

1 **Effects of petting before a brief separation from the owner on dog behavior and physiology: a pilot**  
2 **study.**

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10

11 **Highlights**

- 12 • Human physical contact can induce positive effects on dogs' behavior and physiology
- 13 • The effects of petting before a brief separation from the owner were evaluated
- 14 • During separation dogs who had been petted displayed calmer behavior

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16

## 17 **Abstract**

18 Human physical contact is known to be effective in decreasing dogs' level of stress, assessed through  
19 endocrine, physiological and behavioral parameters. Gentle touching has been found to be beneficial for  
20 dogs while experiencing or after having experienced a stressful event. The aim of the current study was to  
21 assess if dog behavior and physiology during a brief separation from the owner were modified by being  
22 petted before owner's departure. Ten dogs, not affected by separation-related problems, were tested twice  
23 while separated for three minutes from the owner: before separation, dogs once were petted for 1 minute  
24 and once were not petted. During each test, dog behavior was measured by focal animal sampling and saliva  
25 collected 15 min after separation for cortisol determination. Findings show that, during both procedures,  
26 dogs spent a long time seeking for the owner (median 84.5 and 87.5) and did not seem highly stressed by  
27 separation (low salivary cortisol levels and relatively low stress signals). When dogs were petted before  
28 separation displayed behaviors indicative of calmness for a longer period of time while waiting for the  
29 owner's return ( $Z = -1.955$ ;  $P = 0.049$ ), and their heart rate showed a marked decrease after the test ( $Z = -$   
30  $1.682$ ;  $P = 0.073$ ). This pilot study suggests that petting a dog before a brief separation from the owner may  
31 have a positive effect, making the dog calmer during the separation itself. Further studies are needed to  
32 analyze more in depth its effectiveness, especially in dogs affected by separation anxiety.

33  
34 **Key words:** behavior, dog, gentle touching, separation, owner.

## 36 **Introduction**

37 The dog is a highly social species whose individuals have a pronounced need for social contact with  
38 conspecifics (Tuber et al., 1996), as well as with heterospecifics (vonHoldt et al., 2017). An important  
39 characteristic of dogs' sociability is the ability to form an attachment bond towards their owners (Mariti et  
40 al., 2013). Attachment behaviors are part of the normal repertoire of social species, and they are particularly  
41 displayed when the attachment system is activated, e.g. in case of separation from the attachment figure in  
42 an unfamiliar environment. In fact, this kind of condition is artificially created in the Ainsworth's Strange

43 Situation Test (ASST), a validated test used to assess the attachment bond (Ainsworth and Bell, 1970). Not  
44 only young animals, but also adult domestic dogs (Mariti et al., 2013; Topál, Miklósi, Csányi, & Dóka,  
45 1998), during separation from the caregiver in an unfamiliar environment, usually show behaviors indicative  
46 of discomfort and attempt to regain proximity. Although adult dogs showed distress even when separated  
47 from other dogs living in the same household, their behavior in other episodes of the ASST indicates that the  
48 bond with the owner is stronger than the relationship with any other dog, including their own mother (Mariti  
49 et al., 2017).

50 The display of distress at separation in adult dogs during ASST is not necessarily related to separation  
51 anxiety or other separation-related problems (Parthasarathy and Crowell-Davis, 2006). However,  
52 traditionally, owners whose dogs suffered from separation anxiety were advised to reduce affiliative  
53 behaviors towards their dog (called detachment) and to desensitize dogs to the rituals related to departure  
54 (Pageat, 1999; Horwitz, 2009). This kind of behavior modification protocol has been called into question in  
55 recent years, as it may be in contradiction with the current understanding of the importance of predictability  
56 in the stress response (Overall, 2013; Amat, Camps, Le Brech, & Manteca, 2014). A scientific assessment of  
57 the common practices in behavioral medicine is in fact required, considering the strong impact they have on  
58 dog welfare.

59 Dogs' sociability is also reflected in the positive effects of human physical contact and interaction in  
60 decreasing dogs' level of stress, assessed through endocrine (Coppola et al., 2006; Hennessy et al., 1998)  
61 and behavioral parameters (Shiverdecker et al., 2013). Human physical contact also affect dogs' heart rate  
62 and heart rate variability, and its influence depends on the familiarity and on the area petted (Kuhne et al.,  
63 2014). Until now it has been studied the effect of gentle touching on dogs while experiencing or after having  
64 experienced a stressful event. However, its potential preventative effect has not been investigated yet.

