A novel behavioural approach to assess responsiveness to auditory and visual stimuli before cognitive testing in family dogs

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Abstract

Visual and auditory impairments can have a large impact on performance in cognitive tests. It is important to evaluate the sensory capacities of dogs before enrolling them in cognitive tests, in order to exclude sensory impairment as confounding effect. Therefore we designed multiple non-invasive testing paradigms to detect subjects with potential auditory and visual impairment, without requiring extensive training for the dog. Multiple testing was a means to add internal reliability, and to reduce the risk of false positives due to habituation and previous learning or false negatives due to random errors. Our sensory test battery consisted of four subtests: (1) ‘Clapping’ auditory test, (2) ‘Recorded sound’ auditory test, (3) ‘Distance’ visual test, and (4) ‘Darkness’ visual test. The 'Clapping test' was similar to the clapping test used by veterinarians, with the addition that the clapping was performed at various distances from the dog. In the ‘Recorded sound test’, the dogs' reaction to various sounds played back at different volumes. In the ‘Distance test’ we placed a small piece of food on one of four plates placed on the grounds at varying distances from the dogs. In the 'Darkness test’, we measured the dogs' performance in walking through an S-shaped route during artificial dusk and daylight-like conditions.

We were able to design two standardised tests measuring dogs’ responsiveness to visual stimuli and its variance based on a) the distance of stimuli from the dog, and b) the lighting conditions in the room. The performance in our tests requires two elements of the visual function, namely visual acuity and vision in dusk. These tests should be considered for further validation, in order to evaluate their usefulness as screening tools for the decline in dogs’ visual function. In our behaviour tests measuring the response to auditory stimuli, we found that dogs reacted similarly to different sounds. However, older dogs reacted less frequently to sounds with lower decibel, suggesting that older dogs become less reactive to auditory stimuli. The tests we developed are useful to identify subjects who do show a behavioural reaction to the stimuli typically used in cognitive tests (rewards, small objects, barriers, etc.) under various levels of artificial light. It is possible to identify the dogs' baseline level of reactivity to visual/auditory stimuli before these are used in cognitive tests.

Keywords: dog, sensory testing, vision, hearing, sensory impairment

1. Introduction

Ageing dogs are typically affected by a physio-pathological degeneration of the sensory systems. Noticeably, recent findings suggest that impairments in sensory functions (hearing, vision, olfaction) have an effect on age-related behavioral changes, as reported by dog owners (Szabó, Miklósi, & Kubinyi, 2018). Age related cataract might be used as a general biomarker for life expectancy in domestic dogs (Urfer, Greer, & Wolf, 2011). A study involving 240 dogs identified a positive correlation between age and refractive error (ametropia) (Murphy, Zadnik, & Mannis, 1992). Sclerosis of the ocular lenses (age-related cataractS) affects up to one-third of dogs older than 7 years (Baumworcel, Soares, Helms, Rei, & Castro, 2009; Tobias, Tobias, & Abood, 2010). Myopic shift, which likely compromises the visual function, is particularly associated with ageing in Beagles (Hernandez et al., 2016). To our knowledge, only a small number of studies investigated how these eye conditions affect dogs' behaviour. Parry (1953) examined 15 dogs (of various breeds and ages) and found behaviour symptoms in dogs was affected by moderate and severe retinal degeneration. Symptoms of retinal degeneration vary from difficulty in seeing small objects placed on the ground (e.g. failing to mark a prey during gundog training, or overrunning it) to, in the most severe cases, complete blindness (Parry, 1953). Garcia et al. (2010) constructed an obstacle course and reported under high light density (i.e. extremely bright lighting conditions) dogs affected by achromatopsia (a medical syndrome causing colour blindness) were slower in completing the course. Although the condition is genetic, rather than affected by age, achromatopsia causes the individual to have blurred vision at high light densities; similar symptoms are reported by humans affected by age-related cataract. Thus the condition can be considered a good proxy to predict the performance of dogs affected by age-related visual decline.

Hearing loss is another debilitating change affecting aged dogs (ter Haar et al., 2010). Degenerative lesions have been observed in the cochlea (i.e. auditory portion of the inner ear) of dogs as they age (onset of lesions observed between 5 and 12 years of age) and in the (cerebral) cochlear nuclei of dogs over 10 years of age (Shimada, Ebisu, Morita, Takeuchi, & Umemura, 1998). Auditory testing indicated that these morphological changes are accompanied by hearing loss (Johnsson, Felix, Gleeson, & Pollak, 1989; Johnsson & Hawkins, 1972; Liu, Erikson, & Brun, 1996; Shimada et al., 1998), which was measured from dogs' reaction to hand claps of a range of loudness (assessed using a recorder) and recording brainstem auditory-evoked responses test (BAER). The BAER test detects ongoing electrical activity in the brain and records via electrodes placed on the scalp, under the skin (a relatively invasive procedure).

These studies indicate how the sensory perception of ageing dogs may be profoundly altered. It is not surprising that older dogs often show difficulties in navigating the environment, recognising familiar individuals, or responding to commands. A recent survey raised the possibility that such symptoms might be related to the sensory decline of ageing dogs (Szabó et al., 2018). Specifically, in the survey, dogs were classified based on their predicted lifespan (calculated based on size and weight) as 'adult' (up to 50% of their predicted lifespan), 'mature' (50% to 75% of their predicted lifespan), 'senior' (75-100%), and 'geriatric' (> 100%, i.e. they outlived their expected lifespan). According to these data (Szabó et al., 2018), 14% of Hungarian owners of adult dogs reported that their dog had visual and/or acoustic impairment, 11% indicated 'probably yes', 15% 'probably no', while 60% reported that their dog definitely had no sensory impairment. However, in the oldest age group (geriatric), the proportion of definitely unaffected dogs was only 10%, meaning that nearly 90% of the dogs' owners reported some degree of sensory impairment. Such numbers suggest that cognitive ageing researchers should be screening for sensory capacity before any cognitive tests.

