Effect of age and severity of cognitive dysfunction on spontaneous activity in pet dogs. Part 2: Social responsiveness

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Abstract

Changes in social interactions with owners and other dogs are frequently observed in dogs suffering from cognitive dysfunction syndrome (CDS). The aim of this work was to assess the effect of age and severity of CDS on social responsiveness. This is the second part of a two-part report on spontaneous activity in pet dogs. A human interaction and a mirror test were administered at baseline and 6 months later to assess social responses to humans and conspecifics, respectively, to four groups of privately-owned dogs: young (n = 9), middle-aged (n = 9), cognitively unimpaired aged (n = 31), and cognitively impaired aged (n = 36). The severity of cognitive impairment was considered in the last group and dogs were categorised as having either mild or severe CDS. The influence of the person and the mirror on locomotion and exploratory behaviour was moreover studied. Dogs were recorded in the testing room and the video-recordings were subsequently analysed.

Young dogs displayed more interactions involving physical contact with the person. In addition, young and middle-aged dogs showed more vocalisations in response to social isolation. On the other hand, aged animals spent more time in front of the mirror. Suffering from severe CDS influenced social responsiveness so that decreased the response to social isolation and human interaction and increased the time in front of the mirror reflection suggesting a deficit in habituation. Testing on spontaneous activity may help to characterise CDS in aged dogs, a condition increasingly diagnosed in veterinary clinic and considered a useful natural model of Alzheimer's disease.

Keywords: Canine; Aging; Cognitive dysfunction; Human interaction; Mirror test

Introduction

Dogs may naturally suffer age-related neuropathology and cognitive deficits that parallel several key aspects of normal human aging and early Alzheimer's disease (AD). There is evidence of β-amyloid (Aβ) deposits and amyloid angiopathy in the brain of aged dogs, as well as other neuropathological features of AD, such as neurodegeneration and oxidative damage (Colle et al., 2000; Head et al., 2002; Pugliese et al., 2006; Rofina et al., 2006; Bernedo et al., 2009; Insua et al., 2010 and 2011). Moreover, we have recently found changes in plasma Aβ-42 levels during pathological aging in dogs that might exhibit patterns similar to those previously reported for human mild cognitive impairment and AD (González-Martínez et al., 2011).

The so-called Cognitive Dysfunction Syndrome (CDS) in aged dogs includes a constellation of behavioural and cognitive deficits normally affecting four categories: social interactions, sleep-wake cycles, orientation ability and housetraining habits. (Ruehl et al., 1998; Colle et al., 2000; Rofina et al., 2006; González-Martínez et al., 2011). Changes in social interactions include decreased interest in petting or even avoiding contact, decreased greeting behaviour, aggression toward the family members or unfamiliar people, and altered relationship with other household pets (less social or fearful and anxious behaviour) (Landsberg et al., 2011). Human patients suffering from AD also undergo several abnormal changes in social behaviour including aggression, culturally inappropriate behaviours, and affective disturbances (Shinosaki et al., 2000).

Spontaneous activity, including locomotion, exploratory behaviour and social responsiveness has been previously tested in kennel-housed dogs, mostly beagles. These studies show that cognitively impaired dogs display abnormal social responses, often engaging in undirected, stereotypical types of behavioural patterns (Head et al., 1997; Siwak et al., 2001), and it has been suggested that this behavioural profile is consistent with behavioural disruptions occurring in human dementia (Tapp and Siwak, 2006). Interestingly, several studies indicate that behavioural and psychological symptoms of human dementia are not merely an epiphenomenon of cognitive impairment, but could be attributed to specific biological brain dysfunction (Shinosaki et al., 2000).

We have previously tested changes in locomotion and exploratory behaviour as a function of age and cognitive impairment in pet dogs (see companion paper, Part 1). The aim of this work (Part 2) was to further explore spontaneous activity in a human interaction test and a mirror test to assess social responses to humans and conspecifics, respectively. The influence of these introduced stimuli (i.e., human and mirror) on locomotion and exploratory behaviour was moreover studied by comparing these tests with a previously administered OF test (Part 1). The tests were carried out at baseline and 6 months later in pet dogs varying in age and cognitive status. We hypothesised that social responsiveness would be related to age as well as to the severity of cognitive dysfunction.

Materials and methods

Subjects

The same animals participating in the companion paper (Part 1) were included in the present study. Classification of cognitive status was carried out using an owner-based observational questionnaire (see Part 1 or González-Martínez et al., 2011). Briefly, the subjects were sorted into i) young (YG, 1-4 y, *n*=9), ii) middle-aged (MA, 5-8 y, *n*=9), iii) cognitively unimpaired aged (CU, ≥9 y, *n*=31), and iv) cognitively impaired aged (CI, ≥9 y, *n*=36). This last group was further subdivided into mild cognitively impaired (mCI, *n*=20) and severe cognitively impaired (sCI, *n*=16) animals. Six CU and 13 CI (6 mCI and 7 sCI) dogs failed to complete the follow up.

