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2	Affiliative and disciplinary behavior of human handlers during play with
3	their dog affects cortisol concentrations in opposite directions
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- 26 Abstract
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28 It has been shown that cortisol concentrations change characteristically in the course of 29 agonistic interactions; our aim was to find out how a playful situation may affect 30 concentrations of this hormone in the saliva. We studied dogs' behavior and the changes of 31 cortisol concentrations in a play situation, where the dogs played with their handler for 3 32 minutes with a tug toy. In this experiment working dogs were divided into two groups by the 33 type of their work, namely police dogs and border guard dogs. We found that the cortisol 34 concentrations of old police dogs significantly increased, while the adult border guard dogs' 35 hormone levels decreased, which shows that playing with the handler has an effect on both 36 groups, but interestingly this effect was opposite. Behavior analysis showed differences only 37 in the behavior of the human handlers during the play sessions, while the behavior analysis 38 did not reveal significant differences in the two groups of dogs, except that old border guard 39 dogs generally needed more time to begin playing than old police dogs. During the play 40 sessions police officers were mainly disciplining their dogs, while the border guards were 41 truly playing with them (including affiliative and affectionate behavior). Our results are in 42 accordance with those of recent studies, which show that behaviors associated with control, 43 authority or aggression increase cortisol concentrations, while play and affiliative behavior 44 decrease cortisol levels.

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46 Keywords: play, stress, cortisol, communicative signals, working dogs

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49 Introduction

50 Play is a characteristic behavior of young animals (Bekoff, 2001), which is composed of 51 action patterns that are used in various contexts, such as exploration, manipulation, 52 locomotion, predation, fighting and mating (Hol et al., 1994), or combinations of these 53 (Loizos, 1966). Although its evolutionary origin is not clear, many hypothesized functions of 54 play activity have been proposed including its promotional effect on physical and social skills 55 (Byers and Walker, 1995; Drea et al., 1996; Dugatkin and Bekoff, 2003; Holmes, 1995; 56 Soderquist and Serena, 2000).

57 Play is often used as an indicator of well being (for a review see e.g. Boissy et al., 2007) 58 as animals exposed to traumatic stimulation during development, or live in impoverished 59 environments, show reduced play activity. If we define stress as the non-specific response of 60 the body to any demand for change (Selve, 1950), namely as an increase in HPA axis activity 61 caused by any physical, psychological or environmental stimulus (e.g. Sapolsky et al., 2000), 62 then it seems that environmental stress can have an inhibitory effect on the expression of play 63 behavior. However, there could also be an opposite relationship between play and stress. 64 Some recent studies have shown that play activity may contribute to the reduction of stress 65 (Arelis, 2006). Rats stimulated by novel objects increase their play activity and at the same 66 time show reduced anxiety (Darwish et al., 2001), and in chimpanzees the intensity of playing 67 increases before feeding times which are usually associated with high levels of social tension 68 (Palagi et al., 2004). These latter authors assume that this behavior might have a 'preventive' 69 effect to reduce subsequent social stress elicited by the feeding situation. Humans also utilize 70 play routinely to familiarize children with novel situations including research settings.

71 It is often stated that in comparison to their wolf (*Canis lupus*) ancestors, the dog (*Canis familiaris*) is a very playful species, since dogs play levels remain high throughout their life
73 (Bekoff, 1972; Lorenz, 1950). Dogs are valued members of human social groups partly as a

result of their eagerness to engage in inter-specific play (Bekoff, 1972; Fagen, 1981; Mitchell
and Thompson, 1993; Rooney et al., 2001; Russel, 1936), and many owners spend a
considerable amount of their time playing with their dogs (Hart, 1995).

As playful interaction between dogs and humans represents a natural activity for both species (see above), in the present study we used this behavior to investigate the relationship between play and stress by measuring saliva cortisol concentrations before and after doghuman playing interaction.

81 In social situations other than play, it has been shown that human behavior toward the 82 dog has significant effects on its physiological state (e.g. change of cortisol concentrations, 83 heart rate variability). For example, the presence of humans in a shelter (Beerda et al., 1997; 84 Hennessy et al., 1998; Tuber et al., 1996) may be an effective means of reducing the cortisol 85 response of dogs. In a recent study, Jones and Josephs (2006) found that after agility 86 competitions there is a significant correlation between humans' punitive behaviors (physically 87 pushing the dog and yelling at it) and the increase in dogs' cortisol concentrations in the 88 losing teams. We also recently reported that threatening behavior shown by humans resulted 89 in increasing cortisol concentrations in dogs (Horváth et al., 2007).

90 Thus in this study we hypothesized that the actual motor and communicative aspects of 91 behavior of the human partners during play affect playing experience and inner state of the 92 dogs. Since pilot observations showed that there is a variation in the behavior of working dog 93 handlers toward their dog (policemen and border guards, see below), this offered a good opportunity for testing our hypothesis. In our experiments we asked handlers to interact in a 94 95 playful manner with their dog. To reveal how dogs respond to such a situation, we 96 simultaneously gathered behavioral data from both dogs and handlers and measured the saliva 97 cortisol concentrations of dogs before and after the interaction. The experimental protocol was 98 designed to include situations such as playful struggle with the handler (tug-of-war game), but also cooperative behaviors like the retrieval of the toy or giving the toy up to the handler.
Using this method we aimed to document the changes of cortisol concentrations that take
place during the playful episodes in dogs, in parallel to the actual behavior of the handlers.

