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*Title:* Presence and lasting effect of social referencing in dog puppies

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**Abstract**
Social referencing is the process by which individuals utilize cues from emotional displays of a social partner to form their response to a new situation. It can provide advantages, especially to young unexperienced individuals, by favouring an appropriate reaction to novel situations while avoiding the risks of trial and error learning. While there is evidence for social referencing from humans in adult dogs, the ontogeny of this behaviour has not been investigated. Moreover, it is not known whether dogs acquire information during such interactions and recall it later, when encountering a similar situation. We tested 8-week-old companion dog puppies ($N$ = 48) of various breeds by exposing them to a novel stimulus in presence of human or conspecific social partners. With humans, we tested the effect of emotional signals expressed by the informant. With conspecifics, we tested whether the presence of their mother or of an unfamiliar dog affected the subjects' behaviour towards the stimulus. Puppies alternated their gaze between the stimulus and the social partner (referential looking), with all the partners. Puppies tested in presence of a human expressing positive emotional signals towards the stimulus were more likely to approach it than puppies tested with a human expressing neutral emotional signals (behavioural regulation). Importantly, this effect was still apparent after a delay of 1 h, when puppies were tested alone. Puppies tested in presence of their mother were more likely to approach the stimulus than puppies tested alone or with an unfamiliar dog. These results show that the ability for social referencing develops early in the ontogeny of companion dogs as it is already present at 8 weeks. The valence of the emotional cues provided by a human social partner and the presence of the mother affect the behaviour of puppies exposed to novel situations, even after a delay.

**Key words**
Behavioural regulation; dog puppies; emotional signals; referential looking; social referencing

## Introduction
Social referencing is the process by which individuals rely on emotional cues from others upon being confronted with a novel situation (Walden 1993). This process is especially advantageous for young unexperienced individuals, because they are more likely to encounter novel stimuli and situations than more experienced, older individuals. Thus, their survival is very likely enhanced by the ability to refer to others when facing novel situations.
For social referencing to emerge, the individual must be sensitive to the emotional signals expressed by others. Behavioural evidence of social referencing includes two components: referential looking

and behavioural regulation (Russell et al. 1997). Referential looking is defined as a gaze alternation between the social partner and the novel stimulus or situation. This behaviour, which is thought to be aimed at seeking information, has been distinguished from other types of looking behaviours where gaze alternation is not necessarily implied, such as those in which the subject looks at the social partner to seek reassurance (Clyman and Emde 1986). Behavioural regulation refers to the behaviour of the subject being influenced (i.e. modified), consistently with the valence of the emotional signals expressed by the model towards the novel object / situation (Mumme et al. 1966; Morton 1977; Klinnert et al. 1983a). Children perceiving their mother's emotional signals of positive valence are more likely to approach a novel object and interact with it than those receiving emotional signals of negative valence (e.g., Feinman and Lewis 1983; Walden and Ogan 1988; Camas and Sachs 1991). For human infants, the caregiver does not only constitute a secure base for the child (Ainsworth et al. 1970) but it also provides emotional support and serves as a source of information about its environment. (e.g., Klinnert et al. 1983a). While social referencing in human infants and toddlers is abundantly documented (e.g., Klinnert et al. 1983a; Mumme et al. 1996; De Rosnay et al. 2006; Vaish and Striano 2004; Hoehl et al. 2008), there is mixed evidence of its presence in other species. While it has been shown in mammals, including young chimpanzees (Russell et al. 1997) and capuchin monkeys (Morimoto and Fujita 2012), other studies did not find referential looking behaviours in mother-infant pairs of chimpanzees (Russell 1997; Tomonaga et al. 2004) and macaques (Roberts et al. 2008).

Adult dogs are sensitive to the emotional signals of their owners (e.g., Buttelmann and Tomasello 2013; Turcsán et al. 2015) and if such a sensitivity (at least to an extent) develops already during early ontogeny, this may provide the most important pre-requisite of social referencing in puppies. In presence of a novel, potentially fear eliciting stimulus, adult dogs alternated their gaze between the owner and the stimulus (referential looking; Merola et al. 2012a). Based on findings of a recent study (Duranton et al. 2016), this behaviour may also emerge in presence of an unfamiliar human. Duranton et al. (2016) also reported the existence of behavioural regulation, but in this study the owners did not provide any emotional signal. Thus, these results could rather be interpreted as social facilitation (Zajonic 1965). Merola et al. (2012a) did not provide evidence of behavioural regulation in their study; during the tests, the owner started to deliver the message about the object only after some delay, whereas in human studies mothers usually delivered the message immediately and continued to do so for the whole duration of the test (e.g. Kim et al. 2010; Walden and Ogan 1988). Thus, it is possible that, when the informant started to deliver the message, dogs had already acquired some information about the stimulus and formed an appropriate behavioural response, which prevented behavioural regulation to emerge. To elicit behavioural regulation, subsequent studies (Merola et al. 2012b and Merola et al. 2013a) exposed adult dogs to informants providing emotional cues immediately, as soon as the subject was exposed to the novel stimulus. Dogs were found to be more likely to approach the object when they received signals of positive emotional valence with regards to the object, indicating that they regulated their behaviour through social referencing.

In 12-month-old human infants, social referencing seems to occur not only with the mother acting as an informant, but also with a stranger, at least when also the mother is present (e.g., Klinnert et al. 1983b). The role of familiarity of the informant has been investigated in adult dogs. Merola et al. (2012b) tested dogs facing a new object in presence of the owner and a stranger. In different conditions, either the owner or the stranger gave signals of either positive or negative emotional valence. Dogs looked at both informants but regulated their behaviour towards the object only when the emotional signals were given by the owner, while fewer behavioural differences between dogs receiving emotional signals of positive or negative valence emerged when the informant was a stranger. In a different context, Buttlemann and Tomasello (2003) found that dogs were able to discriminate between different emotional signals expressed by a stranger towards an object, at least with regards to some emotional behaviours (expressing happiness and disgust) (Buttleman and Tomasello 2003). Thus, it seems that both the familiarity of the informant and the valence of the emotional signal, affected adult dogs' behaviour when interacting with novel stimuli or facing novel

situations. Nevertheless, it is possible that dogs are able to discriminate different emotional signals even from an unfamiliar human (Nagasawa et al. 2011), but only use it as a relevant information when it comes from their owner.
Social referencing allows individuals to avoid costly mistakes associated with trial and error learning, so it may well bring advantages, especially to young individuals. While studies on apes and monkeys focused mainly on young individuals (e.g. Tomonaga et al. 2004; Roberts et al. 2008; Itakura 1995), no data is available, to our knowledge, about social referencing in dog puppies.
Following the assumption that social referencing is especially advantageous for young, unexperienced individuals, we hypothesised that this skill emerges early in the ontogeny of dogs and we expected to find evidence of it already at 8 weeks of age. Moreover, consistent with the results of studies on social referencing in adult companion dogs (e.g., Merola et al. 2012a; 2012b), we expected puppies to engage in social referencing not only with conspecifics, but also with humans. We also hypothesised that, like in the case of human infants, the presence of the mother, but not that of an unfamiliar conspecific, would affect the puppies' behaviour towards the novel stimulus, because the unfamiliar dog may also cause apprehension and its behaviour might be more difficult to recognize for young puppies. Thus, we expected puppies to be more likely to approach the novel stimulus when exposed to it with the mother than with an unfamiliar dog.
Importantly, given the survival-enhancing function of social referencing, we also expected puppies to be able to retain information acquired through social referencing and to adapt their behaviour accordingly, even after a delay.

