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Smelling themselves: Dogs investigate their own odours longer when modified in an "olfactory mirror" test

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ABSTRACT

While domestic dogs, *Canis familiaris,* have been found to be skillful at social cognitive tasks and even some meta-cognitive tasks, they have not passed the test of mirror self-recognition (MSR). Acknowledging the motivational and sensory challenges that might hinder performance, even before the question of self-recognition is broached, this study creates and enacts a novel design extrapolated from the species' natural behaviour. Given dogs' use of olfactory signals in communication, this experiment presents dogs with various canisters for approach and investigation. Each holds an odorous stimulus: in the critical test, either an "olfactory mirror" of the subject − the dog's own urine − or one in which the odour stimulus is modified. By looking at subjects' investigation times of each canister, it is shown that dogs distinguish between the olfactory "image" of themselves when modified: investigating their own odour for longer when it had an additional odour accompanying it than when it did not. Such behaviour implies a recognition of the odour as being of or from "themselves." The ecological validity of this odour presentation is examined by presenting to the subjects odours of other known or unknown dogs: dogs spend longer investigating the odour of other dogs than their own odour. Finally, in a second experiment, subjects spent longer with the modified stimulus than with the modified odour by itself, indicating that novelty alone does not explain the dogs' behavior. This study translates the MSR study for a species whose primary sensory modality is olfaction, and finds both that natural sniffing behaviour can be replicated in the lab and that dogs show more investigative interest in their own odours when modified.

1. Introduction

The only test of self-recognition that has regularly been used with non-human animals, across taxa, is the so-called mirror self-recognition (MSR) test. In MSR, an individual uses a mirror to touch or attend to a part of its body that would otherwise be out of view (Gallup, 1970). The interest in self-recognition is motivated by a theoretical scheme that, as with humans, self-recognition may be allied with 'self-awareness', a notoriously intractable ability to investigate in non-human, non-verbal animals. As designed by Gordon Gallup, in the MSR test a chimpanzee is surreptitiously "marked" on the brow with an odourless red dye. The animal's behaviour in front of a provided mirror is then observed (the animal having previously been exposed to reflective surfaces and showing few social responses to the mirrors). Those who "pass" the test use the mirror to direct examination of the mark. Sham-marked individuals do not show such self-directed behaviour (Gallup, 1970).

Great apes − chimpanzees and bonobos in particular − reliably pass the MSR test; while humans pass the test, young children tend not to do so until 18 months or two years of age (Amsterdam 1972). Macaques and other monkeys have not been seen to pass the test (Anderson and Gallup, 2015; Ma et al., 2015; Roma et al., 2007; Shaffer and Renner, 2000) though they behave differentially toward mirrors (de Waal et al., 2005). In an intriguing development that emphasizes convergence of abilities more than evolutionary connection to human as the necessary condition for the skill, dolphins (Reiss and Marino, 2001), a single elephant (Plotnik et al., 2006), and a magpie (Prior et al., 2008) also adjusted their position to use the mirror to attend to a mark (variously: ink, tape, or sticker) that had been secretly placed on their bodies. The procedures used with primates were modified to suit research with a non-terrestrial species, in the former case, and non-handed ones, in all cases. The findings suggests that animals who pass the test are using the mirror to orient toward what they may think of as "themselves."

As is the case with many of the species who have passed the MSR test, canids show a high level of social complexity, which has been linked to the self-other distinction needed to pass the test (Plotnik et al., 2006). Domestic dogs, *Canis familiaris*, are highly accomplished at tasks of social cognition, attending to and communicating with other individuals to solve problems (Horowitz, 2014; Miklósi et al., 1998). In fact, in many studies, dogs' performance on social-cognitive tasks

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surpasses that of monkeys and even that of chimpanzees' (Hare et al., 2002). Research has found domestic dogs to be skilled in using others' gaze (Agnetta et al., 2000) or communicative pointing (Miklósi et al., 2000; Pettersson et al., 2011; Soproni et al., 2001) to gather information, and to attend to others' attentional states (Call et al., 2003; Horowitz, 2009; Schwab and Huber, 2006). Dogs' extension of the social group to include humans allows for levels of cooperation between human and dog (Kerepesi et al., 2005; Naderi et al., 2001) as well as various dog-handler working teams (Hecht and Horowitz, 2015).

As a result, it is reasonable to hypothesize that domestic dogs may have developed a rudimentary sense of themselves as a strategy to gauge their own position among social compeers, including humans and other dogs. This kind of self-awareness can be defined functionally as a cognitive ability that involves discrimination of oneself from others, especially conspecifics (Broom, 2010). It would subserve any task that requires distinguishing not only oneself and others, but also recognition of the properties of oneself. In addition, dogs may have a rudimentary ability to think about others' perspectives (Catala et al., 2017; Horowitz, 2011; Kaminski et al., 2009; MacLean et al., 2014) or others' needs (Piotti and Kaminski, 2016), which implies a sense of the distinction of 'self' and "other." As these *meta*-cognitive abilities often go hand in hand with self-recognition, the domestic dog is a promising species with which to investigate the question. For these reasons, and on the strength of the abovementioned MSR results with other social non-primates, this research develops and enacts a test analogous to the MSR test with dogs.

While the MSR test is appropriate for some species, the specific apparatuses and stimuli of the paradigm are less suited to dogs. Just as the test needed to be adapted from its original design for use with dolphins and elephants, the test requires revision in its application to dogs. The species' primary sense is not vision, as with humans and chimpanzees; instead, its primary sensory modality is smell (Berns et al., 2015; Horowitz et al., 2013). Given the role of olfaction in dogs' social lives, olfactory stimuli are more ecologically relevant to the species than visual stimuli (Nielsen et al., 2015). Furthermore, dogs do not exhibit the degree of grooming behaviour that many social great apes, in particular, do, which might lend itself to bodily examination in a context such as a MSR test. Thus it is not surprising that dogs show little self-directed engagement with mirrors, though they show interest in mirrors and can use them to acquire information about objects or food behind them (Fox, 1971; Howell et al., 2013).

Dogs do, however, engage in bouts of olfactory investigation of conspecifics, and, too, of their own odours (Sommerville and Broom, 1998). Left to their own devices, dogs regularly investigate the urine odours of other dogs left on prominent locations (Bekoff, 1979, 2001) as well as leaving urine 'marks' themselves, presumably to be investigated and mined for identity, sex, and health information by other dogs (Cafazzo et al., 2012)(or, in non-dog canids, territorially (Gese and Ruff, 1997; Harrington, 1981; Jordan et al., 2013). Research has found that dogs do not investigate their own urine markings at the same rate as the urine markings of other dogs − thus seeming to distinguish their own odours from others' (Bekoff, 2001; Gatti, 2016). Thus it is reasonable to work with this natural behaviour − investigation of urine − when developing an analog of the MSR test applicable to this species: to give dogs an olfactory "mirror" of themselves − using their own body's scent, in urine − and then "mark", or revise, the "smell image" in that mirror by adding another odour to it. Part of what the MSR test gauges is the contingency of the subject's behaviour on a sensory appearance that is different than expected. In the present study, the question of interest is, similarly, whether a subject's investigative behaviour changes when the sensory impression of themselves − via their urine − changes.