While some instrumental tests exist (e.g. retinoscopy, BAER test), these are relatively expensive and uncomfortable for the animal; thus, in the veterinary field, they are only used if the loss of function is already suspected, rather than for screening. Non-instrumental screening of sensory function is performed in veterinary medicine by looking for the presence of specific behavioural reflexes, such as the menace reflex and the orientation reflex. One limitation of such non-instrumental tests is that they do not provide indications on the degree of functional loss that affects the animal, rather they tell whether the function under exam is likely to be either somewhat present or lost completely. Over the years, some experimental (i.e. laboratory) methods have been developed to provide more subtle measures of dogs' sensory functions. However, they either required extensive training or they employ methods detrimental to the animal's welfare. For example, earlier attempts were made to design audiometry exams of dogs, i.e. they measured the animals' response to auditory stimuli that varied systematically in their tone (Hz) and intensity (dB). In order to elicit reliable behaviour responses to the stimuli, the experimental paradigms were based on conditioning with electric shock (Anderson & Wedenberg, 1968), increased room temperature to elicit panting (Van Der Velden & Rijkse, 1976) or required prolonged and extensive training (Culler, Finch, Girden, & Brogden, 1935; Lipman & Grassi, 1942). While these tests provide precise information, and have been extensively used in the past in laboratory settings, due to the ethical implication or the length of the pre-training required (days or weeks), these are not feasible alternatives for routine screening.

We were interested in tests that could be effectively used to assess the sensory function (specifically vision and hearing) of dogs of various ages. The first step to this process was to design paradigms that could detect a variance in the behavioural response of animals, as this could reflect loss of function. Once this is established, future research should further validate the relationship between the response to these tests and the loss of function. In our study, a form of internal validation was provided through the triangulation of (1) a battery of tests that measured dogs' behavioural responses to auditory and visual stimuli (sensory tests) with (2) a veterinary examination and (3) a report from dog owners in relation to their dogs' sensory abilities (vision, hearing). The sensory tests did not require any formal training or special equipment.

It was expected that the overall score of the sensory tests, the owners' score, and the veterinary exam's score would correlate positively while the age would correlate negatively with performance. It was also expected that the different conditions of the sensory tests would provide various levels of difficulty for the dogs.

2. Ethical statement

The procedures comply with national and EU legislation and institutional guidelines. In Hungary, according to Hungarian legislation and the corresponding definition by law ('1998. évi XXVIII. Törvény' 3. §/9. — the Animal Protection Act), non-invasive studies on dogs are currently allowed to be performed without the requirement of any special permission. Owners provided written consent to their dogs' participation. Our Consent Form was based on the Ethical Codex of Hungarian Psychologists (2004). We took special care to ensure that the consent process was understood completely by the owners to allow their dog to participate. In the Consent Form, owners were informed about the identity of the researchers, the aim, procedure, location, expected time commitment of the experiment, the handling of personal and research data, and data reuse. The information included the owner's right to withdraw their consent at any time. Owners could at any point decline to participate with their dog and could request for their data not to be used and/or deleted after collection. The study was performed in accordance with the recommendations in the International Society for Applied Ethology guidelines (www.applied-ethology.org) for the use of animals in research.

3. Methods

Participants

A sample of 53 dogs was included in the study (Mdn_{age} = 11 years, range 1.5 – 16; 28 males and 25 females). Inclusion criteria for the dogs were to be comfortable in the testing environment; if the dog showed signs of fear or anxiety (e.g. excessive salivation, panting, tucked tail, cowering, hiding, growling or barking at the experimenters) or they were unable to relax within few minutes from their arrival in the laboratory, were excluded from testing. All dogs were privately owned and dog owners were recruited through social media.

Procedure

We obtained data from 3 sources:

(1) *Owner assessment.* Owners provided an overall assessment about their dogs' vision and hearing function based on two 9 points Likert scales (one for vision and the other for hearing), where the score 1 was given to blind or deaf animals and 9 indicated perfect sensory abilities. These scores aimed to provide a quantitative measure of the owners' opinion about the dog's sensory abilities.

(2) *Veterinary assessment.* The veterinary assessment consisted of a physical examination by a veterinary surgeon of the eyes and the ears, and standard veterinary tests for vision and hearing (i.e. cotton ball test, menace test, and clap test). The "cotton ball test" (Gelatt, 1998) was performed by dropping a piece of cotton from above the dog and at the edge of the of the dogs' field of view to assess the presence / absence of a voluntary behavioural response, indicating that the dog was following the movement of the object (i.e. eye movement or head movement). Care was taken to avoid producing noises or hair movements while dropping the cotton ball. The "menace test" was performed on each eye of the dog by quickly moving a hand in front and towards one eye of the dog, so to induce a blink reflex, while the other eye was shielded with the other hand. Care was also taken to avoid touching the dogs' hair or move the air in front of the eye. The "clap test" was performed by standing approximately 2 meters behind the dog and clapping once or twice; an orientation reflex (the dog turning around) or Preyer's reflex (the dog moving the pinnae of its ears towards the source of the sound) was recorded.

At the end of the assessment, the veterinary surgeon provided two scores, one for vision and one for hearing, on a 9 points Likert scale identical to the one used by the owner.

(3) *Sensory tests*. As we were designing a screening procedure, the sensory tests were administered to all adogs in the same order and in identical conditions. The test order was (i) ‘Clapping’ auditory test, (ii) ‘Recorded sound’ auditory test, (iii) ‘Distance’ visual test, (iv) ‘Darkness’ visual test (Table 1). We decided to follow the same order for all dogs as we were designing a screening procedure, thus we aimed to expose all participants to the same identical conditions. The carryover effect of the tests was unknown; therefore, we decided that the use of a fixed order would equally affect all dogs.