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104 Materials and methods

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106 Subjects

107 The police dogs were purchased by the Hungarian National Police Training School for Police 108 Dog Handlers (Dunakeszi, Hungary). Dogs were acquired between 1 and 3 years of age. Dogs 109 were tested physically (i.e. for hip dysplasia) and behaviorally (i.e. reaction to gun shot, bite 110 work). Individuals were purchased only if they did not show signs of hip dyplasia and fear of 111 gun shot. Thereafter they participated in a 12 week training course together with their 112 handlers. During this course the dogs were trained for guarding and obedience.

113 Dogs that participated in the present study were purchased between 1997 and 2003, and 114 performed patrol service with their handlers on the streets for a minimum of 1 year. All 84 subjects were male German Shepherd Dogs. The dogs' age ranged from 2 to 11 years (mean 115 116 age±SD: 6.91±2.19 years) and the subjects were categorized following Studzinski et al. 117 (2006): Adult dogs were 2 – 7 years old (43 individuals; mean age±SD: 5.18±1.45 years); Old 118 dogs were 8 - 11 years old (41 individuals; mean age±SD: 8.76 ± 0.94 years). We also divided 119 the dogs into two work groups ('occupation'): Police dogs (53 individuals; mean age±SD: 120 7.26±2.05 years; 25 adult dogs (mean age±SD: 5.52±1.44 years) and 28 old dogs (mean 121 age±SD: 8.82±0.94 years)), and Border Guard dogs (31 individuals; mean age±SD: 122 6.42±2.32 years; 18 adult dogs (mean age±SD: 4.77±1.47 years) and 13 old dogs (mean 123 age±SD: 8.61±0.96 years)). Dogs of both groups were selected according to the same criteria

initially and they also took part in identical training programs. The only differences were that some dogs were handled by policemen and the others by border guards and also in the nature of their work after the training period. Police dogs and their handlers are assigned to patrol duty on the streets, while border guard dogs and their handlers work the borders of Hungary in the countryside. Policemen and border guards were also selected with similar criteria. Eighty two of the handlers were men and 2 were women.

All procedures were approved by the Ethical Committee of Eötvös Loránd University, Department of Ethology and conducted in accordance with the Hungarian State Health and Medical Service (ÁNTSZ). There is a standing agreement with the Hungarian Police Force that permits testing their working dogs.

134

135 *Date and premises*

136 The experiments were carried out in 2005 and 2006 at the Hungarian National Police Training 137 School for Police Dog Handlers (Dunakeszi, Hungary). The police dogs that were tested, 138 participated in a special 2 week training course with their handlers during the time of testing. 139 The experiments were conducted in an empty room (10 m long x 10 m wide x 5 m high), 140 where only the experimenter (who was also the camera person), the handler and the dog were 141 present during the test. The experimental room was familiar for the subjects (some of the 142 training exercises take place at this location), as it has been shown that introduction into a 143 novel environment enhances HPA activity in the dog (e.g. Beerda et al., 1997). All salivary 144 samples were taken between 9 a.m. and 3 p.m. (e.g. Dreschel and Granger, 2005; Jones and 145 Josephs, 2006). The dogs were in their kennels resting for 30 minutes before starting the test.

146

147 Procedure

148 Dogs played with their handler for three minutes in a similar manner to that which is 149 described by Rooney and Bradshaw (2002). The handler could use a rag or a tug toy (a piece

150 of thick rope with two knots on both ends, 20 cm long) for inducing play behavior in the dog. 151 The handlers were instructed to play as intensively as possible with the dog, and to adjust 152 their behavior to the dog's reactions. Before the test, the handler was asked to leave the dog's 153 toys or food items outside the experimental room. The handlers were asked to encourage the 154 dog during the entire trial, even if the dog displayed only slight or no inclination to play. We 155 imposed few restrictions in the type of play; however the handlers were instructed to execute 156 the following acts at least once during a play session: (1) they had to throw the object and 157 encourage the dog to bring it back; (2) they had to try to take the object from the dog's mouth.

158

159 Measurement of saliva cortisol concentrations

160 Saliva samples were collected from the dogs before the play session and 20 minutes after the 161 end of the play session (see also Beerda et al. (1998), Dreschel and Granger (2005), Jones and 162 Josephs (2006), and Vincent and Michell (1992)). Substances to stimulate saliva flow were 163 not used. The saliva was collected with cotton swabs by the handlers near to the location of 164 the experiment. While the dog was standing still, the handler placed the swab into the mouth 165 of the dog and held it there until it absorbed the greatest amount of saliva possible (lasting 166 from 30 to 60 seconds). The soaked cotton swabs were temporarily stored on dry ice in 167 numbered Eppendorf tubes. For long term storage the saliva samples were kept in a deep 168 freezer (-80 °C). Before analysis the tubes were warmed up to room temperature. The saliva 169 was removed from the cotton swabs by centrifugation (3000 rpm for 15 min) using special 170 centrifuge tubes with filters (Corning Spin-X; Sigma-Aldrich Kft., Budapest, Hungary). After 171 separation the saliva samples were analyzed for cortisol concentrations using a highly 172 sensitive (from 0.003 to 3.0 µg/dl) enzyme immunoassay kit from Salimetrics (State College, 173 PA, USA); the intra- and inter-assay coefficients of variation as provided by the 174 manufacturer, are below 10% and 15% respectively (Salimetrics, 2005). The procedures were

175 performed on the basis of the protocol provided by Salimetrics. Before calculating 176 concentrations, log transformations were used to establish normal distributions. All analyses 177 used the log-transformed hormone values. However, non-transformed data are reported in the 178 figures to facilitate interpretation. All participating dogs provided the required amount of 179 saliva and all cortisol measurements could be used for the statistical analysis.

