Analysis of factors affecting the behaviour of both dogs during a Strange Situation Procedure (SSP) to assess intraspecific attachment

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Abstract

Previous studies on the dog intraspecific attachment carried out with the Strange Situation Procedure (SSP) have not been able to clarify the nature of this bond. Several factors may affect the behaviour of the dog dyads involved the procedure. In the current study, fifty-five dyads of adult dogs living in the same household participated in a SSP. The duration of fifteen behaviours was measured. Data were analysed using Generalised Linear Mixed Models considering single behaviours as dependent variables. The predictors were episodes (1, 4, and 7), type of relationship (mother-offspring pairs, non-related cohabitant pairs), sex (female-female, male-female, and male-male), and age difference. Bonferroni Holmes post hoc tests were performed to allow pairwise comparison. Dog dyads spent significantly more time trying to escape from the experimental room in episodes 1 ($p=0.008$) and 4 ($p=0.029$) than episode 7, in passive behaviours in episode 7 compared to episode 1 ($p=0.001$), in environmental exploration in episode 1 compared to both episode 4 ($p=0.001$) and 7 ($p=0.001$), in proximity to each other in episode 4 and 7 compared to

episode 1 (4 vs 1: p=0.001, 7 vs 1: p=0.001), and in locomotion in episode 1 than episodes 4 (p=0.009) and 7 (p=0.001), and in episode 4 compared to 7 (p=0.007). Mother-offspring pairs spent more time in passive behaviours (p=0.028) compared to unrelated cohabitant pairs. Male-male pairs spent more time oriented to the door/window compared to female-male (p=0.030) and female-female pairs (p=0.030). Finally, proximity to the conspecific decreased (p=0.040), while locomotion increased (p=0.027) with age difference. According to our findings, dogs involved in an intraspecific SSP seem to be primarily distressed by the initial separation from the owner. However, they may be able to use the conspecific as a buffer against stress as the test progresses. Other factors related to the subjects involved in the procedure, such as the type of relationship, sex and age difference may also affect their behaviour. Future studies should take these factors into account if they use the SSP to explore dog intraspecific attachment.

Keywords:

attachment; dog; intraspecific; strange situation test; SSP; bond

1. Introduction

While adult dog's capability to form attachment bonds with humans has been extensively studied in recent years (Mariti et al., 2013; Payne et al., 2015; Rehn et al., 2014, 2013; Topál et al., 1998), dog intraspecific attachment has received scant attention. Contrary to the findings on puppy attachment to adult conspecifics (Prato-Previde et al., 2009), the few studies conducted on adult dog intraspecific attachment could not clearly observe those behavioural dimensions that unequivocally identify the dog-human relationship as an attachment bond (Mariti et al., 2017, 2014; Sipple et al., 2021).

For instance, in a study by Mariti et al. (Mariti et al., 2014), adult dogs involved in a Strange Situation Procedure (SPP) in which the role of the attachment figure was played by an older

cohabitant dog, showed a higher contact maintenance effect (i.e. the tendency to maintain physical contact and proximity with the attachment figure) towards the human stranger rather than towards the familiar conspecific. Although such a difference was not observed when dogs were tested with their own mothers (Mariti et al., 2017), neither was the expected preference for the presumed attachment figure over the human stranger.

While these findings do not clarify the nature of dog intraspecific attachment, they do suggest that factors related to the dyads tested may affect dog attachment behaviour. Beside the type of relationship that ties the pair tested, other individual characteristics, such as age and sex, may affect dog attachment behavioural patterns. With regard to age, Mongillo et al. (Mongillo et al., 2013) found that senior dogs (≥ 7 years) showed more contact seeking, as well as more passive behaviours during separations from the owner compared to adult dogs (<7 years), when tested in the SSP. Furthermore, through a similar procedure, Carlone et al. (Carlone et al., 2014) found that dogs under 24 months of age displayed more behaviours indicative of separation distress during isolation and more contact seeking than older dogs, when tested with a conspecific.

With regard to sex, the majority of previous studies on dog-owner attachment found either minor (Prato-Previde et al., 2003) or no differences (Fallani et al., 2007; Gácsi et al., 2001; Topál et al., 1998) in the attachment behaviour of female and male dogs tested in the SSP. However, a very recent study by D'Aniello et al. (D'Aniello et al., 2021) found that female dogs showed higher levels of sociability towards both the stranger and the owner, as well as higher levels of separation-distress when the owner was absent, compared to male dogs.