All experiments were performed indoor, in a room (5 m x 2.5 m) of the Department of Ethology, Eötvös Loránd University.

Table 1. Sensory tests and scoring

<table>
<tr><td rowspan="2">(i) ‘Clapping’ auditory test</td><td colspan="2">Scores:
0 – no reaction
1 – ears movement, head orientation</td></tr>
<tr><td colspan="2">Trials (distance between the experimenter and the dog):
1 – 4 meters
2 – 2 meters
3 – less than 0.5 meter (right behind the dog without touching it)</td></tr>
<tr><td rowspan="6">(ii) ‘Recorded sound’ auditory test</td><td>Trials:</td><td>Volume levels:</td></tr>
<tr><td>1 – unspecified dog crying</td><td>1 - 50 dB
2 - 58 dB
3 - 68 dB
4 - 78 dB</td></tr>
<tr><td>2 – Border Collie dog barking</td><td>1 - 45 dB
2 - 55 dB
3 - 65 dB
4 - 73 dB</td></tr>
<tr><td>3 – plate crashing</td><td>1 - 41 dB
2 - 48 dB
3 - 58 dB
4 - 67 dB</td></tr>
<tr><td>4 – Chihuahua dog yelping and whining</td><td>1 - 53 dB
2 - 63 dB
3 - 71 dB
4 - 81 dB</td></tr>
<tr><td>5 – siren</td><td>1 - 59 dB</td></tr>
</table>

		2 - 69 dB 3 - 73 dB 4 - 85 dB
	6 – Basset hound dog baying	1 - 45 dB 2 - 55 dB 3 - 63 dB 4 - 73 dB
(iii) 'Distance' visual test	**Scores:** 0 – dog visits first a non-baited plate or does not find the treat in 5 seconds 1 – dog goes directly to the baited plate and eats within 5 seconds	
	Trials (distance between the plate and the dog): 1 – 0 meters (plate is right in front of the dog) 2 – 0.5 meters 3 – 1 meter 4 – 1.5 meters 5 – 2 meters	
(iv) 'Darkness' visual test	**Scores:** 0 – dog touches the obstacles or takes longer than 10 seconds 1 – dog does not touch the obstacle and takes between 5-10 seconds 2 – dog does not touch the obstacle and takes less than 5 seconds	
	Trials: 1 – obstacle on the right with dark condition 2 – obstacle on the left with dark condition 3 – obstacle on the right with light condition 4 – obstacle on the left with light condition	

(i) 'Clapping' auditory test

This test aimed to measure the variation in dogs' response to clapping performed at various distances. As it is difficult to regulate the loudness of a clap, we attempted to maintain the loudness of the clap as constant as possible while varying the distance of the experimenter from the dog. As it happens with the test commonly performed during clinical examinations, there is no guarantee that the clapping was identical between trials. In veterinary practice, dogs that consistently fail to show a clear response through repeated trials and decreasing distances, are suspected to have hearing decline or deafness and may be referred for instrumental evaluation).

During our test, the owner sat on a chair and held the dog while the dog was sitting on the ground in front of him/her, so that the dog was looking towards the owner (Fig. 1, top picture). As the experimenter stood in front of the owner, the dog was facing away from the experimenter and, therefore, could only hear but not see her. The experimenter clapped her hands once. The presence or absence of a reaction of the dog was live coded. A reaction was defined as 1) a movement of the pinnae of the ear towards the noise (Preyer's effect), 2) if the dog was panting, as holding the breath (reported by the owner), 3) head orientation towards the noise (orienting reflex; Fig. 1, bottom picture). If the dog did not react to the first clap, the experimenter clapped once more, then the trial was over. The trial was repeated 3 times, once at each distance. Distances were: 4 meters away from the dog-owner dyad, 2 meters away, and within 1 meter. The dog was given a 0-1 score based on presence (score 1) or absence (score 0) of a reaction (Table 1).

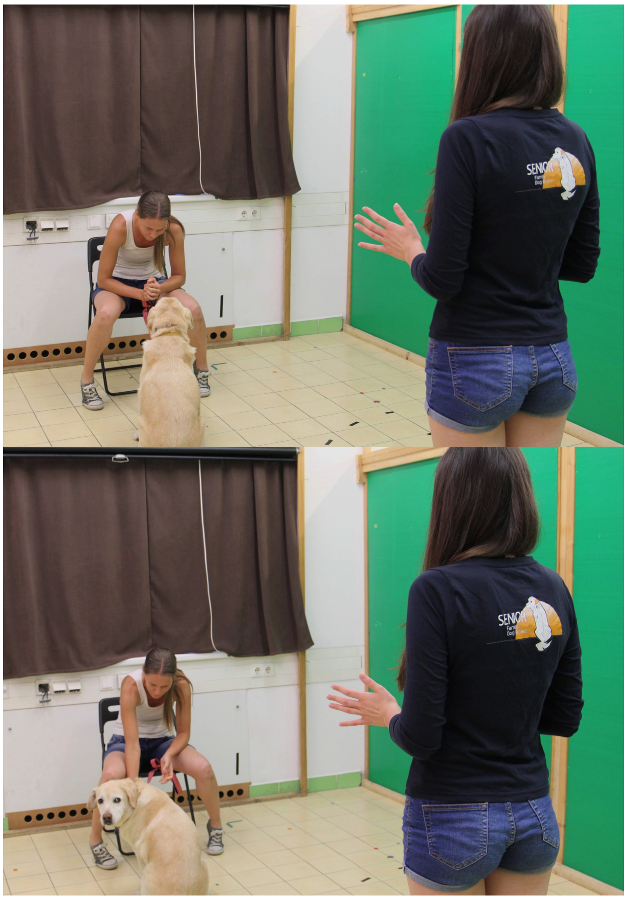

Fig. 1. Clapping test. Set up of the test (top picture) and example of a reaction from the dog (bottom picture).