180

181 Analysis of behavior

The behavior of the dogs was videotaped and analyzed later. From the video recordings, 11 different behaviors were scored based on a subjective assessment of the intensity of the behaviors (frequency), with one variable measured as an absolute latency. The behavioral variables are in Table 1.

186 The subsequent statistical analysis was based on the behavior scoring recorded by an 187 experienced person (Zs. H.), who also coded the entire sample. However 20% of the 188 recordings were also coded by another naïve, independent observer. For data coded by both 189 observers, Kappa coefficients were calculated to measure inter-observer agreement and 190 relatively high values were calculated in all cases. The values of Kappa coefficients are as 191 follows: 'motivation': 1.0; 'playfulness': 1.0; 'willingness to retrieve': 1.0; 'possessivity': 192 0.91; 'latency of starting to play': 0.75; 'control commands': 0.83; 'sound signals': 0.85; 193 'enthusiasm of handler': 0.77; 'praising': 1.0; 'petting head and body': 0.85.

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195 Questionnaire information

After the test, the handlers were asked to fill in a questionnaire which included questions about the dog and the dog-human relationship. Based on this questionnaire we could gain information about the handlers (age, experience with this dog in years), the living arrangements of the dogs, how much time the dog-handler pair spends patrolling and how often the handlers play with the dogs. The exact questions regarding this information arepresented in Table 2.

202

203 Statistical analysis

Shapiro-Wilk's test was used to assess whether or not the sample is consistent with a specified
distribution function.

206 Cortisol concentrations were log transformed for analyses. The salivary cortisol data were 207 analyzed using ANOVAs for repeated measures followed by Bonferroni's post-hoc test. Three-208 way ANOVAs with repeated measures were carried out with 'occupation' (two levels: police 209 dogs, border guard dogs) and 'age' (two levels: adult, old) as between-subjects factors, and 210 'sampling' (two levels: baseline, 20 min post) as within-subjects factor. We used paired t-211 tests for comparing the 'baseline' and '20 min post episode' cortisol concentrations within 212 work groups, and *independent sample t-test* for comparing the 'baseline' between working 213 groups.

The behavior of police and border guard dogs, adult and old dogs, policemen and border guards, was calculated using *Mann-Whitney U*-tests. *Spearman's Rank correlation* was used to search for relationships between non-parametric behavior variables measured in the case of policemen and border guards. We used also *Spearman's Rank correlation* to search for relationships between humans' behavioral variables and dogs' cortisol concentrations. We used the Chi-Square test for analyzing the questionnaire data provided by the policemen and border guards.

Statistical analyses were performed using SPSS (Version 13.0). A significance level of 0.05
was adopted throughout.

223

225 **Results**

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227 The analysis of salivary cortisol concentrations

228 The three-way ANOVA revealed that in general, the 'occupation' did not have a significant 229 effect on the cortisol concentrations (F(1,80)=0.18; p=0.67), nor was there an effect of 230 'sampling' (F(1,80)=0.03; p=0.8), or 'age' of the dog (F(1,80)=0.15; p=0.7). Conversely, we 231 found significant interaction between 'sampling' and the 'occupation' (F(1,80)=9.0; p=0.004). 232 The cortisol concentrations after the test were significantly higher than before the test in 233 police dogs (t=-2.93; p=0.005), while cortisol concentrations after the test were significantly 234 lower than before the test in border guard dogs (t=2.04; p=0.05). When comparing the 235 'baseline' cortisol levels of police and border guard dogs, we found no significant differences 236 between the two groups (t=1.94; df=82; p=0.055). We also found no significant interaction 237 between 'sampling' and 'age' groups (F(1,80)=0.14; p=0.7). The interaction was significant 238 between work ('occupation') and 'age' groups (F(1,80)=4.47; p=0.038), indicating that adult 239 border guard dogs had higher basal cortisol concentrations than the adult police dogs, while 240 the old police dogs had higher post-playing cortisol concentrations than the old border guard 241 dogs (Figure 1). The three-way interaction between 'sampling', the work groups 242 ('occupation') and the 'age' groups, was not significant (F(1,80)=0.28; p=0.6).

243

244 The analysis of behavior

245 <u>Behavior of dogs</u>

When comparing the behavior of all police and border guard dogs, we found no significant differences in any of the behavioral variables (Table 3).

248 Comparisons of the behavior of adult police and border guard dogs did not reveal any 249 significant differences in any of the behavioral variables. However, old border guard dogs generally needed more time to begin playing, than old police dogs ('latency of play': Z=-2.2;
p=0.038).

In the case of police dogs, we found no significant differences in behavior between adult and old dogs. However, old border guard dogs generally needed more time to begin playing than younger border guard dogs (Z=-2.46; p=0.022) (Figure 2).