In this "olfactory mirror" (OM) test, dogs are provided an opportunity to investigate various odour samples in canisters: odours of self (dog's own urine) and odours of marked/modified-self (dog's own urine, with an added odorant). These variables were designed by analogy to the sham-mark and marked conditions in MSR research (Reiss and Marino, 2001). Diverging from previous research, an additional presentation of the "mark" by itself is included in this study, to ensure that the novelty or inherent interest of the "mark" is not responsible for the subjects' behaviour.

Results from neuroimaging work showing differential responses in subject dogs' brains (in the caudate) when exposed to odours of themselves and other dogs supports the use of odour stimuli taken from dogs (Berns et al., 2015). Still, to ensure that subjects' investigative behaviour in the lab is consistent with their behaviour in natural settings, a comparison between the subject's urine and the urine of another dog is also made. Given that canids use urine to leave and receive information about oneself that might be used by friendly conspecifics, dogs are expected investigate the sample of an unknown dog's urine longer than their own urine.

The duration of olfactory investigation of each sample thus serves as a kind of olfactory equivalent of visual close-examination. It is hypothesized that dogs will investigate the self-modified sample longer than the sample of their own urine. Similarly, investigation may take the form of repeated visits to investigate odours in the pairing of self-modified odours and self-odours than in other pairings. Thus, the number of times subjects returned to a previously smelled canister is counted (excluding the first approach to each canister of each pairing).

Evidence of longer investigation of odours from oneself that have been modified would suggest that dogs recognize their own olfactory "image" when it has been altered, in line with self-recognition in other species.

2. Experiment 1

2.1. Materials and methods

2.1.1. Subjects and experimental location

Thirty-six domestic dogs (16 M, 20F) participated in the study, accompanied by their owners. Subject dogs were required to be at least 8 months old, healthy, and comfortable with new people. The mean subject age was 6 years (range: 15 months to 14 years 2 months); 5 dogs (3 M, 2F) were unneutered (intact). Twenty-three dogs were identified as purebred by their owners (3 Labrador Retrievers, 2 German Shepherds, 2 Standard Poodles, 2 Belgian Teruvens, and one each of the following: Border Collie, Small Munsterlander, Cocker Spaniel, Chinese crested, Portuguese Water Dog, Large Munsterlander, Papillion, Smooth Collie, Welsh Terrier, Keeshond, Tibetan Spaniel, Australian Shepherd, Brittany Spaniel, Golden Retriever); 13 were identified as mixed-breeds. The dogs were brought by their owners to a designated room at the Port Chester Obedience Training Club (PCOTC) in White Plains, New York. In preliminary observations, dogs whose owners had not participated with them in nosework, scent games, or scent work did not reliably approach the stimulus canisters nor were some dogs able to separate from their owners to do so. Therefore, only dogs whose owners encouraged their dogs' sniffing behaviour were used in the study; they were recruited via PCOTC electronic mailings. All subjects were familiar with the facility and had participated in at least one class in the sport of nosework with their owners at PCOTC before.

Testing took place from November 2014 to February 2015. Participants were scheduled to arrive in sequence, so no dogs nor owners were present at or witness to trials prior to their own. No other dogs were present in the room while the trials were run. Before the experiment, owners were informed that the stimulus canisters on the floor of the experimental theatre were available to be investigated by the dogs, but were given no other information about the hypothesis of the study, nor were the contents of the canisters revealed. They were instructed as to their role in the trials, as outlined below.

2.1.2. Stimuli, odour collection, and other materials

Owners were asked to collect a sample of urine from their dog the day of their scheduled arrival at the testing facility, or to collect a sample the day before and store it in the refrigerator until their scheduled appointment, at which time it was brought to room temperature before use. Owners were provided with a sterile non-reactive collection cup, a vinyl glove, and were instructed how to collect urine noninvasively by holding the cup in the dog's stream of urine. Given a sizeable number of multi-dog households, these owners also collected urine from the second dog, and this was presented in an additional trial for each of their dogs. Samples were not taken from the day's first urination (Lisberg and Snowdon, 2009).

For a comparative urine sample from a dog unknown to the participants, urine was collected on three occasions from the author's dog, a seven-year-old neutered male mixed-breed dog. The urine was frozen at 4 ° Celsius for up to 14 days until use, at which time it was brought to room temperature. Freezing urine samples has been shown to have no effect on investigation duration (Lisberg and Snowdon, 2009).

As the "mark" added to the subjects' urine sample, a spleen tissue sample was secured from the University of Pennsylvania Veterinary School, from necropsy of a dog with lymphosarcoma. Given the abilities of even untrained dogs to notice diseased tissue, as borne out by the behaviour of dogs who were reported to show olfactory interest in diseased areas on their owners' bodies which turned out to be cancerous (Church and Williams, 2001; Welsh et al., 2005; Williams and Pembroke, 1989), this mark was used for its salience. The approximately 2 gm sample was cut into eighty similar-sized pieces, put into individual tubes, and stored at −70 Celsius until transfer to our possession. At that point the tissue samples were kept at 4 ° Celsius; each was brought to room temperature before use.

An assistant wearing vinyl gloves prepared the samples away from the testing theatre. For each trial, 1.5 ml of the appropriate urine sample − from the subject, the unknown dog, or from a known, family dog − was pipetted into each of two canisters. Tissue sample was added to one of the canister with the subject's urine. One decoy canister with 1.5 ml of water was also prepared for each trial. The canisters were 3.3-cm wide round tin containers with three air holes in their lids (Fig. 1).

2.1.3. Testing theatre

Within the large training facility room, a smaller experimental theatre was established by ringing a 4.7 × 5.7 m area with adjustable accordion fencing; one side ran along a wall of the room. The floor was covered with interlocking Eco-Soft Foam Tiles, which were cleaned with a solution of 70% isopropyl alcohol between each subject's set of trials.

Owners and subject dogs were seated on one end of the fenced theatre and faced an experimenter on the other end (Fig. 2). For each trial, the experimenter placed a new stimulus array of three canisters along an unmarked row 2.5 m from the subjects and owners. Owners were blind to the contents of the canisters. Two canisters held the odour substances to be compared; order and side of presentation were randomized. The decoy canister was placed midway between them (visits to the decoy canister were not coded). It served to mitigate any tendencies a subject may have had to go directly toward the presenting experimenter by instead orienting the dogs toward the canister array. Owners held their dogs by the collar or leash until the experimenter instructed them to release the dog. Care was taken to avoid owner cueing (see Section 2.1.4. below), but as separation from the owner caused subject distress and non-engagement with the canisters, owners were present throughout the trials.

