

Carrots versus sticks: The relationship between training methods and dog-owner attachment

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ABSTRACT

The use of aversive-based training methods has been suggested to negatively affect dog-human attachment. However, the scientific evidence for this claim is relatively limited. Previous studies relied upon owner reports of training methods or on potentially confounded measures of attachment (e.g., eye gaze). The aim of the present study was to comprehensively and objectively investigate the relationship between aversive- and reward-based training methods and dog-owner attachment. Companion dogs (n=34) recruited from 6 different dog training schools (3 reward-based and 3 aversive-based) were given a counterbalanced version of the Ainsworth Strange Situation Test. The presence and absence of the owner and a stranger in a room with the dog was manipulated over different episodes. Dogs' behavior was then analyzed for attachment-related behaviors: contact-maintenance, separation-distress and secure-base effect, as well as following upon separation and greeting upon reunion. Results showed no significant differences between groups for contact-maintenance and separation distress behaviors. However, dogs trained with reward-based methods, but not dogs trained with aversive-based methods, played more in the presence of the owner than in the presence of the stranger, and they also followed and greeted the owner more than the stranger, although these differences were found for only one procedure order. Our study is the first to investigate the relationship between training methods and attachment using a standard and well-validated method for the assessment of dog-owner attachment.

INTRODUCTION

Attachment is an affectional tie that a person or animal forms to another specific individual (Ainsworth, 1969; Ainsworth and Bell, 1970). The foundation of the ethological model of attachment (Ainsworth, 1969; Bowlby, 1969, 1982) is that attachment behavior results in “attaining or maintaining proximity to some clearly identified individual who is conceived as better able to cope with the world” (Bowlby, 1982). Any behavior that increases proximity to the caregiver figure provides immediate physical protection as well as the opportunity to observe and learn survival behavior, thereby increasing infant fitness (Bowlby, 1969). The most relevant characteristics of attachment are thus 1) contact-maintenance with the attachment figure, 2) separation distress in the absence of the attachment figure, and 3) the secure-base effect, i.e., the activation of exploratory and play behavior in the presence of the attachment figure (the attachment figure represents a base from which to explore the world) (Bowlby, 1969; Ainsworth and Bell, 1970; Bowlby, 1982).

For decades, attachment has been discussed in regards to child-mother or child-caregiver bonds but, given the extensive and close history of dogs and humans, more recently attachment research has extended to the dog-human caregiver relationship. The Ainsworth Strange Situation Test (ASST), the hallmark procedure used to study infant-caregiver relationship, has been adapted to study dog-owner bonds (e.g., Topál et al., 1998). In the ASST, the subject is placed in an unfamiliar room where the presence of the caregiver and a stranger is manipulated over a series of episodes (Ainsworth and Bell, 1970). Trained observers then score the subject's behavioral responses to assess attachment-related behaviors. In human infants, there is a predictable general response to the ASST: proximity- and contact-seeking with the caregiver, more exploration and play in caregiver presence, and separation distress in caregiver absence (Ainsworth and Bell, 1970). Within these characteristics, there is still a large amount of variety and complexity in the infant's behaviors (Ainsworth et al., 1978), which can be captured in 'attachment patterns'. Infant attachment patterns are grouped using an insecure-secure spectrum: secure (the infant is clearly more orientated to the caregiver than the stranger, is distressed during separation, positively greets the caregiver upon return, seeks to maintain contact with caregiver, and returns to play or exploration after reunion); insecure-avoidant (the infant shows minimal distress at separation, does not greet the caregiver upon return, actively avoids or ignores the caregiver after reunion, and the stranger is treated similar to the caregiver), insecure-ambivalent/resistant (the infant shows high levels of distress during separation, greets the caregiver upon return but cannot be comforted and may resist contact after reunion) and disorganized (the infant's behavior is not consistent with a pattern, appears disordered or may be contradictory, includes display of stereotypies, confusion, or stilling). Remarkably, the same pattern of attachment behaviors has been shown in dogs, demonstrating that dog-owner attachment can be successfully analyzed using a modified version of the ASST (Topál et al., 1998; Prato-Previde et al., 2003; Topál et al., 2005; Palmer and Custance, 2008; Mariti et al., 2013a, b; Mongillo et al., 2013; Scandurra et al., 2016). In addition, dogs were shown to span a variety of attachment styles along the secure-insecure dimension, which is similar to human attachment classifications (Topál et al., 1998).

One of the main influences on infant attachment patterns is argued to be caregiver reciprocity and sensitivity to infant signals, as high caregiver sensitivity and reciprocity are associated with securely attached infants (Bowlby, 1969; Ainsworth et al., 1978; Egeland and Farber, 1984; Goossens and Van Ijzendoorn, 1990; Bretherton, 1992; Yan-Hua et al., 2012). Related literature in dogs includes some work on attachment in the context of training (Fallani et al., 2006; Mariti et al., 2013a; Scandurra et al., 2016). Specifically, attachment-related behaviors have been studied in companion versus working dogs, namely guide dogs (Fallani et al., 2006), search and rescue dogs (Mariti et al., 2013a) and water rescue dogs (Scandurra et al., 2016). Overall, these studies suggest that the professional training requirements for these dogs do not interfere with attachment. However, these studies did not distinguish between training methods.

Dog training methods differ widely between practitioners, with some using aversive-based methods, which utilize mainly negative reinforcement and positive punishment, and others using reward-based methods, which rely mainly on positive reinforcement and negative punishment (Guilherme-Fernandes et al., 2017), potentially affecting how dogs and humans interact with each other. Indeed, dogs trained with physical punishment played and interacted less with a novel person while in the owner's presence (Rooney and Cowan, 2011), and eye gaze towards the owner, a proposed indicator of dog-owner bond (Nagasawa et al., 2015), was observed more frequently in dogs trained with reward-based methods as compared to aversive-based methods (Deldalle and Gaunet, 2014). However, Rooney and Cowan (2011) used owner reports of training methods instead of directly assessing the methods employed, whereas eye gaze measure potentially confounds results, since many reward-based schools train dogs to gaze towards the owner (Deldalle and Gaunet, 2014).

The aim of the present study was to explore the relationship between the use of aversive and reward-based training methods and dog-owner attachment, using the Ainsworth Strange Situation Test,

recognized as the standard behavioral test of attachment (see Rodriguez et al., 2018), and an objective assessment of the methods used to train the dogs, through the observation of training sessions at the schools from where the dog-owner dyads were recruited. We hypothesized that dogs trained with different methods would display differences in attachment-related behaviors during the ASST. Importantly, in human child-caregiver attachment, physical punishment has been associated with insecure attachment scores (Coyl et al., 2002). Hence, more specifically, we expected that dogs trained with reward-based methods, but not those trained with aversive-based methods, would display a secure-base effect.

METHODS

ETHICAL STATEMENT

All procedures were approved by ICBAS (Abel Salazar Biomedical Sciences Institute) ORBEA (Animal Welfare Body). All owners completed a consent form authorizing the collection and use of the data.

SUBJECTS

The subjects were owner and companion dog pairs ($n=34$) who were recruited directly from six different dog training schools located in the metropolitan area of Porto, Portugal. The schools were classified as reward-based (three) and as aversive-based (three) (see below).