255

256 Handlers' behavior

The police officers used more 'control commands' in the play situation (Z=-2.8; p=0.005), while border guards 'pet' the dog more often (Z=-3.7; p<0.001), and generally showed more 'enthusiasm' in the play situation (Z=-2.35; p=0.018) (Figure 3).

There was no difference between adult and old dogs in either of the behavioral variables measured, and both policemen (P) and border guards (BG) showed similar behavior toward their dog's, independent from their dogs age (Table 4).

263 We found positive correlation between 'control commands' and 'praising' ($r_s=0.349$; 264 p=0.009) in policemen. The policemen gave a command and if the dogs obeyed it, they 265 praised their dogs similarly as they did during training. In the police dyads we found a weak 266 negative correlation between 'enthusiasm of handler' and 'latency of starting to play' of the 267 dog (r_s =-0.302; p=0.025), that is, if the handler was more cheerful, the playing of the dog 268 began sooner. The border guards that were more enthusiastic ('enthusiasm of handler'), 269 tended to pet ('petting head and body') their dogs more often than the others ($r_s=0.413$; 270 p=0.019).

271

272 Relationship between cortisol concentrations and behavior

In case of the police dogs, no significant correlations were found between 'baseline' cortisol concentrations and the dogs' behavioral variables. However, we found a slight positive correlation between dogs' 'post playing' cortisol concentrations and the amount of 'control commands' used by policemen ($r_s=0.272$; p=0.049).

In border guard dogs we found a positive correlation between 'baseline' cortisol concentrations and 'motivation' ($r_s=0.515$; p=0.003), and negative correlation between 'baseline' levels and 'latency of starting to play' ($r_s=-0.428$; p=0.014). Border guard dogs with high 'baseline' cortisol concentrations were more motivated to play than others and they started to play immediately. In addition, we found negative correlation between dogs' 'post playing' cortisol concentrations and the 'frequency of physical praising' ($r_s=-0.37$; p=0.041).

283

284 *Questionnaire information*

285 There were no significant differences between the policemen and border guards in their age 286 (Z=-0.022; p=0.982), or in their experience with their dog (Z=-0.252; p=0.801). We found 287 significant differences in living arrangements, time spent in service and time spent weekly 288 with play. The border guards only kept their dogs at home ('dog's accommodation': 289 Chi²=13.29; df=3; p=0.004), while policemen often kept their dogs in a kennel at the police 290 station. Border guards spent more time with their dogs in service ('patrol duties': Z=-3.619; 291 p<0.001), in contrast to policemen. However, policemen reported that they play more with 292 their dogs (Z=-2.305; p=0.021) than border guards, and we found a negative correlation between 'time in patrol' and 'time spent with play' (r_s =-0.365; p=0.001). 293

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296 **Discussion**

The aim of this study was to examine the effect of human communicative and social behavior on the inner state (measured in terms of saliva cortisol concentrations) of dogs in a play situation. To this end, dogs and their handlers were observed in a playful interaction,before and after which we measured changes of the dogs' cortisol concentrations.

301 We found that on the whole, short term play interaction with the handler did not 302 significantly change the cortisol concentrations of the dogs at a population level. This was in 303 contrast to our previous study in which we observed an overall increase in cortisol 304 concentration after being exposed to an approach by a threatening human (Horváth et al., 305 2007). However, important insights emerged when we compared the results of our two 306 groups, police and border guard dogs, in which the direction of change in the cortisol 307 concentration was the opposite. Moreover, the age of the dogs seemed to also have an 308 influence, as in adult border guard dogs, cortisol concentration decreased after play, whereas 309 in the old police dogs, the cortisol concentration increased by the end of the play session.

310 We also found a relationship between border guard dogs' baseline cortisol 311 concentrations and motivation and latency of starting to play, namely border guard dogs with 312 higher baseline levels are more motivated to play with the handler than those with a lower 313 levels, as they generally start to play immediately. Notably, in contrast to the changes in 314 cortisol concentrations, the play behavior of police dogs was similar to play of the border 315 guard dogs (i.e. they appeared just as playful and motivated as border guard dogs). Although 316 the behavioral analysis of the play behavior may have not revealed initial differences in the 317 motivation, we believe there were different causal factors behind the play behavior of the 318 dogs. That is, police dogs may have executed playful behavior as part of a training exercise 319 ('they were commanded to play'), while border guard dogs may have played spontaneously 320 with their handler. As a result, the social play may have mediated an inner state which was 321 associated with lower concentrations of cortisol.

The analysis revealed that the policemen continually disciplined ('controlled') their dogs and used sound signals to gain their dogs' attention during play sessions, while border

324 guards were more empathetic and enthusiastic, and also pet and praised their dogs more often. 325 The positive correlation between enthusiasm of handler and dogs' latency of starting to play 326 in the case of policemen, also suggested that the more enthusiasm shown by handlers, the 327 sooner their dogs started to play. Thus we suppose that the differences in behavior, mood and 328 motivation of the handlers not only influenced their dogs' motivation and behavior during the 329 play session (O'Farrell, 1997), but also had an effect on the post-play cortisol concentrations, 330 which is in accordance with the findings of previous studies (e.g. Coppola et al., 2006; Jones 331 and Josephs, 2006; Tuber et al., 1996).