Fig. 1. Canister for odour presentation.

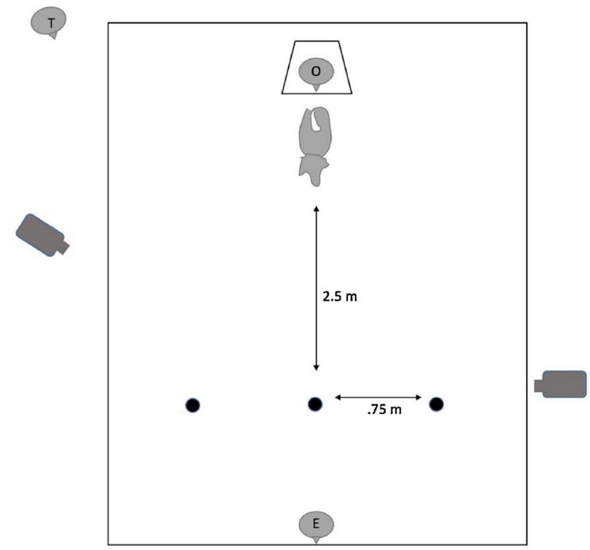

Fig. 2. Experimental theatre. Image shows position of owner (O) and dog, distance to experimental canisters (2.5 m) and distance of canisters from one another (.75 m), position of experimenter (E) after canister placement, location of time-keeper (T), and location of video cameras.

Trials were recorded via two videos cameras. One camera, to the right of the canisters from the subject's perspective, captured a wide view of the testing theatre, including the experimenter and subject; the second, to the left of the canisters, focused on the canisters and served as a backup if the subject's body blocked the first camera's view of the subject's nose.

2.1.4. Experimental design

Subjects participated in a minimum of three trials. Each trial had two elements: simultaneous presentation of canisters, visible to the subjects (Dunbar and Carmichael, 1981); and subject investigation of canisters.

Before each trial, the assistant brought the three canisters on a tray to the presenting experimenter. The experimenter was blind to the contents of each canister. Each trial began with the experimenter kneeling at a pre-determined spot on the floor, placing the water canister in front of her at a designated point, and placing the two experimental canisters (with content as described below) onto the floor at arm's length − approximately 0.75 m − on either side of the center point. Looking straight ahead, the experimenter then said, "What's this?" in a high-pitched voice and simultaneously tapped the two side canisters, repeating the address three times in succession. She then rose, turned around so that her back faced the subject, and stepped to the wall at one edge of the testing theatre (Fig. 2). This was the cue for the owner to release her dog. The experimenter no longer looked in the direction of the subject or canisters and did not interact with the subject if approached.

Owners were instructed not to look at the canisters, in order to avoid cueing their dogs, or to engage socially with the dog during the trial. To help ensure compliance, owners were given a distractor task of counting the number of times the dog looked back at them. When the dog showed directed attention toward the sample through proximity maintenance (within 1 m of the sample), orientation, or engaging in direct sniffing, a timer was started and the dog was allowed to investigate any or all canisters ad lib (e.g., Bekoff, 2001; Dunbar and Carmichael, 1981; Lisberg and Snowdon, 2009). After 30 s, a designated time-keeper outside of the theatre but visible to the experimenter cued the experimenter (whose back was turned), and the experimenter asked the owner to call her dog back. This ended the trial. If the subject was sniffing a canister at the thirty-second mark, the trial ended when the subject moved away from the canister (Lisberg and Snowdon, 2009). As is customary in olfactory discrimination paradigms, an intertrial interval was scheduled (Péron et al., 2014), in which the dog and owner were permitted to rest or to explore the rest of the room at their leisure for 1–3 min.

In the standard three-trial arrangement, subjects were presented with a randomly determined sequence of the following comparisons: S-SM, their own odour ('Self') and their own odour with added "mark" ("Self-Modified"); S-O, Self and the odour of an unknown dog ("Other"); SM-M, Self-modified and the modified substance by itself ("Modified"). The final, "modified" condition was added to ensure that any investigation of self-mark is not only due to interest in the mark odour per se. Left-right placement was also randomized. In the cases when an owner lived with two dogs (n = 17), subjects had a fourth trial: S-F, Self and the urine odour of the known, family dog ("Family"). New Self and Self-modified samples were prepared for each trial.

In the case that the dog did not approach both canisters in a trial, an additional "Owner-enhanced" trial was run with the same comparison stimuli, to encourage approach to the canisters. The owners followed a script in which they were to kneel behind a canister, pick up and hold it at the height of the dog's nose, look only at the canister, and encourage the dog to approach with their voice, saying "What's this?" for ten seconds. They were instructed to repeat this performance with each canister. These trials were done to ensure that investigation time to both samples was collected. If the subject did not approach both canisters, no further trials of that comparison were done, and the experimenter moved to the next trial. N = 29 dogs had at least one owner-enhanced trial.

The maximum number of trials a subject faced was eight: four standard comparisons and four owner-enhanced comparison trials. However, in all cases, only trials in which the subjects investigated both samples were used in analysis: up to a maximum of four trials per dog (S-SM, SM-M, S-O, S-F). As some subjects did not approach one or both of the canisters in some trials, the number of trials for which data were collected varied. One subject did not approach both canisters on any trial.

Data from the owner-enhanced trials were later compared to the data from only the standard trials. In all but one comparison, there was no difference in the trends of investigation-time whether the owner was involved or not. Data were thus collapsed across owner-enhanced and standard trials.

2.2. Data analysis

Each trial was videotaped and coded using frame-by-frame playback (30 fps, iPhone; 60 fps, Flip camera) for accuracy of determination of investigatory behaviour. The dependent variable in this study was gauged primarily by time of olfactory investigation − sniffing − of canisters, a behaviour whose length and frequency can be measured in video playback (e.g., Thompson et al., 2016). Given that the dog nose 'reach' is approximately 4 inches (10 cm) from the nares (Shepherd, 2012), "sniffing" was scored as beginning when the dog's nose was ten centimeters from the canister (Lisberg and Snowdon, 2009) (Fig. 3) and ending when the dog could be seen to visibly move his head away from the canister. Sniffing time was determined in "number of frames" and later converted back to seconds.

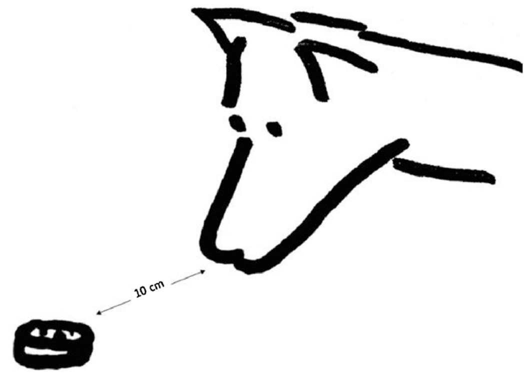

Fig. 3. Olfactory investigation was coded when dog's nose was within 10 cm of canister.

