
461 prevalent behaviours during the search.

462 In some SAGF dogs high baseline values for RBT and HR were recorded that could perhaps
463 reflect an anticipatory response to the excitement of the search mission, indeed HR at T3,
464 recovery, was actually notably below heart rate at T0, baseline. It is common practice for a
465 handler to stimulate an increase in arousal to prepare a dog for the work ahead. The average
466 fluctuations of RBT in the SAGF dogs were always within published normal reference range,
467 as suggested by Steiss et al., [40]. However, we observed a significant relationship between
468 RBT and the proportion of time spent running and walking. This is likely to be a result of the
469 internal physiological adjustments, keeping a constant core temperature to balance the
470 extreme climatic conditions, which would tend to cool external body temperature. The impact
471 of these climatic conditions on the RBT of the dogs is not clearly established. Ambient
472 temperature seemed to not have a marked effect on average rectal temperatures, but might
473 influence their thermoregulatory ability when dogs perform less strenuous behaviours, such as
474 walking. Steiss et al., [40] reported a wider range of fluctuations of values of rectal
475 temperature in Labrador Retrievers dogs during field trial training and competition, but at
476 higher ambient temperatures (29-30°C).

477 In the SAGF dogs, in part the HR response could be also constituted by a secondary effect of
478 increased physical activity involved in the SASR. Similar heart rate responses have been
479 reported in dogs that were subjected to a variety of conditions, such as different types of
480 stimuli [17][23], human-animal interaction effects [53]. Bergamasco et al., [53] indicated that
481 both sympathetic arousal and vagal tone alterations resulting from confrontational,
482 environmental challenges could influence cardiovascular function and subsequent HR in
483 dogs. This might explain the lower HR values recorded in relation to a longer time spent

484 standing still with the head up and exploring with the head up in the SAGF dogs.

485 The behavioural qualitative assessment of the search performance of the SAGF dogs showed
486 that the agreement among the scores of dog performance during search by the handler and the
487 two observers was poor. However, most of the time overall scores tended to slip by only one
488 category, indicating that differences were not large. So it seemed that individual opinions still
489 largely influenced the behavioural assessment of the dogs and that better training of the
490 observers would be required to improve agreement. In a previous study, good agreement was
491 found between subjective ratings of the search performance of the dogs made by dogs’
492 trainers and that of a scientist expert on dog behaviour [31]. These authors pointed out the
493 importance of dogs’ handlers being able to empirically judge the ultimate search performance
494 of their dogs, including potentially critical aspects, which might cause a delay in the discovery
495 of the missing victim.

496 Not one single particular behaviour seemed to be related to the search speed of the dogs.
497 However, all these dogs had been selected and similarly trained to find people. The only
498 exception was signalling: the more intensively dogs did this, the faster they had found the
499 buried victim. Further investigation of these results might be fruitful, to investigate if good
500 signalling could be predictive of better search performance, or whether it was simply
501 correlated with the chance early discovery of the victim.

502 This study encompassed a small group of dogs, which had already been selected by the same
503 military school and trained for the same work tasks using a similar method. So, they were
504 necessarily relatively homogeneous. A high score in signalling might indicate an easy search,
505 however, it could be that a ‘better’ dog would signal more. In this study each dog was only
506 observed during one search, repeated searches by the same dogs would be required to further
507 investigate this effect. A larger sample of dogs should be investigated for highlighting
508 possible breed or sex differences.

509 In our study we could not explore the time-physical effort relationship. However,
510 notwithstanding the arduous environmental and working conditions, all avalanche SAGF dogs

511 completed their search within about 10 minutes, which was remarkably fast considering the
512 size of the simulated avalanche area. This result was particularly relevant considering that
513 there is an earlier and quicker drop in survival at the early stages of burial (10-18 minutes)
514 of avalanche victims [54][55][11].

515 Behavioural and physiological responses could reflect different coping styles to deal with
516 stressful situations [28][56]. From our findings, this group of SAGF dogs seemed to adopt
517 mainly proactive coping styles characterized by active attempts to counteract the stressful
518 stimuli, low HPA-axis reactivity [28] and high activation of the sympathetic-adrenomedullary
519 system [57]. This might be more functional, enabling them to reach higher search
520 performance success, resulting not only from the dogs' personality factors but also from the
521 training process and from other rearing experiences [58][27].