332 Accordingly, the disciplinary behavior of policemen resulted in higher cortisol 333 concentrations in the case of old police dogs, while the friendlier attitude and petting by the 334 border guards, reduced cortisol concentrations in the case of adult border guard dogs. Police 335 dogs might have assessed this situation as a training session due to the behavior of the 336 handlers, rather than play time. Our results are similar to conclusions of Jones and Josephs 337 (2006), who have revealed a relationship between larger increased cortisol concentrations and 338 punitive behavior and lesser increased cortisol concentrations and affiliative behavior, 339 respectively after an agility competition.

340 It is important to stress that other factors might have also contributed to this effect, such 341 as the age of the dogs, previous keeping conditions and the established relationship between 342 dogs and their handler.

In the case of age, we found that adult border guard dogs with higher baseline cortisol concentrations are more motivated to play than old ones, which in general is in agreement with age-related decrease in playing activity in dogs (e.g. Rooney et al., 2000). In our case, the more affiliative behavior of the handler contributed to the reduction of cortisol concentrations in the adult dogs, suggesting a calming effect of social interaction. In contrast, the more disciplinary attitude of the policemen affected the cortisol concentrations in old dogs

in the opposite direction. A recent meta-analysis found greater cortisol response to a challenge
in older people (Otte et al., 2005) and we have reported similar observation in old police dogs
as a response to the approach of a threatening human (Horváth et al., 2007). Notably, the
behavior of the handlers was similar toward adult and old dogs, therefore this cannot explain
the present findings.

According to the handlers (questionnaire), many of the police dogs live in kennels, while all the border guards take their dogs home after work. In the course of the present training course, all of the dogs spent their day in kennels, which might have been a more stressful experience for the border guard dogs, as police dogs are used to living in such quarters. This difference could have contributed to the different reaction of the dogs to the play situation.

360 Furthermore, both previously mentioned factors could have also influenced the nature of 361 the actual social relationship between dogs and handlers, which might in some form, mirror 362 intraspecies relationships in dogs or wolves. In this case, the larger social distance in terms of 363 dominance could also influence the calming effect of the interaction, partly because 364 dominants may use more forceful and physically challenging behavioral actions during the 365 interactions, whereas partners having a similar social status may induce interactions 366 (including play) by using more communicative signals including signals for play invitation 367 (e.g. play bow).

In summary, police dogs playing with their handlers showed some 'similarity to' experience obtained during general training. This could be partly attributed to the behavior of policemen, but other factors cannot be excluded. It follows that despite the session's playfulness, the interaction with police handlers increased cortisol concentrations in the dogs, suggesting increased level of stress. Contrasting processes have been revealed in border guard dogs where we found support for the stress reducing effect of play.

Further studies may reveal whether dogs living with families might be also affected by the play behavior of their owner. It seems that affiliative communicative signals during play might indeed contribute to stress reduction (at least in terms of decreased cortisol concentrations) supporting earlier findings (Arelis, 2006; Palagi et al., 2004). Thus regular play with dogs could contribute to their well being in general and also reduction of stress in certain situations.

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398 **References**

400	Arelis, C.L., 2006. Stress and the power of play. A Thesis Submitted to the School of Graduate
401	Studies of the University of Lethbridge in Partial Fulfillment of the Requirements for the
402	Degree. Department of Neuroscience, University of Lethbridge, Lethbridge, Alberta,
403	Canada.
404	
405	Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., 1997. Manifestations of
406	chronic and acute stress in dogs. Applied Animal Behaviour Science 52, 307-319.
407	
408	Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., Mol, J.A., 1998. Chronic
409	stress in dogs subjected to social and spatial restriction. II. Hormonal and immunological
410	responses. Physiology & Behavior 66, 243-254.
411	
412	Bekoff, M., 1972. The development of social interaction, play, and metacommunication in
413	mammals: an ethological perspective. Quarterly Review of Biology 47, 412-434.
414	
415	Bekoff, M., 2001. Social play behaviour. Journal of Consciousness Studies 8, 81-90.
416	
417	Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C.,
418	Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I., Aubert, A., 2007.
419	Assessment of positive emotions in animals to improve their welfare. Physiology &
420	Behavior 92(3), 375-397.
421	
422	Byers, J.A., Walker, C., 1995. Refining the motor training hypothesis for the evolution of play.
423	American Naturalist 146, 25-40.

425	Coppola, C.L., Grandin, T., Enns, R.M., 2006. Human interaction and cortisol: Can human
426	contact reduce stress for shelter dog? Physiology & Behavior 87, 537-541.
427	
428	Darwish, M., Korányi, L., Nyakas, C., Almeida, O.F.X., 2001. Exposure to a novel stimulus
429	reduces anxiety level in adult and aging rats. Physiology & Behavior 72, 403-407.
430	
431	Drea, C.M., Hawk, J.E., Glickman, S.E., 1996. Aggression decreases as play emerges in infant
432	spotted hyenas: preparation for joining the clan. Animal Behaviour 51, 1323-1336.
433	
434	Dreschel, N.A., Granger, D.A., 2005. Physiological and behavioural reactivity to stress in
435	thunderstorm-phobic dogs and their caregivers. Applied Animal Behaviour Science 95,
436	153-168.
437	
438	Dugatkin, L.A., Bekoff, M., 2003. Play and the evolution of fairness: a game theory model.
439	Behavioural Processes 60, 209-214.
440	
441	Fagen, R., 1981. Animal Play Behavior. Oxford University Press, New York.
442	
443	Hart, L.A., 1995. Dogs as companions: a review of the relationship. In: Serpell, J. (ed.), The
444	Domestic Dog: Its Evolution, Behaviour and Interactions with People. Cambridge
445	University Press, Cambridge 161-179.
446	
447	Hennessy, M.B., Williams, M.T., Miller, D.D., Douglas, C.W., Voith, V.L., 1998. Influence of
448	male and female petters on plasma cortisol and behaviour: can human interaction reduce