65 The aim of the current study was to assess if dog behavior and physiology during a brief separation from the  
66 owner were affected by being petted before owner's departure.

## 68 **Materials and methods**

69 *Participants*

70 The inclusion criteria for dogs involved in this study were:

- 71 ✓ Being more than 1 year old
- 72 ✓ Not displaying behavioral problems, especially those related to separation (excluded with an  
73 interview performed by a veterinary behaviorist)
- 74 ✓ Being generally healthy and not suffering from travel-related problems
- 75 ✓ Having been lived in the current family for at least 6 months
- 76 ✓ Being used to be stroked daily by the owners
- 77 ✓ Not used to be petted by owners before being left alone.

78 The sample was formed by 10 dogs, 6 females (3 spayed) and 4 males (3 neutered), 1-11 years old ( $5.4 \pm$   
79  $3.4$ ), 7 mixed-breeds, 1 Labrador Retriever, 1 Hovawart, and 1 Chihuahua.

80 Each dog was accompanied by the owner, i.e. one member of the family in which the dog lived. Such person  
81 was required to be widely involved in the management of the dog and, according to the family, he/she was a  
82 person to which the dog was particularly bonded. Owners involved in the test were 4 women and 2 men,  
83  $27.3 \pm 5.4$  years old, all volunteers recruited by personal contacts.

84 Together with the owner, some experimenters were involved in the execution of the tests. Test leader 1 was  
85 a person unfamiliar to the dogs; that role was always played by 25-35 years old women. Test leader 2 was  
86 instead a person that dogs had the opportunity to meet before, and he was in charge of measuring heart rate.  
87 A further unfamiliar person was occasionally involved in assisting the owner in taking saliva samples from  
88 the dog.

89  
90 *Behavioral test*

91 Each dog was tested twice, using two slightly modified versions of the same behavioral test, whose detailed  
92 description is reported below. All the test was executed outdoor, in a dog training center in Pisa (Italy) that  
93 was unfamiliar to the dogs. The setting is shown in figure 1. Two video cameras were used to record the  
94 whole tests, and videos were then observed to analyze dog behavior (see section below).

95 Phase 1: Introductory episode. Owners were asked to maintain their dog on the leash and to slowly walk for  
96 5 minutes in a street just out of the dog training center, in which dogs could explore the environment and  
97 evacuate if necessary. The owner changed the leash of the dog, using a 1.5-meter leash provided by the  
98 experimenters. The same leash was used for all tests.

99 Phase 2: Pre-separation. The owner entered the field through gate 1, holding the dog on the leash, and  
100 reached test leader 1 and test leader 2 in point A (two meters far from the fence). Test leader 2 measured  
101 heart rate through a phonendoscope, then went away passing through gate 1. Dog and owner remained in  
102 point A, one meter far from test leader 1. The first minute spent in this condition represented the difference  
103 between the two modified versions of the test. In the NGT test (No Gentle Touch), the owner spent one  
104 minute talking with test leader 1, without touching the dog nor giving him/her any specific attention, and  
105 maintaining a relaxed, neutral attitude. In the WGT test (With Gentle Touch), the owner spent one minute  
106 petting the dog, 30 seconds per part, from the head to the tail; in the meanwhile, the owner was chatting with  
107 test leader 1.

108 Phase 3: Separation. In order to attract dogs' attention on their departure, owners were asked to say a neutral  
109 word, a word that dogs usually do not know, while the use of specific commands or signs was not allowed.  
110 So, in this phase, the owner gave the leash to the test leader 1, said the chosen word, and walked to gate 2  
111 and then to point B. The latter was located behind a wood shed, so that the owner was not visible to the dog.  
112 The owner spent 3 minutes in point B and then he/she was called by the test leader 1. Test leader 1 had to  
113 maintain a neutral attitude, standing still and leaving the dog the opportunity to move within the distance  
114 allowed by the length of the leash. Test leader 1 could not draw dog's attention; whether the dog was  
115 attempting to socialize with test leader 1, she could not move the dog away and then go back to a neutral,  
116 detached state.

117 Phase 4: Reunion. The owner went back from point B to point A, and took the leash from the test leader 1.  
118 At the reunion with the dog, owners were asked not to seek for dog's attention with words nor gestures; in  
119 case the dog greeted the owner, the latter could calmly greet the dog. Then the owner held the dog on the

120 leash for one minute, maintaining a neutral, relaxed attitude, one meter far from test leader 1 and chatting  
121 with her. After that, the test leader 2 came back and measured again the heart rate of the dog.