The head trainers of each dog training school were asked to indicate dogs fitting our inclusion criteria, and we then approached the owners of the first six dogs from each school to ask if they were willing to participate. All the thirty-six owners accepted to participate. However, two dyads (one from each type of training method) withdrew from the study prior to data collection and, as such, we ended with six dog-owner dyads for four schools and five for the remaining two schools. The 17 dogs within the aversive-based training group consisted of 8 female and 9 male dogs (71% neutered) with an average age of 3.4 ± 2.4 years, including 3 Labradors, 2 Weimaraner, 1 Doberman, 1 Portuguese Podengo, 1 Pit Bull Terrier, 1 German Shepherd, 1 Border Collie, 1 Jack Russell Terrier, and 6 mixed breeds. The owners in the aversive-based group were 12 females and 5 males with an average household size of 3.0 ± 1.1 people. The 17 dogs within the reward-based training group consisted of 11 female and 6 male dogs (65% neutered) with an average age of 3.6 ± 1.9 years, including 2 Boxers, 2 Golden Retrievers, 2 German Shepherds, 1 Beagle, 1 Bull Terrier, 1 Labrador, 1 Portuguese Pointing Dog, 1 Bernese Mountain Dog and 6 mixed breeds. The owners in the reward-based group were 12 females and 5 males with an average household size of 2.4 ± 0.7 people.

Recruited dogs had to fit four criteria: 1) owned for at least three months, 2) attended the specific training school for at least two months, 3) free of behavioral problems (e.g., aggression, separation anxiety, as determined by the owner and the first author - ACVC), and 4) used to being left alone and taken outside. In the instances when the dog lived in a multi-member family, the person reported as being the most involved in the training process was the participating owner during the ASST.

TRAINING METHODS

As part of an ongoing study with the same dog training schools, we classified training methods. We videotaped four training sessions at the different schools, using a video camera on a tripod, and afterwards analyzed the videos for the frequency of aversive-based operant conditioning procedures utilized, namely



positive punishment and negative reinforcement (see Table S1 for the specific definitions). The analysis was performed by AVCV using The Observer XT software, version 10.1 (Noldus Information Technology Inc, Wageningen, The Netherlands). The schools were classified as aversive-based if they used some sort of positive punishment and/or negative reinforcement and as reward-based if they did not use any of these techniques. Schools A, C and E were classified as aversive-based and Schools B, D and F were classified as reward-based (see Table S2).

EXPERIMENTAL SETTING

Testing was held in an indoor room (7.7×3 m) within a research building at the Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto in Portugal. The testing room was novel to all subjects and was organized to match ASST requirements including two chairs and six dog toys (one yellow tennis ball, one green rope, one yellow plastic chicken leg, one plastic hot dog, one rubber bone, and a purple Kong® toy) distributed on the floor between the two chairs. Tape was placed on the floor in a circle (1.5-m diameter) around each chair and in a semicircle (1-m radius) around the door to aid in human participants behavior and behavioral coding. There were counters along two walls with a paper taped to one counter in order to designate the location where the owner should place the dog's leash prior to the beginning of the test (see Fig. 1). Two video cameras were located on opposite walls at a high angle to record the testing environment. The location of the toys, chairs, leash, and cameras was controlled for every test. The testing room was cleaned with water and liquid detergent at the end of each test. Tests were conducted between February and August 2018 and were scheduled according to owners' availability, both during weekdays and Saturdays.

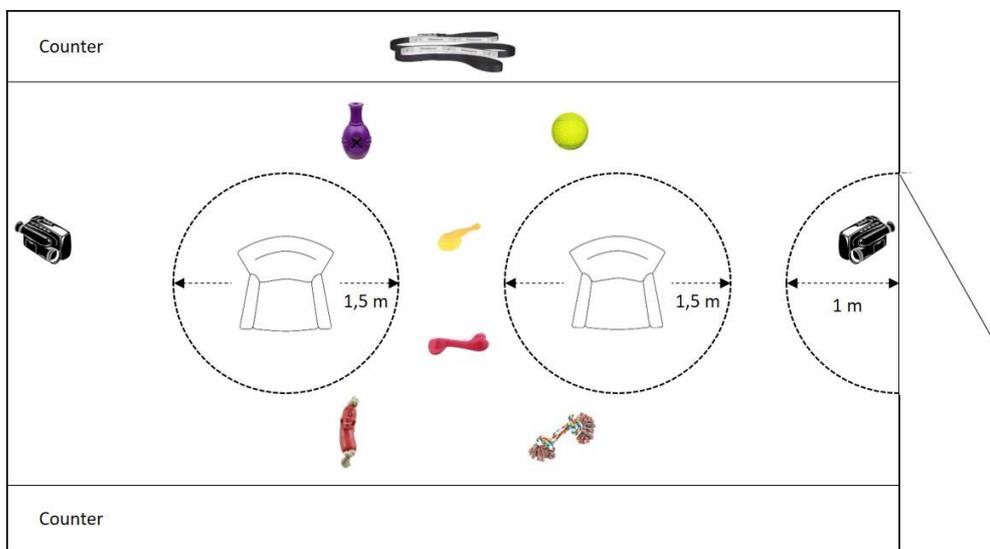


Figure 1. Set-up of the ASST testing room.

EXPERIMENTAL PROCEDURE

The ASST procedure used in the present study was identical to that used by Hall et al. (2015). The presence and absence of the owner and a person unfamiliar to the dog (hereafter the ‘stranger’) in the experimental room was manipulated over six 2-min episodes. To control for potential order effects on the dogs’ behavior (Palmer and Custance, 2008; Rehn et al., 2013; Hall et al., 2015), two counterbalanced orders of the entering and exiting of the owner and the stranger were used. Table 1 describes the two procedure orders and includes a description of each episode. The procedure order was pseudo-randomized so the orders would be counterbalanced across training method group and training school. The chair where the owner or stranger sat—close or far from the door—was also counterbalanced across dogs. The timing of the beginning of each episode started either when someone sat down in the designated chair (if the owner or stranger was entering the room) or when the door closed (if the owner or stranger was exiting the room). Two experimenters (Jennifer Barrett – JB, and Marisa Saraiva), who were blind to the training method groups, played the role of the stranger and the stranger’s identity was also pseudo-counterbalanced across training groups and schools.

Owners were told that the study intended to investigate the exploratory behaviors of dogs in an unfamiliar environment (Mariti et al., 2013b). Before entering the room, each owner was given a detailed set of instructions by ACVC. The owners were instructed to act natural but to only interact with their dog if: 1) the owner was seated in their chair, 2) the dog had at least one part of its body within the taped circle around the owner’s chair, and 3) the dog asked for the owner’s attention by either looking at the owner, making intentional physical contact with the owner, or asking the owner to play. If these three guidelines were met, the owners were asked to interact with their dog by either 1) stroking and talking to the them if the dog looked to the owner or made physical contact with the owner or 2) engaging in play if the dog asked to play. The owners were also asked to keep all interactions brief, around 2–3 s. If the dog sought the owner’s attention again, then the owners could initiate a new 2–3 s interaction with the dog. The owners were asked to never leave the chair during the episodes and to remain neutral if the dog was outside the taped circle surrounding the owner’s chair. During the test, the dogs were allowed to behave

naturally and freely. When the owner or the stranger exited the room, they said “adeus” (goodbye in Portuguese) to the dog.