522 Notwithstanding some individual variability, overall high levels of fitness and performance
523 were observed in the dogs. This could reflect good dog selection and training conditions, and
524 a positive handler-dog relationship, predisposing dogs' wellbeing in working conditions.

525 The SAGF dogs' quick recovery indicates the possibility for their safe use in more extended,
526 multiple search and rescue missions. Improving the performance of avalanche search and
527 rescue dogs implies improving rescue times and increasing the number of successful rescues.

528 However, the recovery of the dogs following more extended, multiple search and rescue
529 missions should form the basis of further study to explore the limits of their performance and
530 endurance. The group of working dogs used in this study were in fine tune with their handlers.
531 Their success as a team of two was in large part due to extended training, itself a highly time-
532 consuming and demanding task. Great attention should be paid to the wellbeing of these dogs
533 given the value of their work and the potential of their work environment to degrade their
534 welfare. Overall the findings of this study suggest that the dogs and the training that they have
535 received have resulted in an animal well adapted to the task that they are required to perform.

536

537 **4. Conclusion**

538 The simulated avalanche search and rescue mission induced temporary and adaptive
539 physiological and behavioural responses in the SAGF dogs. Overall, this group of military
540 dogs carried out high performance search tasks under extreme conditions. However, broader
541 studies would be needed for better investigating the impact of search and rescue missions on
542 avalanche military dogs globally. All SAGF dogs successfully found the buried victim within
543 10 minutes, which coupled with their quick physiological recovery, suggest their safe use in
544 multiple avalanche search and rescue missions. Findings of this study indicated SASR did not
545 compromise military dogs' welfare, notwithstanding the highly demanding task imposed in
546 extremely difficult environmental conditions.

547

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561

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754 Fig. 1 Experimental design

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756 Figure 2: Duration (in seconds) of the simulated avalanche search and rescue mission
757 (SASR) (including the last 10 seconds of digging) in individual SAGF dogs.

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759 Figure 3: Repeated physiological data recorded on SAGF dogs at different sampling times
760 (T0, T1, T2 and T3).

761 Graphs show the physiological observation (Temperature, Heart Rate, Cortisol, Creatine
762 Kinase, Lactate Dehydrogenase, NEFA, Aspartate Aminotransferase) on each SAGF dogs
763 (n=15) at different sampling time (T0, T1, T2, T3). Gray lines represents the repeated
764 measures for the physiological data with the best fit regression line superimposed (in black).

765

766 Figure 4. Duration and frequency of behaviours in SAGF dogs during the simulated avalanche
767 search and rescue mission (SASR).

768 a) time spent (duration) for each behavioral variable from SAGF dogs during search mission.

769 b) total number of occurrences (frequency) for each behavioral variable from SAGF dogs
770 during search mission.

771

772 Table 1: Demographic of the SAGF dogs

773

774 Table 2: Behavioural variables measured from dogs during the simulated avalanche search
775 and rescue mission (SASR)

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777 Table 3: Simulated avalanche search and rescue mission (SASR) ability: SAGF dog's
778 characteristics rated by the handler and two observers after the SASR.

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780 Table 4: Simulated avalanche search and rescue mission (SASR) ability - Qualitative
781 Behaviour Assessment: Agreement Kappa Test between the handler (H) and the two
782 observers (O1 and O2)