(ii) 'Recorded sound' auditory test

A chair was placed in the room between the loudspeakers of a built-in audio system (Fig. 2). A set of 2 Technics SB-M300M2 loudspeakers were placed on a shelf behind the dog-owner dyad, connected to a PC set to maximum volume. The PC was not placed in the room, but could be controlled using a remote. During the test, the owner was sitting on the chair and held the dog in front of him/her, so that the dog could see the experimenter, who was sitting on the ground in front of dog-owner dyad, while holding a video camera and the pointer.

Fig. 2. Recorded sounds. Set up of the dog, owner, and experimenter (left picture) and camera (right picture).

Before playing each sound, the experimenter directed the dog's attention towards herself by calling the dog, clapping or waving her hands. Once the dog looked at the experimenter, she started a power point presentation with embedded sounds. A set of 6 recorded sounds was played in the following order: 1) an unspecified dog whining, 2) a Border Collie barking, 3) the noise of a plate crashing, 4) a Chihuahua yelping and whining, 5) a siren, and 6) a Basset Hound baying. All sounds were set up to play at four increasing levels of loudness (the levels were obtained with the software Audacity 1.3 Beta; details are in Table 1). For each sound, the software played the recording for 5 seconds consecutively, each time at a different volume, starting from the lowest volume up to the loudest one. Before each sound playback, there was a silent slide for the experimenter to call the dog If the dogs reacted to the sound, the experimenter stopped the slides and played the following sound thus skipping the remaining (louder) volume levels.

For each sound, we recorded the volume the dog where showed the first reaction (see volume levels in Table 1). If the dog did not react to a sound at any volume , we marked it as 'censored data' for the survival analysis (described in the statistical analysis paragraph) and we gave an arbitrary 100 dB as the maximum volume level. A reaction was defined as a movement of the pinnae of the ear towards the noise, holding breath (if the dog was panting), or head orientation towards the noise. The dog's reaction was recorded with a hand camera held by the experimenter.

In order to obtain an overall score for this test, we scored trials based on the volume (in dB) of the stimuli at first reaction:

- 40dB < *reaction* ≤ 45 dB: 6 points,
- 45dB < *reaction* <55dB: 5 points,
- 55 ≤ *reaction* <60: 4 points,
- 60 < *reaction* <70: 3 points,
- 70 < *reaction* <75: 2 points,
- 75< *reaction* ≤ 85: 1 point,
- No reaction: 0 points.

This scoring system yielded a points-range between 0 (i.e. no reaction in any of the 6 trials) and 32 (i.e. the dog always reacted to lowest volume) . Subsequently, we normalized this score for further comparisons using the following formula: $X_{normalized}=X-X_{min}/(X_{max}-X_{min})$.

At the time of statistical analysis it was decided *post hoc* to include only the playback test, as this was the only test where we could adequately control the volume of the stimuli. We therefore excluded the clap test from further analysis, as the experimenter may not have clapped with the same intensity each time (datasets including the clapping test are available upon request from the authors).

(iii) 'Distance' visual test

The test set up with a chair at one side of the room. The owner was asked to sit on the chair while holding the dog in front of him/her, so that the dog was to face the rest of the room. Four identical white plates (diameter 20 cm) were placed on the floor in front of the chair at 4 pre-determined distances from the chair, i.e. 0.5m, 1m, 1.5m, and 2m (Fig. 3).