Different from the dog-human relationship, an important aspect to consider when exploring adult dog intraspecific attachment is the lack- or reduced degree- of asymmetry in the roles, as well as in the dynamics of interactions that characterize the relationship between the two individuals (Riggio, 2021; Savalli and Mariti, 2020). For this reason, previous authors have suggested that adult dog intraspecific attachment may better fit a peer-to-peer relationship model, such as the one between

human friends (Savalli and Mariti, 2020) or siblings (Mariti et al., 2014; Sipple et al., 2021), which may be harder to investigate by applying the same paradigm and methodology used for dog-owner attachment.

For instance, similar to what has sometimes been reported for human siblings (Stewart and Marvin, 1984; Teti and Ablard, 1989), when two dogs are tested in the SSP, the separation from the primary attachment figure (i.e. the owner in the case of dogs and the mother in the case of human infants), which occurs from the beginning to the end of the procedure, may lead both subjects to activate their attachment system and consequently manifest distress for the owner's absence (Mariti et al., 2018) and seek comfort from the stranger. Moreover, contrary to what usually occurs with owners, the behaviour of dogs that play the role of the attachment figure in the SSP cannot be even partially standardized, generating dynamics of interactions that are difficult to code and interpret.

The aim of the current study was to assess the effect of test-related (episode) and pair-related variables (relationship, sex combination and age difference) on dog behaviour in an intraspecific SSP. Taking into account the absence of an attachment behaviour pattern that scientifically confirms the presence of an attachment bond between adult conspecifics by means of the SSP (Mariti et al., 2017), as well as previous authors' suggestion that adult dog intraspecific bond may be characterized by a lack of clear asymmetry in the dynamics of interaction between the subjects involved in the relationship (Sipple et al., 2021), the behaviours displayed by the two dogs in the current study were grouped together and analyzed as behaviours of the dyad.

2. Material and Methods

Dog owners were informed about the procedure and asked to sign a consent, as required by standard ethics protocols. However, being observational in nature, this study did not require an additional approval of an animal ethics committee.

2.1.Subjects

Fifty-five dyads were recruited by contacting dog owners among personal contacts of the senior authors (C.M. and A.G.). Inclusion criteria were the same as in Mariti et al. (Mariti et al., 2014), in particular dogs had to be older than 14 months and younger than 11 years, and had to be living in the same household for at least 9 months.

Each dyad was composed by a dog tested as the "attached individual" and another dog representing the supposed "attachment figure", as required in a SSP. Seventeen of these dyads were formed by the mother and her adult offspring; whilst the remaining 38 were unrelated dogs.

Since 18 dogs were involved twice (e.g. once as tested dog and once, the second time, as presumed attachment figure), a total of 92 individual dogs were involved (in the case of unrelated cohabitant pairs the attachment figure was always played by the older dog): 63 females (34 entire and 29 spayed) and 28 males (14 entire and 15 castrated); 57.1 ± 31.8 months old (mean ± standard deviation); 28 mixed-breeds, 14 Labrador Retrievers, 6 German Shepherds, 5 Bearded Collies, 5 Border Collies, 5 Flat Coated Retrievers, and the remaining 28 belonging to breeds with less than 5 individuals. The dyads had different sex composition: 27 female-female, 24 female-male, and 4 male-male.

None of the female dogs was in oestrus, nor were they pregnant at or around the time of testing.

2.2.Experimental setting

The study took place in a bare room within the Department of Veterinary Sciences at the University of Pisa (Italy). The room (4.50 m X 4.30 m) was prepared to match the fundamental requirements described for the original SSP. It was equipped with one chair for the person who played the stranger, one water bowl, and two cameras that offered a full view of the experimental setting. Only

one door gave access to the room. Contrary to the original procedure, no toys were provided in order to avoid possible aggressions between the dogs.

2.3.Experimental Procedure

The experimental protocol was the same as that reported in Mariti et al. (2014, 2017).
Although some modifications were made to the original SSP because of the involvement of a dog instead of a person in the role of the attachment figure, the procedure was conceived to be as similar as possible to the one described by Ainsworth and Bell (1970) for its original application on human infants and their caregivers. Like in the original protocol, the SSP consisted of 7 episodes that included two bouts of separations (episode 3 and episode 5) and reunions (episode 4 and episode 7) from the presumed attachment figure, as well as one single bout of separation (episode 4) and reunion (episode 6) from the stranger; each episode lasted 2 minutes. Table 1 reports a more detailed description of each episode.
The stranger was played by five 25 to 35 years old female researchers, unfamiliar to all the dogs involved. A second researcher helped with moving the dog who played the attachment figure in and out of the room, according to the protocol. Different from some previous studies on dog-owner attachment (Palmer and Custance, 2008; Prato-Previde et al., 2003; Topál et al., 1998), but similar to others (D'Aniello et al., 2021; Mariti et al., 2017, 2014), our protocol required the human stranger to remain passive in the interactions with the dog. This is because the behaviour of dogs towards a human stranger is not easily predictable by the way the latter behaves (Tan et al., 2018), possibly making passive behaviours preferable for the role of the stranger in the current study.
Dogs' behaviours were videotaped throughout the procedure and were analysed using a continuous sampling technique in order to measure their duration (in seconds). The complete list of analysed behaviours is reported in Table 2.