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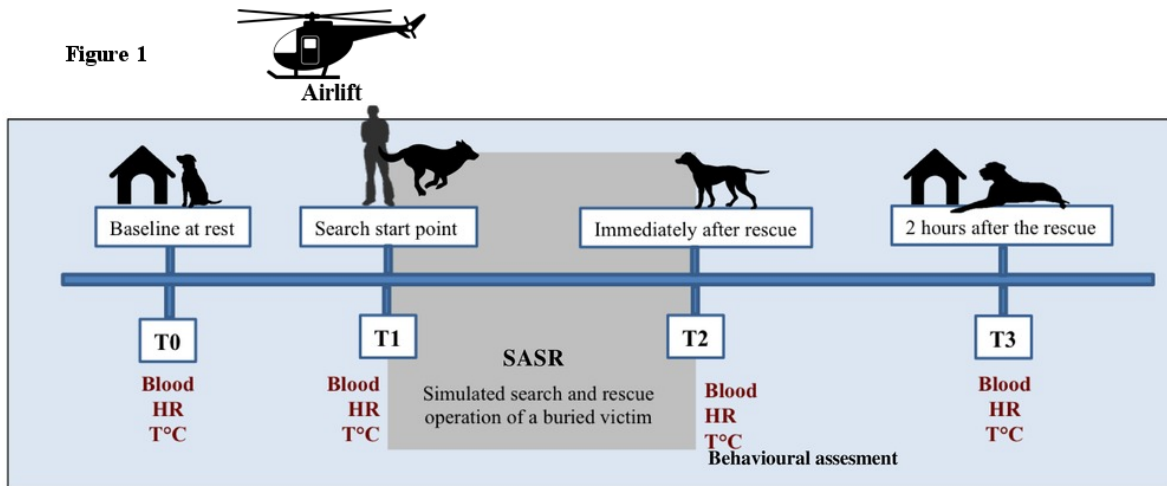
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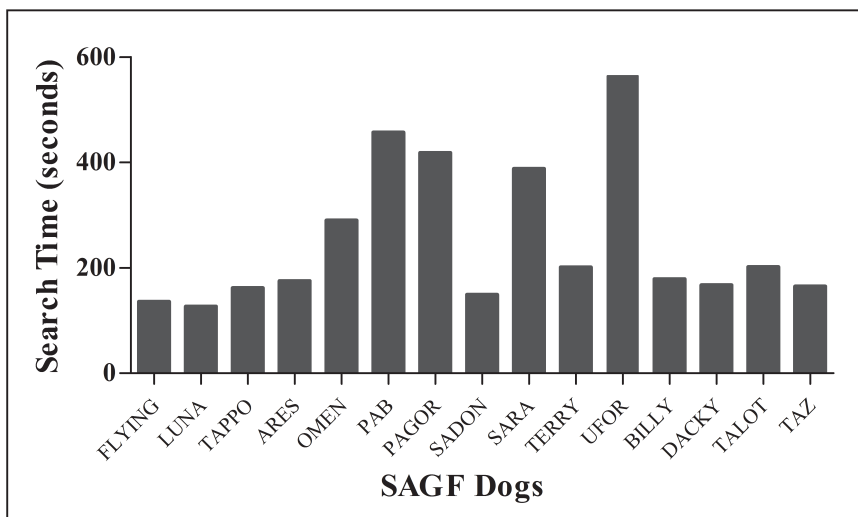
Figure 1