Secondarily, exploratory behaviour was gauged by number of visits to the canisters. While a subject had to visit each of the pairs of canisters in each trial for the trial to be analyzed, all additional, return visits to investigate the canisters (above and beyond the threshold trial visits) in the 30 s of the trial were also noted; a count was thus made of the number and order of visits to each canister. Dogs were determined to have "left" and "returned" to a canister per the scoring criteria above. Any distinctive behaviours by the subject were also noted.

The author coded all trials, blind to canister content, for length of investigation and order and number of canister approaches. A second blinded individual independently coded 33% (n = 36) of the trials. Agreement was high for length of investigation (Pearson's correlation: $r(70) = 0.99$, $p < 0.001$) and order and number of canister approach (Pearson's correlation: $r(70) = 1.0$, $p < 0.001$). For each comparison, the Wilcoxon signed-rank test was used to compare investigation duration.

2.3. Results

Data were gathered from 109 trials from n = 35 dogs (one dog failed to approach both canisters in any trial).

2.3.1. Investigation duration

Dogs spent significantly more time investigating the canister holding their own odour that had been modified (SM) than the one that held their own odour alone (S), (SM-S, Wilcoxon signed-rank test, $Z = -3.85$; $N = 32$; $P = 0.0001$ (two-tailed)) (Fig. 4). Cohen's effect size ($d = 0.90$) suggests high practical significance.

Dogs also spent significantly more time at the canister with an unknown dog's odour (O) than that with their own (S) (O-S, Wilcoxon signed-rank test, $Z = -3.119$; $N = 33$; $P = 0.002$ (two-tailed)).

Duration of investigation of the self-modified (SM) and modified (M) canisters was not significantly different (SM-M, Wilcoxon signed-rank test, $Z = 1.76$, $N = 31$, $P = 0.0784$ (two-tailed); Cohen's $d = 0.32$). Nor was there any difference in the amount of time dogs spent investigating the canister with their own (S) and that holding a known dog's odour (F) (S-F, Wilcoxon signed-rank test, $Z = -0.525$, $N = 13$; $P = 0.600$ (two-tailed)).

2.3.2. Number of visits

The number of visits that subjects made to each pair of canisters varied by trial type. Data were gathered for subjects who made at least one return trip to either canister in addition to visiting both canisters. Subjects made significantly more visits to the canisters in the S-SM trial than in the SM-M trial (Wilcoxon signed-rank test: $Z = -1.73$; $N = 22$; $P = 0.042$) but not more than in the S-O trial (Wilcoxon signed-rank test: $Z = -0.97$; $N = 23$; $P = 0.166$). (The small sample of S-F trials did not enable comparison.)

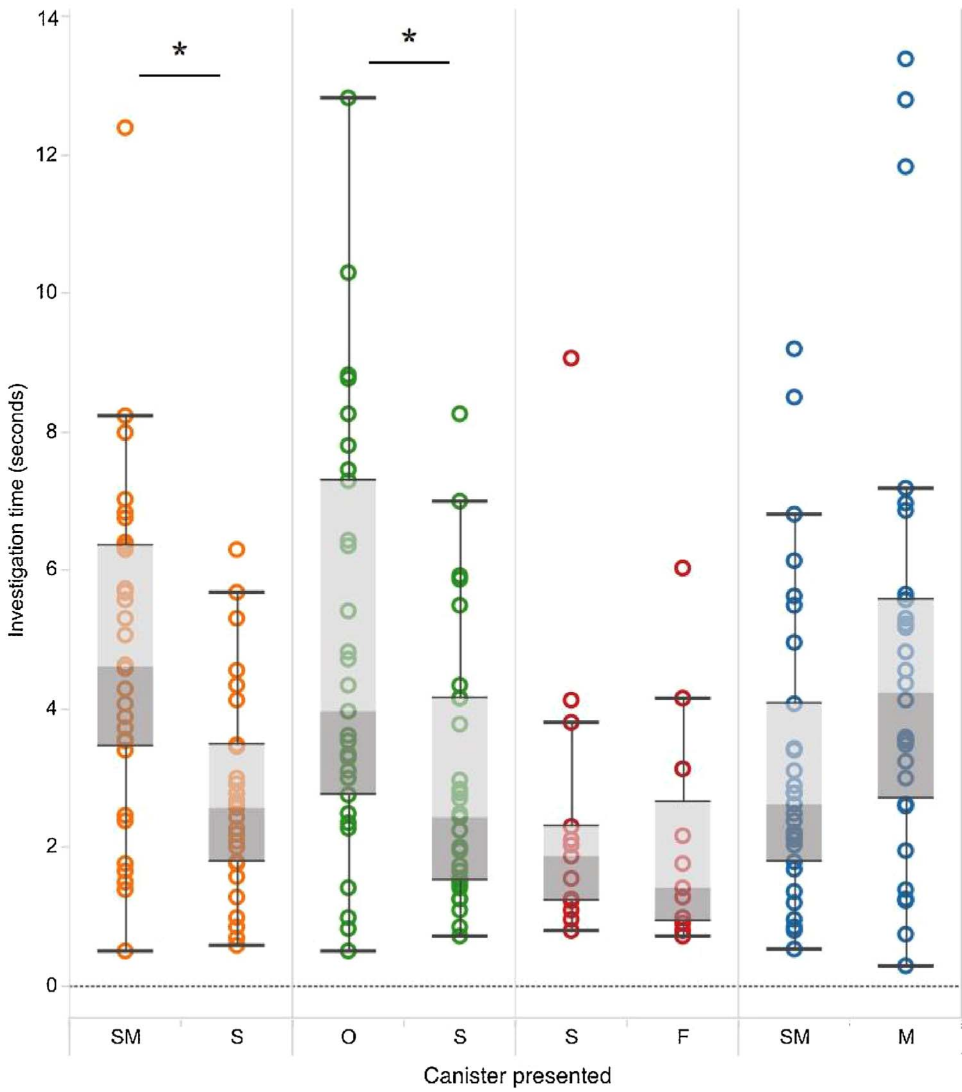

Fig. 4. Tukey box-and-whisker plot of olfactory investigation time by trial type (SM-S, O-S, S-F, SM-M). Box covers middle 50%, with whiskers with maximum 1.5 interquartile range. All data points shown, except for the following 5 extreme pair of outliers: (SM-S) = (18.23, 3.9), (SM-S) = (16.73, 5.67), (O-S) = (37.70, 2.70), (O-S) = (43.27, 3.73), (SM-M) = (4.97, 3.25).

2.4. Discussion

As hypothesized, dogs explored their own odour for longer when it had an additional odour accompanying it than when it did not. The addition of an unexpected odour changed their sniffing behaviour toward their own odour.