	Order 1	Order 2
Episode 1	‘O’ Owner enters the room and releases the dog in the center of the room, leash is placed on the right counter, owner walks to their chair and sits down	‘S’ Owner enters the room where the stranger is already sitting, owner releases the dog in the center of the room, leash is placed on the right counter, owner exits the room and the stranger remains seated
Episode 2	‘OS’ Stranger enters, joining the owner in the room, and sits in the adjacent chair	‘OS’ Owner enters, joining the stranger in the room, and sits in the adjacent chair
Episode 3	‘S’ A sound (“horn”) cues the owner to exit, the stranger remains seated	‘O’ A sound (“whistle”) cues the stranger to exit, the owner remains seated
Episode 4	‘A’ A different sound (“whistle”) cues the stranger to exit, the dog is left alone in the room	‘A’ A different sound (“horn”) cues the owner to exit, the dog is left alone in the room
Episode 5	‘S’ Stranger enters and sits in their chair	‘O’ Owner enters and sits in their chair
Episode 6	‘OS’ Owner enters, joining the stranger in the room, and sits in their chair	‘OS’ Stranger enters, joining the owner in the room, and sits in their chair

Table 1 - Description of the procedure orders and episodes. The labels on each cell display whether the owner (O), the stranger (S), both the owner and the stranger (OS) or none of them (A – dog alone) were present with the dog in the experimental room.

QUESTIONNAIRE

Following the ASST procedure, owners were asked to complete a brief written questionnaire regarding their own demographics and the dog’s demographics, living conditions, and background.

BEHAVIOR CODING

The dogs’ behavior was video recorded during the entire ASST procedure. The duration of behaviors that were related to contact-maintenance, separation distress and secure-base effect were coded during the 2-minute episodes (see Table 2 for the specific behaviors and definitions). These behaviors were not considered to be mutually exclusive with the exception of ‘exploration’, ‘play’, ‘attention to door’ and ‘behaviors towards door’. That is, the dog’s behavior could not be simultaneously coded as more than one of these four behaviors but could be coded concurrently with any of the four remaining ethogram behaviors. For example, the dog could simultaneously be coded for ‘proximity-seeking’ and ‘exploration’ or for ‘attention to door’ and ‘stand by the door’, but not for ‘exploration’ and ‘attention to door’.

Furthermore, each behavior was coded for whether it was directed to or performed in the presence of the owner (O) and the stranger (S). Contact-maintenance behaviors but not secure-base related and



separation-related behaviors were mutually exclusive as regards owner vs. stranger. That is, a dog could not be simultaneously in ‘proximity-seeking’ or ‘physical contact’ with the owner and the stranger, but could be playing in the presence of both, if the two were inside the test room.

We also scored greeting and following behaviors towards the entering and the exiting person, respectively. Greeting behavior was scored in the transitions from Episode 4 to Episode 5 and from Episode 5 to Episode 6, while the owner or the stranger was entering the room after the dog was in complete isolation (“pure” reunion episodes, see Rehn et al., 2013). More specifically, it was scored between the moments the entering person opened the door until five seconds after they sat down in their chair. For procedure Order 1, greeting the owner was scored in the transition from Episode 5 to 6 and greeting the stranger in the transition from Episode 4 to 5; for procedure Order 2 it was the reverse. The behavior of the dog towards the entering person was evaluated with five criteria (approach initiation, full approach, durable physical contact, delay of approach, avoidance; see Table 3) and the scores for these five criteria were summed. The final score could range from -1.5 to 2.5 . Following behavior was scored in the transitions from Episode 2 to Episode 3 and from Episode 3 to Episode 4, while the owner or the stranger was exiting the room. More specifically, following behavior was scored between the moments when the exiting person left their chair until five seconds after the door closed behind them (see Table 3). For procedure Order 1, following the owner was scored in the transition from Episode 2 to 3 and following the stranger in the transition from Episode 3 to 4; for Order 2 it was the reverse. Because the exact duration of the episodes could vary, all results are presented as relative (to the actual episode duration) time, except for greeting and following behaviors, which were rated categorically.

Two observers analyzed the videos (ACVC and JB). Before commencing actual data analysis, both observers coded videos in parallel and only when a Cohen’s $Kappa > 0.8$ was reached, they started coding independently. Overall, ACVC coded 10 videos, and JB coded 22; 2 videos were coded by both observers during inter-observer reliability training (after review and agreement). All behavioral analysis were performed using The Observer XT software, version 10.1 (Noldus Information Technology Inc, Wageningen, The Netherlands).

Previous studies have shown that procedure order can significantly influence dog behavior in the ASST (e.g., Prato-Previde et al., 2003; Palestrini et al., 2005; Palmer and Custance, 2008; Rehn et al., 2013). Importantly, Rehn et al. (2013) found that dogs displayed attachment-related behaviors to an unfamiliar person simply as a function of the order in which she entered and exited the experimental room. Hence, results are presented for each procedure order separately (see also Hall et al., 2015).



Behavior	Definition	Relevant References
<i>Contact-maintenance behaviors (coded only in episodes when at least one person is present)</i>		
Proximity-seeking	Length of time during which the dog has any part of their body within the 1.5-m circle the owner/stranger is sitting in	(Hall et al., 2015)
Physical Contact	Length of time during which the dog engages in physical contact with the owner/stranger	(Topál et al., 2005; Hall et al., 2015)
<i>Secure-base related behaviors (coded in all episodes)</i>		
Exploration	Time spent in any activity directed towards the physical environment, including sniffing, distal or close visual inspection (staring or scanning), and gentle oral examination (such as licking)	(Topál et al., 2005; Palestrini et al., 2005)
Play	Time spent in any vigorous toy- or social partner-related behavior, including chewing, biting, shaking, scratching or batting with the paw, chasing, rolling, and tossing using the mouth (toy-related) or running, jumping or any physical contact with or without toys (social partner-related)	(Topál et al., 2005; Mariti et al., 2013b)
<i>Separation-related behaviors (coded only in episodes when at least one person is absent)</i>		
Whining	High pitched vocalization	(Mariti et al., 2013b)
Attention oriented to the door	Time spent staring fixedly at the door, either close to it or from a distance for at least two seconds	(Mariti et al., 2013b)
Stand by the door	Time spent within 1m of the door regardless of gaze orientation	(Topál et al., 2005; Mariti et al., 2013b)
Behaviors oriented to the door	Time spent in active behaviors resulting in physical contact with the door, including scratching, jumping on the door, pulling the door handle with forelegs or mouth, excludes sniffing	(Mariti et al., 2013b)

Table 2 - Ethogram for behaviors coded during the episodes of the Ainsworth Stranger Situation Test.