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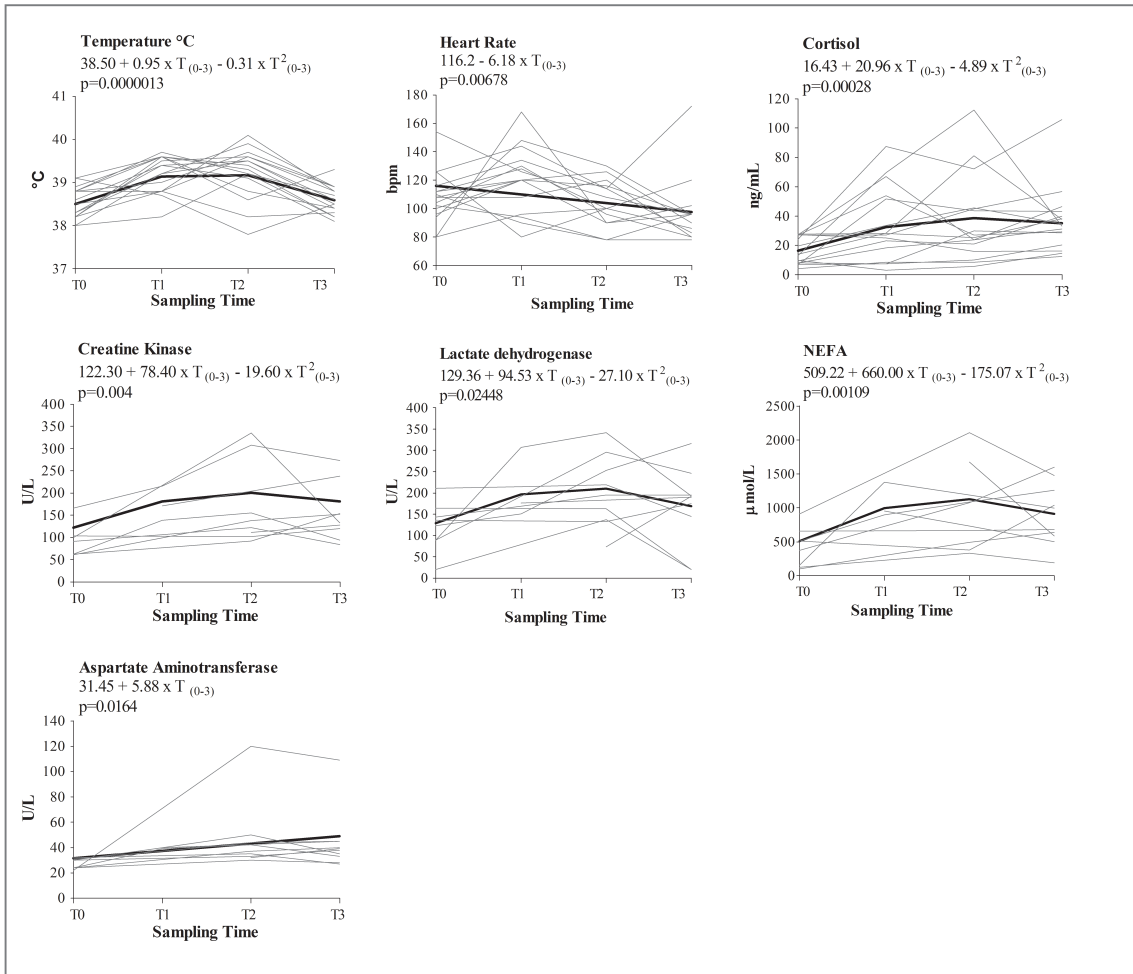
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Figure 3



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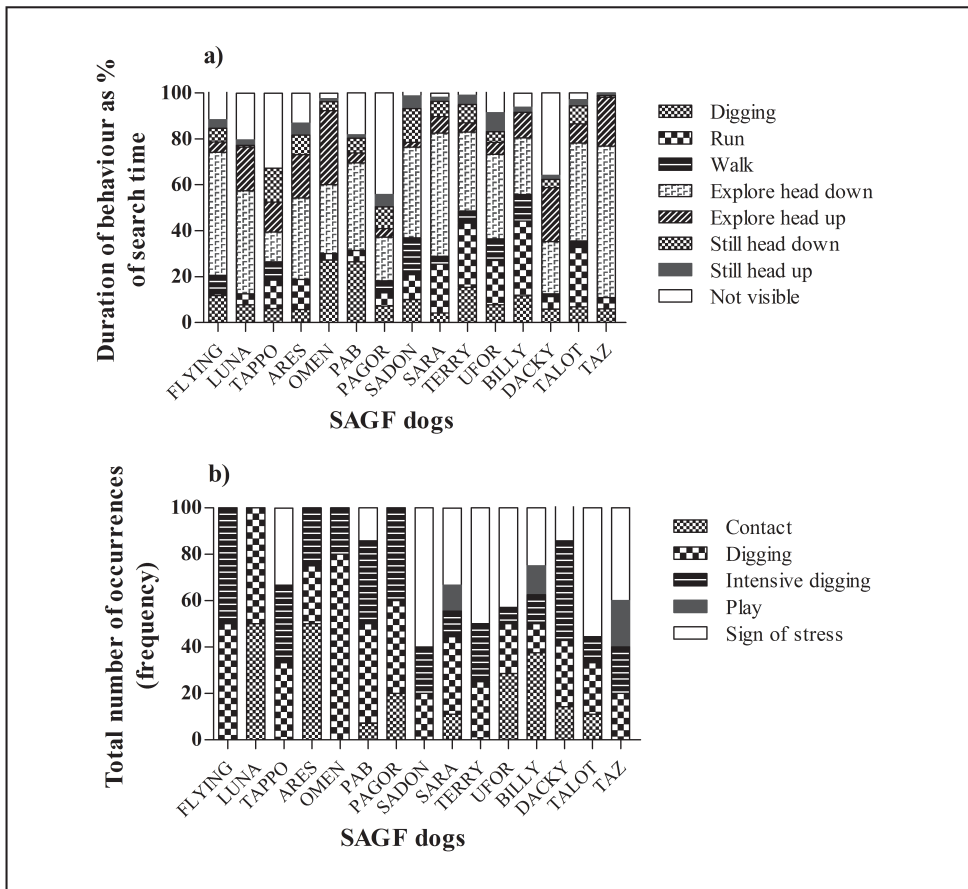
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Figure 4



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849 Table 1: Demographic data of the SAGF dogs.