2.4.Statistical Analysis

We ran all of the analyses with R v 4.1.0. Generalised Linear Mixed Models (GLMMs) were used to test whether the behaviour of both dogs (considered as sums of the time spent by both dogs showing a certain behaviour) varied in relation to episodes (1, 4, and 7), relationship between dogs (cohabit or mother/son), sex of dogs (male-male, male-female, and female-female), and age difference between dogs. Single investigated behaviours (recalculated as proportions over the total time, i.e., sum of the total time of observation on both individuals) were used as dependent variables. The ID of each couple of dogs was considered as subject and random factor in the analysis. Episode, relationship between dogs, sex of dogs, and age difference in years between dogs were considered as predictors in the model. We additionally included a Heterogeneous diagonal covariance structure to consider the repeated measures (i.e., episodes) for each individual.

We used the "glmmTMB" function in the "glmmTMB" package as this function includes several fit families that are suitable to deal with proportions and zero-inflated distributions (Brooks et al., 2017). We tested beta and tweedie families and included or excluded a zero-inflation term based on the QQ plot residuals and residual vs predicted plot from the package "DHARMa". We ran pairwise contrasts using a Bonferroni-Holm post hoc correction via the function "emmeans" in the package "emmeans". We considered p = 0.05 as level of significance.

3. Results

Several behaviours of the dyads tested were affected by the variables analysed through GLMMs (Table 3; Figure 1). More in detail, the SSP episode had a statistically significant effect on the expression of escape attempts (χ^2=13.3, p=0.001), vocalizations (χ^2=9.8, p=0.007), passive behaviours (χ^2=27.0, p=0.001), environmental exploration (χ^2=53.3, p<0.001), proximity (χ^2=26.3, p<0.001) and locomotion (χ^2=56.4, p<0.001).

Post-hoc tests for pairwise comparisons revealed that the dog dyads spent significantly more time in trying to escape from the experimental room in episode 1 (odds ratio: 73.21 ± SE 17.65, t=3.0, p=0.008) and 4 (odds ratio: 11.22 ± SE 4.28, t=2.6, 0.029) compared to episode 7, in vocalizations in episode 4 compared to episode 1 (odds ratio: 1.45 ± SE 0.18, t=3.0, p=0.008), in passive behaviours in episode 7 compared to episode 1 (odds ratio: 6.75 ± SE 2.65, t=4.9, p<0.001), in exploring the environment in episode 1 compared to both episode 4 (odds ratio: 2.94 ± SE 0.46, t=6.8, p<0.001) and 7 (odds ratio: 2.88 ± SE 0.50, t=6.1, p<0.001), in proximity to the conspecific in episode 4 (odds ratio: 2.46 ± SE 0.43, t=5.1, p<0.001) and 7 (odds ratio: 2.17 ± SE 0.39, t=4.3, p<0.001) compared to episode 1, and more time in locomotion in episode 1 than episodes 4 (odds ratio: 1.50 ± SE 0.13, t=4.8, p<0.001) and 7 (odds ratio: 2.05 ± SE 0.21, t=7.2, p<0.001), and in episode 4 than 7 (odds ratio: 1.37 ± SE 0.14, t=3.0, p=0.007).

Furthermore, the type of relationship between the two dogs had a significant effect on the amount of time spent in passive behaviours (Estimate=-1.78 ± SE 0.08; Z=-2.2, p=0.028), which was shorter in non-related pairs. As for the sex of the dyads, it had a significant effect on social exploration (χ^2=6.8, p=0.035) and on being oriented to the door/window (χ^2=7.1, p=0.029). Post-hoc comparisons showed that male-male pairs spent more time being oriented to the door/window compared to female-male (odds ratio: 2.47 ± SE 0.87, t=2.6, p=0.030) and female-female pairs (odds ratio: 2.51 ± SE 0.90, t=2.6, p=0.030). Instead, only non-significant trends were found for social exploration when comparing pairs with different sex combinations (female-female vs female-male: odds ratio: 1.52 ± SE 0.31, t=2.1, p=0.100; female-female vs male-male: odds ratio: 2.46 ± SE 1.05, t=2.1, p=0.093). Finally, age difference between the two dogs had a significant effect on proximity to the conspecific (negative relationship; Estimate=-0.06 ± SE 0.03; Z=-2.1; p=0.040) and locomotion (positive relationship; Estimate=0.10 ± SE 0.05; Z=2.2; p=0.027).