## The observation of social referencing in dog puppies

We tested 8-week-old dog puppies facing a novel stimulus in presence (or absence) of different social partners: with humans expressing emotional signals towards the stimulus, either with positive or with neutral valence and with (or without) conspecifics showing a comfortable attitude in presence of the stimulus. In the case of the conspecific, we also aimed at investigating whether the presence of an unfamiliar dog affected the puppies' behaviour towards the novel stimulus similarly to the presence of their mother. Thus, we tested dog puppies with their mother and with an unfamiliar dog. Importantly, previous studies did not address the lasting effects of social referencing, i.e. whether such socially acquired information is retained to be recalled later upon encountering again similar stimuli and situations. Thus, we also tested dog puppies upon facing the same stimulus after a 1-hour delay. Different physical properties of acoustic signals emitted by the human social partner talking with a happy or neutral voice may have affected the puppies' general level of activity (McConnell 1990) and, consequently, exploration. We controlled for this possibility by comparing the subjects' activity levels when exposed to the experimenter talking with a happy or neutral voice.

## Methods

### *Subjects*

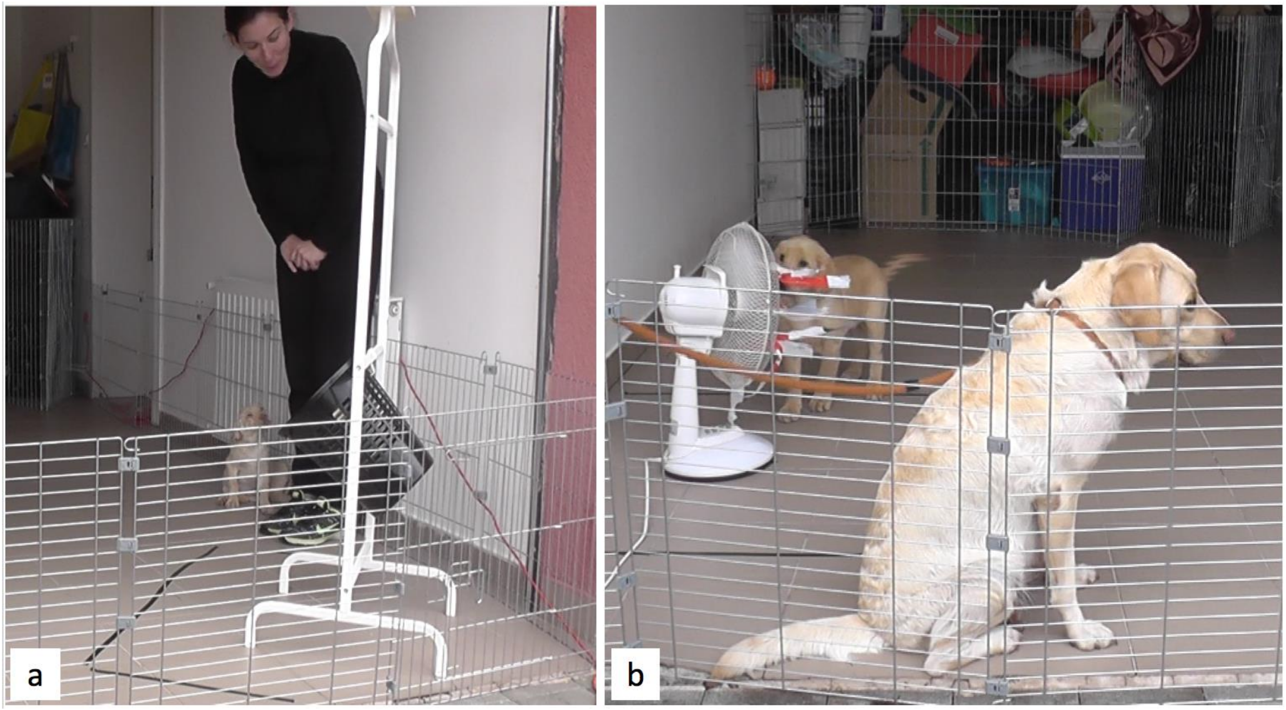
The tests were conducted in Italy ($N$ = 32) and Hungary ($N$ = 16) between August 2016 and March 2017. We enrolled 48 dog puppies originating from 8 litters of various breeds (Swiss hound $N$ = 3, Border collie $N$ = 5, Shetland shepherd $N$ = 4, Belgian tervueren $N$ = 6, Mudi $N$ = 8, Labrador mix $N$ = 8, Schnauzer $N$ = 7 and a mixed breed litter $N$ = 7). Puppies were tested at 8 weeks of age, before adoption, while still living with their mothers at the breeders' facilities. They were kept in kennels or fenced-off indoor areas to which their mother had access and they received daily interactions with the breeders taking care of them.

### *Experimental setup*

The tests were carried out at the breeder's facilities, in an area familiar to the puppies. We delimited the testing area using a puppy-fence (3.50 x 3.50 m). We used two stimuli that, based on the owner's report, were novel for the puppies:

- *Fan*: an electric fan with plastic ribbons attached to it. This stimulus was chosen because it was already successfully used in studies on social referencing in adult dogs (Merola et al. 2012a; Merola et al. 2012b; Merola et al 2013b);
- *Noise*: a plastic bin, hanging from a stand at the height of the puppies' head with a loudspeaker in it, emitting noises (a loud creaking noise and a siren, one after another, for 1 minute each).

During the tests, the stimulus was placed in a corner of the fenced-off area (Figure 1)




***Testing procedure***

All subjects participated in two conditions: 1) human and 2) conspecific, in randomized order. Between the two conditions an interval of minimum 1 h, maximum 2 h elapsed. In both conditions puppies of the same litter were allocated to different experimental groups (in the human condition: human positive, human neutral, control; in the conspecific condition: mother, unfamiliar, control, see below).

Allocation of the stimuli (fan or noise) to condition 1 or 2 was counterbalanced (i.e., if litter 1 was tested with the fan in the conspecific condition and with the noise in the human condition, then litter 2 was tested with the noise in the conspecific condition and with the fan in the human condition).

In the human condition, the human informant (i.e., the experimenter) expressed positive (human positive group) or neutral emotional signals (human neutral group) towards the stimulus, while alternating her gaze between the stimulus and the puppy. For the conspecific condition, we habituated the adult dogs (mothers and unfamiliar dogs) to the stimuli and trained them to associate those with food rewards (see below), so that they would be comfortable and not express behavioural signs of fear or distress in the presence of the stimuli during the tests. In the conspecific condition, dogs (mothers and unfamiliar dogs) did not specifically look at the stimulus nor alternated their gaze between the stimulus and the puppy. Due to the differences in gaze alternation and emotional displays of the social partners, the two conditions (human and conspecific) were not directly comparable.