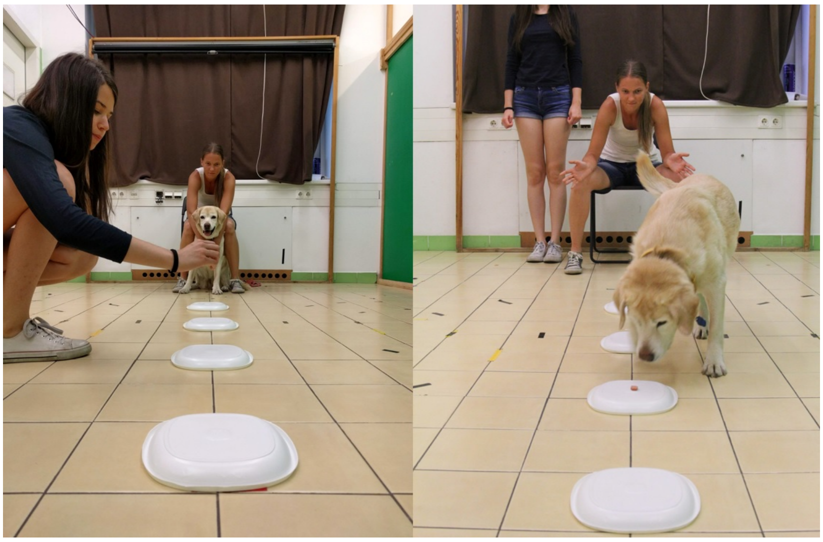

Fig. 3. Distance test. Positioning of the food (picture on the left),

The task, based on the paradigm by Parry et al. (1953), measured the dog's efficiency in locating a small object (a piece of frankfurter) on a single plate from varying distances. The linear placement of the plates prevents side biases and reduce the overall space required for the test. It is known that sniffer dogs trained for explosives' detection are more prone to rely on olfactory signals and visibility does not affect their performance (Gazit & Terkel, 2003). However, research indicates that, in similar situations, untrained family dogs do not rely on olfactory cues, but rather on visual cues, even when relying on olfaction would be more successful (Polgár, Miklósi, & Gácsi, 2015; Szetei, Miklósi, Topál, & Csányi, 2003). For this reason, the dogs in the current study were not expected to follow the smell of the food. All plates were rubbed with test food to avoid possible odour confounders. Prior to the test, the experimenter offered the dog a piece of the food to ensure that the dog was motivated to eat it. If the dog did not eat the food they were excluded from the test. At the beginning of each trial, the experimenter placed a piece of food on one of the four white plates, according to a pre-determined order, making sure the dog was watching and calling dog's name if necessary. The experimenter then walked up to the dog-owner dyad and stood next to them, this was the cue for the owner to release the dog. The dog had 5 seconds to find the food and eat it. If the dog did not go towards the food straight away, the owner was allowed to encourage the dog verbally but was asked to avoid gestures directing the dog towards any specific location (e.g. pointing at the plate). Once the dog ate the food, the trial ended. The trial was repeated 4 times: on the first trial the food was placed on the plate positioned 0.5 meters from the dog; during trial 2 the food 1 meter away from the dog; in trial 3 the food was 1.5 meters away; finally in trial 4 the food was 2 meters away. During each trial, the experimenter gave a binary score based on whether the dog walked directly to the baited plate within the 5 seconds (1 point), or not, i.e. it did not walk directly to the baited plate or took more than 5 seconds (0 points).

(iv) 'Darkness' visual test

There were two doors on two opposite sides of the room (Fig. 4, D1 and D2). The doors were also connected through an external corridor. Therefore, it was possible to move from one corner of the room to the other by walking along the external corridor, rather than across the room. Two opaque barriers, a light brown wooden barrier (200 cm wide and 75 cm high) and a dark green plastic barrier (140 cm wide and 100 cm high), were placed roughly halfway across the room. Each barrier was aligned to one side of the room; one barrier was slightly closer to the owner (who stood at corner A or B, see later) while the other was closer to the opposite side of the room (starting point). The placement of the two barriers created an S-shaped course within the room, so that the dogs needed to be able to see the barriers in order to avoid them as it walked across the room. The setup was similar for all subjects: they had to walk from the starting point to corner A when the gap was on the left hand-side and to corner B when the gap was on the right hand-side. Te distance between the barriers was calculated based on the dog's body size: specifically, the gap was wide just enough for the dog to pass through it.

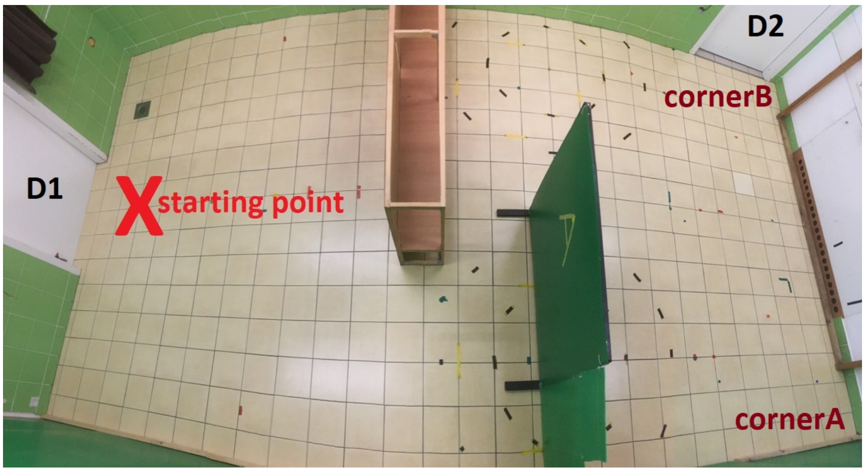

Fig. 4. The obstacle of the 'Darkness' visual test. Two opaque barriers created an S-shaped course in the room. D1 and D2 are the doors through which the owner changed positions. Corner A and corner B are the starting positions of the owner. Starting point is the dog's starting point.

After the "Distance" visual test the dog was given a short break, while the experimenter set up the room; then the "Darkness" visual test begun. The experimenter held the dog by the collar while standing at the starting point (Fig. 4). The owner left the room, walked through the external corridor and reached corner A. The owner then called the dog's name and the experimenter let the dog go. The upper body of the owner was visible to the dog; if necessary, the owner could clap, wave, and call the dog repeatedly in order to obtain the dog's attention. Once the dog reached the owner , the owner rewarded it with a piece of frankfurter and walked it back to the starting point, which ended the trial. At this point, the experimenter shifted the two barriers across the room, so that the gap was on the opposite hand-side of the room (i.e. if during the first trial the gap was on the left hand-side, it was now on the right hand-side; Fig. 4) and the trial was repeated. We repeated trials four times: the first two trials (one for each side) were performed with the light of the room switched off to recreate dusk lightening conditions; during the following two trials, the light was on. An EuroVideo EVC-TG-IC380A28 video-camera, placed on the ceiling opposite to the starting point, was used to record the trials in the dark condition. Not all tests were performed at the same time of the day, therefore, when the test was performed during the day, the laboratory room's windows were blinded to block light and heat coming from outside, creating a dusk-like lighting condition. When tests were performed after sunset, the laboratory room's door was kept slightly ajar to maintain the lighting conditions as consistent as possible. Light intensity (luminance) was measured to be 4 lux in the dark condition and 770 lux in the light condition. Each trial was coded from video and dogs were given a score based on their ability to move through the barriers: the dog walked across the course without touching the barriers and within 5 seconds (score 2); the dog walked across the course without touching the barriers between 5 and 10 seconds (score 1); the dog touched the barriers or crossed the course after the 10 seconds mark (score 0).

4. Statistical analysis

Results were analysed using the statistical software R (R Development Core Team, 2015), with the packages: “reshape2” (Wickham, 2007), “Hmisc” (Harell & Dupont, 2016), “RVAideMemoire” (Hervé, 2017), “corpcor” (Schäfer et al., 2017), “survival” (Therneau, 2015), “survminer” (Kassambara, Kosinski, Biecek, & Fabian, 2018), “ordinal” (Christensen, 2019).