This is consistent with a thesis that dogs notice their odour when it is changed, and move to more fully examine it. Such behaviour implies a recognition of the odour as being or from themselves. The increase in return visits to re-smell the canisters in the S-SM trials, compared to the SM-M trials, is consistent with a hypothesis that the subjects were perceiving the modified sample as interesting with respect to the odour of "themselves" (rather than being itself worthy of investigation).

Dogs also spent more time investigating an unknown dog's odour than their own, as expected, given the information about conspecifics that urine holds. They spent less time with known dogs' odour, which may indicate that, since the animals live together, their companion's odour is familiar, just as each dog's own odour is to itself.

That subjects investigated the modified canister and the self-modified canister for similar durations, however, makes any definitive interpretation of the main S-SM result premature. Had subjects spent less time with the M canister than with the SM, one could conclude that the fact of their own odour changing was the reason for the main result. Given the data, though, we cannot determine whether (a) dogs' interest in the SM canisters was simply an interest in the odour of the material used for marking, or (b) dogs were interested both in their own, changed odour and, additionally, in the odour of M.

Given that the odour sample used for M was a biological sample of diseased tissue, and especially given the recent evidence that dogs can be trained to identify small amounts of diseased tissue (see Wells, 2012, e.g., for a review), it is possible that the choice of "mark" was *too* salient to distinguish the above possibilities. Relatedly, given dogs' preference for novelty (Kaulfuß and Mills, 2008), neophilia may have driven the subjects' responses.

Thus, a second experiment was designed using a novel but detectible "neutral" odour – a mark more on par with the inked mark or tape used in prior MSR studies – and the ambiguous trials (but not the S-O and S-F trials) were run again.

3. Experiment 2

3.1. Materials and methods

3.1.1. Subjects and experimental location

Twelve domestic dogs (9 M, 3F) participated in the follow-up study, accompanied by their owners. The mean subject age was 4 years 11 months (range: 12 months to 10 years); all but one male dog were neutered. Eight dogs were identified as purebred (1 each of Beagle, German Shepherd, Boxer, Sheltie, Standard Poodle, Miniature Dachshund, Small Munsterlander, Wire Fox Terrier) by their owners; 4

were identified as mixed-breeds. The dogs were brought by their owners to Dog Days of New York, a dog day-care facility in New York City, New York, after normal business hours. Subjects were recruited via Dog Cognition Lab electronic mailings to owners who had expressed interest in participating in studies. Only dogs who owners designated as "sniffy" or as having taken a beginning (but not more advanced) nosework class participated in the study.

Testing took place from February 2016 to April 2016. Participants were scheduled to arrive in sequence, so no dogs nor owners were present at or witness to trials prior to their own. Before the experiment, owners were informed that the stimulus canisters on the floor of the experimental theatre were available to be investigated by the dogs, but no other information about the hypothesis of the study was mentioned, nor were the contents of the canisters revealed. As in Experiment 1, owners were instructed as to their role in the trials.

3.1.2. Stimuli, odour collection, and other materials

As with the previous experiment, owners brought a sample of urine from their dog, collected that day or collected the prior day and refrigerated. As the "mark" added to the subjects' urine sample, cotton exposed to anise essential oil (*Pimpinella anisum),* an odorant used in intermediate and advanced nosework practice, was used (National Association of Canine Scent Work, 2016). Pieces of cotton of 0.5-cm were cut from the end of Q-tips. Per odour preparation for nosework studies, the cotton pieces were secured in a jar with six drops ($<$ 1 ml) of anise, a quantity extrapolated from nosework preparation sources and prior canine olfactory research (Gunderson, 2016; Hall et al., 2013) and the jar was shaken so that each piece was in contact with the odorant.

An assistant wearing vinyl gloves prepared samples away from the testing theatre. For each trial, 1.5 ml of the subject's urine was pipetted into each of two 3.3-cm wide tin canisters. Anise-cotton samples were added to one of the canisters with the subject's urine, as well as to one canister with 1.5 ml water. A decoy canister of 1.5 ml of water was also prepared for each trial.

3.1.3. Testing theatre and experimental design

Within the facility, a 2.75 × 3.3 m room with half-height walls was used for the experiment. The floor was covered with interlocking Eco-Soft Foam Tiles, which were cleaned with a solution of 70% isopropyl alcohol between each subject.

The details of the theatre set-up were otherwise identical to that of the previous experiment. Similarly, the experimental design was conceptually and methodologically identical. However, the comparisons were slightly different: S-SM, their own odour ("Self") and their own odour with added "mark" ("Self-modified"); SM-M, Self-modified and the modified substance by itself ("Modified"); S-M, Self and the mark. This design assured that the subjects would be exposed to each kind of canister the same number of times (3) across trials. Subject behaviour was scored for length of investigation to each canister.

3.2. Results

Data were gathered from 31 trials from n = 11 dogs. As before, one dog failed to approach both canisters in any trial.

3.2.1. Investigation duration

Dogs spent significantly more time investigating the canister holding their own modified odour (SM) than the one that held their own odour alone (S), (SM-S, Wilcoxon signed-rank test, $Z = 2.02$; $N = 11$; $P = 0.0434$ (two-tailed); Cohen's $d = 0.58$) (Fig. 5). This was consistent with the results of Experiment 1.

Unlike Experiment 1, the subjects spent significantly more time investigating the self-modified (SM) canister than the modified (M) canister (SM-M, Wilcoxon signed-rank test, $Z = 2.37$, $N = 10$, $P = 0.0178$ (two-tailed); Cohen's $d = 1.15$). Duration of investigation of the self (S) canister and the modified (M) canister was not significantly different (S-M, Wilcoxon signed-rank test, $W = 10$; $N = 8$; $P > 0.5$).

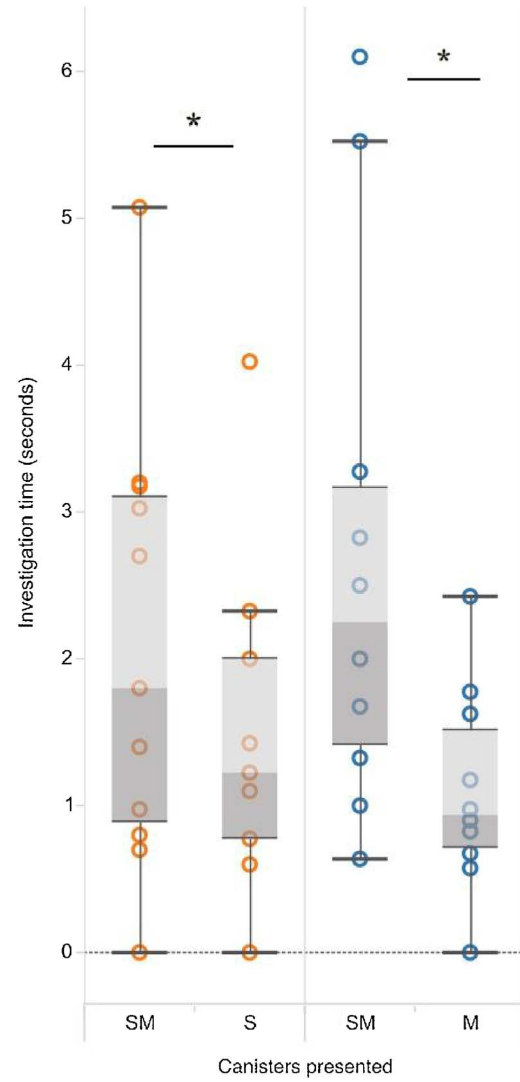

Fig. 5. Tukey box-and-whisker plot of olfactory investigation time for trials SM-S and SM-M; box covers middle 50%, with whiskers with maximum 1.5 interquartile range. All data points shown.