Behavior	Definition	Relevant References
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Greeting	Approach initiation (+1): Dog moves towards the entering person Full Approach (+1): Dog approaches the entering person until physical contact occurs Durable Physical Contact (+0.5): Dog spends more than 3 seconds of uninterrupted physical contact with the entering person Delay of Approach (-0.5): Dog hesitates to initialize any approach towards the entering person for more than 5 seconds Avoidance (-1): Dog displays any avoidance behavior towards the entering person (e.g. backing, getting out of the way)	(Topál et al., 1998; Topál et al., 2005; Mariti et al., 2013b)
Following	0 – Dog does not orient towards the leaving person at all, or only for less than 1 second 1 – Dog orients towards the leaving person for more than 1 second 2 – Dog follows the leaving person to the door 3 – Dog tries to get through the door or stands by the door for more than 1 second	(Topál et al., 2005)

Table 3 - Ethogram for behaviors coded in the transitions between the episodes of the Ainsworth Strange Situation Test.

STATISTICAL ANALYSIS

All statistics were conducted using the software SPSS® Statistics 25.0. A first analysis with the Shapiro-Wilk test suggested the data were not normally distributed; hence, non-parametric tests were used. Namely, Wilcoxon signed-rank tests were used to compare:

- The relative duration of contact-maintenance behaviors (proximity-seeking and physical contact) towards the owner (O) and towards the stranger (S), in Episodes 2 and 6, when both were present in the room with the dog (Episode 2: O vs S, Episode 6: O vs S);
- The relative duration of separation distress-related behaviors (stand by the door, attention to door, whining, behaviors towards door) in the episodes when the dogs were alone with the owner (O) and when they were alone with the stranger (S) (Episode 1 vs 3; Episode 1 vs 5, O or S present, depending on the procedure order, see Table 1);
- The relative duration of secure-base related behaviors (exploration and play) in the episodes when the dogs were alone with the owner and when they were alone with the stranger (Episode 1 vs 3; Episode 1 vs 5, O or S present, depending on the procedure order);
- The greeting score when the owner returned (in the transition from Episode 5 to 6 within procedure Order 1 and in the transition from Episode 4 to 5 within procedure Order 2) and when the stranger returned (in the transition from Episode 4 to 5 within procedure Order 1 and in the transition from Episode 5 to 6 within procedure Order 2);
- The following score when the owner left (in the transition from Episode 2 to 3 within procedure Order 1 and in the transition from Episode 3 to 4 within procedure Order 2) and when the stranger left (in the transition from Episode 3 to 4 within procedure Order 1 and in the transition from Episode 2 to 3 within procedure Order 2);

Each of the aforementioned comparisons was performed for procedure Order 1 and for procedure Order 2 separately. Because two planned comparisons were performed for each behavior related to contact-maintenance, separation distress and secure-base effect, the significance level was adjusted with Bonferroni's correction ($\alpha=0.025$) for these behaviors. For greeting and following behaviors, the significance level was set at $\alpha=0.05$.

RESULTS

CONTACT-MAINTENANCE BEHAVIORS

PROXIMITY SEEKING

In both the Reward and the Aversive groups, dogs spent more time in proximity-seeking towards the owner than towards the stranger in Episodes 2 and 6 within procedure Order 2 [Reward group – Episode 2 (O vs S): $z=-2.52$, $p=0.013$; Episode 6 (O vs S): $z=-2.52$, $p=0.012$; Aversive group – Episode 2 (O vs S): $z=-2.24$, $p=0.025$; Episode 6: $z=-2.52$, $p=0.012$; see Table 4). Within procedure Order 1, dogs from both groups preferred the owner to the stranger in Episode 6 [Reward group (O vs S): $z=-2.49$, $p=0.012$; Aversive group: $z=-2.37$, $p=0.008$], but not in Episode 2,

with dogs from the Aversive group showing a marginally significant difference [Reward group (O vs S): $z=-0.84$, $p=0.400$, Aversive group (O vs S): $z=-2.07$, $p=0.038$].

PHYSICAL CONTACT

For the Reward group, a comparison of the relative time spent in physical contact with the owner and the stranger in Episodes 2 and 6, when both the owner and the stranger were present in the room with the dog, showed that dogs within procedure Order 1 preferred the owner in Episode 6 ($z=-2.52$, $p=0.012$) but not in Episode 2 ($z=-0.41$, $p=ns$), whereas dogs within procedure Order 2 preferred the owner in Episode 2 ($z=-2.54$, $p=0.011$) but not in Episode 6 ($z=0.52$, $p=ns$). For the Aversive group, although dogs always tended to prefer the owner over the stranger, this preference was only statistically significant in Episode 6 of procedure Order 1 ($z=-2.37$, $p=0.018$, remaining comparisons $p=ns$; see Table 4).

SEPARATION DISTRESS

STAND BY THE DOOR

A comparison of the episodes when the dogs were alone with the owner with the episodes when they were alone with the stranger revealed that dogs from the Reward group spent more time standing by the door when they were alone with the stranger for both procedure orders [Order 1 - Episode 1(O) vs Episode 3(S): $z=2.52$, $p=0.012$; Episode 1(O) vs Episode 5(S): $z=2.52$, $p=0.012$; Order 2: Episode 1(S) vs Episode 3(O): $z=-2.31$, $p=0.021$; Episode 1(S) vs Episode 5(O): $z=-2.55$, $p=0.011$]. For the Aversive group, although dogs always tended to spend more time standing by the door when they were alone with the stranger, this difference was only statistically significant when comparing Episode 1 and Episode 5 within procedure Order 1 ($z=2.67$, $p=0.008$, remaining comparisons $p=ns$; see Table 4).

ATTENTION TO DOOR

Dogs from the Reward group spent more time attending to the door when they were alone with the stranger than when they were alone with the owner for both procedure orders [Order 1 - Episode 1(O) vs Episode 3(S): $z=2.54$, $p=0.012$; Episode 1(O) vs Episode 5(S): $z=2.52$, $p=0.012$; Order 2: Episode 1(S) vs Episode 3(O): $z=-2.31$, $p=0.021$; Episode 1(S) vs Episode 5(O): $z=-2.31$, $p=0.021$]. For the Aversive group the results were similar, although the comparison between Episode 1 and Episode 3 of Order 2 did not reach statistical significance [Order 1 - Episode 1(O) vs Episode 3(S): $z=2.67$, $p=0.008$; Episode 1(O) vs Episode 5(S): $z=2.67$, $p=0.008$; Order 2: Episode 1(S) vs Episode 3(O): $z=-1.79$, $p=ns$; Episode 1(S) vs Episode 5(O): $z=-2.20$, $p=0.028$; see Table 4].