We did not include informants expressing emotional signals with a negative valence due to ethical considerations, namely to ensure that puppies, in such a sensitive period, would not experience fear in relation to the test situation and to avoid conditioning fear responses in the adult demonstrator dogs.

The presence or absence of the social partners in the testing area and their interactions with the subjects were experimentally manipulated across conditions, but the experimenter and a helper were always present (but not interacting with the subjects), outside of the testing area to supervise the experiment and the welfare of the subjects.

*Experimental protocol*

1. Training of the adult dogs: adult dogs, used as social partners in the conspecific condition, were the mothers of the subjects and other adult dogs, owned by the breeder, but unfamiliar to the puppies (i.e., they never interacted with them before). The unfamiliar dogs were of a size similar to the mother dogs and were chosen by the breeder based on their friendly behaviour with puppies. For all but one litter (the Swiss hound litter), they were female dogs. Before the test started, the adult dogs were habituated to the stimuli (fan or noise). The dogs were exposed to the stimuli initially from a distance of several meters, then gradually decreasing the distance, ensuring that they did not show fear or stress-related reactions to the stimuli. Once the dogs did not show a fear reaction when close enough to the stimuli to be able to touch it, they were allowed to eat pieces of food placed on it. This way we ensured that the adult dogs were calm, did not show signs of stress or fear and did not try to avoid the stimulus during the tests. Duration of the training varied from approximately 15 min to 1 h. We planned to exclude all trials from the analysis in which the experimenter detected any sign of stress- or fear-related behaviours by the adult dog, but none of the dogs expressed such behaviours.
2. Before the test started, the stimulus (fan or noise) was placed in a corner of the fenced-off area and turned on (Figure 1);
3. The informant (experimenter or adult dog, depending on the condition) entered the testing area and remained at 1.5 m from the stimulus throughout the duration of the test. In the conspecific condition, the dog was held on a 1.5 m leash to ensure that it would not move far away from the location of the stimulus. The leash was held by a helper who stayed outside of the fence and did not interact with nor looked at the subjects. In the control groups, the informant was not present, so that the subject was exposed to the stimuli alone;
4. A helper carried the puppy to the testing area and placed it in the corner that was furthest from the stimulus, approximately 3 m from it;
5. The subject was then allowed to move freely in the testing area for 2 minutes (*first trial*);
6. The same procedure was repeated after a short break (few s), during which the puppy was taken out of the testing area by the helper and was held in her arms out of the view of the testing area, while the stimuli were turned on again after their automatic stop at the end of the trial (*second trial*);
7. To test whether puppies retained the information obtained in the first and second trial, after 1 h delay, all subjects were placed again, one at a time, in the testing area with the stimulus, but in absence of the informant, for 1 minute (*retention trial*).

Table 1 shows the subsequent exposures to the stimuli throughout the whole experiment.

***Experimental groups***

In the human condition, puppies of the same litter were divided into 3 experimental groups:

- **Human positive** (N = 15): Puppies were exposed to the stimulus in presence of the experimenter expressing positive emotional signals towards it. The experimenter spoke using a happy voice directed at the stimulus and displayed a happy facial expression, while alternating her gaze between the puppy and the stimulus during the test;
- **Human neutral** (N = 16): Puppies were exposed to the stimulus in presence of the experimenter expressing neutral emotional signals towards it. The experimenter spoke using a neutral voice and displayed a neutral facial expression directed at the stimulus, and alternated her gaze between the puppy and the stimulus during the test;
- **Control** (N = 17): Puppies were exposed to the stimulus in absence of the experimenter.

In the conspecific condition, puppies of the same litter were divided into 3 experimental groups:

- **Mother** (N = 15): Puppies were exposed to the stimulus in presence of their mother showing a confident attitude (i.e., the dog was calm, did not show any signs of stress or fear and did not try to avoid the stimulus);

- **Unfamiliar** (N = 17): Puppies were exposed to the stimulus in presence of an unfamiliar dog showing a confident attitude;
- **Control** (N = 16): Puppies were exposed to the stimulus in absence of the adult dog.

All subjects had participated in an experiment on social learning before the present study (Fugazza et al. 2018). In that experiment, they were exposed to the experimenter and to a conspecific demonstrator solving a manipulative task to obtain food, before having the possibility to solve the task themselves. The duration of the experimental trials ranged from 2 to 3 h (depending on litter size), during which puppies of the same litter were tested in turns. Before and during the test trials of that experiment, the puppies had the opportunity to acquaint with the Experimenter who familiarized with them, carried them to the testing area and gave them some pieces of food, but they had no experience and interaction with the unfamiliar dog, apart from observing it solving tasks. After a break of at least 2 h, puppies were exposed to the present test.

***Behavioural variables***

All tests were video recorded for later behavioural analysis using Solomon Coder (v. 090913; András Péter http://solomoncoder.com). From the videos, we measured frequency and latency in seconds (s) from the start of the test, i.e., from releasing the puppy in the testing area, until first occurrence, of the following behaviours:

Gaze alternation: number of times per minute when the subject shifted its gaze between the stimulus and the social partner or between the social partner and the stimulus, within 2 s; (i.e., referred to as 'referential looking' in Merola et al. 2012a; 2012b);

Approaching the stimulus: latency of the subject to approach the stimulus by entering (with any body part) a circle of 50 cm radius from it, measured from when the subject was released in the testing area;

Interacting with the stimulus: latency of the subject to contact the stimulus (using any body part), measured from when the subject was released in the testing area;

Activity: (only in the human condition): time spent performing any behaviour other than being static (i.e., except from standing still, sitting or lying down) (Merola et al. 2012b). This behavioural variable was analysed in the human positive and in the human neutral groups, with the aim of assessing whether the experimenter talking with a happy tone of voice stimulated more activity in general in the puppies (McConnell 1990) and consequently, also more exploration of the stimulus.

Interacting with the informant: (in the conspecific condition): latency of the subject to contact with the social partner (using any body part). This was coded only in the conspecific condition to investigate the difference between interaction with the mother or unfamiliar dog as social partners.

***Data analysis***

We used the R statistical environment (v. 3.2.3; R Development Core Team) to analyse our data. Assumptions of statistical tests were considered prior to the analyses. Referential looking (i.e. the number of gaze alternation sequences), was analysed in all but the control groups (in which there was no opportunity for referential looking) using Linear Mixed Models (R package 'lme4', Bates et al. 2015).

Probability to approach the stimuli and to interact with those and with the informants were analysed in separate Cox Mixed Models (R package 'coxme', Therneau, 2015). The models included latencies (in s) until approaching or interacting, and occurrence of approaching or interacting as terminal events, respectively. Puppies that did not approach the stimuli or did not interact with those or with the experimenter were treated as censored observations. All Cox Mixed Models included experimental group and type of stimulus as fixed effects and subject nested in litter as random term. In all initial models, the possible confounding effect of trial (factor with three levels: 1$^{st}$ trial, 2$^{nd}$ trial and retention trial) was investigated and was removed from final models when it did not have significant effects. Model selection was based on AIC values, and the effects of explanatory variables

were analysed by likelihood ratio tests: we provide $\chi^2$ and P values of likelihood ratio tests of models with and without the explanatory variable. Parameter estimates (for LMM) and hazard ratios (Exp[β]; for Cox Mixed Models) with 95% CI between levels of a given fixed effect are also given.
To determine whether the experimenter talking with a happy tone of voice stimulated more activity in general in the puppies than the experimenter talking with a neutral tone, we compared the activity of the puppies in the human positive group to the activity of puppies in the human neutral group using two-sample *t* test.