122 Phase 5: Post-test. Owner and dog went out of the field. Dogs were leashed in order to avoid excessive  
123 locomotion, eating and drinking.

124 Half of the sample underwent test NGT first, while the other half underwent the WGT first, in order to  
125 reduce a possible order effect. The two tests were carried out 5-9 days apart ( $8.0 \pm 2.7$  days), during  
126 springtime (8<sup>th</sup> April-6<sup>th</sup> May 2014) and in the time range 3:00-6:00 p.m. with a maximum deviation, for  
127 each individual dog, of 30 minutes between the two tests.

128

### 129 *Physiological and behavioral parameters*

130 Fifteen minutes after the end of separation (Mongillo et al., 2013), owners were asked to take a saliva  
131 sample from the dog using Salivette<sup>®</sup> cortisol. In case of need, owners were helped by an assistant who  
132 showed the owners how to use it. The swab was gently put under the dog's tongue and in the cheek pouches  
133 for 60 s. Then the cotton roll was placed back in the labelled Salivette tube, placed on ice and brought to the  
134 laboratory within 1 h from the collection. In the ETOVET laboratory at the University of Pisa (Italy), the  
135 Salivette<sup>®</sup> samples were centrifuged at 3000 rpm for 25 minutes and the saliva obtained was stocked at -  
136 20°C. Once thawed, saliva cortisol was measured using a Diametra<sup>®</sup> Cortisol Enzyme Immunoassay Kit  
137 according to the manufacturer's instructions. Due to possible interferences with the procedure, dogs were not  
138 allowed to get any food in the 3 hours preceding the tests, and they could not drink in the 20 minutes  
139 preceding the procedure.

140 The behavior of dogs during the 3-minute separation episode (phase 3) was analyzed through a continuous  
141 sampling method. Videos were analyzed using Adobe Premiere Pro CC<sup>®</sup>, in order to measure the duration  
142 (in seconds) of each behavior reported in the synopsis (table 1). According to their meaning, behaviors were  
143 grouped into behavioral categories.

144

### 145 *Statistical analysis*

146 The values of saliva cortisol and the duration of behavioral categories in the two tests were compared using  
147 the Wilcoxon test ( $P < 0.05$ ) on SPSS<sup>®</sup> 17.0. The same statistical test was used to compare heart rate before  
148 and after each behavioral test.

149

## 150 **Results**

151 Results are summarized in table 2.

152 Heart rate did not show any changes when comparing before and after NGT test, whilst it showed a marked  
153 decrease after WGT test. Saliva cortisol after the two tests did not differ.

154 Dogs showed behaviors indicative of calm for statistically longer durations during WGT test. The other  
155 behavioral categories were not displayed for statistically different durations during the WGT and NGT  
156 versions of the test.

157

## 158 **Discussion**

159 The aim of this study was to investigate the possible preventative effect of gentle touching in reducing stress  
160 in dogs undergoing a brief separation from the owner. Although findings cannot be considered definitive,  
161 and their interpretation should be cautious due to the novelty of the study and to the limited number of dogs  
162 involved, the strict inclusion criteria and the use of dogs as their own control allow us to provide some  
163 tentative explanations.

164 Social isolation has been shown to be one of the most reliable and potent stimuli for producing a stress  
165 response, and it is widely used as an experimental model for inducing stress, e.g. in the Ainsworth Strange  
166 Situation Test. The protocol of this study can be regarded as a simplified version of this test. As expected  
167 from previous research, in the current study dogs displayed some stress signals and vocalizations,  
168 confirming that being separated from the owner represented a mildly stressful event. In addition, dogs  
169 showed a clear preferential attention towards their owner, spending most of the time during separation  
170 oriented towards the place where they saw the owner leaving (Mariti et al., 2013; Mongillo et al., 2010).  
171 Dogs instead spent short time oriented to the stranger (similarly to Mongillo, Bono, Regolin, & Marinelli,



2010). However, none of these behaviors resulted to be differently displayed by dogs when tested in the gentle touching versus no gentle touching condition. In the same way, salivary cortisol levels after separation did not differ according to the treatment received before the owner left. However, it must be noted that in both cases dogs showed low levels of cortisol (Cobb et al., 2016), suggesting that separation from the owner did not result in a high activation of the hypothalamus-pituitary-adrenal cortex system, as expected during a brief separation from the owner in dogs not affected by separation anxiety. This is not in contrast with studies measuring cortisol after ASST, in which salivary cortisol levels were increased (Mongillo et al., 2013), because the ASST consists in a more active (play) and stressful (two separations) procedure. It is, therefore, possible that a longer period of separation would have led to different results.