Behavior	Comparison	Reward Group [mean±standard error of mean (statistical results)]	Aversive Group [mean±standard error of mean (statistical results)]
Proximity-seeking	<u>Order 1</u>		
	Episode 2: O vs S	0.32±0.10 vs 0.13±0.08 (z=-0.84, p=0.400)	0.47±0.09 vs 0.17±0.04 (z=-2.07, p=0.038)
	Episode 6: O vs S	0.53±0.07 vs 0.03±0.01 (z=-2.49, p=0.012)*	0.57±0.08 vs 0.04±0.02 (z=-2.37, p=0.008)*
	<u>Order 2</u>		
	Episode 2: O vs S	0.43±0.06 vs 0.13±0.05 (z=-2.52, p=0.013)*	0.39±0.10 vs 0.08±0.03 (z=-2.24, p=0.025)*
	Episode 6: O vs S	0.40±0.11 vs 0.26±0.10 (z=-2.52, p=0.012)*	0.41±0.10 vs 0.07±0.02 (z=-2.52, p=0.012)*
Physical contact	<u>Order 1</u>		
	Episode 2: O vs S	0.07±0.04 vs 0.06±0.05 (z=-0.41, p=0.686)	0.14±0.09 vs 0.08±0.01 (z=-0.53, p=0.593)
	Episode 6: O vs S	0.25±0.08 vs 0.00±0.00 (z=-2.52, p=0.012)*	0.18±0.10 vs 0.00±0.00 (z=-2.37, p=0.018)*
	<u>Order 2</u>		
Episode 2: O vs S	0.06±0.01 vs 0.00±0.00 (z=-2.54, p=0.011)*	0.12±0.07 vs 0.01±0.01 (z=-1.80, p=0.072)	
Episode 6: O vs S	0.05±0.02 vs 0.10±0.05 (z=0.52, p=0.600)	0.09±0.02 vs 0.00±0.00 (z=-1.75, p=0.080)	
Stand by the door	<u>Order 1</u>		
	Episode 1(O) vs Episode 3(S)	0.19±0.05 vs 0.70±0.11 (z=2.52, p=0.012)*	0.15±0.04 vs 0.60±0.08 (z=2.67, p=0.008)*
	Episode 1(O) vs Episode 5(S)	0.19±0.05 vs 0.71±0.13 (z=2.52, p=0.012)*	0.15±0.04 vs 0.39±0.10 (z=1.84, p=0.066)
	<u>Order 2</u>		
Episode 1(S) vs Episode 3(O)	0.43±0.08 vs 0.16±0.08 (z=-2.31, p=0.021)*	0.52±0.05 vs 0.19±0.11 (z=-1.69, p=0.092)	
Episode 1(S) vs Episode 5(O)	0.43±0.08 vs 0.04±0.02 (z=-2.55, p=0.011)*	0.52±0.05 vs 0.18±0.09 (z=-2.10, p=0.035)	

Attention to door	<u>Order 1</u>		
	Episode 1(O) vs Episode 3(S)	0.12±0.04 vs 0.49±0.07 (z=2.54, p=0.012)*	0.04±0.02 vs 0.51±0.04 (z=2.67, p=0.008)*
	Episode 1(O) vs Episode 5(S)	0.12±0.04 vs 0.44±0.11 (z=2.52, p=0.012)*	0.04±0.02 vs 0.41±0.04 (z=2.67, p=0.008)*
	<u>Order 2</u>		
	Episode 1(S) vs Episode 3(O)	0.38±0.05 vs 0.09±0.03 (z=-2.31, p=0.021)*	0.27±0.07 vs 0.15±0.04 (z=-1.79, p=0.074)
	Episode 1(S) vs Episode 5(O)	0.38±0.05 vs 0.08±0.03 (z=-2.31, p=0.021)*	0.27±0.07 vs 0.10±0.05 (z=-2.20, p=0.028)*
Whining	<u>Order 1</u>		
	Episode 1(O) vs Episode 3(S)	0.04±0.02 vs 0.13±0.04 (z=1.57, p=0.116)	0.01±0.00 vs 0.18±0.03 (z=2.67, p=0.008)*
	Episode 1(O) vs Episode 5(S)	0.04±0.02 vs 0.10±0.04 (z=1.27, p=0.204)	0.01±0.00 vs 0.13±0.04 (z=2.50, p=0.013)*
	<u>Order 2</u>		
	Episode 1(S) vs Episode 3(O)	0.06±0.02 vs 0.02±0.01 (z=-2.38, p=0.018)*	0.13±0.05 vs 0.06±0.03 (z=-1.86, p=0.063)
	Episode 1(S) vs Episode 5(O)	0.06±0.02 vs 0.03±0.02 (z=-1.76, p=0.079)	0.13±0.05 vs 0.06±0.03 (z=-1.61, p=0.107)
Behaviors towards door	<u>Order 1</u>		
	Episode 1(O) vs Episode 3(S)	0.01±0.01 vs 0.03±0.02 (z=1.84, p=0.066)	0.00±0.00 vs 0.03±0.02 (z=2.06, p=0.039)
	Episode 1(O) vs Episode 5(S)	0.01±0.01 vs 0.02±0.02 (z=1.00, p=0.317)	0.00±0.00 vs 0.02±0.02 (z=6.00, p=0.109)
	<u>Order 2</u>		
	Episode 1(S) vs Episode 3(O)	0.02±0.01 vs 0.03±0.03 (z=-0.37, p=0.715)	0.05±0.04 vs 0.00±0.00 (z=2.74, p=0.068)
	Episode 1(S) vs Episode 5(O)	0.02±0.01 vs 0.00±0.00 (z=-1.60, p=0.109)	0.05±0.04 vs 0.00±0.00 (z=-2.02, p=0.043)
Exploration	<u>Order 1</u>		
	Episode 1(O) vs Episode 3(S)	0.66±0.06 vs 0.25±0.05 (z=-2.38, p=0.017)*	0.79±0.04 vs 0.32±0.05 (z=-2.67, p=0.008)*
	Episode 1(O) vs Episode 5(S)	0.66±0.06 vs 0.17±0.06	0.79±0.04 vs 0.27±0.04

			(z=-2.52, p=0.012)*	(z=-2.67, p=0.008)*
	<u>Order 2</u>			
		Episode 1(S) vs Episode 3(O)	0.47±0.04 vs 0.32±0.05 (z=-2.67, p=0.008)*	0.58±0.08 vs 0.36±0.10 (z=-1.68, p=0.093)
		Episode 1(S) vs Episode 5(O)	0.47±0.04 vs 0.24±0.08 (z=-2.43, p=0.015)*	0.58±0.08 vs 0.31±0.006 (z=-1.96, p=0.050)
	<u>Order 1</u>			
Play		Episode 1(O) vs Episode 3(S)	0.00±0.00 vs 0.00±0.00 (z=N/A, p=1.000)	0.01±0.01 vs 0.00±0.00 (z=-1.34, p=0.180)
		Episode 1(O) vs Episode 5(S)	0.00±0.00 vs 0.00±0.00 (z=N/A, p=1.000)	0.01±0.01 vs 0.02±0.02 (z=0.37, p=0.715)
	<u>Order</u>			
		Episode 1(S) vs Episode 3(O)	0.01±0.01 vs 0.33±0.07 (z=2.52, p=0.012)*	0.00±0.00 vs 0.15±0.10 (z=1.60, p=0.109)
		Episode 1(S) vs Episode 5(O)	0.01±0.01 vs 0.40±0.13 (z=2.37, p=0.008)*	0.00±0.00 vs 0.25±0.11 (z=1.83, p=0.068)
Greeting	<u>Order 1</u>	O vs S	2.38±0.08 vs 1.44±0.22 (z=2.26, p=0.024)*	2.22±0.09 vs 1.78±0.26 (z=1.84, p=0.66)
	<u>Order 2</u>	O vs S	1.28±0.23 vs 1.17±0.34 (z=0.43, p=0.670)	1.75±0.37 vs 1.25±0.23 (z=1.20, p=0.230)
Following	<u>Order 1</u>	O vs S	2.88±0.13 vs 3.00±0.00 (z=-1.00, p=0.317)	3.00±0.00 vs 3.00±0.00 (z=N/A, p=1.000)
	<u>Order 2</u>	O vs S	3.00±0.00 vs 1.56±0.34 z=2.41, p=0.016*	3.00±0.00 vs 2.25±0.37 z=1.73, p=0.083

Table 4 - Results of the planned comparisons for the relative duration of contact-maintenance, separation distress and secure-base-related behaviors and for the scores for greeting and following behaviors. O – owner; S – stranger; *Statistically significant differences (p<0.025 for contact-maintenance, separation distress and secure-base-related behaviors and p<0.05 for greeting and following behaviors).