Animals were treated according to the European and Spanish legislation on animal protection (Directive 86/609/EEC, Real Decreto 1201/2005), and the experiments and procedures were approved by the Ethical Committees of both participating universities.

Test procedures

The testing room is illustrated in Fig. 1. A 3 min human interaction test and a 3 min mirror test were conducted at both baseline and follow-up periods. Dogs were also tested in an OF test and a curiosity test which are described in the companion paper (Part 1). A modified version of testing procedures conducted by Siwak et al. (2001) in beagle dogs was used as described below.

Human interaction test - A non-familiar person was seated in a fixed position in the central area of the testing room prior to the dog's entrance (Fig. 1). The person was instructed neither to respond nor to interact with the dog. This test was conducted to assess the reaction of each dog to the presence of a person.

Mirror test - A mirror (90 x 90 cm) was cleaned before each session and securely fixed to one wall of the testing room (Fig. 1). This test was aimed to examine the reaction of the dog to the presence of the mirror.

Dogs were continuously recorded during sessions and video recordings were subsequently analysed by two observers (BR and AG-M). A Fortran-77 software program was designed to assist in the calculation of the activity duration and frequency of occurrence from the data originally collected.

Behavioural measures

Behavioural measures included those previously analysed in the OF test (see Part 1), briefly: (1) vocalisations; (2) total time in the door area (s) and average duration in this area each time the animal entered it (s per occurrence); (3) locomotor activity (LA; number of squares crossed); (4) LA into the central area (proportion of LA displayed at the central squares); (5) time (s) in immobility (i.e., sitting or lying); (6) door and corner-directed behaviours (door-DB and corner-DB). Additional measures for the human interaction and the curiosity tests are described in Table 1.

Statistical analysis

Average differences in quantitative variables between the general study groups (YG, MA, CU and CI) in each test were assessed either by ANOVA or Kruskal-Wallis test when the parameter distribution was normal or non-normal, respectively (normality assessed with the Kolmogorov-Smirnov test). Tukey's HDS post-hoc analysis or Mann-Whitney U test was used afterwards for multiple comparisons when a significant main effect of group was detected. Furthermore, Student's *t* test for paired samples or Wilcoxon signed-rank test was conducted to assess inter-test variations. A subsequent identical analysis was carried out considering only the three aged groups (i.e., CU, mCI and sCI). Distribution of qualitative variables was assessed by Chi-square test.

Calculations were carried out using the statistical program SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA), and $P<0.05$ denoted statistical significance.

Results

The frequency of behavioural measures in the human interaction and the mirror tests within the general study groups as well as in the aged groups is summarised in Tables 2a and 2b, respectively, whereas duration results are shown in Tables 3a and 3b. For one dog (1 MA) in the human interaction test and for eight dogs (2 MA, 5 CU and 1 mCI) in the mirror test, testing was not performed according to procedures and these animals were excluded from analyses. With a few exceptions, which are noted for each test, we found no significant differences between results at baseline and the follow-up across groups and tests, and data from both periods were analysed jointly. The frequency and durations results in the OF test are described in the companion paper (Part 1). A description of the main features follows below.

Human interaction test

In the presence of a person in the testing room, sCI dogs displayed less door-DB and spent less time in immobility than the mCI and the CU group (Tables 2b, 3b). Interestingly, we found that the frequency of corner-DB in this test increased after 6 months only in the sCI group ($P < 0.05$). On the other hand, YG animals displayed more active interactions with the person than the aged groups (Fig. 3). They also showed more immobility-cohesion episodes than the rest of the groups (Table 2a), but the frequency of these episodes decreased after 6 months ($P < 0.05$). Furthermore, YG and CU dogs showed higher LA into the central area, where the person was sitting down, compared to the CI animals (Table 2a). In particular, the sCI group showed less immobility-cohesion episodes than their healthy homologous CU (Table 2b).

When comparing the results in this test with those previously obtained in the OF test, we found that the presence of a person was accompanied by a decrease in vocalisations ($P < 0.001$), and an increase in the LA into the central area ($P < 0.05$) in YG and CU groups. Moreover, YG dogs decreased the time spent in the door area ($P < 0.001$), whereas CU decreased the amount of time spent in immobility ($P = 0.05$). In the presence of a person, the frequency of door-DB ($P < 0.01$) as well as corner-DB (YG and MA, $P < 0.05$; CU and CI, $P < 0.001$) decreased in all groups with respect to the OF test.

Mirror test

In this test, YG dogs vocalised more frequently than CU dogs (Table 2a), whereas sCI animals vocalised less than any other group (Table 2b, Fig. 2a). Moreover, YG dogs spent a longer amount of time in immobility than aged dogs (Table 3a). Again, sCI animals spent less time in immobility and showed less door-DB than the mCI and CU groups (Table 2b, 3b). On the other hand, aged groups (CU and CI) spent more time in the mirror area than the younger groups (YG and MA) (Table 3a). In addition, younger groups showed less LA into the mirror area than CU dogs (Table 2a).

