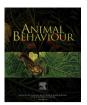
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Presence and lasting effect of social referencing in dog puppies

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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. Social referencing can provide advantages, especially to young, inexperienced 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, Canis familiaris, the ontogeny of this behaviour has not been investigated. Moreover, it is not known whether dogs acquire some 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 the presence of human or conspecific social partners. With humans, we tested the effect of different emotional signals expressed by the informant. With conspecifics, we tested whether the presence of the subject's mother or an unfamiliar dog affected behaviour towards the stimulus. Puppies alternated their gaze between the stimulus and the social partner (referential looking) with all the partners. Puppies tested in the 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 the presence of their mother were more likely to approach the stimulus than puppies tested alone or with an unfamiliar dog. The results of this study 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

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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, inexperienced individuals, because they are more likely to encounter novel stimuli and situations than older, more experienced individuals. Thus, their survival is very probably 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, Bard, & Adamson, 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,

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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 & 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 or situation (Klinnert, Campos, Emde, & Sorce, 1983; Morton, 1977; Mumme, Fernald, & Herrera, 1966). 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. Camas & Sachs, 1991; Feinman & Lewis, 1983; Walden & Ogan, 1988). For human infants, the caregiver not only constitutes a secure base for the child (Ainsworth & Bell, 1970) but also provides emotional support and serves as a source of information about the environment (e.g. Klinnert, Campos, et al., 1983). While social referencing in human infants and toddlers is abundantly documented (e.g. De Rosnay, Cooper, Tsigaras, & Murray, 2006; Hoehl, Wiese, & Striano, 2008; Klinnert, Campos et al., 1983;

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Mumme et al., 1966; Vaish & Striano, 2004), there is mixed evidence of its presence in other species. It has been shown in mammals, including young chimpanzees, *Pan troglodytes* (Russell et al., 1997) and capuchin monkeys, *Cebus apella* (Morimoto & Fujita, 2012), but other studies did not find referential looking behaviours in mother—infant pairs of chimpanzees (Russell et al., 1997; Tomonaga et al., 2004) and Barbary macaques, *Macaca sylvanus* (Roberts, McComb, & Ruffman, 2008).

Adult dogs, Canis familiaris, are sensitive to the emotional signals of their owners (e.g. Buttelmann & Tomasello, 2013; Turcsán, Szánthó, Miklósi, & Kubinyi, 2015) and if such a sensitivity develops during early ontogeny (at least to an extent), this may provide the most important prerequisite of social referencing in puppies. In the presence of a novel, potentially fear-eliciting stimulus, adult dogs alternated their gaze between their owner and the stimulus (referential looking; Merola, Prato-Previde, & Marshall-Pescini, 2012a). The findings of a recent study (Duranton, Bedossa, & Gaunet, 2016) suggest this behaviour may also emerge in the 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 stimulus object only after some delay, whereas in human studies mothers usually delivered the message immediately and continued to do so for the whole test (e.g. Kim, Walden, & Knieps, 2010; Walden & Ogan, 1988). Thus, it is possible that in the study by Merola et al. (2012a), when the informants started to deliver the message, the dogs had already acquired some information about the stimulus and formed an appropriate behavioural response, which prevented the emergence of behavioural regulation. To elicit behavioural regulation, subsequent studies (Merola, Prato-Previde, & Marshall-Pescini, 2012b; Merola, Prato-Previde, Lazzaroni, & Marshall-Pescini, 2014) 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 regard 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 the mother is also present (e.g. Klinnert, Emde, Butterfield, & Campos, 1983). The role of familiarity of the informant has been investigated in adult dogs. Merola et al. (2012b) tested dogs facing a new object in the 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, Buttelmann and Tomasello (2013) found that dogs were able to discriminate between different emotional signals expressed by a stranger towards an object, at least with regard to some emotional behaviours (expressing happiness and disgust; Buttelmann & Tomasello, 2013). Thus, it seems that both the familiarity of the informant and the valence of the emotional signal affect adult dogs' behaviour when interacting with novel stimuli or facing novel situations. Nevertheless, it is possible that while dogs are able to discriminate between different emotional signals even from an unfamiliar human (Nagasawa, Murai, Mogi, & Kikusui, 2011), they only use these signals as relevant information when they come 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 have focused mainly on young individuals (e.g. Itakura, 1995; Roberts et al., 2008; Tomonaga et al., 2004), no data are available, to our knowledge, about social referencing in dog puppies.

Following the assumption that social referencing is especially advantageous for young, inexperienced individuals, we hypothesized 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 hypothesized that, as 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.

METHODS

Observation of Social Referencing in Dog Puppies

We tested 8-week-old dog puppies facing a novel stimulus in the presence (or absence) of different social partners: (1) with (or without) a human, expressing emotional signals towards the stimulus, either with positive or with neutral valence; (2) with (or without) a conspecific showing a comfortable attitude in the presence of the stimulus. In the case of the conspecific, we also aimed to investigate 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 have not addressed the lasting effects of social referencing, i.e. whether such socially acquired information is retained to be recalled later upon encountering similar stimuli and situations again. Therefore, we also tested dog puppies facing the same stimulus after a 1 h delay.

Different physical properties of acoustic signals given by the human social partner talking with a happy or a 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.

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 eight litters of various breeds (Swiss hound N=3, border collie N=5, Shetland sheepdog 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.

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