WHINING

A comparison of the episodes when the dogs were alone with the owner versus the episodes when they were alone with the stranger revealed that, although dogs tended to spend more time whining when they were alone with the stranger, only one comparison was significantly difference for the Reward group (Episode 1 vs Episode 3 of procedure Order 2), and two for the Aversive group (Episode 1 vs Episode 3 of procedure Order 1: $z=2.67$, $p=0.008$ and Episode 1 vs Episode 5 of procedure Order 1: $z=2.50$, $p=0.013$; see Table 4).

BEHAVIORS TOWARDS DOOR

Although dogs from both groups tended to spend more time performing behaviors towards the door when they were alone with the stranger than when they were alone with the owner, the differences never reached statistical significance ($p=ns$ for all comparisons, see Table 4).

SECURE-BASE EFFECT

EXPLORATION

Dogs from the Reward group spent more time exploring during the episodes when only the owner was present than when only the stranger was present within procedure Order 1 [Episode 1(O) vs Episode 3(S): $z=-2.38$, $p=0.017$, Episode 1(O) vs Episode 5(S): $z=-2.52$, $p=0.012$; see Fig. 2]. However, within procedure Order 2, the reverse was true, with dogs spending more time in exploration when only the stranger was present [Order 2 - Episode 1(O) vs Episode 3(S): $z=-2.67$, $p=0.008$, Episode 1(O) vs Episode 5(S): $z=-2.43$, $p=0.015$, see Fig. 2]. As with the Reward group, also dogs from the Aversive group spent more time exploring in the presence of the owner within procedure Order 1 [Episode 1(O) vs Episode 3(S): $z=-2.67$, $p=0.008$, Episode 1(O) vs Episode 5(S): $z=-2.67$, $p=0.008$, see Fig. 2]. Within procedure Order 2, although there was also a tendency for dogs to explore more in the presence of the stranger, the differences were not statistically significant [$p=ns$; see Table 4 and Fig. 2].

PLAY

In the Reward group, dogs within procedure Order 1 did not play at all (see Table 4 and 2). Dogs within procedure Order 2 spent significantly more time playing when they were alone with the owner than when they were alone with the stranger [Episode 1(O) vs Episode 3(S): $z=2.52$, $p=0.012$, Episode 1(O) vs Episode 5(S): $z=2.37$, $p=0.008$, see Fig. 2]. In the Aversive group, no statistically significant differences were found for time spent playing in the company of the owner versus in the company of the stranger, although for procedure Order 2 there was also a tendency for dogs to play more in the presence of the owner (see Table 4 and Fig. 2)

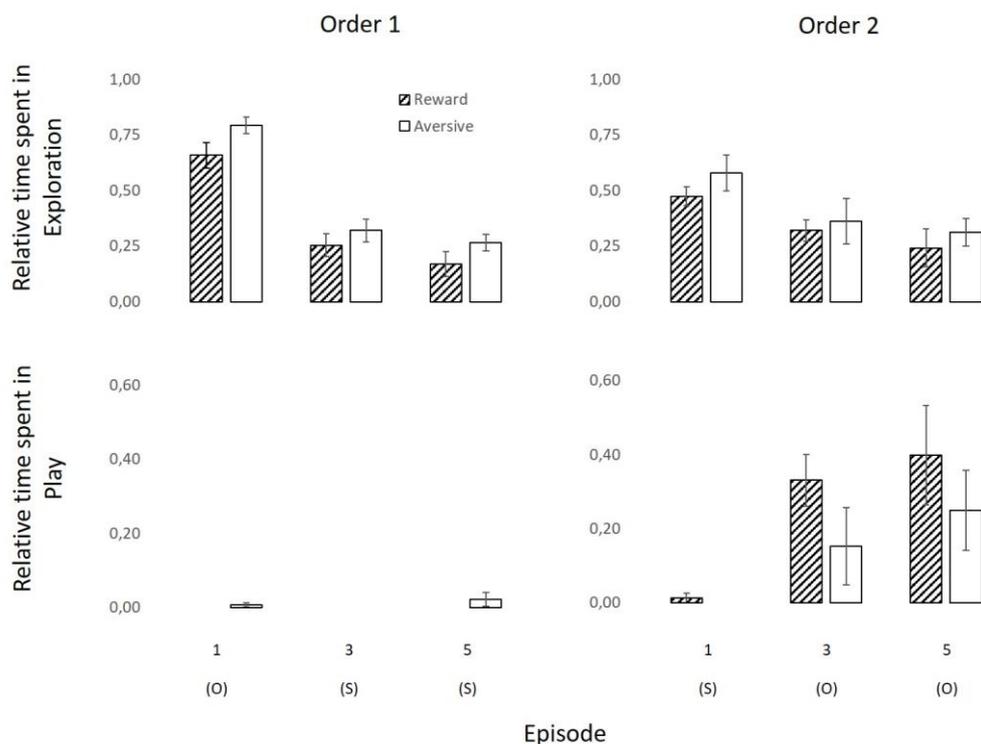


Figure 2 - Average relative time spent in exploration (top panels) and playing (bottom panels) in Episodes 1, 3 and 5 of the ASST by dogs trained with reward-based methods (dashed bars) and dogs trained with aversive-based methods (blank bars) for Order 1 (left panel) and Order 2 (right panel). Vertical bars show the SEM. O – owner; S – stranger.

GREETING

Dogs in the Reward group greeted the owner more than the stranger within procedure Order 1 ($z=2.264$, $p=0.024$; Fig. 3) but not within procedure Order 2, whereas dogs from the Aversive group did not show differences in the greeting scores towards the owner and towards the stranger ($p=ns$ for both procedure orders).

FOLLOWING

Dogs in the Reward group followed the owner more than the stranger within procedure Order 2 ($z=2.41$, $p=0.016$) but not within procedure Order 1 ($z=-1.00$, $p=ns$, Fig. 3). In the Aversive group no statistically significant differences were found in the following scores, although dogs within procedure Order 2 tended to follow the owner more than the stranger (Order 2: $z=1.73$, $p=0.083$).

