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# Behaviors expressed by whippets and Italian greyhounds when in two positive emotional states, as perceived by Italian caretakers

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**Abstract:** Given the importance of scientifically investigating signs of good welfare and positive emotional states in dogs, the present pilot study aimed to investigate behaviors expressed by whippets and Italian greyhounds when in two positive emotional states, as perceived by their caretakers. A dedicated questionnaire was developed and uploaded on a digital platform after pre-testing. The questionnaire included a section asking whether the respondents thought that there any postures or expressions signaling when their dogs were “happy and excited” or “happy and relaxed”. In case of a positive answer, respondents were asked how often their dog showed 13 behaviors (e.g., tail wagging, running up and forth, bowing, spinning, jumping on the caretaker) when in each of the two positive emotional states, and whether there were other possible behavioral signs of those two states. The survey yielded answers for 329 dogs (165 whippets, 162 Italian greyhounds, two whippet mixes) given by a convenience sample of 266 respondents. Among the tested 13 behaviors, the only one reported to happen at least often in more than 90% of the dogs was tail wagging when “happy and excited”. Most of the investigated behaviors were expressed more often when the dog was “happy and excited” than when “happy and relaxed” (e.g., tail wagging, spinning), and some were expressed with a different frequency in the two breeds (e.g., Italian greyhounds bowed more often than whippets when “happy and excited”). It is concluded that further studies are needed to identify possible behavioral correlates of different positive emotional states in different types/breeds of dogs.

**Key Words:** positive welfare, sighthounds, tail-wagging, arousal, individual differences.

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## Introduction

The welfare of animals is important from both a scientific and an ethical point of view. Therefore much research has been devoted to it. However, up to now, such research has focussed more on signs of a negative welfare status than on those of a positive one, although the importance of the latter has been widely recognized (Boissy et al., 2007; Csoltova & Mehinagic, 2020; Mellor & Beausoleil, 2015). The aforementioned general trend can be found also concerning an intensively researched upon species, such as the domestic dog (*Canis familiaris*), (Csoltova & Mehinagic, 2020). Most studies have investigated indicators of compromised dogs' welfare (e.g., Beerda et al., 1997, 1998, 2000; Rooney et al., 2007; Mariti & Bein, 2015; Mariti et al., 2015), whereas few have tried to investigate correlates of good welfare and positive emotional states (e.g., Gygax et al., 2015; for a review, please see Csoltova & Mehinagic, 2020). One of the problems of the study of emotions in animals is the low specificity of the behaviors, and the dog is no exception in this regard, most canine behaviors having been found to have multiple meaning depending on the context (Csoltova & Mehinagic, 2020; Rehn & Keeling, 2011; Westerback, 2011). The dog,

however, also presents a further, more specific problem: the great variety of breeds, differing not only in morphology but also in behavior (e.g., Goodwin et al, 1997; Turcsán et al., 2011). In this regard, a group of breeds that have received clinical and scientific attention mainly due to their (perceived) differences from other dogs is that of the sighthound (e.g., Wagner & Ruf, 2019). Although the FCI (Federation Cynologique Internationale) and KC (Kennel Club) recognized sighthound breeds were codified mainly in the 19<sup>th</sup> century, sighthounds are considered ancient breeds because depictions of sighthound-like dogs can be found as far as 6000 BC (Bartel et al., 2007). Sighthounds have been selected to hunt by sight and chase their prey relying on their speed (Bartel et al., 2007). They are known to differ both physiologically (e.g., Mesa-Sánchez et al., 2016; Zaldívar-López et al., 2011) and, to some extent, behavior-wise (Elliot et al., 2010; Normando et al., 2021), from other breeds, although most of the studies have been done on specific subpopulations of sighthounds, such as rescued ex-racer greyhounds (Elliot et al., 2010; Thomas et al., 2017; Zaldívar-López et al., 2011).

Hence, this explorative pilot study aimed to investigate behaviors expressed by two genetically near (Parker et al., 2017) sighthound breeds, whippets, and (miniature) Italian greyhounds, when in two positive emotional states, differing in arousal level, as perceived by their caretakers. Our hypotheses were that:

1. some of the listed behavior was going to be reported as happening “always” in the vast (i.e., 90%) majority of the dogs in the specified positive state, thus being a promising candidate to being a behavioral correlate of that positive emotional state;
2. the frequency of expression of most of the listed behaviors would be different between the two states, thus highlighting the importance of the arousal component;
3. there would be differences in the frequency of expression of at least some of the listed behaviors in the specified emotional state between the two breeds, morphologic and genetic similarity notwithstanding.

## Materials and methods

### Survey

The results detailed in the present paper are part of a larger survey on the behavior of the smaller two of the sighthounds breeds: whippets and (miniature) Italian greyhounds (Contalbrigo et al., 2020). Based on the literature, the topic, and the authors' experience, a dedicated questionnaire was developed for the survey. The survey targeted whippets' and Italian greyhounds' caretakers, although the question about the breed of the dog also included “other, please specify”, for control reasons. Apart from dogs' and caretakers' demographics, the part of the survey presented here concerns possible behavioral correlates of positive emotional states in the dogs as perceived by their caretakers. In the first set of questions of that part, the respondents were asked whether they thought their whippet or Italian greyhound had any postures or expressions signaling when they were happy and excited. The possible answers were “yes”, “no”, or “I do not know”. If the respondent selected “yes”, then (s)he was asked how often his/her dog was showing each of 13 behaviors when “happy and excited”. The behaviors were (in the order they were asked): tail wagging, bowing by flexing the front legs, jumping on the respondent, eyes wide open, eyes half closed, spinning, rolling on the ground, running back and forth without any apparent goal (running crazy/aimlessly), smiling (lips relaxed and teeth showing), ears pricked/straightened, ears laid back, sighing, smiling (lips relaxed, but teeth not showing). The possible answers were “always”, “often”, “sometimes”, “rarely”, “never”, and “I do not know”. A further question asked whether the respondent thought that his/her dog had other postures or expressions signaling when the dog was happy and excited. In case of a positive answer, the respondent was asked to specify which. Also, the latter two questions were asked only to respondents having answered “yes” to their dog having behaviors signal-

ing that they were happy and excited. The second set of questions was identical to the first, but the situation it referred to was “happy and relaxed” instead of “happy and excited”. The decision to ask separately about the two emotionally positive states differing in arousal was done mainly on the basis of the literature finding different behavioral responses to stimulus maintaining the same emotionally positive valence, depending on the dog getting used to it (i.e., on decreased arousal; Gygas et al., 2015), and that owners could recognise the difference (Buckland et al., 2014). Given the preliminary and exploratory nature of the present study, the choice of the behaviors to be included was done partially based on the published literature (Buckland et al., 2014; Csoltova & Mehinagic, 2020) and partially based on the authors’ experience with sighthounds. For example, based on their experience, authors deemed running back and forth “as crazy” (aimlessly) was an example of locomotor play in this breeds. As play has been described in the literature as related to positive emotional states (Csoltova & Mehinagic, 2020) the behavior was included among those investigated.

Once pre-tested for clarity of the questions, the questionnaire was uploaded on the platform “Limesurvey” (LimeSurvey GmbH, Hamburg, Germany) and tested for the functioning of the electronic version. The survey was then publicized via word of mouth, breed clubs, and social media (i.e., Facebook groups dedicated to whippets and Italian greyhounds), using a virtual snowball sample technique.

### *Data analysis*

Descriptive statistics were done on how many dogs the caretaker answered that there were behavioral signs of the two positive emotional states, how many other behaviors were suggested, and how often dogs were reported as showing each behavior in each state.

In order to investigate differences between the two breeds in the number of dogs reported as showing each behavior in each situation with different frequency, three levels of frequency were created: always, often, and less than often (i.e., the sum of sometimes, rarely and never). Then the Freeman-Halton extension of Fisher’s exact test (Freeman & Halton; 1951; Soper, 2020) was used to assess possible differences between the two breeds.

Wilcoxon rank tests were run to investigate whether there were differences among the two states (i.e., “happy and excited” vs “happy and relaxed”) in how often the dogs were reported showing a specific behavior. In order to run the Wilcoxon rank tests on the reported frequency of the behaviors in the two states, the answers were converted into a numeric scale, from “never” = 1 to “always” = 5, whereas “I do not know” was considered a missing answer. For those behaviors differing in frequency between breeds in at least one of the two states, the statistical analysis was repeated also breed by breed.

## Results and Discussion

The survey yielded viable answers for 329 dogs (165 whippets, 162 Italian greyhounds, one whippet x Italian greyhound and one whippet x greyhound) given by a convenience sample of 266 respondents (216 female, 49 male; mean age  $\pm$  SD = 45.5  $\pm$  11.0 years). For three dogs (two whippets and one Italian greyhound), the respondents failed to identify behaviors expressed by their dogs both when “happy and relaxed” and when “happy and excited” (i.e., they answered that there were no signs or that they did not know). The three cases were eliminated from the analyses. For two dogs the respondent answered that the dog showed behaviors signalling when he/she was “happy and relaxed” but not/they did not know when “happy and excited”. For 40 the reverse was true.

Among the tested 13 behaviors, none was reported as always happening in the specified emotional state in the vast majority of the dogs. However, tail wagging when “happy and excited” was reported as always happening at least in the majority (>50%) of the dogs, and as happening at least

often in more than the 90% of the dogs. Tail wagging was described by around 30% of caretakers as a sign of a “happy and excited” dog in Buckland et al. (2014), with another almost 25% indicating wagging fast as a sign of the state. An increase in tail wagging was found during palatable treat offering (Travain et al., 2016), when expecting a food treat (McGowan et al., 2014), and during reunion after separation from the caretaker (Rehn & Keeling, 2011) especially when vocal, but not tactile, interaction was enacted (Rehn et al., 2014).

The results in terms of the percentage of dogs reported as showing the behavior always, often or less than often, when in that particular positive state (“happy and relaxed” or “happy and excited”), are summarised in table 1. The percentage is calculated on the total number of dogs whose caretakers answered there were behavioral correlates of that state (i.e., 324 for “happy and excited”, 286 for “happy and relaxed”). Please note that the answers related to the two “longdogs” (i.e., crosses between two sighthound breeds) were only included in the overall columns of table 1, and then removed from any further analyses. Although “ears pricked” was one the most often described correlate of dogs being “happy and excited” in Buckland et al. (2014), in the present study almost half of the dogs, especially Italian greyhounds, were reported to show it less than often when “happy and excited”. This difference could be due to many factors, including differences in the method of the study (open questions in Buckland et al. (2014), closed questions with a frequency Likert scale in the present study), type of dog (any in Buckland et al. (2014), only two sighthound breeds in the present study) or language of the survey. Another often described behavior in in Buckland et al. (2014) was carrying the head high when “happy and excited”, but in present study it was reported (in the open question about the other behaviors the dog could show when “happy and excited”) only by one respondent.

As for our second hypothesis, there were significant differences between the two breeds, and they are reported in table 1.

**Table 1.** Percentage of dogs reported as always or often or less than often (i.e., never, rarely, sometimes) showing the listed behavior when in the specified positive emotional state. A stands for “always”; O for “often”; L for “less than often”, \* marks behaviors differing between the two breeds at  $p < 0.05$ ; \*\* at  $p < 0.01$ ; \*\*\* at  $p < 0.001$ . When the sum of A+O+L is less than 100%, it is because of respondents selecting the answer “I do not know” for that behavior.

Happy and excited									
Sample	Italian Greyhound			Whippet			Overall		
Behavior	A	O	L	A	O	L	A	O	L
Bowing*	21.25	39.38	39.38	13.58	31.48	53.70	17.59	35.19	46.60
Ears back	15.00	32.50	48.75	16.67	32.10	49.38	16.05	32.41	48.77
Ears pricked*	11.88	35.63	49.38	16.67	45.68	35.19	14.20	40.74	42.28
Eyes half closed	6.25	27.50	60.00	6.79	22.22	64.20	6.48	24.69	62.35
Eyes wide open	11.88	24.38	54.38	16.67	30.25	48.15	14.81	27.16	50.93
Jumping on***	55.63	28.75	15.63	27.16	33.95	38.89	41.36	31.48	27.16
Rolling on the ground*	3.13	10.63	86.25	6.79	18.52	74.69	4.94	14.81	80.25
Running aimlessly	30.00	32.50	36.88	20.99	41.36	37.65	25.93	36.73	37.04
Sighing	1.88	15.63	76.25	4.32	12.35	82.10	3.09	13.89	79.32
Smiling (teeth not visible)	6.88	19.38	65.00	7.41	19.14	69.75	7.10	19.75	66.98
Smiling (teeth visible)**	3.75	21.88	68.13	7.41	10.49	77.16	5.56	16.36	72.53
Spinning*	5.00	11.88	82.50	1.85	22.84	74.69	3.70	17.28	78.40
Tail wagging*	68.13	28.13	3.75	58.64	29.63	10.49	63.27	29.01	7.10

Happy and relaxed									
Sample	Italian Greyhound			Whippet			Overall		
Behavior	A	O	L	A	O	L	A	O	L
Bowing*	6.90	46.21	46.90	8.63	30.22	60.43	8.04	38.11	53.50
Ears back	13.79	29.66	53.79	14.39	34.53	48.92	13.99	31.82	51.75
Ears pricked	5.52	20.69	70.34	6.47	25.90	67.63	5.94	23.08	69.23
Eyes half closed	12.41	35.86	47.59	11.51	41.73	43.88	12.59	38.46	45.45
Eyes wide open	4.14	17.24	71.03	4.32	15.11	76.98	4.20	16.08	74.13
Jumping on**	26.21	26.90	45.52	13.67	23.02	63.31	19.93	24.83	54.55
Rolling on the ground**	2.76	7.59	89.66	5.04	19.42	75.54	3.85	13.29	82.87
Running aimlessly	8.97	26.90	63.45	12.23	20.86	66.91	10.49	23.78	65.38
Sighing	4.14	26.21	68.28	5.04	24.46	69.06	4.55	25.87	68.18
Smiling (teeth not visible)	4.14	24.14	64.14	6.47	17.27	71.22	5.59	20.63	67.48
Smiling (teeth visible)	2.07	17.24	75.17	2.88	15.11	78.42	2.45	16.43	76.57
Spinning	2.07	8.28	88.97	3.60	12.95	82.73	2.80	10.49	86.01
Tail wagging	37.24	38.62	23.45	29.50	42.45	27.34	33.57	40.56	25.17

There were differences between the two states (i.e., “happy and relaxed” vs “happy and excited”) in how often the dogs were reported to show a behavior, as for our third hypothesis, and they are shown in table 2. Most of the investigated behaviors were expressed more often when the dog was “happy and excited” than when “happy and relaxed”.