As for the display of stress-related behaviors during separation, the number seems to be relatively low. However, a valid evaluation of their intensity is hard, as no data are available on a possible range of normality. A comparison with other studies that investigated the display of some of the stress signals analyzed in the current study reveals that their frequency was comparable to that of dogs before and after a session of animal-assisted intervention (Pirrone et al., 2017), lower than that of rescue dogs during a search operation (Diverio et al., 2016) and apparently higher than that of dogs left home alone (Scaglia et al., 2013), although in the latter fewer behaviors were analyzed and for a longer time.

The most remarkable finding of the current study is represented by the longer time spent by dogs in calm behaviors if petted before being separated from the owner. This result was confirmed by a marked decrease in heart rate in such experimental condition, although the trend did not reach a statistically significant difference. Such findings are in agreement with previous studies showing that positive social interactions, through oxytocin release, may dampen stress responses and increase relaxation (DeVries et al., 2003; Uvnäs-Moberg, 1998).

Most studies using behavioral and physiological measures have investigated the effects on dogs of stressful situations. A lower number of studies have focused on pleasant conditions. For instance, endogenous oxytocin levels increase in dogs after they engage in affiliation with conspecific partners, indicating a stimulation of the oxytocin system during social interactions (Romero et al., 2014). In domestic dogs, the

198 same stimulation seems to be the result of affiliative interactions with people; e.g. it has been found that a  
199 calm and positive interaction, such as the dog being stroked by a human, induces a decrease in cortisol levels  
200 (Tuber et al., 1996) and blood pressure (Odendaal and Meintjes, 2003), while oxytocin is increased (Handlin  
201 et al., 2011; Odendaal and Meintjes, 2003). Rehn et al., 2014 found that the mere return of a familiar person  
202 after separation had a positive effect on oxytocin levels and induced contact-seeking behavior in dogs,  
203 whereas physical contact was necessary in order to induce a sustained increase in oxytocin levels and to  
204 decrease cortisol levels in the period following reunion. The two findings are strongly linked one to the  
205 other, since the excretion of oxytocin can inhibit HPA responses and hence lower cortisol release (Neumann  
206 et al., 2000). In the current study the dogs' cortisol levels after the two treatments, being or not being gently  
207 touched before separation, did not differ. The discrepancy with the findings of (Rehn et al., 2014) may be  
208 due to the lower length of the physical contact (one versus four minutes), as well as to other factors, e.g. the  
209 different experimental conditions (reunion versus before separation).

210 Rehn et al., (2014) also found that dogs who were touched by their owners at reunion showed elevated levels  
211 of oxytocin even after the interaction had ended and a decreasing curve of cortisol levels throughout the test.  
212 However, this decrease was most pronounced if, at the reunion, owners greeted the dog with a combination  
213 of physical and verbal contact in a calm and friendly way. The owner attentional state involved in petting the  
214 dog may also play a role, since it has been shown to promote successful coping styles in dogs (Diverio et al.,  
215 2017). Future studies should assess whether a longer time spent stroking the dog before separation and/or  
216 the combination of gentle physical, verbal and gazing contact before separation have a higher calming effect  
217 on dogs.

218 Summarizing, based on the findings of the current study, it seems that dogs during the procedure were  
219 waiting for the owner return (long time spent seeking for the owner), they were not highly stressed by  
220 separation (low salivary cortisol levels and relatively low stress signals), and being petted before separation  
221 made them calmer while waiting for the owner's return.

222 The restricted number of dogs assessed in the current study limits the possibility to generalize the findings to  
223 the whole canine population. For instance, dogs with specific characteristics, such as old age, are likely to

224 show a physiological and behavioral response to separation that differs from the response of younger  
225 individuals (Mongillo et al., 2013). At the same time, the inclusion of dogs with separation-related problems,  
226 especially separation anxiety, may have led to very different results. However, gently touching dogs with  
227 separation anxiety before being left alone may be taken into account in a behavior modification protocol that  
228 attempts to put the dog in a positive emotional state. In addition, if petting would become part of a departure  
229 ritual (positive discriminative stimulus), it may increase the predictability and therefore the dog's perceived  
230 control over the environment that, as suggested by Amat et al. (2014), is crucial when treating an anxious  
231 subject. Finally, stroking the dog may be regarded as a feature of owners' warmth, which is known to have  
232 beneficial effects on the dog-owner relationship in threatening situations (Cimarelli et al., 2016). The use of  
233 petting should therefore be encouraged both in preventative and therapeutic conditions.