We performed correlations and analysis of variance tests. As some of the data were not normally distributed, non-parametric tests were used. In the presence of multiple comparisons, *p*-values of post-hoc tests were adjusted with the Benjamini & Hochberg method (Benjamini & Hochberg, 1995). Cochran's Q test was used with the scores of the ‘Clapping’ and the ‘Distance’ tests to assess the effect of distance. For the ‘Darkness’ tests, the scores were analysed with Cumulative Link Mixed Model to assess the effect of conditions (lighting conditions, different sides) and age in years. Correlations between the overall scores were assessed using the Spearman rho test.

Since right censoring occurred during the ‘Recorded sound’ test, survival analysis was used. To compare the volume level (dB) corresponding to the first reaction Kaplan Meier estimates were used; we analysed both the effect of the volume and age, an age group was included as main factor. To compare younger and older subjects in this analysis, we created two groups: dogs up to 10 years of age (N=13) and dogs over 10 years (N=13). Mixed Effects Cox Regression Models were used to analyse the effects of volume level (dB) and age group (old vs young) on the score. Therefore trial order and age group were included in the model as main factor, while subjects were included as random factors to control for repeated testing .

5. Results

Dogs with missing data were excluded from the corresponding test’s analysis. Therefore, the number of dogs included in the test has been specified separately for each analysis. We used normalized scores of the dogs’ performance/evaluation for calculating correlations. As described in the Analysis section, the scores were normalized using the following formula: $X_{normalized}=X-X_{min}/(X_{max}-X_{min})$.

For the assessment of response to visual stimuli, Spearman *rho* test indicated a very strong positive correlation between the veterinary exam and the owner’s assessment; the test had a strong positive correlation both with the owner’s assessment and the veterinary exam (N = 10, Mdn_{Vet} = 87%,

Mdn_{Owner} = 75%, Mdn_{Test} = 85%; $r_{Vet\text{-}Owner}$ = 0.88, p = 0.002; $r_{Vet\text{-}Test}$ = 0.77, p = 0.010, $r_{Test\text{-}Owner}$= 0.73, p = 0.016).

Cochran's Q indicated a significant difference across conditions (distances) in the scores of the ‘Distance’ test (N = 47; percentages of successful dogs: 0 m = 98%, 0.5 m = 91%, 1 m = 74%, 1.5 m = 55%, 2m = 40%; Q = 66, df = 4, p < 0.001).

For the ‘Darkness’ test Cumulative Link Mixed Model (LR test: $\chi^2(3)$ = 42.184, p < 0.001) indicated that dogs performed significantly better in the light, compared to the dark condition, and also on the left, compared to the right side (N = 43; ß±SE: $\beta_{Lighting}$ = 3.731 ± 0.001, z = 2720.4, p < 0.001; β_{Side} = -1.060 ± 0.001, z = -777.5, p < 0.001; Fig. 5), as well as younger dogs performed better, than older dogs (β_{Age} = -0.665 ± 0.001, z = -470.7, p < 0.001).

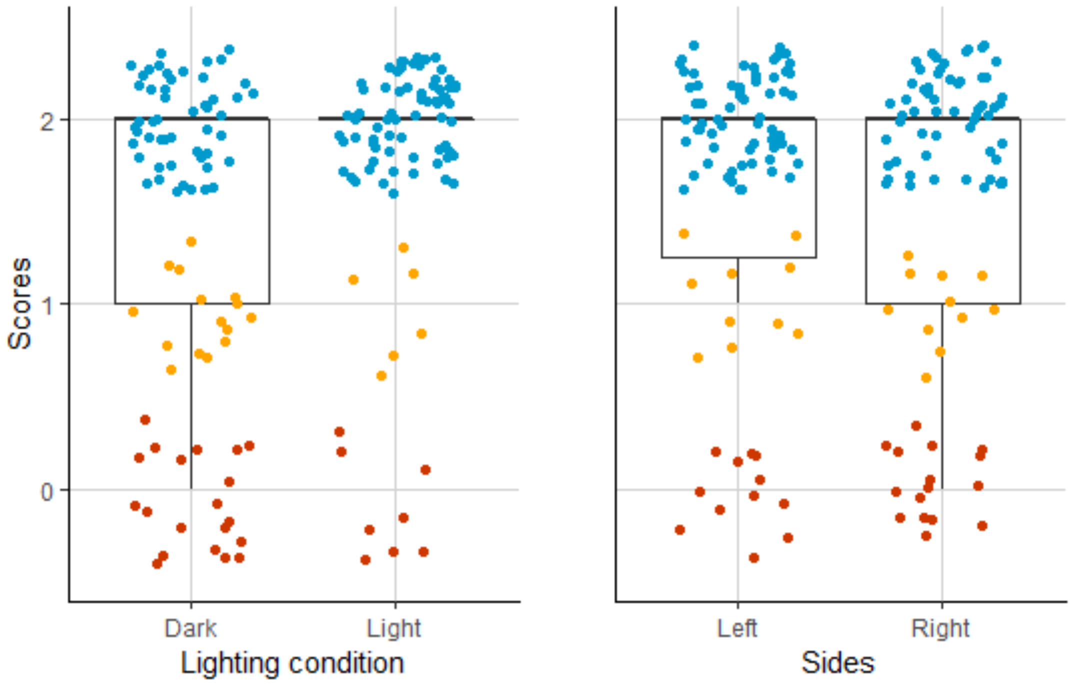

Fig. 5. Box-plots for the ‘Darkness’ test. The scores could be 0, 1 and 2. For visualization a random jitter was added to avoid overstacking; the colour represents the aggregated score value for each individual dog (red = 0, yellow = 1, blue = 3).