**Table 2.** Differences in the frequency the dogs are reported as showing the behavior depending on the investigated situation (i.e., “happy and excited” versus “happy and relaxed”)

Behavior	Sample	Z	p	Direction of the difference
Bowing	Whippets + Italian Greyhounds	3.97	<0.001	excited>relaxed
	Whippets	2.15	0.032	excited>relaxed
	Italian Greyhounds	3.45	0.001	excited>relaxed
Ears back	Whippets + Italian Greyhounds	0.88	0.381	n. s.
	Whippets	6.47	<0.001	excited>relaxed
	Italian Greyhounds	5.77	<0.001	excited>relaxed
Ears pricked	Whippets + Italian Greyhounds	8.67	<0.001	excited>relaxed
	Whippets	6.47	<0.001	excited>relaxed
	Italian Greyhounds	5.77	<0.001	excited>relaxed
Eyes half closed	Whippets + Italian Greyhounds	5.63	<0.001	relaxed>excited
	Whippets + Italian Greyhounds	8.30	<0.001	excited>relaxed
	Whippets + Italian Greyhounds	9.83	<0.001	excited>relaxed
Jumping on	Whippets + Italian Greyhounds	9.83	<0.001	excited>relaxed
	Whippets	6.77	<0.001	excited>relaxed
	Italian Greyhounds	7.14	<0.001	excited>relaxed
Rolling on the ground	Whippets + Italian Greyhounds	0.79	0.432	n. s.
	Whippets	0.96	0.338	n. s.
	Italian Greyhounds	0.07	0.943	n. s.
Running aimlessly	Whippets + Italian Greyhounds	9.93	<0.001	excited>relaxed
	Whippets + Italian Greyhounds	5.58	<0.001	relaxed>excited
	Whippets + Italian Greyhounds	0.80	0.422	n. s.
Smiling (teeth visible)	Whippets + Italian Greyhounds	2.99	0.003	excited>relaxed

	Whippets	1.44	0.151	n. s.
	Italian Greyhounds	2.74	0.006	excited>relaxed
Spinning	Whippets + Italian Greyhounds	5.07	<0.001	excited>relaxed
	Whippets	3.25	0.001	excited>relaxed
	Italian Greyhounds	3.90	<0.001	excited>relaxed
Tail wagging	Whippets + Italian Greyhounds	8.33	<0.001	excited>relaxed
	Whippets	6.15	<0.001	excited>relaxed
	Italian Greyhounds	5.65	<0.001	excited>relaxed

The majority of the respondents (229 for “happy and excited”; 222 for “happy and relaxed”) did not suggest other behavioral signs of the investigated positive emotional states. The most often suggested other behaviors in case of the dog being “happy and excited” were: vocalizations (e.g., barking, howling, whining), jumping (repeatedly) into the air, licking/kissing the caretaker, hugging the caretaker, carrying the tail high, taking a toy in the mouth and either running around or taking it to the caretaker, mouth wide open, wriggling one’s butt, touching the caretaker with a paw. The most often suggested other behaviors in case of the dog being “happy and relaxed” were: roaching (Figure 1, i.e., lying in a relaxed position on a soft surface on the back - or partially on the back, with the legs usually extended, often in the air), stretching, seeking contact, lying down in lateral recumbence. Overall more extra behaviors were suggested for “happy and excited” than for “happy and relaxed”, agreeing with the findings of Buckland et al. (2014), who found that “caretakers described happy and excited” states in more detail than “happy and relaxed” ones, suggesting the former may be less well understood or difficult to interpret.



**Figure 1.** A whippet “roaching” and “smiling” (teeth visible).



## Conclusions

The present study is a first attempt at investigating possible behavioral correlates of positive emotional states in two sighthound breeds. However, no behavior appeared to be always shown by the vast majority (90%) of the dogs in any of the two investigated states, and only tail wagging when “happy and excited” was reported to happen at least often in more than the 90% of the dogs, suggesting great individual differences in the expression of positive emotional states in this species, at least in the perception of the caretakers. Differences between the two breeds and between “happy and excited” and “happy and relaxed” states were evident as well. Further studies are needed to identify possible behavioral correlates of different positive emotional states in different types/breeds of dogs.

## Conflicts of interest

All authors report no conflicts of interest: none of the authors has any financial or personal relationships that could inappropriately influence or bias the paper’s content.

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## Il comportamento di whippet e levrieri italiani percepito dai caretaker italiani in due stati emozionali positivi

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### *Sintesi*

Data l'importanza di indagare scientificamente i possibili correlati comportamentali di stati emotivi positivi e di benessere positivo nel cane, col presente studio pilota ci si è prefissi di indagare i comportamenti che, secondo la percezione di chi si occupa di cani stessi, vengono espressi dai whippet e dai piccoli levrieri italiani in due stati emotivi positivi. Per l'indagine, è stato sviluppato un questionario *ad hoc*, che è stato caricato su una piattaforma digitale dopo i test preliminari. Nel questionario, una sezione chiedeva se, secondo gli intervistati, ci fossero posture o espressioni che segnalassero quando i loro cani erano "felici ed eccitati" o "felici e rilassati". In caso di risposta positiva, agli intervistati veniva chiesto quanto spesso il loro cane mostrasse 13 comportamenti (ad esempio, scodinzolare, correre avanti e indietro all'impazzata, inchinarsi, girare su se stesso, saltare addosso) quando si trovava in ciascuno dei due stati emotivi positivi, e se ci fossero altri possibili segnali comportamentali di questi due stati. Il sondaggio ha ottenuto risposte per 329 cani (165 whippet, 162 levrieri italiani, due mix whippet) fornite da un campione di convenienza di 266 intervistati. Tra i 13 comportamenti indagati, l'unico che è stato segnalato accadere almeno "spesso" in più del 90% dei cani è stato scodinzolare quando "felice ed eccitato". La maggior parte dei comportamenti proposti sono stati riportati accadere più spesso quando il cane è "felice ed eccitato" che quando è "felice e rilassato" (ad esempio, scodinzolare, girare), e alcuni sono riportati con una frequenza diversa nelle due razze (ad esempio, i piccoli levrieri italiani si inchinano più spesso dei whippet quando sono "felici ed eccitati"). Si conclude che sono necessari ulteriori studi per identificare possibili correlati comportamentali di diversi stati emotivi positivi in diversi tipi/razze di cani.





# Tryptophan, serotonin and dog behavior

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*Abstract:* Serotonin, also known as 5-hydroxytryptamine (5-HT), has been firstly isolated and described by Maurice Rapport and colleagues, after decades of research on a vasoconstrictor substance suspected of being contained in platelets. After its discovery, several studies have been done to identify its location and function and 5-HT was quickly identified in many tissues, including the brain, lungs, kidneys, platelets and gastrointestinal tract. In addition to its role in platelet function, Brodie & Shore (1957), firstly proposed a role of 5-HT as a neurotransmitter, based on the localization of 5-HT receptors in specific areas of the vertebrate brain. The link between TRP metabolism, serotonin and behavior has been the subject of considerable interest and discussion in the dog. The use of TRP in the behavioral clinic of the dog is still controversial. While there is evidence of efficacy of products containing TRP and other substances during anxiety syndrome and stress, the use of only TRP in the control of canine aggression has given, until now, inconclusive results.

*Key Words:* dog, behavior, serotonin, L-tryptophan.

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## Introduction

Serotonin, also known as 5-hydroxytryptamine (5-HT), has been firstly isolated and described by Maurice Rapport and colleagues (Rapport et al., 1948), after decades of research on a vasoconstrictor substance suspected of being contained in platelets (Reid & Brick, 1942; Zucker, 1944). After its discovery, several studies have been done to identify its location and function and 5-HT was quickly identified in many tissues, including the brain, lungs, kidneys, platelets and gastrointestinal tract. In addition to its role in platelet function, Brodie & Shore (1957), firstly proposed a role of 5-HT as a neurotransmitter, based on the localization of 5-HT receptors in specific areas of the vertebrate brain (Twarog & Page, 1952; Amin et al., 1954).

Serotonin is produced in two phases. In the first phase, the essential amino acid L-tryptophan (TRP) is hydroxylated by the enzyme tryptophan hydroxylase (TPH) to 5-hydroxytryptophan (5-HTP).

Unlike other amino acids, TRP circulates in the blood and plasma mainly bound to albumin (Pardridge, 1979); only 10-20% of TRP is present as a free form in the plasma and there is a balance between the forms linked to albumin and the free forms in the peripheral circulation (McMenamy, 1965). At rest, approximately 90% of total plasma TRP is bound to albumin, forming a complex that cannot cross the blood brain barrier (BBB); the remainder circulates in a free form which is available for transport through the BBB in the brain (Madras et al., 1974). A determining factor in the relationship between free TRP and albumin-bound tryptophan is the plasma concentration of fatty acids non esterified (NEFA), which bind to albumin, displacing TRP from its binding site (Curzon et al., 1973), causing an increase in free TRP in plasma (Chaouloff et al., 1986; Blomstrand et al., 1989). Other exogenous factors can also divide TRP from albumin, as

is the case with some drugs (Spano et al., 1974; Muller et al., 1975). It remains debated whether the binding of tryptophan with albumin can modify the availability of TRP for tissue metabolism (Smith & Pogson, 1980; Pardridge, 1983).

In a second step, 5-HTP is decarboxylated to form 5-HT (Mohammad-Zadeh et al., 2008). Studies by Clark and colleagues (1954) have shown that hydroxylation and decarboxylation occur almost instantly in the presence of TRP. Although both enzymes are necessary for the conversion of TRP into 5-HT, tryptophan hydroxylase is considered the rate limiting enzyme for several reasons; in fact, it has a relatively high  $K_m$  and little affinity for other amino acids (Noguchi et al., 1973) and its distribution is limited to those tissues containing serotonin (Noguchi et al., 1973; Champier et al., 1997). The activity of this enzyme is in contrast with the non-specific enzymatic activity of tryptophan carboxylase, an enzyme that has an affinity for many amino acids. The latter is present in most tissues (Clark et al., 1954) and, since it is not a limiting factor in 5-HT synthesis, it is difficult to reduce neurotransmitter levels by inhibiting this enzyme (Mohammad-Zadeh et al., 2008).

In the Central Nervous System (CNS), 5-HT is synthesized and stored in presynaptic neurons, while, outside of it, the synthesis is limited to enterochromaffin cells and, to a lesser extent, to platelets.

The platelets may have a very limited capacity to produce 5-HT, however they represent an important storage site outside the CNS, because they readily absorb 5-HT from plasma (Toh, 1954; Hardisty & Stacey, 1955). 99% of the total body 5-HT has intracellular localization, which implies strict regulation (Mohammad-Zadeh et al., 2008). Its concentration in the tissues depends on the speed of synthesis and metabolism (Tyce, 1990). The primary metabolic pathway of serotonin is that of monoamine oxidase (MAO), which occurs in the cytosol of the neuron (McIsaac & Page, 1959). MAOs are ubiquitous enzymes, belonging to the class of oxidoreductases, which exist in two main forms: MAO-A and MAO-B. Serotonin is mainly inactivated by MAO-A (Sandler et al., 1981).

## TRP, 5-HT and behavior

The link between TRP metabolism, serotonin and behavior has been the subject of considerable interest and discussion in the dog.

The first research dates to 1996 (Dodman et al., 1996) when it was determined the effect of diets containing a low, medium, or high protein content would have on behavior of dogs with dominance aggression, hyperactivity, territorial aggression or without behavioral problems. The results showed that the behavior of the animals with dominance aggression, with hyperactivity and that of control dogs was unchanged by the dietary manipulations. Instead, territorial aggression was significantly reduced when dogs were fed the low- or medium-protein diet, compared with territorial aggression when fed the high-protein diet. This effect was attributable to a marked reduction in aggression in a subset of the group in which aggression was a result of fear. The conclusions of the research were that a reduction in dietary protein content is not generally useful in the treatment of behavior problems in dogs but may be appropriate in dogs with territorial aggression that is a result of fear.