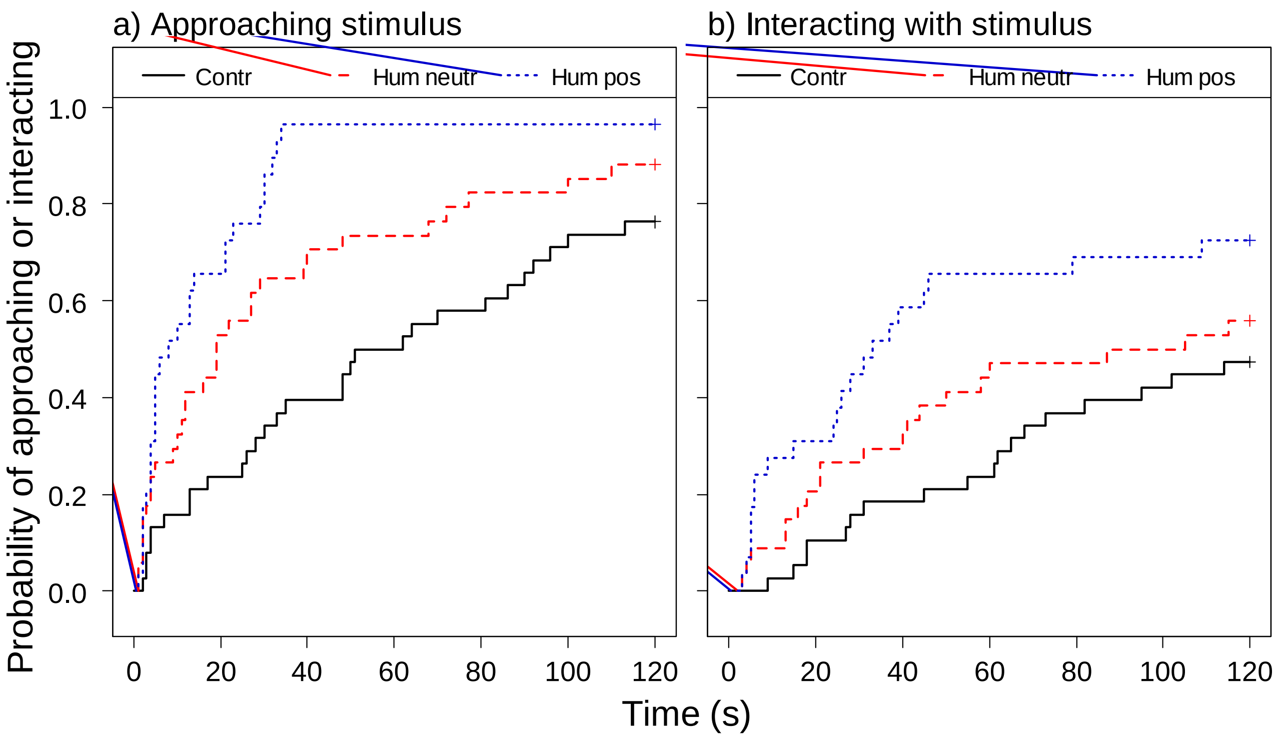
***Ethical note***
The authors confirm that the experiments reported in this paper are in accordance with the current Hungarian laws in regard to animal protection. The experimental protocol was approved by the ethical committee (AWERB) at Waltham Centre for Pet Nutrition on 2nd November 2015.

## Results

### *Human condition*

*Gaze alternation*
Gaze alternation was similar among experimental groups: in the human positive group, all puppies alternated their gaze between social partner and stimulus at least once (mean ± SD gaze alternations per minute: 9.7 ± 5.7), whereas in the human neutral group, 95.2% of the puppies alternated their gaze between social partner and stimulus at least once (mean ± SD number of gaze alternations per minute: 6.8 ± 4.5; LMM of square root transformed frequency of gaze alternation, effect of experimental group: $\chi^2_1 = 2.21$, $P = 0.137$). This analysis also revealed trial differences, as gaze alternations were less frequent in the second trial as opposed to the first trial (effect of trial: $\chi^2_1 = 6.83$, $P = 0.009$; trial 1 → trial 2: b = -0.36 [-0.63; -0.09], t = -2.76, $P = 0.014$).

*Stimulus approach and interaction*
The probability of approaching the stimulus was significantly different between experimental groups (Cox Mixed Model of latency to approach, effect of experimental group: $\chi^2_2 = 23.76$, $P < 0.001$). Puppies in both the human neutral and human positive groups were more likely to approach the stimulus than puppies in the control group, although the difference was more pronounced in the latter group (control → human neutral: Exp(β) = 2.24 [1.31; 3.82], z = 2.95, $P = 0.003$; control → human positive: Exp(β) = 5.56 [3.01; 10.26], z = 5.49, $P < 0.001$; Figure 2a).
Probability of interacting with the stimulus was also significantly different between experimental groups (Cox Mixed Model of latency to interact, effect of experimental group: $\chi^2_2 = 7.11$, $P = 0.029$). Puppies in the human positive group were more likely to interact with the stimulus than puppies in the control and human neutral groups (control → human neutral: Exp(β) = 1.44 [0.72; 2.89], z = 1.03, $P = 0.300$; control → human positive: Exp(β) = 2.64 [1.31; 5.33], z = 2.72, $P = 0.007$; Figure 2b).