4. Discussion

The current study aimed to test the effect of some variables related to both the experimental procedure and the experimental subjects on the behaviour of the dog dyads involved in an intraspecific SSP. Thus, only those episodes of the SSP in which both dogs were simultaneously present in the experimental room were analysed (i.e., episodes 1, 4 and 7). Among those behaviours affected by the test episodes, environmental exploration, locomotion and escape attempts seemed to follow a similar trend, which decreased with the progression of the test. As for exploration, our results are in line with Prato-Previde et al. (2003) that found this behaviour to decrease sharply from the first to the second episode in dogs involved in the SSP. Accordingly, our findings seem to suggest that exploration behaviour patterns across SSP episodes may be affected by the novelty effect that the unfamiliar experimental environment exerts on dog dyads at the beginning of the procedure.

Escape attempts and locomotion, which refers to apparently aimless motor activity, are likely to reflect a state of negative arousal (Beerda et al., 2000, 1999). In fact, in a modified version of the SSP aimed to test adult dog interspecific attachment, Palestrini et al. (2005) reported higher levels of locomotion in both the first episode and the episodes in which the dog was left alone in the experimental room. While Palestrini et al. (2005) suggested that the unfamiliarity of the experimental setting may be stressful in itself for some dogs, in the case of our study, the first episode also corresponds to the necessary separation from the owner that allows the test to begin. However, the separation from the owner does not explain why the time spent by the dog dyads in locomotion and in trying to escape tended to decrease along with the progression of the test – only vocalizations, in terms of stress behaviours, increased from episode 1 to episode 4. In fact, in a recent study by Stephan et al. (2021), levels of activity and behaviours indicative of relaxation of dogs living in single dog-households remained stable over the first three hours after the owner's departures, whereas they tended, respectively, to decrease and increase, in dogs living with conspecifics. These findings point towards a complex and context-dependent behavioural response

to the owner's absence in which the presence of a conspecific may play a key role, rather than a simple habituation process. Therefore, the interpretation we gave to our results is that the activation of the dogs' attachment system induced by the SSP determined a shift in their coping response towards a more effective use of the conspecific as a source of emotional comfort. This explanation is further supported by the increase in proximity in the subsequent episodes of the SSP observed in the current study, as well as in previous studies on both interspecific (Riggio et al., 2021; Solomon et al., 2019) and intraspecific attachment (Mariti et al., 2014).

Passive behaviours were also affected by the SSP episode, as they were higher in the seventh episode compared to the first one and had an overall increasing trend across the procedure. Although the display of passive behaviours during the SSP has been previously associated to physiological indicators of stress, such as pronounced cardiac activation (Palestrini et al., 2005; Valsecchi et al., 2010) and increased cortisol levels (Mongillo et al., 2013), their pattern in the current study seems to support previous authors' interpretation that they are the expression of a calmer emotional state (Palmer and Custance, 2008; Prato-Previde et al., 2003) induced by the progressive activation of the attachment behavioural system towards the conspecific.

This may also explain why mother-offspring dyads displayed higher levels of passive behaviours compared to unrelated cohabitant pairs. In fact, as found by Mariti et al. (2017), mother-offspring dyads tend to be tied by a stronger affective bond than unrelated cohabitant adult dogs. As a consequence, mothers of dogs tested in the SSP seem to exert a stronger ameliorative effect on their siblings compared to older unrelated cohabitant dogs (Mariti et al., 2017). Although further research is necessary to clarify the effect of the type of relationship on dogs' single behaviours during the SSP, it is interesting to notice that the majority of the behavioural variables investigated remained unaffected. Some features of the experimental sample may explain this finding. Firstly, the dogs involved in this study were all adult subjects. In human psychology, it is widely recognized that adult individuals do not express their attachment behaviour with the same intensity

and frequency as infants (Kerns et al., 2015). In fact, the quality of their attachment is investigated through interviews and self-reports aimed at assessing the mental representations of the relationship (Ravitz et al., 2010) rather than the actual attachment behaviour. Therefore, the absence of a significant effect of the type of relationship on many SSP behaviours does not necessarily imply that there is no difference in the quality of the attachment bond between mother-offspring pairs and those composed by unrelated cohabitant dogs.