449	the stress of dogs in a public animal shelter? Applied Animal Behaviour Science 61, 63-
450	77.
451	
452	Hol, T., Koolhaas, J.M., Spriujt, B.M., 1994. Consequences of short-term isolation after
453	weaning on later adult behavioural and neuroendocrine reaction to social stress.
454	Behavioural Pharmacology 5, 88-89.
455	
456	Holmes, W.G., 1995. The ontogeny of littermate preferences in juvenile golden-mantled
457	ground squirrels: effects of rearing and relatedness. Animal Behaviour 50, 309-322.
458	
459	Horváth, Z., Igyártó, BZ., Magyar, A., Miklósi, Á., 2007. Three different coping styles in
460	police dogs exposed to a short-term challenge. Hormones and Behavior 52, 621-630.
461	
462	Jones, A.C., Josephs, R.A., 2006. Hormonal interactions between man and the domestic dog.
463	Hormones and Behavior 50(3), 393-400.
464	
465	Loizos, C., 1966. Play in mammals. Symposia of the Zoological Society of London 18, 1-9.
466	
467	Lorenz, K., 1950. Man meets dog. Routledge Classics, Great Britain.
468	
469	Mitchell, R.W., Thompson, N.S., 1993. Familiarity and the rarity of deception: Two theories and
470	their relevance to play between dogs (Canis familiaris) and humans (Homo sapiens).
471	Journal of Comparative Psychology 107(3), 291-300.
472	

474	Science 52, 205-213.
475	
476	Otte, C., Hart, S., Neylan, T.C., Marmar, C.R., Yaffe, K., Mohr, D.C., 2005. A meta-analysis of
477	cortisol response to challenge in human aging: importance of gender.
478	Psychoneuroendocrinology 30, 80-91.
479	
480	Palagi, E., Cordoni, G., Borgognini Tarli, S.M., 2004. Immediate and delayed benefits of play
481	behaviour: New evidence from chimpanzees (Pan troglodytes). Ethology 110, 949-
482	962.
483	
484	Rooney, N.J., Bradshaw, J.W.S., Robinson, I.H., 2000. A comparison of dog-dog and dog-
485	human play behaviour. Applied Animal Behaviour Science 66, 235-248.
486	
487	Rooney, N.J., Bradshaw, J.W.S., Robinson, I.H., 2001. Do dogs respond to play signals given by
488	humans? Animal Behaviour 61, 715-722.
489	
490	Rooney, N.J., Bradshaw, J.W.S., 2002. An experimental study of the effects of play upon the
491	dog-human relationship. Applied Animal Behaviour Science 75, 161-176.
492	
493	Russell, E.S., 1936. Playing with a dog. The Quarterly Review of Biology 11(1), 1-15.
494	
495	Salimetrics, L.L.C., 2005. Expanded Range High Sensitivity Salivary Cortisol Enzyme
496	Immunoassay Kit [Brochure]. State College, PA.
497	

O'Farrell, V., 1997. Owner attitudes and dog behaviour problems. Applied Animal Behaviour

499	responses? Integrating permissive, suppressive, stimulatory, and preparative actions.
500	Endocrine Reviews 21(1), 55-89.
501	
502	Selye, H., 1950. Stress. Acta, Inc. Medical Publishers, Montreal, Canada.
503	
504	Soderquist, T.R., Serena, M., 2000. Juvenile behaviour and dispersal of chuditch (Dasyurus
505	geoffroii) (Marsupialia: Dasyuridae). Australian Journal of Zoology 48, 551-560.
506	
507	Studzinski, C.M., Christie, LA., Araujo, J.A., Burnham, W.M., Head, E., Cotman, C.W.,
508	Milgram, N.W., 2006. Visuospatial function in the beagle dog: An early marker of
509	cognitive decline in a model of human aging and dementia. Neurobiology of Learning
510	and Memory 86(2), 197-204.
511	
512	Tuber, D.S., Hennessy, M.B., Sanders, S., Miller, J.A., 1996. Behavioral and glucocorticoid
513	responses of adult domestic dogs (Canis familiaris) to companionship and social separation.
514	Journal of Comparative Psychology 110(1), 103-108.
515	
516	Vincent, I.C., Michell, A.R., 1992. Comparison of cortisol concentrations in saliva and plasma of
517	dogs. Research in Veterinary Science 53(3), 342-345.
518	
519 520	

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress

Table 1. Behavioral variables coded in dogs and handlers, where 0 points are given (Score 0)
522 in case of the behavior that is least favorable from the point of view of our cooperative
523 situation, while 2 points (Score 2) are given in case of the most favorable behavior.