For the hearing tests, Spearman *rho* test indicated no significant correlation between the veterinary and owners scores, but there was a significant positive correlation between owners scores and the behaviour tests (N = 10, Mdn_{Vet} = 100%, Mdn_{Owner} = 75%, Mdn_{Test} = 94%; $r_{Vet\text{-}Owner}$ = 0.04, p =

0.912, $r_{Vet\text{-}Test} = 0.22$, $p = 0.547$; $r_{Test\text{-}Owner} = 0.59$, $p = 0.001$). Dog age in years also negatively correlated with the playback test scores ($N = 26$; $r_{Test\text{-}Age} = -0.67$, $p < 0.001$).

The scores of the ’Clapping’ test were identical in all conditions, i.e. the distance between the dog and the noise source did not affect the dogs’ response ($N = 18$; Success rate for all conditions = 83%).

A log-rank test showed that dogs over 10 years (N = 13) responded at higher volume levels (Chi $X_2 = 44.7$ $p<0.001$; Young: (95% CI: 45 dB; 50 dB), Old: (95% CI: 58 dB; 73 dB)) (Fig. 6).

We tested whether age groups and trial number influenced at what volumes the dogs first reacted to the sounds via a Mixed Effects Cox regression model. The cumulative hazard results of the Cox regression showed a significant hazard decrease ($\exp(\beta)=<1$) as the trials progressed of -2.87 ($\exp(\beta)= 0.84$, 95%CI=(0.80-1.00), p=0.004), and a significant hazard decrease for the group over 10 years of -3.47 ($\exp(\beta)= 0.19$, 95%CI=(0.17-0.39), $p<0.001$). An $\exp(\beta)$ below 1 for these factors suggests a higher volume required to elicit reaction.

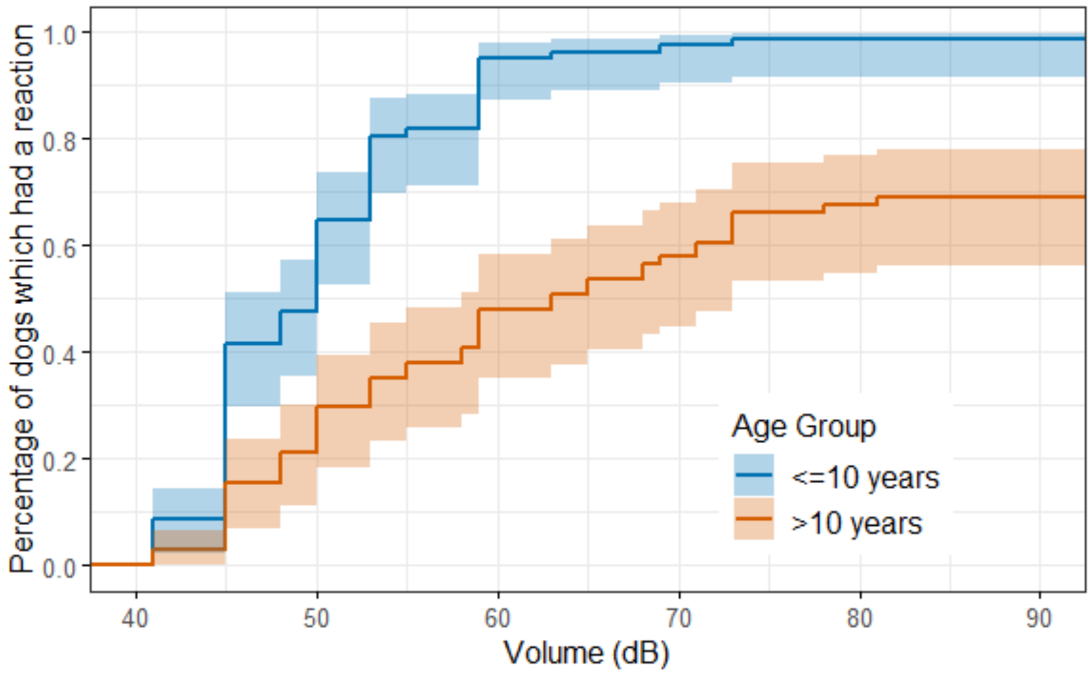

Fig. 6 Survival plot for the ‘Recorded sound’ test. On X axis the dB of the sounds is reported; on Y axis, the percentage of dogs that already had a reaction at a given dB level. For

example, at a 60 dB volume more than 90% of dogs below 10 years reacted, while about 50% of dogs above 10 years reacted.

6. Discussion

In this study, we sought to develop behaviour tests that could separate subjects based on their performance in tasks that relied on auditory and visual skills. Our aim was to develop non-invasive screening tests, that do not require extensive training, to detect subjects with potential visual and/or acoustic impairment. We found that the behaviour tests we used to investigate the response to auditory stimuli were not as informative as expected, i.e. as a group, dogs reacted similarly to different sounds and the volume has not affected their behaviour. However, fewer older dogs reacted to sounds with lower decibel. It is possible that this is due to overall reactiveness to stimuli, or that the relationship between age and hearing decline is not linear, and thus is masked when young and old dogs' data are analysed together. In case of the 'Clapping' test, nearly all dogs performed at ceiling. For some of the dogs hearing decline was reported by the veterinary surgeon and/or the owner. Thus possibly our behaviour test was not sensitive to mild hearing impairment. However, the assessments of owners and veterinary surgeons did not correlate with each other either. One plausible explanation for this lack of correlation is that scores may not measure the same phenomenon. For example, it is possible that owners interpreted a generally decreased responsiveness to external stimuli because of impaired auditory function. Alternatively, it is possible that during their daily life dogs rely more on visual cues, rather than auditory cues. It should be noted that the sample size of the dogs that completed the auditory testing in this study was low, therefore, the implication of these results should be taken carefully.