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Age (years)	N°	Sex	N°	Breed	N°
1-3	6	Male	15	<i>German Shepard</i>	8
3.1-6	5	Female	2	<i>Belgian Shepard Malinois</i>	6
6.1-8	4			<i>Border Collie</i>	3

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854 **Table 2:** Behavioural variables measured from dogs during Simulated Avalanche Search and
855 Rescue mission.

Variable	Definition
Duration DIGGING	Time spent scratching with front paws on snow within the search area (in seconds)
Duration RUN	Time spent running within the search area (in seconds)
Duration WALK	Time spent moving position within the search area (in seconds)
Duration EXPLORE HEAD DOWN	Time spent exploring with head down within the search area (in seconds)
Duration EXPLORE HEAD UP	Time spent exploring with head up within the search area (in seconds)
Duration STILL HEAD DOWN	Time spent stationary with head down within the search area (in seconds)
Duration STILL HEAD UP	Time spent stationary with head up within the search area (in seconds)
Frequency of CONTACT	Number of times dog get in contact with his handler within the search area
Frequency of DIGGING	Number of times dog scratch with front paws on snow within the search area
Frequency of INTENSIVE DIGGING	Number of times dog intensively scratch with front paws on snow within the search area
Frequency of PLAY	Number of times dog plays within the search area
Frequency of SIGN OF STRESS	Number of times dog shows signs of stress* within the search area

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857 * miscellaneous of stress-related behaviours (panting, barking, paw-lifting, snout and lip-
858 licking, circling, body shaking, yawning)

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862 **Table 3:** Simulated avalanche search and rescue mission (SASR) ability: SAGF dog's
 863 characteristics rated by the handler and two observers after the SASR.

No.	Characteristic of the SAGF Dog	Description
Rating scale 1-5		
1	Attention	Attentional state during search
2	Initial Motivation	Motivational state at the onset of the search
3	Conclusive Motivation	Motivational state at the end of the search
4	Signalling	Digging and barking aimed to signal the <i>disperse victim</i>
5	Sign of stress	Dog showing a range of stress-related behaviours*
6	Possessive reward playing	Dog reluctant to return the reward to the handler
7	Collaborative reward playing	Dog playing to leave and take again the reward with the handler
8	Relationship with the handler – Communication	Dog-handler communication
9	Relationship with the handler – Collaboration	Dog-handler collaboration
Answer Yes/NO		
10	Urination	
11	Defecation	
12	Urine marking	
13	Play with the 'disperse victim'	
14	Play with the handler	

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 865 * stress-related behaviours (panting, barking, paw-lifting, snout and lip-licking, circling, body
 866 shaking, yawning)
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877 **Table 4:** Simulated avalanche search and rescue mission (SASR) ability - Qualitative
 878 Behaviour Assessment: Agreement Kappa Test between the handler (H) and the two
 879 observers (O1 and O2).
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Characteristic of the SAGF Dog	Inter-rater H-O1-O2	Kappa coeff	Kappa P<
Initial Motivation	ns	ns	ns
Conclusive Motivation	O1-O2	0.3924	0.0025
Signalling	H-O2	0.2537	0.0319
	O1-O2	0.5479	0.0002
Sign of stress	O1-O2	0.5894	0.0000
Possessive prey playing	H-O2	0.2641	0.0098
Collaborative prey playing	ns	ns	ns
Relationship with the handler – Communication	ns	ns	ns
Relationship with the handler – Collaboration	ns	ns	ns
Urination	ns	ns	ns
Defecation	ns	ns	ns
Urine marking	ns	ns	ns
Play with the disperse victim	ns	ns	ns
Play with the handler	ns	ns	ns

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