Secondly, all the younger subjects within the dyads of cohabitant dogs were adopted/acquired at 2 months of age. This may have led to a relationship with the older conspecific that presented asymmetric features, in terms of interactions, roles and functions similar to those characterizing the mother-offspring bond. While the mere presence of this asymmetry does not imply that the bond formed with a mother is the same as that formed with an older unrelated cohabitant dog, it may have attenuated the effect of the biological nature of the relationship on dog attachment behaviours during the SSP. For pet dogs, it is also possible that the owner may take on the referential function (Cimarelli et al., 2019) that a mother would have in other environmental conditions, altering some aspects of the bond between conspecifics. These hypotheses are not mutually exclusive and they all should be investigated in future studies. Regardless, we agree with Sipple et al.'s (2021) suggestion that functional roles rather than biological ones may determine the quality of the bond that dogs form with other individuals of both the same and different species. Future studies should focus on examining the effect of variables that may provide information on the functional nature of the dog-dog relationship on dog attachment behaviour, such as age at adoption, time spent in company of the older dog, caregiving behaviour of the older dog, etc.

In the current study, sex combination seemed to have a slight effect on social exploration with a non-significant trend for female-female dyads towards a greater amount of time performing this behaviour compared to both male-male and male-female dyads. Although not significant, this trend

seems coherent with previous findings that report female dogs to be generally more sociable with conspecifics compared to male dogs (Kubinyi et al., 2009), as well as to show a higher motivation to social contacts in the specific context of the SSP (D'Aniello et al., 2021). On the opposite, male-male dyads spent more time oriented to the window/door compared to male-female and female-female pairs supporting the notion that male subjects of many mammal species tend to show higher levels environmental alert (Scandurra et al., 2018). Nonetheless, in the specific stress-inducing context of the SSP, the overall effect of sex on the behaviours of the dog dyads may suggest that female dogs may be likely to use their conspecific as a source of emotional support, as it has been previously observed towards humans (Duranton et al., 2016; Horn et al., 2013).

Dog pairs with greater age difference spent more time in locomotion and less time in proximity to each other suggesting a reduced ability of these dogs to use their conspecific as a source of emotional support in the stressful context of the SSP. It is unlikely that locomotion simply reflects levels of activity in relation to age. In fact, age does not seem to affect dogs' levels of locomotor activity in novel environments (Siwak et al., 2003, 2002, 2001) nor in the specific context of the SSP (Carlone et al., 2014; Valsecchi et al., 2010). As previously mentioned, a more plausible explanation is that locomotion represents an indicator of negative arousal (Beerda et al., 2000, 1999).

Furthermore, in the context of the current study, proximity may reflect either caregiving or support-seeking behaviour. Interestingly, age difference seems to be negatively associated with caregiving behaviours of adult dogs towards young conspecifics showing signs of distress (Pongrácz and Sztruhala, 2019). Therefore, age difference may negatively affect the dogs' ability to both use the conspecific as a buffer against stress and operate as a source of emotional support.

This study has limitations. First, this was an opportunistic study. All the demographic data on the dog dyads had already been collected and partially published in previous studies (Mariti et al., 2017, 2014). Therefore, although it may have been interesting to test the effect of other dog-related factors, the corresponding information could no longer be obtained. Second, the relatively small size of the sample may have affected our results, especially in regard to sex combination, for which we only had 4 male-male pairs. Third, to the best of our knowledge, this is the first published study to assess the behaviour of the dog that plays the role of the attachment figure in an intraspecific SSP. As such, we had some difficulties in discussing and interpreting our results in light of previous literature on the topic. Finally, as previously mentioned, in this study the behaviour of both of dogs within the pair was summed to be analysed as the behaviour of the dyad. This is because we assumed that, during the SSP, the behaviour of one dog would equally be influenced by and influence the behaviour of the other dog, in a continuous feedback mechanism. Hence, we did not want to give any conceptual nor statistical prominence to one individual over the other. Nonetheless, it would be interesting for future studies on intraspecific attachment to focus on assessing the behaviour of the dog that plays the role of the attachment figure and that of the attached individual, separately, in order to uncover additional aspects of the dynamics of interaction between two conspecifics during the SSP.

5. Conclusions

This is the first study to assess the behaviour of both dogs during an intraspecific SSP. The behaviour of the dyad was affected by SSP episodes in a way that suggests the activation of the attachment behavioural system. Whether the dyads were composed by mothers with their adult offspring or by unrelated cohabitant dogs affected only one behavioural variable suggesting that other factors related to the functional rather than the biological nature of the relationship may have a more relevant role in shaping dog interactions during the SSP. Female-female pairs showed reduced

vigilance towards the environment and a trend towards higher levels of social exploration compared to male-male pairs possibly suggesting a greater ability of female dogs at using the conspecific as a buffer against the stress generated by the procedure. Finally, locomotion increased and proximity decreased along with age difference suggesting a lower capability of dog pairs with greater age gap to function as a source of emotional support to each other. Being the first of its kind, this study has important limitations, however it offers several interesting hints for future research on intraspecific attachment.