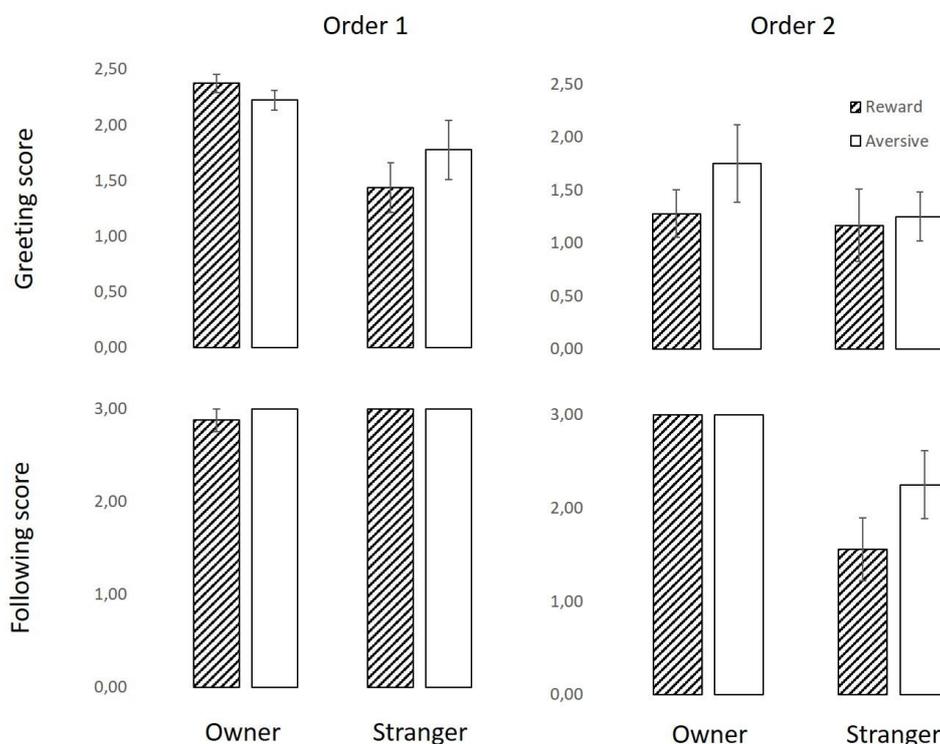


Figure 3 - Average greeting (top panels) and following (bottom panels) scores for dogs trained with reward-based methods (dashed bars) and dogs trained with aversive-based methods (blank bars) for Order 1 (left panel) and Order 2 (right panel). Vertical bars show the SEM.

DISCUSSION

The aim of the present study was to explore the relationship between the use of aversive- and reward-based training methods and dog-owner attachment using the Ainsworth Strange Situation Test. We hypothesized that dogs attending schools employing different methods would display differences in attachment-related behaviors, more specifically in secure-base-related behaviors, with dogs trained with reward-based methods, but not dogs trained with aversive-based methods, showing a secure-base effect.

Both the Reward and the Aversive groups behaved similarly regarding contact-maintenance and separation-related behaviors. Generally, dogs spent more time in proximity-seeking with the owner as compared to the stranger. Interestingly, when dogs were tested with procedure Order 1, the preference for the owner over the stranger was only evident in Episode 6, whereas in Order 2 it was evident for both Episodes 2 and 6. This difference between orders is likely because in Order 2, but not in Order 1, Episode 2 was preceded by a period of separation from the owner, which is expected to make dogs engage more in behaviors aimed at seeking proximity with the owner in Episode 2 (see Hall et al., 2015). The same trend was found for physical contact for dogs tested with procedure Order 1; however, for procedure Order 2, only the dogs from the Reward group showed a preference for the owner (and this occurred only in Episode 2). Time spent in physical contact may have been too short for differences to emerge. Regarding separation-distress behaviors, both groups tended to spend more time standing by the door and attending to the door when they were alone with the stranger than when they were alone with the owner. The time spent whining also tended to be higher in the presence of the stranger; however, the differences

failed to reach statistical significance in some instances. Finally, no differences were found for behaviors towards the door. As was the case with physical contact, the little time spent whining and performing behaviors towards the door can explain the absence of a statistically significant effect.

Playing and exploring more in the presence of the owner than in the presence of the stranger indicates a secure-base effect. Within procedure Order 2, dogs trained with reward-based methods spent more time playing in the presence of the owner than in the presence of the stranger, but this difference was not statistically significant in dogs trained with aversive-based methods. Within procedure Order 1, the absence of a difference in play behavior depending on who was present may have been due to a floor effect (dogs played very little in Order 1). The difference in play levels between orders is, however, difficult to explain. One possibility is that procedure Order 2 results in a greater activation of the attachment system, possibly due to the fact that there are two separation episodes from the owner. Since play is typically used as a reinforcer in reward-based dog training, one could argue that the higher levels of play in the presence of the owner shown by the Reward group could be simply because play is overall a more common behavior in these dogs, instead of a true difference in attachment security. However, and despite in situ observations of training sessions revealed that the three reward-based schools used different levels of play during training: School D did not use play at all, School B used it very occasionally and School F used it consistently, there were no differences between the three schools in the average time dogs spent playing in the ASST (see Table S3). Hence, it is unlikely that the reported differences are due to dogs from reward-based schools being more accustomed to play with their owners. Regarding exploration behavior, both groups explored more in the presence of the owner within procedure Order 1, but not within procedure Order 2. Although this result may at first sight suggest evidence for a secure-base effect within Order 1, the order effect suggests another explanation. The decline in exploration from Episode 1 (when the dog is alone with the owner) to Episodes 3 and 5 (when the dog is alone with the stranger) can be simply due to decreasing interest in exploring the space which is no longer new and unfamiliar, rather than to an effect of whoever is present in the room with the dog (Prato-Previde et al., 2003; Palmer and Custance, 2008; Rehn et al., 2013). In support of this view is the fact that, when the order was reversed, and the dog was with the stranger in Episode 1 and with the owner in Episodes 3 and 5, the effect did not show up (Aversive group) or was even reversed (Reward group), with the dogs exploring more in the presence of the stranger. Other studies have also failed to find a secure-base effect using exploration as the reference behavior (Prato-Previde et al., 2003; Topál et al., 2005; Rehn et al., 2013).

The two groups performed differently regarding greeting and following behaviors. Importantly, reunion behavior (greeting) is proposed to be the most relevant feature of the ASST to assess the style of attachment in the human literature (Ainsworth et al., 1978; Rehn et al., 2013). In the present study (although only for Order 1), dogs trained with reward-based methods, but not dogs trained with aversive-based methods, greeted the owner more than the stranger. Such differences further suggest a secure attachment by dogs trained with reward-based methods, with these dogs performing more comfort-seeking behaviors towards the owner than the stranger after the distress of separation (see Rehn et al., 2013). One difference between the two procedure orders which may contribute to the different reactions is the longer period of separation from the owner that occurs in Order 1 - three consecutive episodes (versus one episode in Order 2), which may result in a heightened desire to regain proximity with the owner after complete isolation in this procedure order. This result, however, seems to be in contradiction with what was found for play behavior, where procedure Order 2 appears to have resulted in a greater activation of the attachment system. As for following behavior, dogs trained with reward-based methods, but not dogs trained with aversive-based methods, followed the owner more than the stranger within procedure Order 2. This result strengthens the contact-maintenance effect shown by dogs trained with reward-based methods, with these dogs seeking contact with the owner more than with the stranger when they were about to leave the room. The explanation for the order difference in this case seems straightforward: in Order 2, when the stranger exits the room, the dog is left in the company of the owner,

whereas in Order 1 the dog is left in complete isolation, which may thus evoke a reaction of seeking contact with whoever is leaving.