## 234

### 235 **Conclusions**

236 This pilot study suggests that petting a dog before a brief separation from the owner may have a positive  
237 effect, making the dog calmer during the separation itself. Further studies are needed to analyze more in  
238 depth its effectiveness, especially in dogs affected by separation anxiety.

### 239

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### 243

### 244 **Authorship statement**

245 The idea for the article was conceived by C. Mariti and B. Carlone. The experiments were designed by C.  
246 Mariti and A. Gazzano. The experiments were performed by C. Mariti, M. Protti and B. Carlone. The data  
247 were analyzed by C. Mariti and A. Gazzano. The article was written by C. Mariti and S. Diverio. All authors  
248 have worked in the interpretation of data and have approved the final article.

250

251 **Conflict of interest**

252 The authors declare that they have no conflict of interest.

253

254 **Ethical considerations**

255 All procedures were performed in full accordance with the Directive 2010/63/EU of the European  
256 Parliament and of the Council of 22/09/2010 on the protection of animals used for scientific purposes and  
257 conformed to the “Guidelines for the treatment of animals in behavioral research and teaching” (Behaviour,  
258 2016). No special permission for this study was needed, being observational and non-invasive in nature.

259

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348 **Tab. 1:** Dog behaviors analyzed by focal animal sampling during separation from the owner and their  
 349 categorization.

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<b>Behavioral categories</b>	<b>Behaviors</b>	<b>References</b>
Stress signals	Nose licking	Beerda et al., 1998; 1997
	Body shaking	Beerda et al., 1999
	Yawning	Beerda et al., 1998
	Nosing sniffing the ground for <3 sec	Beerda et al., 1998
	Paw lifting	Beerda et al., 1998, 1997
Calmness	Lying down	Beerda et al., 1999
	Exploration sniffing the ground for $\geq 3$ sec	Modified from Mariti et al., 2013
Vocalizations	Yelping	Beerda et al., 1997
	Barking	Beerda et al., 1998
Social behaviors towards the stranger	Contact seeking towards stranger	Mariti et al., 2017
	Sniffing the stranger	
	Looking at the stranger	Mongillo et al., 2010
Social behaviors towards the owner	Sniffing in the direction where the owner left	
	Looking at the direction where the owner left	Modified from Mongillo et al., 2010
	Sniffing where the owner was hidden	
	Looking where the owner was hidden	Modified from Mongillo et al., 2010

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**Tab. 2:** Data of the physiological and behavioral parameters analyzed in both tests (NGT = No Gentle Touch test; WGT = With Gentle Touch test) and statistical results (\*:  $P < 0.05$ ; +:  $P < 0.10$ ).