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Table 1: Version of the Strange Situation Procedure used the current study

Episode	Subjects involved	Description
1	Dog 1+ Dog 2	Both dogs are free to move and explore the room.
2	Dog 1+ Dog 2 + Stranger	The Stranger enters the room. She can greet the dogs if they seek attention. Then she sits on the chair and ignores the dogs, although she cannot move them away if they approach her.
3	Dog 1+ Stranger	Dog 2 is taken to another room 20 m away from the experimental room. The Stranger remains seated.
4	Dog 1+ Dog 2	Dog 2 is led into the experimental room. As Dog 2 enters, the Stranger exits the room.
5	Dog 1	Dog 2 is taken to another room 20 m away from the experimental room. Dog 1 remains alone.
6	Dog 1+ Stranger	The Stranger enters the room. She can greet Dog 1 if he/she seeks attention. Then she sits on the chair and ignores Dog 1, although she cannot move him/her away if he/she approaches her.
7	Dog 1+ Dog 2	Dog 2 is led into the experimental room. As Dog 2 enters, the Stranger exits the room.

Dog 1 = Tested dog. Dog 2= Dog acting as the presumed attachment figure.

Table 2: Description of behaviours analysed in the current study

Behaviour	Description	Reference
Visual orientation	Staring fixedly at the stranger or the other dog (at least for 0.5 s), regardless of whether the behavior is reciprocated	Modified from (Palestrini et al., 2005); (Prato-Previde et al., 2003)
Social exploration	Sniffing, close visual inspection, or gentle oral examination (such as licking) of the stranger or the other dog	Mariti et al., 2014

Escape attempts	Every attempt to escape from the experimental room through points of exit other than the main door (e.g. windows)	Current study
Physical contact	Being in physical contact with the other dog	Modified from Mariti et al., 2013
Vocalizations	Any type of vocal sounds emitted by the dog (e.g. barking, whining, yelping, growling, etc.)	Modified from (Parthasarathy and Crowell-davis, 2006); (Palestrini et al., 2010)
Stress	Lip-licking, head-turning, yawning, shaking, self-scratching, self-grooming	Beerda et al., 2000; Riggio et al., 2021
Passive behaviour	Sitting, standing or lying down without any obvious orientation toward the physical or social environment	Modified from Prato-Previde et al., 2003
Approach	Approaching while clearly visually oriented to the stranger or the other dog	Modified from Prato-Previde et al., 2003
Proximity to the door/window	Standing close to the door (<1 m) regardless whether the face was oriented to the exit	Modified from Topál et al., 1998
Behaviour against the door/window	All active behaviors resulting in physical contact with the door, including scratching the door with the paws, jumping on the door, pulling on the door handle with the forelegs or mouth	Modified from Prato-Previde et al., 2003
Oriented to the door/window	Staring fixedly at the door, either when close to it or from a distance	Modified from Palestrini et al., 2005; Prato-Previde et al., 2003
Following	Following the stranger or the other dog around the room or to the door	Modified from Palestrini et al., 2005; Prato-Previde et al., 2003

Environmental exploration	Activities directed toward physical aspects of the environment, such as sniffing, close visual inspection, distal visual inspection, and oral examination	Palestrini et al., 2005; Prato-Previde et al., 2003; Topál et al., 1998
Proximity	Close to (in the range of 1.5 times the length of D1, but not in physical contact)	Modified from Mariti et al., 2013
Locomotion	Every motor activity different from exploration, play, and following	Modified from Palestrini et al., 2005; Prato-Previde et al., 2003

Table 3: Results of the Generalised Linear Mixed Model. Values represent the percentage over the total observation time and are estimated marginal means and standard errors (in brackets).