In 2000, De Napoli et al. evaluated the effect of high and low protein diets with or without TRP supplementation on behavior of dogs with dominance or territorial aggression and hyperactivity.

In each group, 4 diets were fed for 1 week each in random order with a transition period of 3 days between each diet. Two diets had low protein content (approximately 18%), and 2 diets had high protein content (approximately 30%). Two of the diets (1 low-protein and 1 high-protein) were supplemented with TRP (1.45 g/Kg). Owners scored their dog's behavior daily, by use of customized behavioral score sheets. Mean weekly values of 5 behavioral measures and serum

concentrations of 5-HT and TRP were determined at the end of each dietary period. The result showed that for dominance aggression, behavioral scores were highest in dogs fed unsupplemented high-protein rations. TRP-supplemented low protein diets were associated with significantly lower behavioral scores than low-protein diets without TRP supplements. Significant differences were not detected among behavioral groups or diets for plasma TRP or serotonin concentrations. Both of the TRP-supplemented diets produced higher TRP:LNAAs serum ratio, than the 2 unsupplemented diets; the higher ratio might have caused a greater proportion of TRP crossing the blood-brain barrier, increasing brain serotonin concentration and decreasing aggression. A factor that may have been operating regarding the high-protein diets is that increased dietary protein concentration increases plasma concentrations of tyrosine and phenylalanine, which are both catecholamine precursors this change could effectively reduce the threshold for aggression (Haller et al., 1998; Stoddard et al., 1986).

In the study of Bosch and colleagues (2009), for 8 weeks, privately owned dogs were fed a control diet or a diet containing 2.6-fold more TRP than the control diet. A third diet fortified with TRP, beet pulp, salmon oil, soy lecithin, and green tea extract was studied for its potential in other dogs. Owners reported on their dogs' behavior in the home-situation by filling out a web-based questionnaire before the onset of dietary treatment and after 4 and 8 weeks of feeding the diets. The dogs were subjected to behavior tests before and after 8 weeks of dietary treatment. The tests included open-field situations and owner-separation procedures and were set up to measure anxiousness. Blood was collected after 8 weeks from dogs in the control and TRP groups for evaluation of plasma amino acid concentrations. Intake of the TRP supplemented diet significantly increased plasma TRP concentrations by 37.4% and its ratio with large neutral amino acids by 31.2% compared to the control diet but owners did not report on behavioral changes that could be attributed to a specific dietary treatment. Also, the dogs' responses in the behavioral tests, including those in saliva cortisol, were unaffected after 8 weeks of consuming the TRP supplemented food. The authors concluded that intake of diets supplemented solely with TRP or in combination with beet pulp, salmon oil, soy lecithin, and green tea extract does not change (anxiety-related) behavior in privately owned dogs that do not show clear signs of abnormal behavior.

In 2012, Leon and colleagues assessed the suitability of different types of blood samples for measuring circulating 5-HT in canine clinical studies and investigated the relationship between the serotonergic system and canine aggression. The mean 5-HT concentration in aggressive dogs was significantly lower than in non aggressive dogs in all the assayed samples. These findings suggest an inverse relationship between the activity of the serotonergic system and canine aggression.

Kato et al. (2012) tested the effectiveness of a diet supplemented with L-tryptophan and alpha-casozepina, a decapeptide derivative of casein hydrolysis alpha-S1-casein, in situations of acute stress such as a visit to the veterinarian. Stress levels were evaluated by assessing the relationship between cortisol and urinary creatinine and questionnaire C-Bark (Serpell & Hsu, 2001). After seven weeks with the diet, the authors witnessed a significant reduction in the scores for the behaviors: aggression toward strangers, fear of strangers, fear not social and sensitivity to physical contact.

In a study of 2013 (Anzola et al., 2013), fifty-three sheltered dogs exhibiting high anxiety levels were studied for a period of six weeks in order to evaluate clinical responses to treatment with TRP. The effect of the diet on the behavior and fecal cortisol levels were assessed weekly throughout the study. The animals were randomly separated into two groups, according to the amounts of administered TRP; the Trp-1 group received 0.6% of TRP and the Trp-2 group received 1.2%. The social behavior of the two groups, during the final week, was significantly different ( $P < 0.05$ ). The results indicated that the dogs spent less time engaged in abnormal behaviors ( $P < 0.05$  and  $P < 0.001$ ) than in other behaviors. Likewise, significant differences in cortisol levels ( $P < 0.05$ ) were detected between the first and the sixth week. Cortisol values were lower, although not significantly ( $P > 0.05$ ) between the fifth and first week of treatment.

In a same year of the Anzola's research, in a study carried out by Cannas et al. (2013), a supple-

ment of *Valeriana officinalis*, *Melissa officinalis* and L-tryptophan was given to a group of 15 dogs. The symptoms related to anxiety of each dog were identified through specific questionnaires provided to the owners. After two months a follow-up questionnaire was given to the owners. Dogs, treated with the supplement, obtained significantly lower scores than the control group, showing significant improvements in the frequency and intensity of some of the symptoms analyzed, such as: “follow the owner”, “do not answer the call”, “aggressive manifestations” “eliminations inappropriate”, “excessive vocalizations,” “destructions” and “coprophagy” (Cannas et al., 2013).

In 2018, Gazzano and colleagues (Gazzano et al., 2018) conducted a research with the aim to investigate the possibility to augment the serum TRP bioavailability, modifying the diet. In fact, at the current state of knowledge two factors, appear influencing, in a decisive way, the L-tryptophan bioavailability: its relative concentration respect LNAA (large electrically neutral amino acids: tyrosine, phenylalanine, leucine\isoleucine and valine) (Fernstrom & Wurtman, 1972) and its binding to albumin (Chaouloff, 1993). Although TRP is contained in most of the proteins, its real availability depends on the amount of other aminoacids present, and particularly of the amino acids LNAA. Both TRP and LNAA are transported through the blood-brain barrier by the same carrier for which the LNAA have, however, higher affinity. Therefore, in a diet rich in proteins, TRP is available to a lesser degree than the other amino acids. The authors evaluated the plasma ratio between TRP and five large neutral amino acids (isoleucine + leucine + phenylalanine + tyrosine + valine) (5LNAA) after a single meal with high carbohydrates level. Five female Labrador Retrievers were involved. Each dog was fed three different meals: M1 (a mix of puffed rice, minced meat and olive oil), M2 (puffed rice and olive oil) and M3 (commercial dry food usually consumed) once in the morning for one single day every 30 days. Blood was collected right before the first meal (T0) and after 2, 4, 6, 8, 10 and 24 h. Plasmatic TRP concentrations showed no significant difference between M1, M2 and M3 samples at any sampling time. M2 led to a decrease in 5LNAA levels and consequently led to a significant higher TRP/5LNAA ratios in the 6 h period after the provision of carbohydrates, compared to both M1 and M3. In addition, the mean TRP/5LNAA ratio was significantly higher in M2 than in M3 at t8 and t10. These results indicate that meal composition affects TRP/5LNAA ratio and possibly, TRP bioavailability.

The same research group (Gazzano et al., 2019) evaluated, in a pilot study, the effects of a carbohydrate-based diet on serotonin blood concentrations in phobic dogs. For this study were recruited three dogs who have received by a veterinary behaviorist a diagnosis of interspecific social phobia. The dogs were fed 2 daily meals (at 8.00 A.M. and 4.00 P.M.), the first meal was exclusively carbohydrate-based (puffed rice) whereas the second one was composed by the commercial diet. Blood was collected every 21 days after 8 hours from carbohydrate meal to determine the levels of 5-HT, TRP and cortisol. Statistical analysis did not reveal any significative difference between the serum concentrations of 5-HT, TRP and cortisol, at the different times, despite a tendency to increase during the time.

Finally, in 2021 a study of Riggio and colleagues (Riggio et al., 2021) aimed at investigating possible differences in dogs' serum TRP and 5-HT concentrations according to their behavioral response to a potentially stressful procedure. Thirty-nine physically healthy shelter dogs, 15 females and 24 males, mean age = 5.6 years, were categorized by a certified veterinary behaviorist according to their behavioral response to medical examination and blood collection, in: relaxation, stress signals, tension without growling, tension with growling, escape attempts, and aggression attempts. The results showed no significant difference in TRP nor 5-HT serum concentrations among different categories of dogs; however, some categories were underrepresented (relaxation = 20.5%, stress signals = 30.8%, tension without growling = 43.6%, tension with growling = 5.1%, escape attempts = 0%, aggression attempts = 0%). No correlation between serum TRP and 5-HT concentrations was found. Serum 5-HT levels do not seem to be associated with dogs' behavioral response to a stressful situation nor with serum TRP concentrations.



## Conclusion

As pointed out by Dipace (2015), “based on these studies the use of TRP in the behavioral clinic of the dog is still controversial. While there is evidence of efficacy of products containing TRP and other substances during anxiety syndrome and stress, the use of only TRP in the control of canine aggression has given, until now, inconclusive results. Some authors recommend feeding aggressive dogs with high carbohydrate and low protein diets in order to help TRP to overcome the emato-encephalic barrier”. Further studies will be needed to clarify the efficacy of diet modifications on dog behavior.

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## Triptofano, serotonina e comportamento del cane

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### *Sintesi*

La serotonina è stata isolata per la prima volta da Rapport e colleghi, dopo decenni di ricerche su una sostanza con attività vasocostrittrice che si sospettava fosse contenuta nelle piastrine.

Dopo la sua scoperta, alcuni studi hanno permesso di identificare la sua localizzazione e funzione in molti tessuti ed organi (cervello, polmoni, reni, piastrine e tratto gastroenterico).

Oltre al suo ruolo nella funzione piastrinica, Brodie & Shore (1957), proposero in primo luogo un ruolo della 5-HT come neurotrasmettitore, basato sulla localizzazione dei recettori 5-HT in aree specifiche del cervello dei vertebrati. Il legame tra metabolismo TRP, serotonina e comportamento nel cane è stato oggetto di notevole interesse e discussione. L'uso del TRP nella clinica comportamentale del cane è ancora controverso. Mentre ci sono prove dell'efficacia di prodotti contenenti TRP e altre sostanze durante la sindrome d'ansia e lo stress, l'uso del solo TRP nel controllo dell'aggressività canina ha dato, fino ad ora, risultati inconcludenti.





# A new perspective on the bond between human beings and animals: A study on the human-dog and human-horse relationship

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**Abstract:** The aim of this study was to investigate whether and to what extent human attachment theory explains the bond between humans and animals. We examined whether the five main dimensions, outlined by Bowlby also exist in human-dog and human-horse relationships. A sample of 592 Italian adult dog and/or horse owners were tested using the Reciprocal Attachment Questionnaire (RAQ) to analyze their intra-specific relationships, while two adapted versions of the RAQ were used to investigate human-dog (RAQ-HD) and human-horse (RAQ-HH) bonds. The results indicate that the construct of the human-dog and human-horse relationship appears to be based only on three of the five main dimensions of Bowlby’s attachment theory: namely, proximity seeking, separation protest and feared loss. These findings suggest that the bond between adult humans and animals, although long-lasting, intense and psychologically and emotionally important, can be viewed as a bond of affection and not as a real attachment bond.

**Key Words:** attachment bond, human, dog, horse, animal, mental representations.

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## Introduction

Many people choose to share their lives with a pet and several studies have investigated the reasons leading to this choice and particularly the type of psychological bond that people establish with their pets (Anderson, 2008). The literature on the human-animal relationship clearly shows that some pet owners have a strong feeling of affection towards their animals and generally consider and treat them as true members of the family (Katcher et al., 1983; Burnham et al., 2002; Walsh, 2009). Indeed, pets, especially dogs and cats, can be perceived as a source of comfort in situations of emotional stress (Kurdek, 2009) and most owners experience painful long-lasting feelings of grief and discomfort when their pets die, maintaining with them ongoing and meaningful ties even following their death (Davis, 2011; Testoni et al., 2017). It seems that there are clear parallels between the kind of reactions that people exhibit to the loss of a pet and the loss of a meaningful human relationship (McCutcheon & Fleming, 2001; Field et al., 2009).

The relationship with animals can be considered as an inter-personal relationship; therefore, behaviors, actions and beliefs are influenced by the mental representations that people have of these relationships (Zilcha-Mano et al., 2011). Animal owners dedicate time and money to guarantee physical and psychological well-being to their pets and different theories have attempted to explain the benefits of this relationship: the theory of biophilia, the theory of social support and the attachment theory. According to the theory of biophilia, humans tend to establish relationships with animals because they are useful for survival. Animals can warn humans of possible dangers and/or predators; however, in an industrial context like the one we

live in nowadays, this characteristic is less relevant and poorly applicable. For this reason, it is legitimate to think that the benefit of this relationship is also attributable to other aspects that go beyond physical survival. In relation to the theory of social support, companion animals tend to reduce the feeling of loneliness and contribute to a general sense of well-being in their owners (Sable, 1995). The relationship with pets, unlike the one with humans, offers social support because pets offer constant availability, unconditional love and a relationship that is non-judgmental (Friedmann et al., 1980; Kruger et al., 2004). Pets not only have a direct role in providing social support but also an indirect role in favoring a general well-being in their owners. Moreover, animals can help in catalyzing certain dimensions of socialization: getting to know people, forming friendship and creating social support networks (Wood et al., 2015).