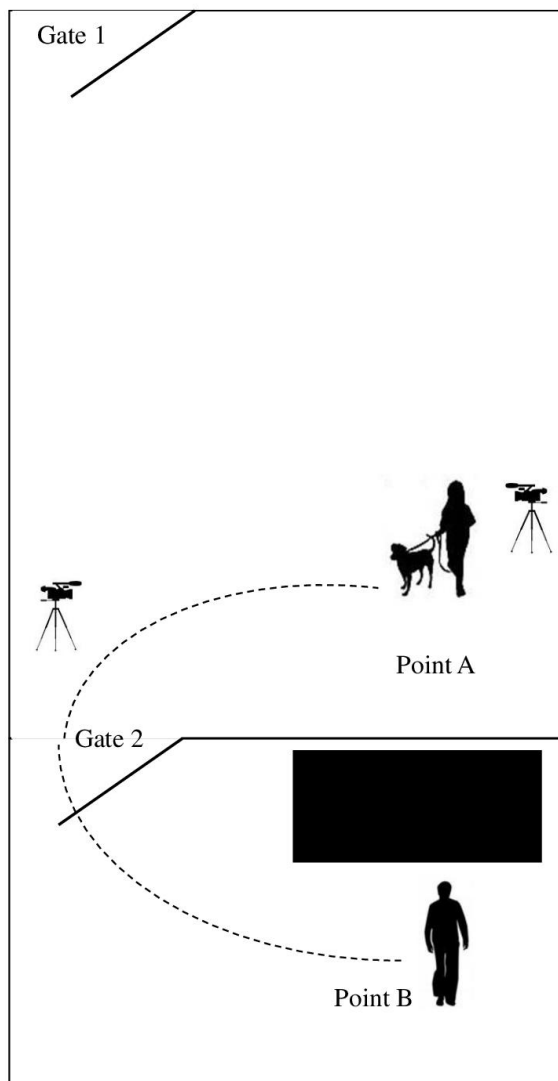
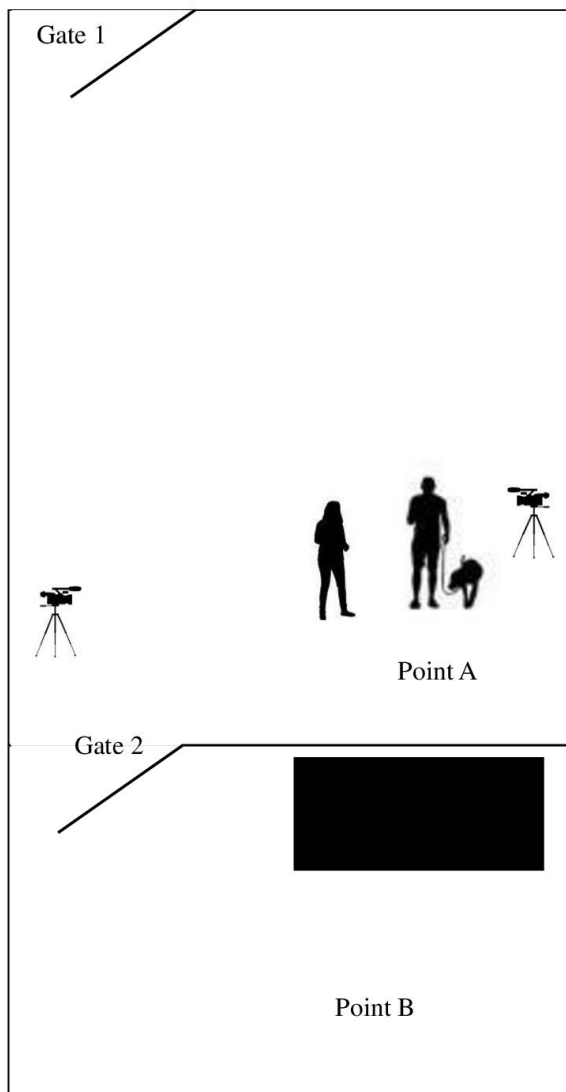
<b>Variables (Units)</b>		<b>Min-max</b>	<b>Median</b>	<b>Statistical values</b>
Heart rate (Bpm)	before NGT	95-130	99.5	$Z = -0.314$ ; $P = 0.753$
	after NGT	96-115	105.0	
	before WGT	90-133	115.5	$Z = -1.682$ ; $P = 0.073^+$
	after WGT	82-126	110.0	
Cortisol ( $\mu\text{g/dL}$ )	after NGT	0.2-2.7	1.0	$Z = -1.125$ ; $P = 0.260$
	after WGT	0.4-0.8	0.9	
Stress signals (sec)	during NGT	0-26	10.0	$Z = -0.665$ ; $P = 0.506$
	during WGT	1-20	7.0	
Calmness (sec)	during NGT	0-79	11.5	$Z = -1.955$ ; $P = 0.049^*$
	during WGT	0-149	38.0	
Vocalizations (sec)	during NGT	0-121	18.0	$Z = -0.676$ ; $P = 0.499$
	during WGT	0-111	26.5	
Behaviors to stranger (sec)	during NGT	0-14	3.0	$Z = -1.174$ ; $P = 0.241$
	during WGT	0-19	7.0	
Seeking owner (sec)	during NGT	0-142	87.5	$Z = -0.510$ ; $P = 0.610$
	during WGT	5-122	84.5	

358 **Fig. 1:** Setting of the test. Fig. 1a shows the disposition of dog, owner and test leader 1 in the pre-separation  
359 and upon reunion episode. In fig. 2b the dashed line represents the path covered by the owner passing from  
360 point A (where the dog was left with test leader 1) to point B (where the owner was hidden behind a shed  
361 and not visible by the dog) and back.  
362

363

fig.1a

fig.2b



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