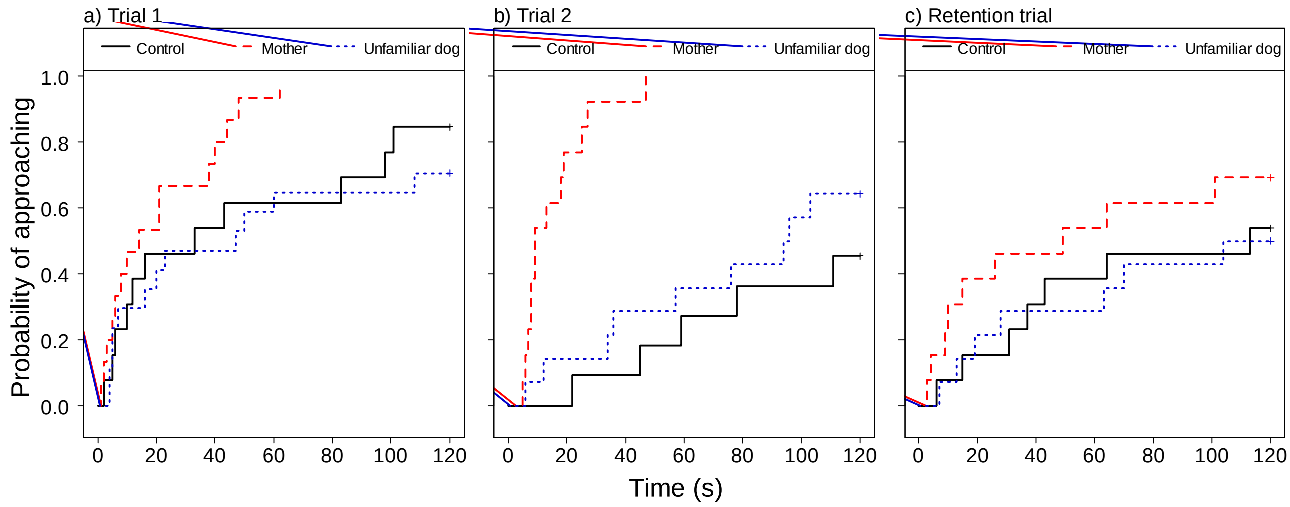



We found a significant interaction between trials and type of stimulus ($\chi^2_2 = 9.32$, $P = 0.009$). Puppies in trial 2 and in the retention trial were more likely to approach the noise stimulus than the fan stimulus, compared to trial 1 (Table 2).
No other interactions emerged from the model.

*Activity*
We did not find a significant difference between the time spent actively by puppies in the human positive group (mean ± SE: 168 ± 51 sec) and in the human neutral group (mean ± SE: 156 ± 80 sec; LMM of activity, effect of experimental group: $c^2_1 = 0.33$, $P = 0.567$).

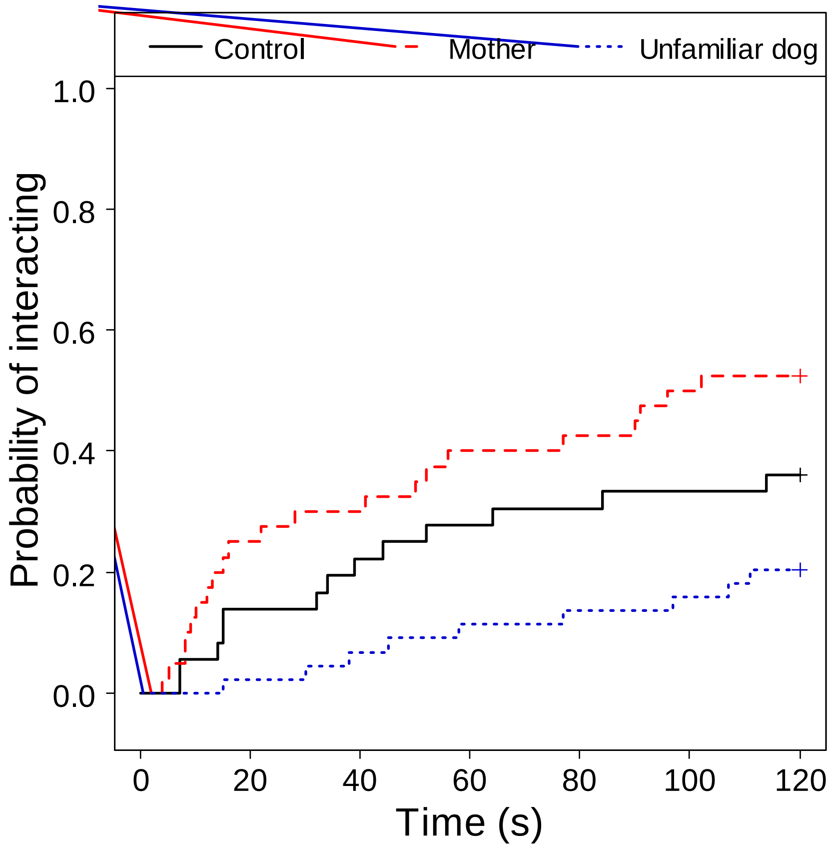
***Conspecific condition***
*Gaze alternation*
In the mother group, 88.8% of the puppies alternated their gaze between social partner and stimulus at least once (mean ± SD referential looks per minute: 5.8 ± 5.4), whereas in the unfamiliar dog group, 80% of the puppies alternated their gaze between social partner and stimulus at least once (mean ± SD referential looks per minute: 5.2 ± 7.3). Therefore, experimental group alone did not have an effect on the frequency of gaze alternation (LMM of log transformed frequency of gaze alternation, effect of experimental group: $\chi^2_1 = 0.86$, $P = 0.355$), although we found gaze alternation to be different based on the combined effects of type of stimulus, trial and experimental group (effect of the 3-way interaction: $\chi^2_1 = 4.11$, $P = 0.043$; Table 3).

*Stimulus approach*
The probability of approaching the stimulus was significantly different between experimental groups and trials (Cox Mixed Model of latency to approach, effect of experimental group: $\chi^2_2 = 21.49$, $P < 0.001$). Puppies in the mother group approached the stimuli more likely than puppies in the control and unfamiliar groups (control → mother: Exp(β) = 3.05 [1.76; 5.32], z = 3.95, $P < 0.001$; Figure 3). In addition, puppies were less likely to approach the stimuli in the retention trial than in the preceding two trials (effect of trial: $\chi^2_2 = 10.68$, $P = 0.005$; trial 1 → trial 2 Exp(β) = 0.66 [0.40; 1.09], z = -1.62, $P = 0.100$; trial 1 → retention trial: Exp(β) = 0.41 [0.24; 0.71], z = -3.22, $P = 0.001$).




*Interaction with the stimulus*

The probability of interacting with the stimuli was different between experimental groups (Cox Mixed Model of latency to interact, effect of experimental group: $\chi^2_2 = 9.22$, $P = 0.010$), because puppies in the mother and control groups were more likely to interact with the stimuli than puppies in the unfamiliar group (Table 4; Figure 4).




In addition, the type of stimulus had a trial-specific effect on the subjects' interaction with the stimuli ($\chi^2_2 = 7.25$, $P = 0.027$), as puppies were more likely to interact with the noise than with the fan stimulus in the second trial, compared to both the first trial and the retention trial (Table 4).

*Interaction with the adult dog*

Interaction with the conspecific partner was different between experimental groups (Cox Mixed Model of latency to interact with partner, effect of experimental group: $\chi^2_1 = 13.09$, $P < 0.001$).

Puppies were more likely to interact with their mother than with an unfamiliar dog (mother → unfamiliar: Exp(β) = 0.11 [0.03; 0.35], z = -3.77, $P < 0.001$). The probability of interacting with the partner was influenced by the type of stimulus dependent on experimental group (experimental group x type of stimulus interaction: $\chi^2_1 = 6.48$, $P = 0.011$). Puppies in the unfamiliar group interacted more likely with the partner when exposed to the fan stimulus compared to the noise stimulus, while this was not the case for puppies in the mother experimental group (fan → noise in unfamiliar vs. mother experimental groups: Exp(β) = 5.38 [1.36; 21.23], z = 2.40, $P = 0.016$).