Finally, several studies, investigating the biological and psychological bases of the human-animal relationship, have considered Bowlby's Attachment Theory (Bowlby 1982, 1983) as a useful framework to explain the emotional and relational complexity of the inter-specific bond between humans and animals and, in particular, the human-dog relationship (Julius et al., 2010; Zilcha-Mano et al., 2011; Cromer & Barlow, 2013).

Besides being considered members of the family, pets are often viewed and treated like children in many aspects: pet owners play with them (Smith, 1983), hold and cuddle them (Serpell, 1986) and also talk to them using baby-talk (Hirsh-Pasek & Treiman 1982; Berryman, 1985; Beebe & Lachmann, 2003, 2015; Prato-Previde et al., 2006), a type of adult-child verbal communication characterized by simplicity (usually only verbs and adverbs) and by a strong non-verbal and emotional component (Schore, 2008). Ellen Noonan has suggested in her work "People and Pets" (Noonan, 2008) that pets can act as attachment figures, transitional objects and even therapists. She questioned whether it was the human being or the animal to turn to the other in moments of need, which one of the two provided comfort, protection and stood as a secure base, promoting exploration. Her studies revealed that the human-pet relationship was reciprocal in that the owner was a figure of attachment to the animal and vice versa. Likewise, Julius et al. (2014) suggested that the human-animal bond is a reciprocal relationship in which, depending on the situation, either the pet or the owner can be a source of comfort and caregiving. This flexible relationship is considered as a core element of the biopsychosocial benefits that humans obtain from a relationship with animals (Julius et al., 2014). Pets act for their owners as mediators in their relationship with the world and they may serve the same roles of traditional transitional objects for children (Triebenbacher, 1998), although they cannot be a real transitional object because they are living and sentient beings.

The literature on the human-animal relationship suggests that the inter-specific human-animal bond is characterized by four specific components of the attachment bond: search for proximity, safe haven, safe base (or secure base) and separation distress (Zilcha-Mano et al., 2011; Prato-Previde & Valsecchi, 2014; Payne et al., 2016).

Several studies have investigated whether the close and individualized relationship between an animal species (i.e. chimpanzees, cats, dogs) and a human partner conforms to an attachment bond using adapted versions of the Strange Situation Test (Ainsworth, 1978), devised to investigate the mother-infant relationship (e.g. Bard, 1991; Topal et al., 1998; Prato Previde et al., 2003; Edwards et al., 2007). Most of these studies have been carried out on dogs and have shown that the human-dog bond conforms to an infant-like attachment (Prato-Previde & Valsecchi, 2014). In particular, it has been shown that dogs engage in behaviors, which are indicative of a form of attachment that is present in human caregiver-infant relationships, namely proximity seeking, protest and separation-related distress, the safe haven effect and secure base effect (Prato-Previde et al., 2003; Gácsi et al., 2011; Horn et al., 2013).

The bond between man and animal has been investigated mainly through questionnaires and scales in which the behavioral component of the strange situation is measured by drawing on its adult psychological equivalent, although not necessarily on the basis of the theory of hu-

man attachment (Albert & Bulcroft 1988; Johnson et al., 1992; Beck & Madresh, 2008). However, the means used in these studies does not really return a psychometric validation of the theoretical construct.

The purpose of our study was not to validate a new scale but rather to investigate if the theory behind the human-animal bond may be explained by the 4 typical dimensions present in the infant-like attachment, if the dimensions are more, i.e. 5, as happens in the adult attachment according to West (West et al., 1987; West, 1994), or, at the opposite, if the dimensions are less.

For example, validated psychological tests were used by Konok and colleagues (2015) to support behavioural data on the human-dog relationship. More specifically, the authors (Konok et al., 2015), employed the Adult Attachment Scale (Collins, 1990; Main, 1991; Main et al., 2005) to capture the core structure, determining the attachment style, the Big Five Inventory (John & Srivastava, 1999) to describe the main personality traits of the owner and, in addition, they developed a Dog Big five Inventory to assess dogs' personality traits.

Finally, a number of studies have shown that, other than psychological aspects, also biological aspects should be taken into account in the human-animal relationship (and in particular the human-dog relationship). For example, oxytocin seems to play a central role in the human-animal bond, as has been demonstrated in mother-infant relationships and romantic relationships (Odendaal & Meintjes, 2003; Miller et al., 2009; Handlin et al., 2011, 2012). Oxytocin can be released after visual and/or physical contact with others (either conspecifics or non-conspecifics) and there is evidence of a correlation between the oxytocin system, the stress system and the attachment-care systems throughout the lifespan of an individual (Schneiderman et al., 2012; Ogi et al., 2020). According to Bowlby (1988) the concept of attachment is not only important during infancy but also throughout the entire life of an individual. In fact, it has recently been claimed that attachment bonds fulfil the same functions across the life span of a person (Hazan & Shaver, 1987), being equally important also in adult relationships. In romantic relationships, even as adults, we show towards the partner proximity seeking and maintenance, separation distress, seeking comfort from him/her in times of stress (safe haven), and deriving from him/her a sense of security (secure base) (Mikulincer & Shaver, 2003; Doherty & Feeney, 2004; Zilcha-Mano et al., 2011). Studies, which have extended the human attachment framework in order to analyze the relationship between attachment to pets (mainly dogs and cats) and attachment to people, have provided an initial evidence of the usefulness of the measures of attachment as tools for the investigation of people's relationship of affection with their pets (Beck & Madresh, 2008; Smolkovic et al., 2012). The style of attachment to humans and the one to animals appear to be independent from each other; there seems to be no transfer of the human attachment style to animals (Beck & Madresh, 2008; Julius et al., 2010).

We decided to investigate on the human-dog and human-horse bonds considering the lack of scientific literature on the mental representations of the human-horse or human-dog bonds, the evolutionary and domestication histories of these two species, as well as their current daily management in human societies (Payne et al., 2016). Both dogs and horses live and interact with humans and both species are kept for specific working purposes and/or as pets. However, dogs have a more central role as human companions than horses and generally they share their life with us to a greater extent. In addition, both species are social, and there is evidence that they form social relationships with conspecifics and humans (Malavasi & Huber, 2016; Fugazza, et al., 2018). Indeed, they both play a different but important role in promoting human health and well-being and are considered the most suitable animals in Animal-Assisted Intervention (American Veterinary Association, 2014; Guidelines of Italian Ministry of Health, 2015). Nevertheless, these two species also differ in their evolution and domestication histories: dogs are predators whereas horses are prey. Therefore, it has been suggested that these two species may possibly differ in their propensity to form attachment or attachment-like relationships with

humans. Payne and co-workers (2016) outlined there is ample evidence for the existence of human-dog attachment (e.g. Prato-Previde & Valsecchi, 2014), whereas the evidence for human-horse attachment is almost negligible (DeAraugo et al., 2014). They also suggested that this difference might depend on the different selection paths of domestic dogs and horses as well as the different contexts in which the two species interact with humans and the contrasting roles these animals occupy in human domains.

Another reason for choosing and comparing these two species in the current study is the different management and living environments of the horse and the dog. In the Italian context horses often stay in stables, while dogs live in homes with their owners. In the literature there are no studies on the impact that the different way of looking after the dog and the horse may have on the attachment bond; however, the differences in intimacy with humans could affect attachment. Therefore, in the present study the theory was investigated separately for dog and horse.

The current study, thus, investigated whether and to what extent the attachment theory developed for human intra-specific relationships could explain the inter-specific bond between humans and animals. More specifically, our aim was to evaluate the mental representations that people have of the bond with their animals and to assess whether the five main dimensions, characterizing the attachment bond (i.e. proximity seeking, separation protest, feared loss, perceived availability, use of the attachment figure) according to West and Sheldon-Keller (West & Sheldon-Keller, 1992), can also be attributed to the human-dog and human-horse inter-specific relationship.

We considered the five dimensions of attachment, instead of four, as we applied the Reciprocal Attachment Questionnaire (RAQ), constructed and validated in English by West (West et al., 1987; West, 1994). We relied on this means because we considered it as the most suitable tool for our study although aware of the fact of it not being totally adherent to the human-animal attachment theory but validated unlike other scales. Our final aim was to study if there was a difference in the human-dog and human-horse bond assuming that the difference between species and the different way in which dog and horse are managed could influence this link.

## Materials and methods

### *Participants and procedure*

The study population consisted of 592 adults (177 males and 415 females), aged between 18 and 78 years old (mean = 34.41 years; SD = 13.41), who participated to the four-day sports event Fiera Cavalli 2018, which was held in Verona (Italy) from 25<sup>th</sup> to 28<sup>th</sup> October 2018. The setting did not allow to calculate the response rate and participation to the survey was voluntary and anonymous.

Eight research assistants, who had been previously trained, directly contacted all participants and each research assistant approached a person at random. The research assistants explained the survey to the participants and assisted in the compilation of the self-report questionnaire if they claimed to be owners of at least one dog and/or one horse. Participants were told that the purpose of the study was to gain knowledge concerning the human-dog and/or human-horse relationship and that their responses would remain anonymous and would be used for scientific research alone. Research assistants had received a specific training and questions were addressed following a precise protocol: in case a participant did not understand a question and asked for help, the question was rephrased by the assistant in a different but standardized way to avoid personal reformulations. Each research assistant further verified the correct compilation upon completion of the questionnaire. Only respondents that completed the questionnaire were considered for statistical analyses.



### *Experimental setting*

The test was administered in the main stand of the Federazione Italiana Sport Equestri (FISE) at the Fiera Cavalli 2018, in Verona (Italy), by research assistants with a specific training in the psychological field (Bachelor or Master's degree in Psychology) and trained by the research team in addressing the questions and in the compilation of the questionnaire. The test administration took place during two of the four opening days of the event (26<sup>th</sup>-27<sup>th</sup> October). Respondents were informed about the privacy and anonymity of the participation according to the National Privacy Law 675/96. Formal ethical approval was not requested but the study was carried out according to the indications of the declaration of Helsinki.

No personal sensitive data were asked. The questionnaire's aim to assess the attachment of the participants towards pets was not explicitly mentioned to avoid the social desirability bias. For the same reason, participants were told that there were no right or wrong answers, as the focus was on their authentic point of view. After completing the questionnaire, participants were fully debriefed on the purpose of the study.

### *Measurement tools*

Following a preliminary analysis of the literature on the human-animal bond, we identified two types of psychological scales: scales created for measuring inter-specific attachment to pets (i.e. Pet Attachment Questionnaire – PAQ: Zilcha-Mano et al., 2011; Lexington Attachment to Pets Scale – LAPS: Johnson et al., 1996) and scales created for measuring intra-specific attachment between humans in adulthood (e.g. self-report measure, Attachment Style Questionnaire – ASQ: Feeney et al., 1994; 15 Item Questionnaire – 15IQ: Mikulincer et al., 1990; or interview, i.e. Adult Attachment Interview – AAI: George et al., 1985; Current Relationship Interview – CRI: Crowell and Owens, 1996). The inter-specific attachment scales (human-pet) were excluded from our research due to the poor methodological framework used in their construction and for the lack of adequate general psychometric properties. Moreover, some intra-specific attachment scales were not utilized since they were specific to intimate relationships and sexual factors when analyzing the pattern of attachment. In addition, unfortunately, only a few of the intra-specific and none of the inter-specific attachment scales were validated in the Italian population.

Thus, the only instrument, satisfying our research requirements, was the Reciprocal Attachment Questionnaire (RAQ). The RAQ scale is based on the attachment theory, which takes into consideration the different features of attachment (proximity seeking, separation protest, feared loss, perceived availability and use of the attachment figure). It was originally developed to evaluate an adult's pattern of attachment to a significant other being with whom the individual had shared a special relationship for at least 6 months. We chose the RAQ scale as a basis to create two different adapted versions of the measurement tool to analyze the human-animal bond: namely, the Reciprocal Attachment Questionnaire Human-Dog (RAQ-HD) and the Reciprocal Attachment Questionnaire Human-Horse (RAQ-HH). We chose to use the RAQ scale as it assesses the quality of attachment towards anyone, who is identified as the most important attachment figure for the subject (e.g. a partner, a friend, etc.) and also because its validity and reliability have been established in different studies on both clinical and nonclinical adult populations (West & Sheldon-Keller, 1994; Perris & Andersson, 2000; Ward, et al., 2000). The Reciprocal Attachment Questionnaire (RAQ) is a self-report questionnaire, constructed and validated by West (West et al., 1987; West, 1994), and designed to measure attachment towards the main human attachment figure. The complete version of the RAQ scale consists of 45 items and each item is rated using a 5-point Likert-type scale, ranging from strongly agree to strongly disagree. Items are grouped into different subscales to assess both clinical and nonclinical aspects of human attachment (West & Sheldon-Keller, 1992). In particular, three of the subscales

describe the criteria of attachment distinguishing it from other social relationships (i.e. Separation Protest, Feared Loss, and Proximity Seeking), while the other two subscales (i.e. Use and perceived Availability of the attachment figure) are related to the unique provisions provided by attachment. The remaining subscales describe identified dysfunctional patterns of adult attachment relationships (i.e. Compulsive Self-Reliance, Compulsive Care-Giving, Compulsive Care-Seeking, and Angry Withdrawal).