3.3. Discussion

Experiment 2 bore out the main result of the first experiment: namely, that dogs spent more time investigating their own odour, "marked", when it was paired with their own odour. In addition, Experiment 2 helped to disambiguate the finding about the salience of the "marking" odour: with a less salient odour, subjects spent significantly *more* time with the self-modified canister than with the canister with the modification odour by itself. This suggests that the longer investigation time is not tied to an interest in the mark, per se, but rather an interest in the mark *when it appears in combination with or on the dog's own odour.* The finding in Experiment 2 that there was no difference in the investigation time of the self and modified canisters (though this sample is small) bears out the relative lack of interest by the subjects in either odour − unless they appear in combination.

4. General discussion

This research introduces a species-relevant analog of the MSR test for dogs: a test asking if dogs distinguish, by investigation time, odours generated from themselves from those odours when altered. As hypothesized, subjects investigated the self-modified (SM) sample longer than the sample of their own urine (S). Such behaviour is suggestive of the subjects' recognition of the odour as being or from themselves. Similarly, dogs investigated the self-modified (SM) sample longer than the modified substance alone (M), once a neutral substance − akin to the "mark" on MSR subjects' bodies − was used. The interest in the "mark" was greater when it represented a change in the subjects' own odour.

Secondarily, subjects also investigated the sample of an unknown dog's urine (O) longer than their own urine (S). This result suggests that in bringing the "S" and "O" odours into the lab, the difference dogs have been observed to pay to these odours in their natural environment has been replicated. While not relevant to the thrust of the MSR test, the S-O comparison was included because of the novelty of the medium − where the medium is "urine" and "olfaction" rather than "mirror image" and "visual inspection." Given previous research showing different investigative behaviour by neutered and intact dogs − higher rates of flehmen-like behaviours in intact males (Berthoud et al., 2013); higher rates of social sniffing, licking, and tooth chattering in intact males than neutered males (Kaufmann et al., 2017); and higher rates of neutered males sniffing intact males (Lisberg and Snowdon, 2009) − any future work examining behaviour toward other dogs' odours should take neutering status into account. There were too few unneutered dogs in this study for any comparison based on neutering status.

It is worth considering the relationship of this 'OM' test to MSR tests. While the MSR test was the jumping-off point for the translation, the OM test is not intended to be a perfect replica to the test. Instead, in translating the MSR test, the salient elements of the experimental design were highlighted and then re-created in a setting more suited to the species at hand, a canid.

The salient elements of the MSR test can be broken down into three groups: stimulus; medium of investigation (environmental); and means of investigation (by animal). For the great apes, for instance, the stimulus is *a reflection of the animal marked on the forehead with a red dot;* the medium of investigation (environmental) is *mirror;* and the means of investigation (by animal) looked for is "*mark-directed responses" (such as exploration of the mark by hand)* (Gallup, 1970).

Notably, the presence or absence of any resultant effect − such as *successful modification or elimination of the mark* − is not noted or required. To pass the test, the subjects need not actually effect a change in their appearance. Thus, while exploratory behaviour may seem to be directed toward modifying the mark, simply having *explored* the mark suffices.

Variations of Gallup's original study with great apes have used, implicitly, these constituents. For instance, dolphin subjects were presented with a stimulus of *a reflection of themselves marked on various parts of the body;* the medium of investigation (environmental) was *a reflective surface on the tank wall;* the means of investigation (by animal) was "*mark-directed" or exploratory body movements* toward the mark − as contrasted to a condition in which the animals had been "sham" marked (Reiss and Marino, 2001). Again, there was no gauge of resultant effect.

Thus, in the present study, subjects were presented with a stimulus: *an olfactory "reflection", altered* − a sample of their own urine with an additional odour "mark". The medium of investigation (environmental) was, instead of a mirror, *a canister holding the olfactory sample*. The means of investigation − the dependent variable − was *increased sniffing time* of the marked canister. As with dolphins and great apes, participant dogs interacted with the stimulus through the means of investigation predicted: they sniffed the marked sample longer than they sniffed the unmarked sample. This result is particularly notable given the fact that in this study, subjects' exposure to the samples was limited in duration; in prior studies showing successful MSR, exposure to the mirror was ad lib over sessions of many minutes or hours (Gallup, 1970; Reiss and Marino, 2001). Consistent with the main result, dogs more often returned to investigate the canisters in the trial with their own sample, marked and unmarked, than in other trials.

Another explanation for some of the subjects' behaviour could be described as an "unfamiliarity preference": a preference for unfamiliar or novel odours. This explanation would require the presumption that one's own odours are "familiar" (itself perhaps indicative of recognition of "self" odours); given that premise, one would expect that dogs would investigate others' and self-modified odours more than one's own odours. That result was seen. However, subjects in Experiment 2 investigated the modified odour *less* when it was presented by itself − counter to the hypothesis of unfamiliarity preference. Still, the explanation is at least *consistent with* some of the results of this study. Interestingly, though, in eleven cases dogs were observed to show an expression that could be called "disgust": after starting to sniff, abruptly drawing the head away from the sample, often accompanied by one of the following behaviours: lip retraction, air-licking (repeated tongue protrusion), raising a single paw, or retreating. The cases included samples of S (n = 1), M (n = 4), SM (n = 5), and O (n = 1) − in this anecdotal observation, more often of "unfamiliar" than "familiar" odours. Disgust is associated with avoidance behaviour (Curtis et al., 2001), so one would expect that one would see *less* investigation of the unfamiliar stimuli in these cases. Future work might compare subject investigation time of SM to investigation time of a marked sample of a family dog (F): this would also help determine if unfamiliarity drives some of the behaviour seen in the current result.