**Discussion**

Our study provided the first experimental evidence for social referencing in 8-week-old companion dog puppies. Precisely, we found that dog puppies alternated their gaze between the novel stimulus and the social partner (conspecific and human) when exposed to this novel situation and that they modified their behaviour towards the stimulus consistently with the emotional signals provided by the human social partner. Puppies facing a novel situation in presence of a human showing positive emotional signals were more likely to approach the stimulus and interact with it than puppies facing it alone or with a human displaying a neutral facial expression and voice. The likelihood to interact with the stimulus was not affected by the retention interval, indicating that the information acquired socially had lasting effects and influenced the behaviour of puppies in similar situations later in time. Finally, puppies were more likely to explore a novel stimulus in presence of their mother than alone or in presence of an unfamiliar adult conspecific. These results support our hypotheses. They confirm that social referencing emerges during early ontogeny in dogs and the puppies' behaviour is influenced by both the emotional valence of the human informant's signals and by the presence of the mother dog.

In all conditions and groups in which a social partner was present, we found evidence for gaze alternation between the partner and the stimulus. This behaviour has been interpreted as referential looking in adult dogs (Merola et al. 2012b; 2013). Puppies looked referentially at their partner more often in the first trial than in the second. This result corroborates the function of referential looking as seeking information about a novel situation, because, as the situation becomes more familiar with time – i.e., in trial 2 - the need of information is reduced. Consistent with this interpretation, 7- and 10-month-old human infants tended to look at a social partner more often right after a novel event occurred, as opposed to after a while, arguably in search for social cues to disambiguate the novel event (Striano and Rochat 2000).

Puppies looked referentially to both their mother and the unfamiliar conspecific. This is consistent with the results obtained by previous studies on adult dogs, which demonstrated that referential looking occurs not only with familiar human social partners but also with strangers (Merola et al. 2012a; Merola et al. 2012b; 2013a). Merola et al. (2012b) reported that although adult dogs looked referentially to the social partner, regardless of his/her familiarity to the subject, they explored the stimulus more likely when the social partner was familiar. Our results in the conspecific condition are in line with these previous findings. We found that puppies looked referentially both to their mother and to an unfamiliar dog; however, only the presence of the mother enhanced exploration of the novel stimulus. Merola et al. (2013a) found that adult dogs reacted more to the emotional signals expressed by their owner than those expressed by a stranger. Our subjects showed a behavioural regulation that was consistent with the valence of the emotional signals provided by the experimenter, as puppies were more likely to approach the stimulus when the valence was positive than when it was neutral. In our study, the experimenter, who fulfilled the role of the unfamiliar social partner, spent half a day testing the subjects for another study, immediately before the tests of this study were run. It is possible that this acquaintance period was enough for the puppies to familiarize with her, especially considering that 8-week-old dogs are in a period of their development during which they are particularly predisposed to form social relationships (e.g., Fox 1971; 1972; Miklósi 2014). The puppies' ability to recognize emotional signals expressed by a (relatively) unfamiliar human would be consistent with the results of Buttleman and Tomasello (2013), who found that adult dogs discriminated between emotional signals displayed by an unfamiliar person.

We did not find activity differences between puppies in the human positive and human neutral groups. This result rules out the possibility that puppies simply reacted to different physical properties of acoustic signals emitted by the experimenter in the two experimental groups with different activity levels (McConnell 1990) and, as a mere consequence of increased activity, they explored more in the positive group. As the differences in the subjects' behaviour cannot be explained by the general level of motor activity, we suggest that 1) puppies were able to recognize the emotional valence of the signal of the human informant and 2) they regulated their behaviour accordingly. To ensure that puppies can recognize an emotional signal as directed towards a specific object, future studies are needed in which e.g., the informant directs its display towards one of multiple objects simultaneously present (e.g., for adult dogs, Merola et al. 2013a).

When tested alone in the retention trial, puppies in the human positive and human neutral groups were more likely to approach the stimulus than puppies in the control group. This effect was more pronounced in the human positive group. Thus, the effect of the emotional signals provided by the human informant on the puppies' behaviour towards the stimulus could still be observed after a delay of 1 h. To our knowledge this is the first evidence that, in social referencing situations, dogs (more specifically, dog puppies) acquire and retain some information and that this information is used to regulate their behaviour not only immediately, but also after a delay and when the informant is no longer present. This result suggests that even a very short exposure to such situation constitutes a social learning occasion. If so, this has applied consequences, because the owners' attitude in novel situations may affect the puppies' future behaviour in those situations. Therefore, we suggest further research into social referencing to focus on longer term effects (i.e. over days or weeks following exposure).

Puppies tested with conspecifics varied their behaviour according to whether they were tested with their mother or with an unfamiliar dog. The presence of the mother dog showing a confident behaviour in the new situation facilitated approaching and interacting with the stimulus, but the presence of the unfamiliar dog did not elicit similar behaviours. While in the human condition the social partner communicated positive or neutral emotional signals, resulting in the puppies behaving consistently with the signals received, in the conspecific condition the social partner was merely present and, being habituated to the stimulus, it displayed a confident behaviour in the situation. The underlying processes behind the puppies' behaviour, therefore, may be different between these two conditions. Although in all conditions the presence of a social partner may facilitate exploration of the stimulus, in the human condition the different behaviour of the puppies according to the emotional signals expressed by the social partner cannot be explained by social facilitation alone (Zajonic 1965; Gardner and Engle 1971). Thus, the most congruent process is social referencing. In the conspecific condition the mere presence of the mother might have enhanced the likelihood of exploration of the stimulus. Thus, in the latter case, the behaviour of the puppies might be explained by social facilitation. Consistent with this line of reasoning, while in the retention trials of the human condition the puppies' behaviour varied between experimental groups (i.e., based on emotional valence in the preceding trials), in the conspecific condition, puppies were less likely to approach the stimuli alone (regardless of whether they were tested previously with their mother or with an unfamiliar dog).

The fact that the puppies behaved differently towards the stimulus when the mother was present as compared to when the unfamiliar dog was present is in line with studies on the secure base effect provided by the presence of the mother for human infants (e.g. Ainsworth et al. 1970). In fact, the puppies were also more likely to interact with their mother than with the unfamiliar dog. So far there is no evidence of attachment - a behavioural system which also includes a secure base effect - between adult dogs of the same household (e.g., Mariti et al. 2014; 2017), which may further explain why only the presence of the mother, but not the presence of another dog, facilitated approach to the stimulus. Moreover, our subjects were tested with conspecifics with which they never had previous interaction. Thus, the difference in our subjects' behaviour in the mother and unfamiliar group might be explained by the mother, but not the unfamiliar dog, functioning as a secure base for the puppies. In addition, the unfamiliar dog itself can also be regarded as a novel stimulus so that this might have influenced approaching and interacting with the stimulus too.