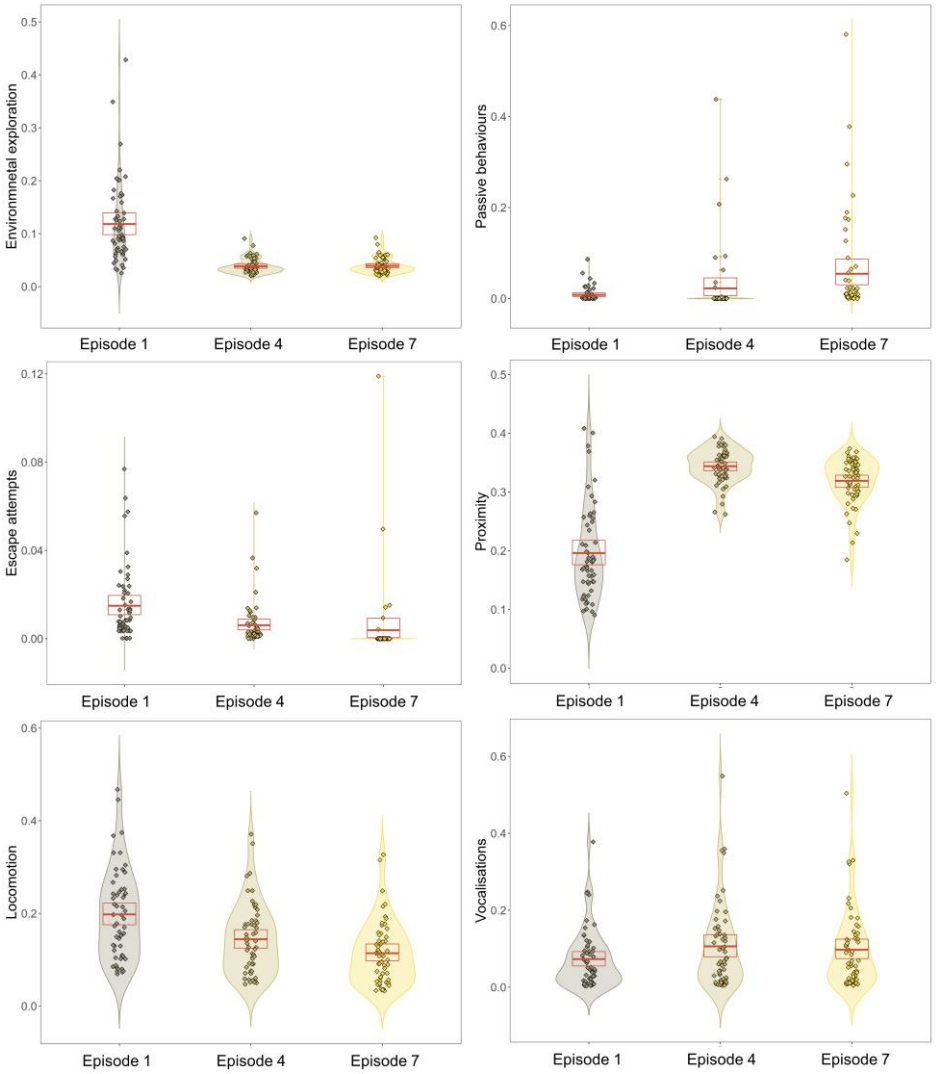
	Episodes				**Relationship**			**Sex**				**Age difference**
Response variable	**1**	**4**	**7**	**Test**	**Cohabitant**	**Mother/ Offspring**	**Test**	**F-F**	**F-M**	**M-M**	**Test**	**Test**
Visual orientation	8.6 (1.2)	10.0 (1.4)	10.4 (1.5)	χ^2=3.8 p=0.153	9.9 (1.4)	9.3 (1.6)	χ^2=0.1 p=0.701	9.2 (1.2)	9.5 (1.3)	10.2 (3.0)	χ^2=0.1 p=0.943	χ^2=3.5 p=0.060
Social exploration	1.0 (0.2)	0.7 (0.1)	0.8 (0.2)	χ^2=2.2 p=0.328	1.0 (0.1)	0.8 (0.2)	χ^2=0.9 p=0.343	1.3 (0.2)	0.9 (0.1)	0.5 (0.2)	**χ^2=6.8 p=0.035***	χ^2=0.3 p=0.585
Escape attempts	0.33 (0.28)	0.10 (0.10)	0.00 (0.00)	**χ^2=13.3 p=0.001***	0.01 (0.02)	0.03 (0.03)	χ^2=3.4 p=0.064	0.05 (0.04)	0.07 (0.06)	0.00 (0.00)	χ^2=3.0 p=0.225	χ^2=0.0 p=0.927
Physical contact	0.2 (0.1)	0.3 (0.1)	0.4 (0.2)	χ^2=1.3 p=0.533	0.3 (0.1)	0.3 (0.2)	χ^2=0.0 p=0.920	0.5 (0.2)	0.4 (0.1)	0.1 (0.1)	χ^2=1.8 p=0.414	χ^2=0.2 p=0.630