In the 'Distance' test, dogs' performance decreased when the dogs were required to find an object placed 2 meters away, compared to a close by object (0 or 0.5 meters). Similarly, dogs' performance in the obstacle course (Darkness test) was worse when this was in the dark rather than in the light. Furthermore, the overall "owner", "veterinary" and "sensory tests" scores all positively correlated with each other for vision, this consistency suggests that the three measures regard indeed about the same phenomenon. Dog owners also appear to be fairly reliable about subjectively recognising the decline of their dog's performance relying on vision . In the case of visual stimuli, our behaviour tests were also able to provide information about performance related to two phenomena, not specifically addressed by veterinary examinations, i.e. distance and luminosity. One study used a similar protocol to our "Darkness test" to investigate vision impairment under different lighting conditions (Garcia et al., 2010). This study focused on changes in speed, therefore required a relatively long course (at least 3 meters); our focus on contact with the obstacle allowed us to shorten the obstacle course. Moreover, the study by Garcia et al. (2010) focused only on a genetic condition (achromatopsia), which affects vision under bright lights. Thus, we present the first study relying on the vision function under everyday conditions (i.e. day or room light and dusk), where the performance is affected by any form of decline in vision, including age-related changes. Our test can therefore be considered relevant for a wide range of individuals and further validations should be sought in the future.

We believe that the possibility that dogs relied on their olfaction during the "Distance" test was adequately controlled for by the test design. However, it is not impossible that some of the dogs had a worse performance in our visual studies due to reasons other than sensory impairment, such as lack of interest in the tasks. To ensure that the tasks really measure vision acuity, it is necessary to validate the current task with a full ophthalmological examination and electrophysiological measures indicating, for example, the presence of refractive errors. We stress that, at present, these results should be interpreted as measures of responsiveness to visual stimuli. Moreover, we cannot fully exclude an order effect, as the test started with easiest condition (food placed on the closest location) and ended with the most difficult condition (food placed on the most distant location). Nevertheless, the dogs were motivated to find and eat the food for the duration of the test, suggesting that motivation should not have impacted their performance. It is also important to consider that, in the case of the "Distance visual test", the actual distance between the dog and the plates was affected by the size of the dog, as a larger dog is closer to the plates. Similarly, based on a dog's height, the angle at which the dog can see the plates can be different, too, which can cause difference in their performance (e.g. Helton & Helton (2010) reported that larger dogs are more successful in following human visual gestures). There is also a link between head shape and the distribution of retinal ganglion cells, the cells form a horizontally aligned visual streak in longer headed dogs, while a strong area centralis in shorter headed dogs (McGreevy, Grassi, & Harman, 2004). Therefore shorter headed dogs might have been more successful in following human visual gestures (Gácsi, McGreevy, Kara, & Miklósi, 2009), might have paid more attention to projected faces (Bognár, Iotchev, & Kubinyi, 2018) and might have formed eye contact sooner with humans (Bognár et al., in prep), than longer headed dogs. Most of the dogs in our test were medium sized and medium headed, we had only a few small or large dogs, and only a few short or long headed dogs, thus the individual dog's size and head shape effect could not be statistically analysed in this study and the role of head shape should be investigated in further research.

We could not design a test where the dogs' response related to the decibles of the auditory stimuli, although age affected the response to decreasing decibels. Age may have a role on responsiveness to auditory stimuli but we cannot exclude causes unrelated to hearing. For example, older dogs might have less interest in certain stimuli. In fact, ecologically relevant stimuli yielded variable reactions in dogs: these might depend on the valence of the sound, the pitch, or individual differences in reactivity. Moreover, previous research showed that decline in the hearing function occurred very late in the life of the dogs (above 13 years of age, Shimada et al., 1998). Therefore, it may simply be that dogs in our sample were not severely affected by hearing decline.

Previous findings showed that the duration of behavioural orientation towards the source of a recorded conspecific vocalisation declines with repetitions of the same recording and may be increased by playing a vocalisation from a different social context (Molnár, Pongrácz, Faragó, Dóka, & Miklósi, 2009). Therefore, close presentation of varying sounds should be a successful approach to elicit spontaneous stimulus-orienting behaviours in dogs, as they may quickly habituate to non-social, mechanical sounds, irrespectively of their nature (Maros et al., 2008; Molnár et al., 2009). Such habituation may potentially lead to lack of behavioural responses in a situation where mechanical sounds are presented repeatedly to dogs, even if the type of sound is changed.

We were able to design two non-invasive standardised tests, which do not require training, to measure dogs' responsiveness to visual stimuli and its variation based on a) the distance of the stimuli from the dog, b) the lighting conditions. The ability to measure vision-based performances is particularly important because performance in such tests requires that two elements of the visual function are intact, namely visual acuity and vision in dusk. Therefore, these findings indicate that the vision tests should be considered for further validation, in order to evaluate their usefulness as screening tools for the decline in dogs' visual function. These tests may have wider implications for the welfare of dogs, as these are simple behaviour procedures that dog owners, dog trainers, staff at dog shelters could routinely perform to red-flag dogs with potential sensory impairment. Following further validation, the tests could be used for monitoring the sensory decline of ageing dogs. For example, the performance of the vision tests could be compared to the results of a retinoscopy performed on the same subjects, a technique to obtain an objective measurement of the refractive error of a patient's eyes, also known as near-sightedness, far-sightedness, astigmatism, and presbyopia. Results of the auditory tests could be validated through comparison with an audiogram (i.e. audible threshold for standardized frequencies as measured by an audiometer) produced by a BAER test.

In conclusion, sensory testing is essential before cognitive assessments. We stress that cognitive and behaviour researchers should routinely query owners regarding the visual impairments of their dogs before cognitive testing, especially with at risk populations (e.g. ageing dogs). When available, behaviour tests should also be employed in order to obtain baseline response levels to stimuli that share physical properties similar to those used in subsequent cognitive tests.

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Compliance with Ethical Standards

Ethical approval: All applicable international, national, and institutional guidelines for the care and use of animals were followed. All the procedures were in line with the guidelines of the International Society for Applied Ethology.

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