Our results revealed that the two different stimuli had different effects across trials. It is possible that the puppies habituated sooner to one stimulus than to the other one (e.g. one stimulus might have been more interesting to explore or less frightening than the other). Marshall-Pescini et al. (2017) exposed 6- and 8-week-old wolf and dog puppies to two different novel objects - a toy dog and remote-controlled car - and found that all animals interacted more with the toy dog. Thus, different types of stimuli are likely to elicit different exploratory behaviours. The specific effect of the two different stimuli in our experiment is difficult to interpret because, while in the human condition puppies in trial 2 and in the retention trial approached more likely the noise stimulus than the fan stimulus, in the conspecific condition puppies interacted more likely with the fan in the same two trials. The effect and comparison of different types of stimuli is beyond the scope of the present study. However, our results and those of Marshall-Pescini et al. (2017) suggest caution should be taken when comparing data of studies in which different stimuli were used.

In conclusion, this study shows that social referencing is already developed in 8-week-old dog puppies. Puppies show both referential looking and behavioural regulation when exposed to novel stimuli in presence of a human social partner expressing emotional signals towards it. Moreover, puppies acquire some information about the stimulus and recall it using their memory, to behave appropriately when later exposed to the same situation in absence of the social partner.

Puppies also show referential looking in presence of conspecifics, regardless of their identity. The presence of the mother – but not the presence of an unfamiliar dog – enhances puppies approach to the stimulus, suggesting that the mother acts as a secure base for exploration of novel stimuli.

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**References**

Ainsworth, M.D., Bell, S.M. (1970). Attachment, exploration and separation illustrated by behaviour of one year olds in a strange situation. *Child Development*, *41*, 49–67.

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*, 1-48. doi:10.18637/jss.v067.i01.

Buttelmann, D., Tomasello, M. (2013). Can domestic dogs (*Canis familiaris*) use referential emotional expressions to locate hidden food? *Animal Cognition, 16*, 137–145. doi:10.1007/s10071-012-0560-4

Camas, L.A., and Sachs, V.B. (1991). Social Referencing and Caretaker expressive behavior in a day care setting. *Infant Behavior and Development, 14,* 27–36.

Clyman, R.B., Emde, R.N. (1986). Social referencing and social looking among twelve-old infants. In T.B. Brazelton & M. W. Yogman (Eds.), *Affective development in infancy* (pp. 75–94). Norwood, NJ: Ablex.

De Rosnay, M., Cooper, PJ., Tsigaras, N., Murray, L. (2006). Transmission of social anxiety from mother to infant: an experimental study using a social referencing paradigm. *Behavior Research and Therapy, 44*, 1164–1165.

Duranton, C., Bedossa, T., Gaunet, F. (2016). When facing an unfamiliar person, pet dogs present social referencing based on their owner's direction of movement alone. *Animal Behaviour*, *113,* 147-156.

Feinman, S., Lewis, M. (1983). Social referencing at ten months: a second order effect on infant

responses. *Child Development, 54,* 878-887.
Fox, M. (1971). Integrative Development of Brain and Behavior in the Dog. University of Chicago Press, Chicago, IL, 225-233.
Fox, M. (1972). Understanding Your Dog. Coward, McCann and Geoghegan, New York, NY, 74, 100-108.
Fugazza, C., Moesta, A., Pogány, Á., Miklósi, Á. (2018). Social learning from conspecifics and humans in dog puppies (under review).
Gardner EL, Engel DR (1971). Imitational and social facilitatory aspects of observational learning in the laboratory rat. *Psychonomic Science, 25,* 5–6.
Hoehl, S., Wiese, L., Striano, T. (2008). Young infants' neural processing of objects is affected by eye gaze direction and emotional expression. *PLoS One, 3;* e2389
Itakura, S. (1995). Snakes as agents of evolutionary change in primate brains. *Journal of Human Evolution, 51,* 44–48.
Kim, G., Walden, T.A., Knieps, L.J. (2010). Impact and characteristics of positive and fearful emotional messages during infant social referencing. *Infant Behavior and Development, 33,* 189–195.
Klinnert, M.D., Campos J.J, Emde, R.N., Sorce, J.F. (1983a). Emotions as behaviour regulators. Social referencing in infancy. In R Plutchik and H Kellerman (Eds) The Emotions New York Academic Press vol 2, 57–59.
Klinnert, M.D., Emde, R.N., Butterfield, P., Campos, J.J., (1983b). Social referencing: the infant's use of emotional signals from a friendly adult with mother present. *Developmental Psychology, 22,* 427–432.
Mariti, C., Carlone, B., Ricci, E., Sighieri, C., Gazzano, A. (2014). Intraspecific attachment in adult domestic dogs (*Canis familiaris*): preliminary results. *Applied Animal Behaviour Science, 152,* 64–72.
Mariti, C., Carlone, B., Votta, E., Ricci, E., Sighieri, C., Gazzano, A. (2017). Intraspecific relationships in adult domestic dogs (*Canis familiaris*) living in the same household: A comparison of the relationship with the mother and an unrelated older female dog. *Applied Animal Behaviour Science, 194,* 62-66.
Marshall-Pescini, S., Viranyi, Z., Kubinyi, E., Range, F. (2017). Motivational factors underlying problem solving: comparing wolf and dog puppies' explorative and neophobic behaviors at 5, 6, and 8 Weeks of Age. *Frontiers in Psychology, 8,* 180. doi:10.3389/fpsyg.2017.00180.
McConnell, P.B. (1990). Acoustic structure and receiver response in domestic dogs, *Canis familiaris*. *Animal Behaviour, 39,* 897-904.
Merola, I., Prato-Previde, E., Marshall-Pescini, S. (2012a). Social referencing in dog-owner dyads? *Animal Cognition, 15,* 175–185.
Merola, I., Prato-Previde, E., Marshall-Pescini, S. (2012b). Dogs' social referencing towards owners and strangers. *PLoS ONE, 7,* e47653.
Merola, I., Prato-Previde, E., Lazzaroni, M., Marshall-Pescini, S. (2013a). Dogs' comprehension of referential emotional expressions: familiar people and familiar emotions are easier. *Animal Cognition*, doi:10.1007/s10071-013-0668-1.
Merola, I., Marshall-Pescini, S., D'Aniello, B., Prato-Previde, E. (2013b). Social referencing: water rescue trained dogs are less affected than pet dogs by the stranger's message. *Applied Animal Behaviour Science, 147,* 132-138.
Miklósi, A. (2014). Dog, behaviour, evolution and cognition. Oxford University Press.
Morimoto, Y., Fujita, K. (2012). Capuchin monkeys (*Cebus apella*) use conspecific' emotional expressions to evaluate emotional valence of objects. *Animal Cognition, 15,* 341–34.
Morton, E. (1977). On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. *The American Naturalist, 111,* 855-869.
Mumme, D.L., Fernald, A., Herrera, C. (1966). Infants' responses to facial and vocal emotional signals in a social referencing paradigm. *Child Development, 67,* 3219–37.