While olfaction may be the most appropriate means to consider the dogs' understanding of their world, including themselves (Hecht and Horowitz, 2015), the move from visual stimuli to olfactory stimuli has challenges that also change the nature of the test. Perception of volatile organic compounds is difficult to study (Nielsen et al., 2015): for instance, odours move erratically in a noncontrolled space, and their concentration varies based on context. A redirection of a test of self-recognition from one sense to the other is also complicated theoretically: for instance, while among humans one's own odour may be acknowledged as emanating from oneself, it would not be considered identical with one's "self". Still, the results of this study argue for use of species-relevant means in testing self-recognition.

The question of whether a mirror − or olfactory mirror − test is in fact a good test of the subjects' understanding of self − including but not limited to self-awareness − is still debated (Bendl, 2016; de Waal et al., 2005; Hauser et al., 1995; Ristau, 2013). And even if passing the MSR test is determined to be sufficient to gauge a species' self-recognition, there are many built-in prerequisites that need to be met to pass the MSR test apart from the ability to recognize oneself, per se. For instance, the species must have the perceptual capacity to appreciate the test stimuli and the subject must have an interest in dynamically adjusting its appearance and/or grooming itself or others. Even the sociality of a species may be relevant to its likelihood to pass the test (see, e.g., the failure of the solitary giant panda to pass the MSR test (Ma et al., 2015)).

In the present study, as in prior MSR tests, the subjects were not asked to effectively change the state of their visual or olfactory appearance − by, for instance, removing the mark. Unlike an adult human's self-regard in a mirror − in which the observation of an unwanted "mark" on one's forehead would typically be followed by an attempt to remove the mark − no species has been asked to pass a test in which the action of investigation changes the state of their visual or olfactory appearance. It is notable that the analogy from human mirror-directed behaviour to non-human primates' and other mammalian behaviour is not perfect in this regard. It is an open question whether such a "resultant effect" requirement should be necessary for a test of self-awareness.

Instead, this and previous MSR research show repetitive examination of "self": selective sensory pursuit of a stimulus that reflects or comes from oneself. Such repetitive examination of a changed visual or olfactory image, notably, does appear to distinguish species cognitively. These studies could be considered "partial" self-recognition studies − where the notion of partial self-recognition is acknowledged to be a state of development of humans, too: an understanding of self almost certainly develops gradually in humans, not all at once (Brandl, 2016).

In all cases, it is clear is that the perceptual abilities of the tested species should be − and can be − accounted for in the design of comparative-cognition studies.

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References

Agnetta, B., Hare, B., Tomasello, M., 2000. Cues to food locations that domestic dogs (*Canis familiaris*) of different ages do and do not use. Anim. Cognit. 3, 107–112.

Amsterdam, B., 1972. Mirror self-image reactions before age two. Dev. Psychobiol. 5, 297–305.

Anderson, J.R., Gallup Jr., G.G., 2015. Mirror self-recognition: a review and critique of attempts to promote and engineer self-recognition in primates. Primates 56, 317–326.

Bekoff, M., 1979. Scent-marking by free-ranging domestic dogs: Olfactory and visual components. Biol. Behav. 4, 123–139.

Bekoff, M., 2001. Observations of scent-marking and discriminating self from others by a domestic dog: tales of displaced yellow snow. Behav. Proc. 55, 75–79.

Berns, G.S., Brooks, A.M., Spivak, M., 2015. Scent of the familiar: an fMRI study of canine brain responses to familiar and unfamiliar human and dog odors. Behav. Proc. 110, 37–46.

Berthoud, D., Nevison, C., Waterhouse, J., Hawkins, D., 2013. The effect of castrating male dogs on their use of the vomeronasal organ when investigating conspecific urine deposits. J. Vet. Behav. 8, e40.

Brandl, J.L., 2016. The puzzle of mirror self-recognition. Phenomenol. Cognit. Sci. 1–26. http://dx.doi.org/10.1007/s11097-016-9486-7.

Broom, D.M., 2010. Cognitive ability and awareness in domestic animals and decisions about obligations to animals. App. Anim. Behav. Sci. 126, 1–11.

Cafazzo, S., Natoli, E., Valsecchi, P., 2012. Scent-marking behaviour in a pack of free-ranging domestic dogs. Ethology 118, 1–12.

Call, J., Bräuer, J., Kaminski, J., Tomasello, M., 2003. Domestic dogs are sensitive to the attentional state of humans. J. Comp. Psychol. 117, 257–263.

Catala, A., Mang, B., Wallis, L., Huber, L., 2017. Dogs demonstrate perspective taking based on geometrical gaze following in a Guesser–Knower task. Anim. Cognit. 20, 581–589.

Church, J., Williams, H., 2001. Another sniffer dog for the clinic? Lancet 358, 930.

Curtis, V., de Barra, M., Aunger, M., 2001. Disgust as an adaptive system for disease avoidance behaviour. Phil. Trans. R. Soc. B. 366, 389–401. http://dx.doi.org/10.1098/rstb.2010.0117.

de Waal, F.B., Dindo, M., Freeman, C.A., Hall, M.J., 2005. The monkey in the mirror: hardly a stranger. Proc. Natl. Acad. Sci. U. S. A. 102, 11140–11147.

Dunbar, I., Carmichael, M., 1981. The response of male dogs to urine from other males. Behav. Neural Biol. 31, 465–470.

Fox, M.W., 1971. Behaviour of Wolves, Dogs and Related Canids. Harper and Row, New York.

Gallup Jr., G.G., 1970. Chimpanzees: self-recognition. Science 167, 86–87.

Gatti, R.C., 2016. Self-consciousness: beyond the looking-glass and what dogs found there. Ethol. Ecol. Evol. 28, 232–240.

Gese, E.M., Ruff, R.L., 1997. Scent-marking by coyotes, *Canis latrans*: The influence of social and ecological factors. Anim. Behav. 54, 1155–1166.

Gunderson, A., 2016. Preparing Odour Scent. (Retrieved from from http://www.nosework.ca/preparing-odourscent.html).

Hall, N.J., Smith, D.W., Wynne, C.D.L., 2013. Training domestic dogs (*Canis lupus familiaris*) on a novel discrete trials odor-detection task. Learn. Motiv. 44, 218–228. http://dx.doi.org/10.1016/j.lmot.2013.02.004.

Hare, B., Brown, M., Williamson, C., Tomasello, M., 2002. The domestication of social cognition in dogs. Science 298, 1634–1636.

Harrington, F.H., 1981. Urine-marking and caching behavior in the wolf. Behaviour 76, 280–288.

Hauser, M.D., Kralik, J., Botto-Mahan, C., Garrett, M., Oser, J., 1995. Self-recognition in primates: phylogeny and the salience of species-typical features. Proc. Natl. Acad. Sci. U. S. A. 92, 10811–10814.

Hecht, J., Horowitz, A., 2015. Introduction to dog behavior. In: Weiss, E., Mohan-Gibbons, H., Zawitowski, S. (Eds.), Animal Behavior for Shelter Veterinarians and Staff. Wiley-Blackwell., pp. 5–30.