The RAQ scale has a short version, which consists of 15 items referring to the 5 dimensions of attachment: 1. Proximity seeking with the attachment figure, 2. Separation protest, 3. Feared loss, 4. Perceived availability and, 5. Use of the attachment figure. Each dimension is measured by 3 items on a 5-point Likert-type scale (strongly agree: score=1; strongly disagree: score=5).

The original English version of the short RAQ scale was translated into Italian by Busonera and co-workers (2011) and was used in the current research as a basis to create the adapted versions of the Reciprocal Attachment Questionnaires (RAQ-HD and RAQ-HH). We obtained permission to apply the Italian short RAQ scale in the present study directly from the authors of the translated test.

The final questionnaire, which was presented to the participants in the study, included a few preliminary socio-demographic questions (i.e. age, gender, marital status, education, occupation, presence of children) with the purpose of gaining background information on the participants themselves that could be relevant to their responses. In addition, it contained the original RAQ scale (intra-specific human-human), and the adapted versions RAQ-HD (inter-specific human-dog) and RAQ-HH (inter-specific human-horse).

### *Statistics analysis*

Descriptive statistics (frequency distributions, central trend and variability indicators) were used to analyse the participant's characteristics (age, gender, study title, occupation, marital status, presence of children and the scores in the RAQ scale), especially in relation to owning a dog, a horse or both. These three groups of participants were compared in order to evaluate the homogeneity of the examined characteristics: the non-parametric test *Chi-square* was used for the categorical variables (gender, study title, occupation, marital status, presence of children), while Kruskal-Wallis and Mann-Whitney tests were applied for numerical variables (age and the scores at the RAQ scale), since the null hypothesis of normal distribution for these measures was rejected according to the Kolmogorov-Smirnov test.

Exploratory factor analysis was used to test the factorial structure of the version of the RAQ scale adapted to the human-dog and human-horse relationships. The sample size was established according to the rule of thumb that sets adequate numerosity at 300 participants when the participants/items ratio is also between 5 and 10. The violation of normality suggested the application of the principal axis method of factor extraction. The number of factors to be extracted was determined using the scree plot analysis, together to the criterion involving the extraction of factors showing an eigenvalue greater than 1. The extracted factors were rotated according to the Oblimin method since we assumed the correlation between the factors. The value of the sample size adequacy was examined by the KMO index and the sphericity hypothesis of the correlation matrix was verified by the Bartlett Test. The reliability of the extracted factors was assessed by Cronbach's alpha, taking into account that this index is influenced by the items numerosity and that its interpretation is quite controversial (Bland and Altman, 1997; Tavakol and Dennick, 2011): values between 0.7 and 0.95 are acceptable to assess for the internal consistency of a group of items, while higher values highlight the presence of redundant items. The significance level was set at  $p < 0.05$  and the analyses were performed with the SPSS-IBM v.23 software.

## Results

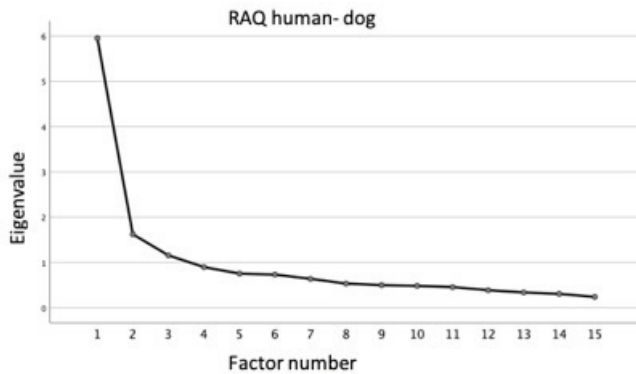
The characteristics of the three groups (dog owners, horse owners, and both dog and horse owners) are illustrated in Table 1. The characteristics showed to be homogeneous within the three groups. None of the tests rejected the null hypothesis, except for the variable gender: in this case men seemed to be more prone to own a dog (36.6%), while women were more likely to own both a dog and a horse (75.8%), or only a horse (72.3%), and the difference between genders was significant ( $p=0.012$ ; refer to Table 1 for details).

**Table 1.** Comparison between the characteristics of dog owners, horse owners and both dog and horse owners; absolute frequencies and relative frequencies (%) are indicated for categorical variables, while means $\pm$ standard deviation for numerical variables.

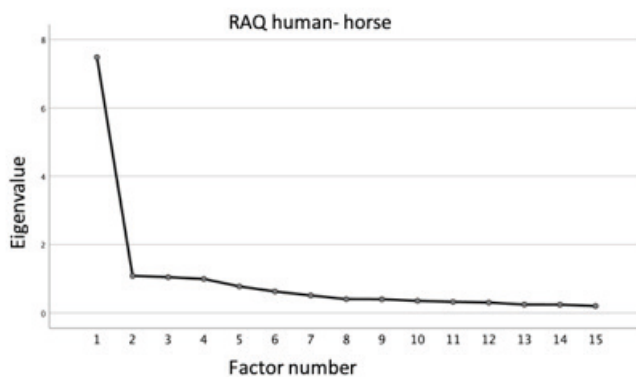
		Dog owners (n=232)	Horse owners (n=137)	Dog and horse owners (n=223)	Statistics	P
Gender	M	85 (36.6)	38 (27.7)	54 (24.2)	Chi-square(2) = 8.769	0.012
	F	147 (63.4)	99 (72.3)	169 (75.8)		
Age		34.72 $\pm$ 13.451	34.18 $\pm$ 13.446	33.79 $\pm$ 13.339	KW** H(2) = 0.744	0.689
Qualification	First or second degree	43 (18.5)	23 (16.8)	27 (12.1)	Chi-square(4) = 4.302	0.367
	Diploma	132 (56.9)	75 (54.7)	136 (61.0)		
	Graduation or other titles	57 (24.6)	39 (28.5)	60 (26.9)		
Profession	Student	47 (20.3)	34 (24.8)	56 (25.1)	Chi-square(6) = 7.474	0.279
	Worker	151 (65.1)	93 (67.9)	139 (62.3)		
	Unemployed/hous ewife/retired	27 (11.6)	7 (5.1)	25 (11.2)		
	Other	7 (3.0)	3 (2.2)	3 (1.3)		
Marital status	Single	71 (30.6)	45 (32.8)	65 (29.1)	Chi-square(6) = 0.772	0.993
	In pairs/cohabitant	88 (37.9)	50 (36.5)	89 (39.9)		
	Married	62 (26.7)	35 (25.5)	59 (26.5)		
	Other	11 (4.7)	7 (5.1)	10 (4.5)		
Presence of children	Yes	70 (30.2)	34 (24.8)	69 (30.9)	Chi-square(2) = 1.705	0.426
	No	162 (69.8)	103 (75.2)	154 (69.1)		
RAQ Points	Human-dog	53.89 $\pm$ 10.28		54.58 $\pm$ 9.43	MW* U = 25.366,500	0.661
	Human-horse		55.13 $\pm$ 12.38	55.99 $\pm$ 11.44	MW* U = 14.729,500	

\*: Mann-Whitney test; \*\*: Kruskal-Wallis test.

The scree plots (Figure 1 and Figure 2), resulting from the factorial analysis made with the RAQ scale human-dog (RAQ-HD) and human-horse (RAQ-HH), showed a completely overlapping factorial structure. When looking at the two criteria (i.e. eigenvalues greater than 1 and variation of the slope of the curve), the number of useful dimensions explaining the factorial structure of the scales appeared to be equals to 3, as previously indicated (Figure 1 and Figure 2).



**Figure 1.** Scree plot of factor analysis: assessment of the human-dog attachment (RAQ-HD).



**Figure 2.** Scree plot of factor analysis: assessment of the human-horse attachment (RAQ-HH).

The two exploratory factor analyses showed an adequate sample size (RAQ-HD:  $KMO=0.898$ ; RAQ-HH:  $KMO=0.928$ ) and non-spherical covariance matrices (RAQ-HD:  $p<0.000$ ; RAQ-HH:  $p<0.000$ ). The structure matrix and the distribution of the items related to each factor are described in Table 2 and Table 3. In both scales, the first factor was the one collecting the largest number of items (10), while the other two dimensions, containing only two items, showed an insufficient number of items to provide an adequate description of the construct below. In addition, one of the scale's items ("I'm not in trouble if I walk away for a few days from my dog/horse") did not fit well in any of the extracted factors from the RAQ-HD or RAQ-HH's scale. Therefore, it was not placed in any of the factors as its factorial score was less than 0.3 (details are given in Table 2 and Table 3). The factorial structure of the two scales resulted almost completely overlapping: 8 of 10 items that were descriptive of the first factor of the RAQ-HD scale were also found in the first factor of the corresponding RAQ-HH scale. Interestingly, the items of the third factor of the RAQ-HD scale were the same to those corresponding to the second factor of the RAQ-HH scale.

The factor structure describing the psychological attachment construct between humans and animals did not seem to follow the traditional attachment theory between humans, although the three factors identified gave a glimpse of their specificity with a meaning that remains unclear. For example, as shown in the structure matrix of RAQ-HD (Table 2), an important level of cross loading was revealed between the items of factor 1 and the items of factor 3, highlighting the confusion regarding the definition of the items: this occurs when the informational content is not correctly identified by the respondents of the questionnaire. Likewise, even more elevated and widespread cross loadings were noticed in the RAQ-HH scale (Table 3).

**Table 2.** Structure matrix of the factor analysis on the human-dog RAQ scale (RAQ-HD); extraction method: principal axis factoring; rotation method: oblimin; Factorial scores below 0.300 were omitted.

	Factor		
	1	2	3
I turn to my dog when I am in serious trouble	0.808		0.317
I feel lost and worried if my dog is not near me	0.741		0.559
When I'm anxious, I desperately need to stand next to my dog	0.737		0.413
When I'm upset, I need to have my dog close	0.728	0.338	0.543
I talk about things and I'm confident with my dog	0.721		0.422
When I'm upset, I'm sure my dog is there to listen to me	0.66	0.433	0.469
I feel sad when I spend time away from my dog	0.585		0.501
If I walk away for a few days from my dog I feel to have abandoned it	0.555		0.555
I address my dog for many things, including comfort and reassurance	0.548	0.4	0.345
I'm afraid I can lose my dog's love	0.486		0.372
I'm sure my dog will love me forever	0.322	0.589	0.406
I'm sure my dog perceives my mood	0.303	0.55	0.311
I feel scared to think that my dog can die	0.467		0.732
It saddens me to think that my dog will die	0.38	0.353	0.732
I'm not in trouble if I walk away for a few days from my dog			

**Table 3.** Structure matrix of the factor analysis on the human-horse RAQ scale (RAQ-HH); extraction method: principal axis factoring; rotation method: oblimin; Factorial scores below 0.300 were omitted.

	Factor		
	1	2	3
When I'm upset, I'm sure my horse is there to listen to me	0.847	0.56	0.399
I turn to my horse when I am in serious trouble	0.822	0.501	0.488
When I'm upset, I need to have my horse close	0.822	0.506	0.458
I address my horse for many things, including comfort and reassurance	0.808	0.468	0.349
When I'm anxious, I desperately need to stand next to my horse	0.795	0.5	0.579
I talk about things and I'm confident with my horse	0.761	0.532	0.538
I'm sure my horse will love me forever	0.666	0.518	0.309
If I walk away for a few days from my horse I feel to have abandoned it	0.647	0.48	0.605
I feel sad when I spend time away from my horse	0.643	0.496	0.399
I'm sure my horse perceives my mood	0.476	0.427	
I feel scared to think that my horse can die	0.586	0.832	0.334
It saddens me to think that my horse will die	0.512	0.815	
I feel lost and worried if my horse is not near me	0.654	0.488	0.844
I'm afraid I can lose my horse's love	0.453	0.391	0.612
I'm not in trouble if I walk away for a few days from my horse			

The reliability analysis of the extracted factors showed heterogeneous findings. Very high values of Cronbach's alpha were found for the first factor in both scales (RAQ-HD:  $\alpha=0.880$ ; RAQ-HH:  $\alpha=0.920$ ), although scale implementation strategies could be used. In the dog scale, the reliability value was increased by removing the item "I'm afraid I can lose my dog's love", while in the horse scale, Cronbach's alpha could be increased by removing the item "I'm sure my horse perceives my moods". Regarding the other dimensions, the second factor of the RAQ-HD scale showed a Cronbach's alpha of  $\alpha=0.597$ , while the same factor of the RAQ-HH scale highlighted a value of  $\alpha=0.814$ . Lastly, Cronbach's alpha of the third factor was similar for the dog scale (0.760) and the horse scale (0.721).