	BEHAVIORAL VARIABLES	SCORE 0	SCORE 1	SCORE 2
DOGS	'Motivation'	there is no physical contact between the dog and the play object (e.g. the dog never holds or chews the object during the session)	the dog makes physical contact (with his mouth or paw) with the object at least once	there are two or more physical contacts made between the dog and the object
	'Playfulness'	the dog is passive, pays no attention to play object	the dog shows interest (looks at, contacts, etc.) in playing with the object at least one time	the dog pays attention to the play object during the entire session or more than one time
	'Willingness to retrieve'	the dog never brings the object to the handler or there is no physical contact between the dog and the object (e.g. the dog never holds or chews the toy during the session)	the dog makes steps to move towards the handler with the object in its mouth, but the handler cannot get the object without approaching the dog	the dog usually brings the object back to the handler
	' Possessivity ' (this behavior variable was not coded when the dog was given Score 0 at 'Motivation' and/or 'Playfulness')	the handler is unable to take the object from the dog during the session even with force	there are visible signs of resistance when the handler tries to take the object or the dog shows avoidance with the object in its mouth, but finally the handler can take it from the dog	the handler can take the object from the dog's mouth (without any visible signs of force)
	'Aggression' (signs of aggression after Kim et al. (2006), Kroll et al. (2004), McLeod (1996), Netto and Planta (1997) and Pal et al. (1998): tail is not tucked, the	no signs of aggression can be observed during the play session	the dog shows at least one of the behaviors described during the play session	

	ears are forward or ambivalent, stiff body posture; growling, barking, snarling, showing the teeth) 'Fear' (signs of fear after Dreschel and Granger (2005) and Kroll et al. (2004): the dog's tail is tucked between its hind legs, hides behind the human's leg, backs away or retreats, ears are	no signs of fear can be observed during the play session	the dog shows at least one of the behaviors described during the play session	
	pinned or drawn back or down) 'Latency of starting to play' (sec; from the handler's first call to play till the dog takes the tug toy)			
H A N	'Control commands' (number of verbal orders issued by the handler during the 3 minute session, e.g. using words of command: sit - <i>ül</i> , stand - <i>áll</i> , lay - <i>fekszik</i> , heel - <i>lábhoz</i> , listen - <i>figyelj</i>)	does not occur	occurs between 1 and 5 times	occurs 6 or more times
	'Sound signal' (number of non-verbal sound signals used by the handler during the 3 minute session to attract the dogs' attention, e.g. whistle, clapping, etc.)	does not occur	occurs between 1 and 5 times	occurs 6 or more times
D L E	'Enthusiasm of handler'	the handler does not laugh or smile during the play session	the handler is cheerful during the play session	
R S	'Praising ' (handler praises the dog or speaks to the dog kindly, in a high pitched or fluctuating voice)	does not occur	occurs between 1 and 5 times	occurs 6 or more times
	'Petting head and body' (handler extends hand to touch or pet the dog anywhere on the head or body)	does not occur	occurs between 1 and 5 times	occurs 6 or more times

527 Table 2. Summary table for the questionnaires filled in by the handlers. Accommodation and 528 playing with the dog are exclusive categories (tick boxes on the questionnaire) and duration of 529 the duty had to be judged by the handler. The bold numbers signify that more border guards 530 kept their dogs at the family house with a garden than policemen, while more policemen kept 531 their dogs in the kennel at the police station than border guards. Also border guards played 532 three times per week with their dogs which was more than policemen, while more policemen 533 played with their dogs daily which was more than border guards. The star (*) shows that 534 border guards spend significantly more time on duty with their dogs than policemen do.

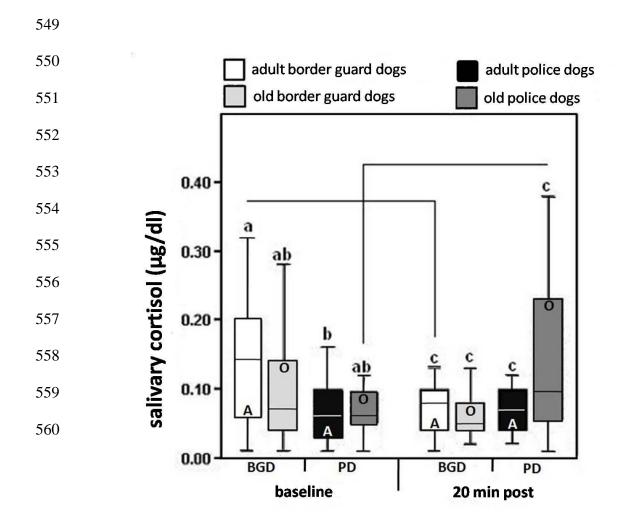
ANSWERS TO THE QUESTIONNAIRE			Border
		Police	Guard
Where is the dog kept (dog's accommodation	ı (%))?		
	block of flats	0	0
	flat with common garden	5.66	3.22
	family house with garden	62.26	96.77
	farm	3.77	0
	kennel at the police station	28.3	0
How many times per week does the handler	play with the dog? (%)		
	never	3.77	3.22
	once/week	11.32	12.9
	three times/week	16.98	38.7
	five times/week	41.5	35.48
	every day	26.41	9.67
How much time is spent with the dog in patro hours/day)	ol duties? (mean±SD	5.87±2.87	9.26±3.83*

Table 3. Statistical results of behavioral variables in the case of police and border guard dogs.
The stars for the significance show that old border guard dogs needed significantly (*) more time to begin playing, than old police dogs; and old border guard dogs needed significantly (**) more time to begin playing than younger border guard dogs. We have not mentioned the results concerning fear and aggression, as fear was not coded in any of the subjects (—), while aggressive behavior was shown by only one subject (—). BGD - border guard dogs; PD - police dogs.