Horowitz, A., Hecht, J., Dedrick, A., 2013. Smelling more or less: investigating the olfactory experience of the domestic dog. Learn. Motiv. 44, 207–217.

Horowitz, A., 2009. Attention to attention in domestic dog (*Canis familiaris*) dyadic play. Anim. Cognit. 12, 107–118.

Horowitz, A., 2011. Theory of mind in dogs? Examining method and concept. Learn. Behav. 39, 314–317.

Horowitz, A. (Ed.), 2014. Domestic Dog Behavior and Cognition: The Scientific Study of Canis Familiaris. Springer-Verlag, Heidelberg, Germany.

Howell, T.J., Toukhsati, S., Conduit, R., Bennett, P., 2013. Do dogs use a mirror to find hidden food? J. Vet. Behav. Clin. App. Res. 8, 425–430.

Jordan, N.R., Golabek, K.A., Apps, P.J., Gilfillan, G.D., McNutt, J.W., 2013. Scent-mark identification and scent-marking behaviour in African wild dogs (*Lycaon pictus*). Ethology 119, 644–652.

Kaminski, J., Bräuer, J., Call, J., Tomasello, M., 2009. Domestic dogs are sensitive to a human's perspective. Behaviour 146, 979–998.

Kaufmann, C.A., Forndran, S., Stauber, C., Woerner, K., Gansloßer, U., 2017. The social behaviour of neutered male dogs compared to intact dogs (*Canis lupus familiaris*): Video analyses, questionnaires and case studies. Vet. Med. Open J. 2, 22–37. http://dx.doi.org/10.17140/VMOJ-2-113.

Kaulfuß, P., Mills, D.S., 2008. Neophilia in domestic dogs (*Canis familiaris*) and its implication for studies of dog cognition. Anim. Cognit. 11, 553–556.

Kerepesi, A., Jonsson, G.K., Miklósi, Á., Topál, J., Csányi, V., Magnusson, M.S., 2005. Detection of temporal patterns in dog-human interaction. Behav. Proc. 70, 69–79.

Lisberg, A., Snowdon, C., 2009. The effects of sex, gonadectomy, and status on investigation patterns of unfamiliar conspecific urine in dogs (*Canis familiaris*). Anim. Behav. 77, 1147–1154.

Ma, X., Jin, Y., Luo, B., Zhang, G., Wei, R., Liu, D., 2015. Giant pandas failed to show mirror self-recognition. Anim. Cognit. 18, 713–721.

MacLean, E.L., Krupenye, C., Hare, B., 2014. Dogs (*Canis familiaris*) account for body orientation but not visual barriers when responding to pointing gestures. J. Comp. Psychol. 128, 285–297.

Miklósi, Á., Polgárdi, R., Topál, J., Csányi, V., 2000. Intentional behaviour in dog-human communication: an experimental analysis of showing behaviour in the dog. Anim. Cognit. 3, 159–166.

Miklósi, Á., Polgárdi, R., Topál, J., Csányi, V., 1998. Use of experimenter-given cues in dogs. Anim. Cognit. 1, 113–121.

Naderi, S.Z., Miklósi Á, Dóka, A., Csányi, V., 2001. Cooperative interactions between blind persons and their dog. Appl Anim. Behav. (Sci) 74, 59–80.

National Association of Canine Scent Work, (2016) Target odors. Retrieved from http://www.nacsw.net/target-odors.

Nielsen, B.L., Jezierski, T., Bolhuis, J.E., Amo, L., Rosell, F., Oostindjer, M., Christensen, J.W., McKeegan, D., Wells, D.L., Hepper, P., 2015. Olfaction: an overlooked sensory modality in applied ethology and animal welfare. Front. Vet. Sci. 2, 69. http://dx.doi.org/10.3389/fvets.2015.00069.

Péron, F., Ward, R., Burman, O., 2014. Horses *(Equus caballus)* discriminate body odour cues from conspecifics. Anim. Cogn. 17, 1007–1011.

Pettersson, H., Kaminski, J., Herrmann, E., Tomasello, M., 2011. Understanding of human communicative motives in domestic dogs. Appl. Anim. Behav. Sci. 133, 235–245.

Piotti, P., Kaminski, J., 2016. Do dogs provide information helpfully? PLoS One 11, e0159797. http://dx.doi.org/10.1371/journal.pone.0159797.

Plotnik, J.M., de Waal, F.B., Reiss, D., 2006. Self-recognition in an Asian elephant. Proc. Natl. Acad. Sci. U. S. A. 103, 17053–17057.

Prior, H., Schwarz, A., Güntürkün, O., 2008. Mirror-induced behavior in the magpie (*Pica pica*): Evidence of self-recognition. PLoS Biol. 6, e202.

Reiss, D., Marino, L., 2001. Mirror self-recognition in the bottlenose dolphin: a case of cognitive convergence. Proc. Natl. Acad. Sci. U. S. A. 98, 5937–5942.

Ristau, C.A., 2013. Cognitive ethology: advanced review. Wiley Interdiscipl. Rev. Cogn. Sci 4, 493–509. http://dx.doi.org/10.1002/wcs.1239.

Roma, P.G., Silberberg, A., Huntsberry, M.E., Christensen, C.J., Ruggiero, A.M., Suomi, S.J., 2007. Mark tests for mirror self-recognition in capuchin monkeys (*Cebus apella*) trained to touch marks. Am. J. Primatol. 69, 989–1000.

Schwab, C., Huber, L., 2006. Obey or not obey? Dogs (*Canis familiaris*) behave differently in response to attentional states of their owners. J. Comp. Psychol. 120, 169–175.

Shaffer, V.A., Renner, M.J., 2000. Black-and-white colobus monkeys (*Colobus guereza*) do not show mirror self-recognition. Int. J. Comp. Psychol. 13, 154–160.

Shepherd, G.M., 2012. Neurogastronomy: How the Brain Creates Flavor and Why It Matters. Columbia University Press, New York.

Sommerville, B.A., Broom, D.M., 1998. Olfactory awareness. App. Anim. Behav. Sci. 57, 269–286.

Soproni, K., Miklósi, Á., Topál, J., Csányi, V., 2001. Comprehension of human communicative signs in pet dogs (*Canis familiaris*). J. Comp. Psychol. 115, 122–126.

Thompson, H., Riemer, S., Ellis, S.L.H., Burman, O.H.P., 2016. Behaviour directed towards inaccessible food predicts consumption − A novel way of assessing food preference App. Anim. Behav. Sci. 178, 111–117.

Wells, D.L., 2012. Dogs as a diagnostic tool for ill health in humans. Altern. Therap. 18, 12–17.

Welsh, J.S., Barton, D., Ahuja, H., 2005. A case of breast cancer detected by a pet dog. Community Oncol. 2, 324–326.

Williams, H., Pembroke, A., 1989. Sniffer dog in the melanoma clinic? Lancet 734.