Vocalizations	3.1 (0.9)	4.5 (1.3)	4.1 (1.2)	**χ^2=9.8 p=0.007***	5.2 (1.5)	2.8 (1.2)	χ^2=2.2 p=0.140	6.2 (1.7)	3.0 (0.9)	3.0 (2.2)	χ^2=3.4 p=0.180	χ^2=0.0 p=0.984
Stress	2.2 (0.3)	2.3 (0.3)	2.5 (0.4)	χ^2=1.0 p=0.607	2.3 (0.3)	2.3 (0.4)	χ^2=0.0 p=0.845	1.9 (0.2)	2.5 (0.3)	2.6 (0.8)	χ^2=3.1 p=0.217	χ^2=0.0 p=0.905
Passive behaviour	0.9 (0.2)	1.0 (0.2)	5.9 (1.1)	**χ^2=27.0 p<0.001***	1.9 (0.4)	3.2 (0.1)	**χ^2=4.8 p=0.028***	1.6 (0.2)	1.2 (0.1)	1.0 (0.2)	χ^2=0.1 p=0.947	χ^2=0.7 p=0.401
Approach	0.9 (0.2)	0.8 (0.2)	1.0 (0.3)	χ^2=0.8 p=0.686	1.0 (0.2)	0.8 (0.3)	χ^2=0.8 p=0.371	0.9 (0.2)	1.0 (0.2)	0.8 (0.5)	χ^2=0.2 p=0.899	χ^2=0.0 p=0.991
Proximity to the door/window	39.2 (4.2)	38.7 (4.2)	41.4 (4.8)	χ^2=0.6 p=0.755	41.4 (3.9)	38.2 (5.2)	χ^2=0.4 p=0.557	41.3 (3.9)	42.1 (4.1)	36.2 (8.7)	χ^2=0.4 p=0.836	χ^2=0.4 p=0.536
Behaviour against the door/window	1.8 (0.7)	1.5 (0.7)	0.9 (0.4)	χ^2=3.7 p=0.157	1.2 (0.4)	1.6 (0.8)	χ^2=0.4 p=0.548	0.8 (0.3)	1.0 (0.4)	3.0 (2.5)	χ^2=2.2 p=0.341	χ^2=2.6 p=0.109
Oriented to the door/window	56.5 (3.6)	62.0 (3.4)	59.6 (4.0)	χ^2=4.6 p=0.099	55.8 (3.3)	62.9 (4.5)	χ^2=2.4 p=0.122	51.7 (3.3)	52.1 (3.4)	72.9 (6.7)	**χ^2=7.1 p=0.029***	χ^2=0.7 p=0.411
Following	1.6 (0.3)	2.1 (0.5)	2.0 (0.4)	χ^2=1.4 p=0.498	1.7 (0.3)	2.2 (0.5)	χ^2=2.0 p=0.157	1.7 (0.3)	1.9 (0.3)	2.2 (0.9)	χ^2=0.3 p=0.868	χ^2=0.0 p=0.966
Environmental exploration	10.3 (1.6)	3.8 (0.6)	3.8 (0.7)	**χ^2=53.3 p<0.001***	5.7 (0.8)	5.0 (1.0)	χ^2=0.5 p=0.491	5.6 (0.8)	6.3 (0.9)	4.2 (1.4)	χ^2=1. 5 p=0.474	χ^2=0.4 p=0.530
Proximity	18.1 (2.7)	35.2 (2.6)	32.5 (2.6)	**χ^2=26.3 p<0.001***	27.8 (1.9)	27.9 (3.0)	χ^2=0.0 p=0.751	28.5 (2.0)	29.4 (2.2)	25.9 (4.6)	χ^2=0.6 p=0.751	**χ^2=4.2 p=0.040***
Locomotion	17.8 (2.2)	12.6 (1.7)	9.6 (1.4)	**χ^2=56.4 p<0.001***	12.2 (1.6)	13.8 (2.5)	χ^2=0.5 p=0.502	12.2 (1.5)	14.8 (1.9)	12.1 (3.9)	χ^2=1.3 p=0.530	**χ^2=4.9 p=0.027***

Significant results are written in bold

* $p<0.05$

Figure 1. Significant results based on Generalised Linear Mixed Models showing the difference in the behaviours of the dyads between SSP episodes. Data are predicted response values based on the model outcome, crossbars indicate means and 95% confidence intervals.



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Contributions

☒ Each author declares substantial contributions through the following:

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content,

Highlights

- Several factors may affect the behaviour of dogs involved in a Strange Situation Procedure aimed to assess intraspecific attachment
- We assessed the effect of test-related and pair-related variables on the behaviour of the dog dyads
- The effect of the test episode suggests that dogs may be primarily stressed by the initial separation from the owner
- Dogs may be able to use the conspecific as a source of emotional support as the test progresses
- Pair-related variables, such as type of relationship, sex and age difference may affect the dogs' behaviour during the test