As already said, reliability measures showed heterogeneity between the factors. The lowest reliability values were recorded for factors with a low number of items, as the value of Cronbach's alpha is directly influenced by the number of items describing the factor. Nevertheless, the acceptable reliability values obtained from a small number of items, allowed to hypothesize the

possibility that these items could be the first building blocks to be used for a better description of the construct, which was the aim of the current study.

## Discussion

The aim of this study was to investigate whether the attachment theory, developed to explain intra-specific human relationships, could provide an explanation for the inter-specific human-animal bond as well. In addition, possible differences between the human-dog and the human-horse bond were also examined. More specifically, we investigated whether the five main dimensions characterizing the attachment bond, as outlined by Bowlby (i.e. proximity seeking, separation protest, feared loss, perceived availability, use of the attachment figure), also existed in the human-dog and human-horse relationships. Two adapted versions of The RAQ scale (Boneria et al., 2011) were used to assess the human-dog (RAQ-HD) and human-horse (RAQ-HH) bonds, respectively.

The factor analyses revealed interesting differences between the human-human relationship and the inter-specific human-dog and human-horse bonds. Indeed, a three-factors structure was revealed by the exploratory factor analysis of the RAQ-HD and RAQ-HH scale rather than the five-factors structure known in the RAQ scale describing adult human attachment (West, 1994). As shown by the structure matrix of the factor analysis of the human-dog RAQ and the human-horse RAQ, both dog and horse owners appeared to search proximity to their animal, mainly in case of difficulty or distress. Moreover, the fear of the loss of the animal, such as in case of death of the animal but also in the case of a temporary separation, led the owners to negative thoughts and to behaviours aimed at seeking contact to their animal or at limiting the separation with it. The results, thus, obtained do not contradict the validity of the 5 dimensions recognized in the adult attachment but simply suggest they are not valid in the human-animal attachment.

Overall, our results indicate that the inter-specific bond with animals, specifically with dogs and horses, appears to be characterized by the dimensions “proximity maintenance”, “separation protest” and “fear of loss”, and, in contrast to other studies, not by the dimensions of “perceived availability” and “use” (Kurdek, 2008). This appears to be consistent with the fact that, as opposed to a human reference figure, an animal cannot engage in operative and concrete behaviours to solve problematic or difficult situations. However, it is worth noting that an animal can provide comfort and reassurance in case of difficulty or stress: Kurdek (2009) reported that owners turn to their pet dogs in times of emotional distress and prefer turning to their dog rather than to other figures of reference. Thus, additional studies are needed to further investigate the impact of individual differences on human-animal bonds and to better understand the role played by variables related to both the owner (i.e. age, gender, marital status etc.) and the dog.

Considering the informative content expressed by the dimensions identified by the RAQ scale, the interpretation of the first factor suggests the possibility of the hypothesis of an almost “pathological” bond with the animal: the items that were lumped together expressed a situation of individual feelings of discomfort or disturbance, associated with the use of affection or closeness of the dog/horse as the preferred solution. A factor (second and third factor in the human-horse and human-dog relationship, respectively), describing negative thoughts concerning the death of the animal, was also identified by the analysis; finally, the third factor, although not having the same representation in the two scales (human-dog, human-horse), expressed a sense of detachment from the animal and a sense of lacking due to the animal’s absence.

Another interesting finding, which emerged in the study, was the lack of significant differences between the human-dog and human-horse bonding. This finding deserves further investigation as the literature on the human-horse and horse-human bond is still very limited compared to that regarding the human-dog and dog-human relationship. It has been suggested that due to

the different domestication and selection histories of dogs and horses and the different contexts in which they live and interact with humans, some differences in the propensity to form reciprocal attachment or attachment-like relationships would be detectable (e.g. Payne, 2016).

To summarise, the results suggest that the theory of human intra-specific attachment does not explain appropriately the inter-specific human-dog and human-horse bonds, which, instead, seem rather to have their own form, peculiarities and dimensions. Adult humans form their intra-specific relationships on the basis of their relational experiences from childhood and of the internal operative models learned from the relationship with their significant reference figures. Therefore, it is probable that also the inter-specific human-dog and human-horse bonds in adulthood are influenced by the person's internal operative models and by the style of attachment, though still with the assumption of a proper configuration.

A limitation of our study was attributable to the use of a scale that, although widespread in Italian contexts (Bonera et al., 2011), has not undergone a rigorous process of adaptation and validation elsewhere. Furthermore, although only a few aspects of the original scale were modified (for example the substitution of the term "person" with "dog/horse") to adapt the RAQ scale to the study of an inter-specific bond, the effect (and consequences) of this adaptation have yet to be investigated and should be further examined.

## Conclusions

This study is a further step in the investigation and measurement of the human-animal inter-specific bond and provides a potentially useful tool to verify existing theories.

Our findings suggest that, as already demonstrated in other studies, the bond between humans and dogs/horses is a strong and meaningful affective bond, but it does not conform to a real attachment bond that meets the requirements, as identified by Bowlby (Bowlby, 1982 and 1983; Ainsworth, 1989). However, it is worth mentioning that the psychopathological variable was not evaluated in the current work: we assumed the participants to be healthy, although it might be interesting to investigate the effect of this variable in future studies.

Moreover, the use of the adapted versions of the RAQ scale (RAQ-HD, RAQ-HH) in the current study has provided data to work on the validation process in the Italian population of a new instrument devised to investigate the inter-specific bonds with both horses and dogs: namely the Reciprocal Human And Animal Questionnaire scale (RHAAQ).

Thus, although promising, the results of the present work highlight the need for further research on this topic and, in particular, on the bond between human beings and pets, and especially on the bond established between humans and dogs and humans and horses.

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## Una nuova prospettiva sul legame tra uomo e animale: uno studio sul rapporto uomo-cane e uomo-cavallo

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### *Sintesi*

Lo scopo di questo studio è stato quello di indagare se e in che misura la teoria dell'attaccamento umano spieghi il legame tra uomo e animale. A tal fine si è verificato se le cinque dimensioni principali, delineate da Bowlby, esistano anche nelle relazioni uomo-cane e uomo-cavallo. Un campione di 592 proprietari italiani di cani e/o cavalli adulti è stato testato utilizzando il Reciprocal Attachment Questionnaire (RAQ) per analizzare le loro relazioni intra-specifiche, mentre due versioni adattate del RAQ sono state utilizzate per indagare il legame uomo-cane (RAQ-HD) e uomo-cavallo (RAQ-HH).

I risultati indicano che il costrutto della relazione uomo-cane e uomo-cavallo sembra essere basato solo su tre delle cinque dimensioni principali della teoria dell'attaccamento di Bowlby: vale a dire, ricerca di prossimità, protesta per la separazione e timore della perdita. Questi risultati suggeriscono che il legame tra umani adulti e animali, sebbene duraturo, intenso e psicologicamente ed emotivamente importante, può essere visto come un legame di affetto e non come un vero e proprio legame di attaccamento.



# Separation-related disorder management through the COVID-19 pandemic: A case report

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**Abstract:** The closure related to the COVID-19 pandemic impacted on the management of separation-related disorder in a dog. Eight weeks before the first COVID-19 pandemic closure, the patient, an 8-year-old female neutered cross breed dog weighing 6 kg, was presented for nonstop barking when separated from her owners, 8 hours a day, 5 days each week. Before the first consultation, 4 years of training based on desensitization without medication helped the patient but never lowered the barking beyond two hours a day, which remains way too high to provide a correct quality of life to this dog. The patient welfare was at stake, and the neighborhood complains were growing. The patient was prescribed fluoxetine at 20 mg (3.3 mg/kg) PO q24 h. and trazodone PRN before separation at 25 mg (4.2 mg/kg) PO. A behavioral modification plan based on extinction and calm reinforcement was prescribed. Eight weeks after the treatment onset, just before the first COVID-19 pandemic closure, the dog improved significantly, lowered daily barking up to 15 minutes. During all the successive closures (i.e., one year), the dog was never left alone. When the closures ended, the barking relapsed, straight at the first separation event, reaching 1 to 2 hours daily, even though the fluoxetine had never been interrupted. Therefore, gabapentin was prescribed PRN before separation at 100 mg (16.6 mg/kg) in place of trazodone that triggered excitation in the patient when it was previously tried. The behavioral plan was completed with additional conditioning learning before separation. The dog improved quickly to a short tolerable time of barking (i.e., 5 to 10 minutes). This outcome remains stable by the time the paper is written i.e., 3 months after the end of the closure. The patient's evolution emphasizes two important topics in the treatment of separation-related disorder: firstly, medication is needed for most cases to lower the level of emotional reaction, and secondly, interruption in the exposition to the fearful context may have rebound effects when the context will be encountered again. The long-term effects of the COVID-19 pandemic remains unknown on both human and dog's welfare. More extensive studies should be conducted to measure its impact on separation-related disorder in dogs.

**Key Words:** dog, separation related disorder, COVID-19 pandemic, separation anxiety, Fluoxetine, Gabapentin, Trazodone.

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## Presentation

Influence of the COVID-19 pandemic on separation related behavioral disorder in a dog and its management.

## Signalment

The patient is an 8-year-old female neutered cross breed dog weighing 6 kg.

## Demographic

The patient was adopted 4 years ago from a shelter. She was relinquished there a year before the adoption and her previous history or the reason for abandoning her are completely unknown to the owners. During her year spent in the shelter, she had been adopted several times. Each time, she came back to the shelter under a few weeks. The shelter staff did not provide the reasons for these returns to the current owners. The current owners live in an apartment in a large city with a two-year-old child.

## Medical and behavioral history

When adopted 4 years ago, the patient was not cared of, she had diarrhea and a bad body condition. She improved within a month with a good alimentation and antibiotic treatment.

Immediately after adoption the patient was extremely fearful and for this reason most of the time inhibited. She was not playful at home and her main behavior was sleeping. It was exceedingly difficult to walk the dog in the street. She would react to all the noises of the urban surrounding by freezing or pulling back to the house direction. She was avoiding all the strangers in the street and the visitor's proximity. She showed extreme fear with shaking, panting and restlessness when exposed to thunderstorm, fireworks, or explosions. After a few months, with the resolution of her diarrhea, her body shape condition got back within the normal ranges, and her behavior improved. She got rapidly used to the urban surrounding, and progressively her signs of fear decreased. Four months after the adoption when left alone at home the patient began to bark and howl. The situation worsened rapidly and 5 months after adoption, when left alone, the patient barked non-stop until reunion with her owners, 8 hours daily. The owners got the help of four different trainers, which slightly improved the situation of fears. Four years after adoption the patient was able to walk in the street without fear, the proximity of strangers did not trigger avoidance apart from some individuals with a loud voice and rapid movement towards her. In this situation, the patient would avoid, but never reacted in an aggressive way. However, the dog never ceased barking when left alone at home. The best improvement after training reached one hour of barking on and off. The neighborhood complains increased and for this reason the owners sought the help of the behaviorist veterinarian. It was a month before the beginning of the COVID-19 pandemic.

## Presenting physical and behavioral signs

The patient was friendly and not fearful at all. She approached when invited, tail and pelvis waving. She was easy to touch and pet. Her physical examination was easy to perform and revealed no abnormal findings. The patient weighed 6 kg with a good body condition score of 5/9 ("Body Condition Score," 2013). The results of the serum chemistry panel and complete blood count (CBC) were within the limits of the laboratory reference range.

Based on owner's video recordings, the main behavioral symptoms were non-stop barking and howling for 1 to 8 hours daily, five days a week when the patient was left alone. She could also scratch the apartment main door or the walls around the door. According to the owner's video recording, the patient exhibited autonomic signs (e.g., panting and shaking) and restlessness that owners also described by the time they would prepare themselves to go out. The owners also reported that the patient showed a high tendency to seek contact with them during all their preparation routine. Once the owners were out, the dog immediately started to

bark. The reunion with the owners were also highly emotional with the patient jumping, crying, and being overjoyed. Most of the time, minutes after the reunion, the dog went to drink. It seemed according to the water amount, that the patient did not drink at all for 8 hours when she was staying alone. Regularly, owners found puddles of saliva next to the main door of the apartment. When the owners were present in the house, the dog sought for the owner's proximity more than normal. She frequently asked for attention and tended to follow the owners in the flat. The separation from only one of the owners could trigger a light expression of distress for a short time, essentially crying next to the door then the dog would settle down.

The dog was overreacting to loud noises. During storm, fireworks, or alarm, she started shaking, panting, and could not settle down. In this context, she sought contact with the owners, even though their proximity did not bring any comfort, despite their tries to reassure her. The dog did not express any other phobia. She was friendly and comfortable with humans and dogs. She had never been aggressive. She could be startled by a toy and play during the consultation, but she rapidly lost interest and settled down.

## Diagnosis

Based on the clinical signs and the video recording contributions (Ogi, 2018), the veterinarian using the French psychiatry model concluded to intermittent anxiety and secondary autonomy disorder due to deprivation syndrome (Mège et al., 2003). This pathology is also called separation-related disorder or separation anxiety (Denenberg, 2020; Landsberg et al., 2013; Overall, 2013) in the Anglo-Saxon literature. Immediately after adoption, the dog showed signs of severe phobias with inhibition that improved with the onset of the separation-related disorder. A secondary hyper attachment probably developed while the phobias improved (Sherman, 2008) as a compensatory strategy to cope with the fears.