Our findings further motivate reflections on two aspects of the ASST: the role of exploration as an indicator of the secure-base effect, and the role of different procedure orders. Criticisms have been raised against the use of exploratory behavior as a measure of the secure-base effect in the ASST, because the order of appearance of the owner versus the stranger is confounded with the passage of time, which can naturally lead to a decrease in exploration levels (Prato-Previde et al., 2003; Palmer and Custance, 2008; Rehn et al., 2013). The use of counterbalanced versions of the ASST, as was the case in the current study, has been proposed as a way of overcoming these order effects (e.g., Rehn et al., 2013). However, similar to what was reported by Rehn et al. (2013) using this approach, we found no differences in exploration that would indicate a security-providing role by the owner. In contrast, results on play behavior are more consistent in the literature, with only one study (Valsecchi et al., 2010) showing no preference for the owner over the stranger, suggesting that play behavior is a more reliable measure of the secure-base effect than exploration when evaluating dog-owner attachment using the Ainsworth Strange Situation Test (see also Mongillo et al., 2013). Our results provide further evidence that dog behavior in the Ainsworth Strange Situation Test is highly sensitive to order effects, and furthermore suggest that different procedure orders may indeed reveal different aspects of dog attachment behavior. In the present study, procedure Order 1 (but not procedure Order 2) elicited a preference for greeting the owner in the Reward group. On the contrary, procedure Order 2 (but not procedure Order 1) elicited a preference for playing with and following the owner in this same group. With the counterbalanced design used in the present study, the order of appearance of the owner and the stranger is not the only distinguishing factor between the two procedure orders. The two procedure orders are also asymmetric as regards the number of separation events from the owner (one in Order 1 versus two in Order 2) and the time spent in the absence of the owner (three episodes in Order 1 and one episode in Order 2). It is possible that the number of separation events has a stronger effect on play and following behavior and the duration of the separation has a stronger effect on greeting behavior, although the small sample size of our study requires caution in interpreting these differences. Nevertheless, it seems clear that when counterbalanced versions of the ASST procedure are used, the data of the two procedure orders should be analyzed and interpreted separately as they may reveal different aspects of behavior.

In summary, the present study shows that, whereas the results are similar on several measures, the evidence for a secure attachment to owners is more consistent in dogs trained with reward-based methods than in dogs trained with aversive-based methods. Dogs trained with reward-based methods played more in the presence of the owner as compared to the stranger, and they greeted and followed the owner more than the stranger, although these differences were found only for one procedure order. Hence, using a standard and well-validated method for the assessment of dog-owner attachment (see Rodriguez et al., 2018), the present study points to a relationship between training methods and dog-owner attachment.

Former studies that addressed the effects of training on dog-owner attachment using the ASST failed to find any significant differences in attachment behaviors (Fallani et al., 2006; Mariti et al., 2013b; Scandurra et al., 2016). Importantly, dogs tended to display a secure attachment regardless of training. However, these studies compared formally trained (working) and untrained (companion) dogs rather than different training methods. Both Mariti et al. (2013b) and Scandurra et al. (2016) report that the dogs in their studies were trained using reward-based methods, but still they failed to find differences in relation to untrained dogs. Together with our results, this suggests it is not the reward-based training in itself that generates a secure attachment, but rather the aversive-based training that may be related to the absence of a secure-base effect.

Some limitations of the present study need to be recognized. The first relates to the small sample size. Although we found statistically significant differences for play, greeting and following behavior for the



Reward group, but not for the Aversive group, the latter also tended to play more in the presence of the owner than in the presence of the stranger and to greet and follow the owner more than stranger. It is possible that with a bigger sample, statistically significant differences could also have emerged in the Aversive group. Secondly, this is a study investigating an association rather than a causal relationship between dog training methods and dog-owner attachment. Because we did not randomly allocate the subjects to the two treatments (training methods), we cannot discard the possibility that owners enrolled in training schools that matched their pre-conceived preferences for certain types of methods or ways of relating to their dogs. Importantly, future studies should look at reasons for choosing training schools and training methods among dog owners. To investigate a causal relationship between training method and attachment would require a randomized control trial. However, this is not without ethical concerns, as aversive-based methods have been associated with some indicators of stress in dogs (see Guilherme-Fernandes et al., 2017 for a review).

The nature of the relationship between dogs and their owners has been proposed to have an important impact on the well-being of both members of the dyad (e.g., Crawford et al., 2006; Payne et al., 2015). Identifying the factors that affect the dog-owner relationship will contribute to the understanding of how the successful relationship is achieved and thus help to bring benefits for both species. The present study shows some evidence that dog training methods may play a role in the success of the relationship. However, more studies need to be conducted in order for the association between training methods and dog-owner attachment to be fully understood.

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APPENDIX A - SUPPLEMENTARY DATA

Procedure	Definition
Positive punishment	Any (unpleasant) stimuli that was applied to the dog after the exhibition of an undesirable behavior. Common examples were leash jerks, hitting or yelling at the dog.
Negative reinforcement	Any (unpleasant) stimuli that was applied to the dog and which was stopped only after the dog exhibited the desirable behavior. A common example was pulling the collar upward, choking the dog, and releasing the pressure only when the dog sat.

Table S1 - Definition of the aversive-based operant conditioning procedures used for the classification of dog training schools as aversive-based or reward-based. The schools were classified as aversive-based if they used some sort of positive punishment and/or negative reinforcement and as reward-based if they did not use any of these techniques.

Session	Dog training school					
	A	B	C	D	E	F
1	138	0	166	0	38	0
2	101	0	50	0	23	0
3	93	0	76	0	37	0
4	73	0	37	0	8	0
Mean	101.25	0.00	82.25	0.00	26.5	0.00
Std Dev	27.18	0.00	58.14	0.00	14.11	0.00

Table S2. Frequency (mean \pm standard deviation) of positive punishments and negative reinforcements used during the four training sessions videotaped at the six dog training schools. Schools A, C and E were classified as aversive-based and Schools B, D and F were classified as reward-based.

Episode	Dog training school			Statistical results
	B	D	F	
1	0.00 \pm 0.00	0.02 \pm 0.05	0.00 \pm 0.00	H=1.83, p=0.400
3	0.22 \pm 0.25	0.15 \pm 0.23	0.15 \pm 0.21	H=0.36, p=0.835
5	0.25 \pm 0.39	0.22 \pm 0.38	0.14 \pm 0.28	H=0.38, p=0.825

Table S3. Relative time (mean \pm standard deviation) spent playing in Episodes 1, 3 and 5 of the Ainsworth Strange Situation Test by dogs of the reward-based schools. Comparisons between the different schools with Kruskal-Wallis tests showed no statistically significant differences in the time spent playing for either episode.