Nagasawa, M., Murai, K., Mogi, K., Kikusui, T. (2011) Dogs can discriminate human smiling faces from blank expressions. *Animal Cognition, 14*, 525 – 533. doi:10.1007/s10071-011-0386-5
Roberts, S.G., McComb, K., Ruffman, T. (2008). An experimental investigation of referential looking in free-ranging Barbary macaques (*Macaca sylvanus*). *Journal of Comparative Psychology*, *122,* 94–99.
Russell, C.L., Bard, K.A., Adamson, L.B. (1997). Social referencing by young chimpanzees (*Pan troglodytes*). *Journal of Comparative Psychology, 111,* 185–93.
Striano, T., Rochat, P. (2000). Emerging of selective social referencing in infancy. *Infancy*, *1*, 253-264.
Tomonaga. M., Tanaka, M., Matsuzawa, T., Myowa-Yamakoshi, M., Kosugi, Mizuno, Y., Okamoto, S., Yamaguchi, M.K., Bard, K.A. (2004). Development of social cognition in infant chimpanzees (*Pan troglodytes*): Face recognition, smiling, gaze and the lack of triadic interactions. *Japanese Psychological Research*, *46,* 227–235.
Turcsán, B., Szánthó, F., Miklósi, Á., Kubinyi, E. (2015). Fetching what the owner prefers? Dogs recognize disgust and happiness in human behaviour. *Animal Cognition*, *18*, 83-94.
Vaish, A., Striano, T. (2004). Is visual reference necessary? Contributions of facial versus vocal cues in 12-month-olds' social referencing behavior. *Developmental Science*, *7,* 261– 269.
Walden, T. (1993). Communicating the meaning of social events through social referencing. In: Kaiser A, Gray D, editors. Enhancing children's communication: Research foundations for intervention. Baltimore, MD: Brooks Publishers; pp. 187–199.

Walden, T.A., Ogan, T.A. (1988). The development of social referencing. *Child Development, 59,* 1230–1240.
Zajonc, R.B. (1965). Social facilitation. *Science*, *149,* 269–274.

## Tables

**Table 1.** Experimental protocol.

| **1st Condition (human or conspecific, counterbalanced)** | **Duration** |
|---|---|
| 1st exposure to stimulus | 2 min |
| 2nd exposure to stimulus | 2 min |
| Break | 1 h |
| 3rd exposure to stimulus (retention trial) | 1 min |
| Break | 1 to 2 h |
| **2nd Condition (conspecific or human, counterbalanced)** | |
| 1st exposure to stimulus | 2 min |
| 2nd exposure to stimulus | 2 min |
| Break | 1 h |
| 3rd exposure to stimulus (retention trial) | 1 min |

Every subject was tested in two conditions: human and conspecific condition. Both conditions consisted of three trials. The third (retention) trial was carried out after a 1-h break and in absence of the social partner.

**Table 2.** Cox Mixed Model of probability of approaching the stimulus in the human condition.

| **Explanatory variables in final model** | **Exp (*β*) [95% CI]** | ***z*** | ***P*** |
|---|---|---|---|
| TS[a] [noise] | 0.47 [0.19; 1.21] | -1.56 | 0.120 |
| Trial[b] [2nd] | 0.22 [0.10; 0.49] | -3.71 | < 0.001 |
| Trial [retention] | 0.12 [0.05; 0.28] | -4.97 | < 0.001 |
| Trial [2nd] x TS [noise] | 3.95 [1.40; 11.14] | 2.60 | 0.010 |
| Trial [retention] x TS [noise] | 4.74 [1.58; 14.16] | 2.78 | 0.005 |

[a]TS: type of stimulus, factor with two levels [fan and noise]
[b]Trial: experimental trial, factor with three levels [1st, 2nd and retention trial]

Besides experimental group, the final model included the interaction of trial x type of stimulus following stepwise model selection based on AIC values. Hazard ratios Exp($\beta$) with 95% CI between levels of fixed factors are provided.

**Table 3.** Linear Mixed Model of frequency of gaze alternations, model summary.

| Explanatory variables in final model | Parameter estimate [95% CI] | *df* | *t* | *P* |
|---|---|---|---|---|
| TS[a] [noise] | -0.79 [-1.34; -0.24] | 27 | -2.75 | 0.010 |
| Trial[b] [2nd] | -1.02 [-1.54; -0.51] | 22 | -3.82 | < 0.001 |
| EG[c] [unfamiliar] | -0.19 [-0.74; -0.35] | 27 | -0.67 | 0.511 |
| TS [noise] x Trial [2nd] | 0.92 [0.14; 1.70] | 22 | 2.26 | 0.034 |
| TS [noise] x EG [unfamiliar] | 0.10 [-0.66; 0.86] | 27 | 0.24 | 0.809 |
| Trial [2nd] x EG [unfamiliar] | 0.65 [-0.11; 1.41] | 22 | 1.65 | 0.113 |
| TS [noise] x Trial [2nd] x EG [unfamiliar] | -1.10 [-2.17; 0.02] | 22 | -1.97 | 0.062 |

[a]TS: type of stimulus, factor with two levels [fan and noise]
[b]Trial: experimental trial, factor with two levels [1st and 2nd]
[c]EG: experimental group, factor with two levels [mother and unfamiliar dog]

**Table 4.** Cox Mixed Model of probability of interacting with the stimulus in the conspecific condition.

| Explanatory variables in final model | Exp (β) [95% CI] | z | *P* |
|---|---|---|---|
| TS[a] [noise] | 0.09 [0.02; 0.50] | -2.75 | 0.006 |
| Trial[b] | | | |
| Trial [2nd] | 0.45 [0.18; 1.11] | -1.73 | 0.083 |
| Trial [retention] | 1.05 [0.44; 2.51] | 0.12 | 0.910 |
| EG[c] | | | |
| EG [mother] | 1.53 [0.73; 3.22] | 1.12 | 0.260 |
| EG [unfamiliar] | 0.44 [0.18; 1.09] | -1.78 | 0.076 |
| Trial x TS | | | |
| Trial [2nd] x TS [noise] | 8.44 [1.33; 53.57] | 2.26 | 0.024 |
| Trial [retention] x TS [noise] | 1.32 [0.18; 9.73] | 0.27 | 0.780 |

[a]TS: type of stimulus, factor with two levels [fan and noise]
[b]Trial: experimental trial, factor with three levels [1st, 2nd and retention trial]
[c]EG: experimental group, factor with three levels [control, mother and unfamiliar dog]

Besides the main effect of experimental group, the final model included the interaction of trial x type of stimulus following stepwise model selection based on AIC values. Hazard ratios (Exp[$\beta$]) with 95% CI between levels of fixed factors are provided.

## Figure captions

**Figure 1.** Experimental stimuli that were placed in the testing area. At the beginning of the trial, the puppy was released from the corner opposite to that of the stimulus and was then free to move in the testing area for the whole duration of the trial. (a) Noise stimulus in the human condition. A loudspeaker emitting noises was placed in a plastic bin, hanging from a stand. The human displayed emotional signals of positive valence towards the stimulus (Human positive group). (b) Fan stimulus, with plastic ribbons attached to it, in in the conspecific condition. The mother dog was ca. 1.5 m from the stimulus and was habituated to it (mother group).

**Figure 2**. Probability of puppies to approach (a) and to interact with (b) the stimuli between experimental groups in the human condition.

**Figure 3.** Effects of experimental group and trial on stimulus approach by puppies in the conspecific condition.

**Figure 4.** Probability of puppies to interact with the stimulus when tested alone (control), with their mother or with an unfamiliar dog.