## Treatment

### 1. Behavioral modification

All the previous desensitization protocols advised by the dog trainers failed. Owners were therefore advised to simply limit their emotional expressions during their regular departure and reunion routine. To do so, before leaving for their regular activities or work, the owners were advised to delay for 20 minutes the separation from the end of their departure routine and to fill this time with neutral occupations without having any communication with the dog. The goal was to limit the effects of the previous and strong classic conditioning between the departure routine and the dog anxiety. During reunion, they were advised not to greet the dog until she was able to answer to a "sit" cue. Then they could greet her in a calm manner.

### 2. Medication

The dog was prescribed fluoxetine at a dose of 20 mg (3.3 mg/kg) every 24 hours (Bleuer-Elsner et al., 2021), which is above the widely recommended dosages (e.g., 1-2 mg/kg) (Landsberg et al., 2008). Additionally, the owners were asked to give the dog trazodone *PRN* at a dose of 25 mg (4.2 mg/kg) 90 minutes before departures. Trazodone was also given *PRN* at a dose of 50 mg (8.4 mg/kg) *PO* before storm, fireworks, or alarms, 90 minutes before the stressful event when it is predictable and at the event onset if not. A gap of 8 hours minimum had to be respected between 2 administrations of trazodone (Harting et al., 2018; Gruen & Sherman, 2008).

### 3. *Environment modification*

A safe place where the dog could feel secure was proposed. She already had a carpet. The central localization of the carpet was changed for a more peripheric one in order not to give the patient the possibility to watch her owners' movements from her resting place and therefore lower her vigilance.

A basic training using a stay cue was used to habituate the dog to be relaxed in her resting place. Toys and treats were used to increase the interest of the dog for her resting place, in order make it reassuring, even when the owners would not be at home. Finally, the owners were advised not to play, nor to pet, nor to have any interaction with the patient when she was resting on her mat. The goal was to provide a very private and secure place where the dog can settle down and be certain that she would not be disturbed.

### Follow-up

The patient presented rare episodes of shaking without identified trigger one week after the treatment onset probably induced by the fluoxetine (Simpson et al., 2007). This adverse effect disappeared during the next two weeks without any dose adjustment. The barking intensity and frequency decreased significantly 2 days after the treatment onset. The dog stopped to scratch the door during the first week and the autonomic signs before separation decreased gradually during the first two weeks of treatment. During the reunion, the dog still jumped and showed overjoy but tended to settle down faster.

Six weeks after starting the treatment, a closure was just declared to prevent the spread of the COVID-19 pandemic. It was decided with the owners to perform a remote follow up consultation. Just before the closer, the patient showed a significant improvement in her behavior during the owner separation routine. She was able to keep calm, even lay on her mat without signs of hypervigilance. Panting and shaking were not observed anymore. The barking improved and stayed limited between 15 to 45 minutes daily. The salivation near the door disappeared. The reunions were easier, the dog was able to sit to be greeted after 2 to 5 minutes. Since the closure start, the separation ceased completely, the dog was never left alone. The owners stopped to give the trazodone 2 weeks before the follow up because the dog began to be too excited and was jumping a lot. When they stopped, this adverse effect ceased.

The owners reported a good influence of the treatment on the general level of the dog's fears. For this reason, it was decided to continue fluoxetine treatment daily even if the dog was not left alone. One year later, with the containment of the COVID-19 pandemic and the return to a work routine, separation situations came back. The patient worsened again immediately after the first separation. She was left alone 5 hours daily and barked randomly from 1 to 2 hours. She did not scratch the door as she did before treatment but the previous hypervigilance during the owners' preparation routine, overjoy during reunion and proximity seeking came back. The autonomic symptoms (i.e., panting and shaking) did not reappeared.

#### 1. *Behavioral modification*

Because the owners applied the behavioral modification only a few weeks before the start of the pandemic, the veterinarian had to explain it again to be sure everything was understood.

In addition, the owners were asked to create a new ritual using a specific object, such as a colored water bottle. They were asked to put the bottle in a specific place, and make sure that the dog saw them do it. Each time the bottle was in this place, they were asked not to interact with the dog. After a few minutes, they had to hide the bottle and then the dog was allowed to interact with them. They were advised to start this training only for very short times, and once the



dog would be able to respond to it for one hour, they were allowed to get the bottle out on real departures.

## 2. Medication

The dog was still under fluoxetine at a dose of 20 mg (3.3 mg/kg) *PO* every 24 hours. In addition, the veterinarian prescribed gabapentin 100 mg (16.6 mg/kg) *per PO PRN* one hour and a half before separation (Piotti et al., 2019).

Ten days after the treatment onset the dog improved. The barking was limited to 10 minutes after separation. Then the dog would settle down and rest until the owners return.

At the time this paper is written, the dog is still under the same treatment. The barking did not disappear completely but are limited in time (i.e., 5 to 10 minutes). No other signs of distress are reported by the owners and the neighborhood did not complain any more.

## Discussion

Separation-related disorder is a common disorder in dogs, particularly when adopted from shelters (Sargisson, 2014). Still, its etiology, its treatment, and its prevention remain elusive (Ogata, 2016). In this case, the dog's anxiety is probably the result of a hyper attachment secondary to extensive phobia. This insecure attachment (Konok et al., 2019; Solomon et al., 2019) is also a common behavioral profile in dogs adopted from shelters (McCraive, 1991).

Most of the authors agreed that the onset of separation treatment should include medication, at least for the first weeks (Takeuchi et al., 2000). The main and FDA approved drugs for this indication are fluoxetine (Landsberg et al., 2008) and clomipramine (King et al., 2000).

In the case of the patient, since the main complaint was non-stop barking, fluoxetine was preferred for its anxiolytic (Reisner, 2003) and anti-compulsive effects (Irimajiri et al., 2009). Higher dose helps the regulation of uncontrolled behaviors (Bleuer-Elsner et al., 2021), therefore fluoxetine was prescribed above its widely recommended dosages of 1-2 mg/kg.

Trazodone is an off label drug legally prescribed in dog anxiety (Chea & Giorgi, 2017) and suggested stand alone or as adjunctive (Gruen & Sherman, 2008). In this case, after a month of trazodone treatment, the dog showed symptoms of excitation. Given that trazodone is an antidepressant medication with sedative effects, this sudden adverse effect after a month of treatment might be the result of the antidepressant onset of the molecule. For this reason, and given its efficiency on acute expression of fear (Bleuer-Elsner et al., 2021), gabapentin was prescribed instead of trazodone with good results.

Since the adoption of the dog, 4 years of treatment attempts only based on desensitization and counter conditioning did not improve the patient. Systematic desensitization is indeed the standard treatment (Butler et al., 2011) with better results when coupled to counter conditioning (Poppen, 1970). The main source of difficulties is the owner's commitment. In a standard family busy day, owners lose rapidly patience and interest in such a long procedure (Takeuchi et al., 2000).

For this reason, the behavioral modifications for the patient were based on extinction and anxiety control more than on desensitization.

Learning theory tells us that the delay between an unconditional stimulus and the anticipation onset is primary to classical conditioning (Davis, 1970). Then, once the conditional and unconditioned stimulus are unlinked, the conditional behavior is extinct (Gluck et al., 2014). For those reasons, the goal of the therapy was to postpone the separation from all its cues (e.g., owners' preparation routine, closure of parts of the house).

In addition, such a delay between the conditional stimulus (i.e., preparation routine) and the unconditional one (i.e., dog left alone), helps the dog to control his own anxiety since the dog will not be left alone at the anxiety peak.

To do so, the owners were advised to end all their pre-separation routine 20 minutes before going out for their regular activities. During this time, the owners were advised to shut down all their communication channels to avoid reactivating any separation related cue.

Conditioning and anxiety are intimately linked (Carpenter et al., 2019). On one hand, an extinction protocol helps anxiety control, and on the other hand, medication that lowers vigilance and fear makes the extinction learning more efficient. In this case, this extinction protocol led to better results than the previous desensitization. But probably both would have brought improvements once the anxiety was lowered by the effectiveness of the medication.

During reunion, a protocol of calm reinforcement was preferred with a sit cue as the first condition for the dog to be greeted. This way, the patient and the owner would be more able to control their emotions.

The patient did not show any signs of disorder during the pandemic closure since owners never left the dog alone. When the closure ended and the owners got back to their work routine, the symptoms reappeared immediately, with more severity than they were after the first weeks of treatment.

This is a good illustration of the fact that a conditioned behavior cannot be erased. Extinction leaves previously conditioned association intact (Bouton & Nelson, 1998; Falls, 1998). When the environment and routine will change, retrieving a context associated with fear memory reactivates neurons in the hippocampus, amygdala, and cortex (Tayler et al., 2013). In the case of the patient, the previous extinction procedure seemed to help, then during the pandemic closure the context authorized a complete avoidance of the anxious circumstances (i.e., being left alone). With the return of the anxiogenic situation, the previous symptoms came back immediately.

After the relapse, the object-cue therapy was prescribed to replace the classical conditioning based on the owners' routine before separation, by another one with a larger context. Replacing a classical conditioning with undesirable outcome with another one that leads to a different outcome is another way of therapy (Gluck et al., 2014).

Finally, the patient seemed stabilized again. His history shows lots of vulnerability and future context change may probably cause relapse.

## Conclusion

The COVID-19 pandemic is a major event of the early 21st century. Its final influence on humans and animals remains uncertain at the time this paper is being written. More and more cases of phobia and anxiety in dogs emerge that could be related to the pandemic. Indeed, due to the closure lots of fearful dogs avoided their fear-related context and their symptoms apparently disappeared. With the end of the closure and the reactivation of the fearful contexts, the symptoms may reappear with a higher intensity due to a rebound effect. This case underlines the fact that therapy of anxiety is a long process that should be primarily supported by medication, and secondarily not interrupted even if the fearful context is removed temporarily. As far as possible, a regular exposition is necessary to complete the treatment and prevent this rebound effect when back to a normal routine. These optimal conditions were not easily reached under the COVID-19 pandemic conditions and many similar cases may be seen in the months coming.

## Authorship

The idea for the article was conceived by Stephane Bleuer-Elsner.

The case was handled by Stephane Bleuer-Elsner and discussed with Sylvia Masson.

The article was written by Stephane Bleuer-Elsner and Sylvia Masson.

## Conflicts of interest

The authors have no conflicts of interest to declare.

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## Gestione dei disturbi legati alla separazione durante la pandemia di COVID-19: un caso clinico

Stephane Bleuer-Elsner, Sylvia Masson

### *Sintesi*

La chiusura correlata alla pandemia COVID-19 ha avuto un impatto sulla gestione del disturbo legato alla separazione nel cane. Otto settimane prima della chiusura della prima pandemia di COVID-19, la paziente, una cagnolina meticcica, sterilizzata, di 8 anni di età e del peso di 6 kg, è stata portata alla visita comportamentale a causa dell'abbaiare ininterrottamente quando era separata dai proprietari, 8 ore al giorno, 5 giorni alla settimana.

Il cane, prima del consulto, aveva seguito, per 4 anni, corsi di addestramento basati sulla desensibilizzazione, senza l'uso di farmaci. Tali esperienze avevano ottenuto un miglioramento del paziente ma non avevano mai ridotto l'abbaiare oltre le due ore al giorno. Al paziente è stata prescritta fluoxetina alla dose di 20 mg (3,3 mg/kg) PO ogni 24 h e trazodone PRN prima della separazione alla dose di 25 mg (4,2 mg/kg) PO. È stato prescritto un piano di modificazione comportamentale, basato sull'estinzione e sul rinforzo della calma.

Otto settimane dopo l'inizio del trattamento, poco prima della fine del primo lock-down, il cane è migliorato significativamente, riducendo l'abbaiare quotidiano fino a 15 minuti. Durante tutto il successivo periodo di lock-down (cioè un anno), il cane non è mai stato lasciato solo. Terminato il lock-down, l'abbaiare si è ripresentato, proprio al primo evento di separazione, raggiungendo da 1 a 2 ore giornaliere, anche se la fluoxetina non era mai stata interrotta. Pertanto, il gabapentin è stato prescritto PRN prima della separazione alla dose di 100 mg (16,6 mg/kg) al posto del trazodone che ha innescato l'eccitazione nel paziente quando è stato precedentemente provato. Il piano comportamentale è stato completato con un condizionamento aggiuntivo prima della separazione. Il cane è migliorato rapidamente fino a un breve tempo tollerabile di abbaio (cioè da 5 a 10 minuti). Questo risultato è rimasto stabile al momento della stesura del documento, ovvero 3 mesi dopo la fine del lock-down. L'evoluzione del paziente pone l'accento su due temi importanti nel trattamento del disturbo da separazione: in primo luogo, nella maggior parte dei casi, i farmaci sono necessari per abbassare il livello di reazione emotiva e, in secondo luogo, l'interruzione dell'esposizione al contesto pauroso può avere effetti di rimbalzo quando il contesto sarà sperimentato di nuovo. Gli effetti a lungo termine della pandemia di COVID-19 rimangono sconosciuti sul benessere sia dell'uomo che del cane. Dovrebbero essere condotti studi più ampi per misurare il suo impatto sul disturbo correlato alla separazione nei cani.