	Statistical results				
Behavioral variables	PD vs.	adult PD vs.	old PD vs.	adult PD	adult BGD vs.
	BGD	adult BGD	old BGD	vs. old PD	old BGD
MOTIVATION	Z=-1.05,	Z=-0.49,	Z=-1.49,	Z=-0.72,	Z=-1.51,
MOTIVATION	p=0.293	p=0.62	p=0.13	p=0.47	p=0.13
PLAYFULNESS	Z=-1.94;	Z=-1.21;	Z=-1.78;	Z=-0.86;	Z=-1.32;
PLAIFULNESS	p=0.052	p=0.22	p=0.07	p=0.39	p=0.18
WILLINGNESS TO	Z=-0.65,	Z=-0.58,	Z=-1.7,	Z=-0.22,	Z=-1.90,
RETRIEVE	p=0.511	p=0.56	p=0.08	p=0.83	p=0.06
POSSESSIVITY	Z=-0.30,	Z=-0.28,	Z=-0.53,	Z=-0.73,	Z=-1.09,
1033E3517111	p=0.75	p=0.77	p=0.59	p=0.47	p=0.27
LATENCY OF	Z=-1.9;	Z=-0.88;	Z=-2.19;	Z=-1.76;	Z=-2.46;
STARTING TO PLAY	p=0.057	p=0.38	p=0.03*	p=0.08	p=0.02**
AGGRESSION		_	_	_	—
FEAR	—	—	—	—	—

Table 4. Statistical results of behavioral variables in the case of policemen (P) and border547 guards (BG).

Behavioral variables	Statistical results		
	Р	BG	
CONTROL COMMANDS	Z=-0.02; p=0.18	Z=-1.66; p=0.09	
SOUND SIGNALS	Z=-1.44; p=0.15	Z=-1.13; p=0.26	
ENTHUSIASM OF HANDLER	Z=-0.32; p=0.75	Z=-1.2; p=0.23	
PRAISING	Z=-0.13; p=0.89	Z=-0.89; p=0.37	
PETTING HEAD AND BODY	Z=-0.99; p=0.32	Z=-1.0; p=0.31	



561 Figure 1. The cortisol concentrations after the test of adult border guard dogs was 562 significantly lower than before, while the cortisol concentration increased significantly in old 563 police dogs (the connecting lines indicate the interactions). Post hoc analysis showed that the 564 baseline cortisol concentrations were significantly higher in adult border guard dogs than in 565 police dogs. Non-transformed data are presented as median \pm quartiles in μ g/dl. Sample size 566 of the subgroups: APD: N=25 (mean age±SD: 5.52±1.44 years); OPD: N=28 (mean age±SD: 567 8.82±0.94 years); ABGD: N=18 (mean age±SD: 4.77±1.47 years); OBGD: N=13 (mean 568 age±SD: 8.61±0.96 years). BGD - border guard dogs; PD - police dogs; A - adult dogs; O -569 old dogs.

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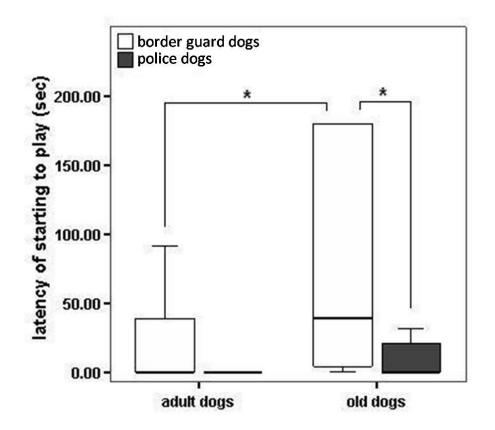


Figure 2. We found significant (*) differences in the latency of starting to play between adult and old dogs within the groups of border guard dogs (\Box white boxes), and between old border guard dogs (\Box white box) and old police dogs (\blacksquare rey filled box), as well. The old border guard dogs needed more time to begin playing. Sample size of the subgroups: APD: N=25 (mean age±SD: 5.52±1.44 years); OPD: N=28 (mean age±SD: 8.82±0.94 years); ABGD: N=18 (mean age±SD: 4.77±1.47 years); OBGD: N=13 (mean age±SD: 8.61±0.96 years). Data are presented as median \pm quartiles and differences are considered statistically significant if p<0.05.

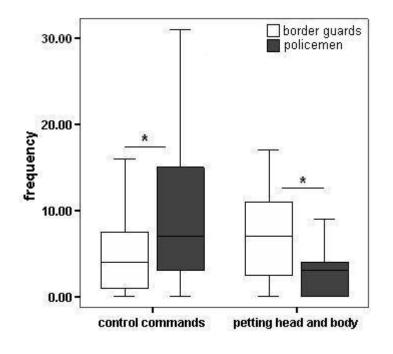




Figure 3. The policemen (grey filled boxes) use significantly (*) more 'control commands' with their dogs (white boxes), while the border guards pet their dogs significantly (*) more. Data are presented as median \pm quartiles and differences are considered statistically significant if p<0.05.