# Behavioral management of puppies in training as military dogs

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*Abstract:* Military dogs, besides being exposed to stressful events during operations (loud noises, transport, exposure to high and low temperatures, etc.), can be involved in explosions and/or gunshot wounds.

Breeding, selection and management of working dogs are specific activities that involve a deep knowledge of different disciplines such as genetics, animal husbandry, internal medicine and applied ethology.

This study aimed to provide specific guidelines on the breeding and behavioral management of German Shepherd and Belgian Malinois military dogs, from birth to the seventh month of age.

The deep knowledge of the dog from the point of view of applied ethology and the psychology of learning, beyond a natural predisposition to interact correctly with the dog, are the most important topics for a good canine trainer; education and dog training are only a direct and natural consequence of this.

*Key Words:* puppy, behavioral management, military dogs.

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## Introduction

Military dogs, besides being exposed to stressful events during operations (loud noises, transport, exposure to high and low temperatures, etc.), can be involved in explosions and/or gunshot wounds. They could be injured or witness the injury of other dogs or of their own handler.

In these conditions, some dogs may develop phobia and panic attacks, i.e. similar to conditions reported in humans as post-traumatic stress disorder.

However, not all dogs develop behavioral pathologies, and this could be related to genetic factors, breed predispositions, previous experiences during the socialization period and to the specific relationship with their handlers.

Breeding, selection and management of working dogs are specific activities that involve a deep knowledge of different disciplines such as genetics, animal husbandry, internal medicine and applied ethology.

Few scientific studies have dealt with the breeding and selection of working dogs for public utility (Tidu et al., 2002), but many studies have investigated the effect on behavior of the physical and social environments in which the puppy is managed daily (Lezama-Garcia et al., 2019; Guardini et al., 2016; 2017).

This study aimed to provide specific guidelines on the breeding and behavioral management of German Shepherd and Belgian Malinois military dogs, from birth to the seventh month of age.

## Management of bitches

The reproduction and breeding area must be separated from other divisions of training and adult kennels.

The separation of this area provides correct sanitary isolation and guarantees a suitable environment for gestating/lactating females and growing puppies.

Generally, puppies spend a lot of time sleeping (90% of the time in the first week of life) and much of their sleep is paradoxical sleep (95% of total sleep) (Gazzano, 2013).

Growth hormone (GH) secretion in different animal species occurs with spontaneous secretion peaks during paradoxical sleep. In this phase, the inhibition of the release of somatostatin occurs and results in the release of GH. The release of GH is therefore linked to sleep and not to the light–dark cycle and for this reason, is conditioned by management factors (Van Cauter & Refetoff, 1985). Isolation and quiet space, therefore, allow puppies to grow better.

The bitches must be housed in single kennels, possibly with a linear disposition. Inside the kennels, there is an antechamber area, in which food and water are administered; laterally and further inside, there is a more protected area: the ‘birth area’, made of waterproof and washable wooden material covered with non-toxic paint.

Ambient heating is provided by an infrared lamp, secured to the ceiling.

The bitches’ kennels are equipped with a closed-circuit camera for remote monitoring of the behavior of the bitch during labour and of the puppies.

In front of the kennels are fenced lawn areas, suitable for hosting dogs for a period in which the daily cleaning and cleansing of the kennels is carried out.

The playful, training and educational activities of the puppies take place in a large area of lawn, adjacent to the area where the kennels and walkways are located.

These areas are equipped with masonry rubble to simulate disastrous urban environments, circuits and paths for overcoming physical obstacles and are enriched by both known and unknown objects.

Two months before the start of the estrous cycle, the bitch is transferred to the facilities of the reproduction and breeding area.

After mating, the bitch receives two vaccine doses of canine herpesvirus antigen (F205 strain). The first dose is given about ten days after mating, while the second dose is administered one or two weeks before the expected date of delivery.

The diagnosis of pregnancy is made around the twenty-fifth day of ovulation, with an abdominal ultrasound examination.

The bitch continues to carry out normal basic training and play activities even during the gestation period, as this allows her to maintain her mental and physical fitness. On the other hand, any type of training in searching for explosive substances is prohibited as there are currently no data suggesting the absence of a teratogenic effect of substances absorbed by contact, inhalation or ingestion by a pregnant bitch.

## Handling of puppies

The puppy is handled by a specialized staff member for five minutes a day, from the second week to the end of the third week of life; the fingers are gently passed over the gums, the auricles are massaged, the limbs and paws are palpated. This practice has the purpose of obtaining a habituation to handling that should allow better management of the dog, also for when the adult dog will be managed during medical examinations and common practices (Gazzano



et al., 2008). This form of manipulation of cutaneous and mucosal tissues aims to guarantee a sensory homeostasis at higher levels allowing the adult dog to wear, without discomfort, some devices used during work, such as protective shoes for rubble or anti-UV glasses, for dogs that are more predisposed to develop keratoconjunctivitis sicca.

During this period, the dedicated staff must ensure and guarantee that: the puppy sucks milk regularly, the bitch produces enough milk for all the puppies in the litter, and defecation and urination with perineal stimulation (Ogi et al., 2021).

## Socialisation of puppies

From the third week to the fourth month of age, the puppy is ready to deal with the 'socialization period'.

The tendency to isolate the puppy before completing the vaccination cycle, to prevent the onset of infectious diseases, is not an advisable practice. Puppies, born and nursed by regularly vaccinated bitches, enjoy, up to the eighth week, the coverage of colostral antibodies and therefore, can and must carry out the social and environmental experiences necessary in this phase of development.

The weaning of the puppies, which coincides with dental eruption (the period in which the 'first detachment' occurs), begins around the fourth week of age. The feed used for weaning is the same used for feeding the bitches during pregnancy and lactation. However, prenatal dietary orientation in the dog is well known (Pageat, 2009).

The puppies are kept separate from bitches for gradually increasing times, starting from the fourth week of age. However, it is important to keep the puppies with the siblings and the bitch until the fifth week, as in this period they learn to modulate the bite. Contact with the bitch is also primary after weaning for learning basic social rules and the acquisition of self-control. In this period, the puppies engage in pseudo-competitive and aggressive forms of play, in which they use signals of dominance or submission; these activities also allow them to learn to inhibit, through the mutual use of negative punishments, their ability to inflict pain (Dehasse, 1994). Around the sixth week of life, the puppies are permanently removed from their mother. The litter remains together until the third month of life to guarantee the puppy's intraspecific play.

The breeder, a marginal figure in the 'neonatal period' and in the 'transition' period, becomes a point of reference for the puppy at the time of weaning. There are different times and ways in which detachment occurs for puppies of different sexes (Verga, 1993).

Once the puppy has gained confidence with the breeder, interspecific socialization can begin. The puppy has the maximum ability to socialize with humans between the fifth and seventh week and the tenth week of age.

The breeder, after weaning, becomes a 'safe base' for the puppy (Riggio et al., 2021; Mariti et al., 2013; 2018; 2020; Carlone et al., 2019) who can explore the environment, interact with other individuals and learn new social skills (Tidu et al., 2002).

At the beginning of the socialization phase, puppies tend to approach social stimuli, while later, from the eighth week, this response is reduced, leading to an avoidance reaction to unknown stimuli (Bradshaw, 1990). For this reason, it is desirable that the breeder's socialization activity starts early, at the beginning of the recommended period.

From the second half of the third month until the puppy passes to the training section, the dog is exposed regularly and always in the presence of the breeder, to new environments and new situations.

## Training protocol

### *Training module 1* (getting the puppy used to travelling uneven surfaces and training it to overcome small obstacles)

The puppy, as a childlike type, creates a gradually increasing emotional bond with the *role model*: the breeder. The puppy, in the presence of the breeder, can explore, interact with the external environment and is more predisposed to play with its brothers/sisters and with strangers in different, new or unusual environments.

In this way, it is possible to teach the puppy to move in environments with different types of surfaces or to overcome physical obstacles with increasing degrees of difficulty.

The puppy learns, in particular, to overcome physical obstacles such as small stairs and small tilting platforms, to walk on uneven or wet surfaces, to enter dark or closed places.

To attract the puppy to new environments and to encourage it to overcome the proposed obstacles, the attractive power of the role model, food or the 'social facilitation' provided by the presence of the litter siblings is generally exploited.

This training module begins to be implemented starting from the fourth week but continues, from the didactic point of view, up to the tenth week.

### *Training module 2* (teaching the puppy how to use the sense of smell)

During the period, in which the puppy is performing exercises of training module 1, it can simultaneously begin training module 2.

This module involves the use of food reinforcements, administered when the puppy has been able to perform the intended task, for example, to identify dog treats through the use of smell alone.

### *Training module 3* ('push and pull' game)

From the third month of age, the puppies learn to play with the breeder in a competitive way. This type of game exploits the possession and predatory motivation of the puppy, so that it enters competition with the handler to conquer a toy.

The push and pull exercise can be later associated with the 'take and leave' exercise.

### *Training module 4* (progressive habituation to strong and sudden acoustic stimuli)

When the puppy has reached the age of four weeks it is exposed to loud sounds to which it must learn not to direct its attention or to have reactions of fear.

This state of emotional stability is reached gradually while the puppy is concentrating on a specific activity, that completely occupies its attention (for example, the olfactory search for food).

When the puppy has learned to completely orient itself towards the human model (handler) and when it is able to perform what is foreseen in training module 3, then, as a distracting activity, it is desirable that food be replaced by social play.

### *Training module 5* (getting used to new sensory stimuli using social games)

Social play is a method used as a reward, also in the training of adult dogs, as it has a very powerful reinforcing ability.

For this reason, the distracting use of social play is indicated for learning not to react in contexts where it is necessary for the dog to remain emotionally calm and relaxed or when, in the work environment, where annoying and high-volume acoustic and distracting stimuli are present.

This way of learning without reaction (habituation) is not just about acoustic stimuli. All described concepts must also be applied in other contexts, such as for habituation to travel in aircraft with rotating and fixed wings, on vehicles or on boats.

### *Training module 6* (search for objects in new environments)

The dog training module to operate in closed and new environments has to be faced by dogs that have reached at least ten weeks of age.

To have an optimal performance, it is essential that the puppies are motivated to interact with the reference model (human model) and to play in a competitive way with them.

In this module, the puppy is taught to search for its favorite toy through the sense of smell in dark or new places. These environments represent a further difficulty for the dog in carrying out the exercise as the dog feels uncomfortable in the transition from light to dark or in an unfamiliar place.

If the dog has a strong social play motivation, it will be able to overcome these difficulties more easily. For this reason, this specific activity must be proposed only after a strong emotional bond with the reference model has been established, such that its presence represents a safe basis for the dog and gives value to social play.

Specific exercises of this training module continue until the puppy passes from the breeder to the handler, who will activate the specific training work. During this long period, exercises will be modified and intensified, increasing in difficulty. For this reason, this phase ends when the dog has reached seven months of age.

## Conclusions

Breeding and management of dogs in kennels and the modality of activity administration being conducted, can affect the well-being and performance achieved during training.

However, there are still doubts on the specific role that the breed plays in the onset of behavioral pathologies. It is not possible to remove the working dog from the stressors to which it is exposed or to act on the elimination of these in the environment in which the dog has to operate or live. It is necessary that the dog can perceive stressors in the correct modality, so that these do not alter its equilibrium (Weiss, 1972).

The presence of the 'secure base', which generally coincides with the figure of the breeder, first, and the handler, subsequently, helps to lower the level of discomfort in dogs exposed to high-level stressful stimuli.

The observation for the same type of stress to act in different ways in different animals, and how this can happen, is related to behavioral development and building the emotional bond between the dog and its handler. However, it cannot be excluded that breed contributes greatly to the acquisition of this ability.

Ultimately, the handler plays a fundamental role in the management of the dog as well as the breeder who is important in influencing the behavioral development of the puppy.

The deep knowledge of the dog from the point of view of applied ethology and the psychology of learning, beyond a natural predisposition to interact correctly with the dog, are the most important topics for a good canine trainer; education and dog training are only a direct and natural consequence of this.

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## Behavioral management of puppies in training as military dogs

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### *Sintesi*

I cani militari, oltre ad essere esposti ad eventi stressanti durante le operazioni (rumori forti, trasporto, esposizione ad alte e basse temperature, ecc.), possono essere coinvolti in esplosioni e/o ferite da arma da fuoco.

L'allevamento, la selezione e la gestione dei cani da lavoro sono attività specifiche che comportano una profonda conoscenza di diverse discipline come la genetica, la zootecnia, la medicina interna e l'etologia applicata.

Questo studio mira a fornire linee guida specifiche sull'allevamento e la gestione comportamentale dei cani militari Pastore Tedesco e Malinois Belga, dalla nascita al settimo mese di età.

La profonda conoscenza del cane dal punto di vista dell'etologia applicata e della psicologia dell'apprendimento, oltre ad una naturale predisposizione ad interagire correttamente con il cane, sono gli argomenti più importanti per un buon addestratore cinofilo; l'educazione e l'addestramento del cane sono solo una conseguenza diretta e naturale di questo.