With respect to the OF test, the introduction of the mirror was accompanied by an increase in vocalisations in CU ($P < 0.01$) and mCI groups ($P < 0.05$) and a decrease in LA in all aged groups (CU and mCI, $P < 0.001$; sCI, $P < 0.05$). In addition, the frequency of door-DB and corner-DB in the mirror test decreased significantly in all groups with the exception of MA animals. Similarly, the time spent in the mirror area increased significantly in all animals with respect to the OF test, except for the MA and the mCI groups.

Compared to baseline, at the follow-up assessment the mCI animals showed a decrease in the time (total and average) spent in the door area ($P < 0.05$). In addition, YG dogs decreased the time spent in the mirror area ($P < 0.05$), and also the amount of entrances into this area ($P < 0.001$). This latter result was also observed in CU dogs ($P < 0.05$).

Discussion

Dogs are a highly social species and establish strong relationships with their conspecifics and humans (Lindsay, 2001). In this regard, Tuber et al. (1996) found kennel dogs exposed to a novel environment had lower activity (and glucocorticoid levels) when in the company of their human caretaker than with their kennel mates. This finding points to a difference in the nature of dog-human and inter-dog social relationships that we assessed by the human interaction test and the mirror test, respectively, in a cohort of companion dogs varying in age and cognitive status. All of them had been previously tested in an OF situation (see companion paper, Part 1).

The YG dogs showed more active (i.e., displaying attention-seeking behaviours) and passive (i.e., remaining in physical contact) interactions with the person. In some instances these YG dogs climbed upon the person´s lap for the total duration of the test. In agreement with these results, Siwak et al. (2001) found that young beagles spent more time in contact with the person. The rest of the cognitively intact dogs (i.e., MA and CU groups) also varied their activity pattern in the presence of a person but to a lesser extent, and a decline in door- and corner-DB was observed in all groups with respect to the results in the OF test. Moreover, CU dogs and YG dogs decreased their frequency of vocalisations, and increased the times they entered the central area compared to the OF test, suggesting that being close to an unfamiliar person may attenuate the response to isolation from the owner in these animals.

We have previously reported sCI dogs showed the highest LA in an OF and a curiosity test (Part 1). This also occurred in the present mirror test but not in the human interaction test. Thus, when the person was present, those differences previously observed in locomotion between CI dogs, sCI animals in particular, and CU dogs disappeared. In this sense, it could be argued that CI dogs also reacted to the presence of the person, but to a lesser extent. However, sCI dogs appeared to be quite refractory to this strong social influence and displayed less passive interactions directed to the person than their healthy homologs. Furthermore, sCI dogs continued spending shorter time immobile, which resembles the reduced sociability combined with increased activity recently described in a transgenic mouse model of AD (Filali et al., 2011).

Mirror self-recognition responses have been observed in some primates, dolphins and elephants (Plotnik et al., 2006). The reaction toward the mirror reflection in dogs is initially not a self-recognition response but an alien-directed response (Gallup, 1968; Tapp and Siwak, 2006). In this study, we used the time spent in the mirror area as well as the times (frequency) the dog entered this area as surrogate measures of the time spent reacting to the reflection. Since the mirror reflected the self-image, and therefore, a false conspecific image, habituation to the reflection would be expected after a while. Interestingly, mCI animals spent a shorter time reacting to the reflection than sCI animals. We also found that the aged dogs (CU and CI) spent a longer time than the younger ones (YG and MA) in the mirror area, although they showed extensive variability in the way they reacted to the reflection (data not shown). It is possible that a more detailed analysis of the mirror-directed behaviour (instead of just the time spent in front of it) may yield stronger differences among groups. Regardless of this, the mirror test in our aged dogs revealed certain similarities with the inability of self-recognition observed in AD patients with severe degrees of dementia (Biringer et al., 1988; Biringer and Anderson, 1992; Mendez et al. 1992). Misidentification and facial misrecognition in human dementia have been related to right hemispheric pathology (Forstl et al., 1991; Silva et al., 1993; Ellis, 1994; Breen et al., 2001).

A typical context for barking in domestic dogs occurs when they are left alone in a room isolated from their owner, and these barks may play a role in the human-dog communication (Yin and McCowan, 2004; Pongracz et al., 2010). Considering this, one may use the OF test to obtain information not only on locomotor and exploratory behaviours, but also on the dog's response to social isolation in a novel environment. Besides vocalising, the previously described door-oriented activities may also reflect the response to social isolation. In this study, younger dogs vocalised more than aged dogs during the OF test. Among the aged groups, animals suffering from sCI showed the lowest frequency of vocalisations in all tests, even during the mirror test, in which they spent a lot of time reacting to the reflected image. A weak response to isolation, as measured by little vocal communication behaviours together with other door-oriented activities, might denote disorientation and not a rapid habituation to separation from their owner.

Taken together the results from the two reports (Part 1 and 2) on locomotor and exploratory behaviours, as well as on social responsiveness, it is possible to depict group behavioural profiles. In particular, the CU group, similar to younger dogs, was characterised by exhibiting goal-directed activities in most cases. However, social and curiosity responses in this group were moderate with regard to YG animals, as they might prefer proximity to the person or the novel stimuli instead of physical contact. Dogs suffering from sCI were hyperactive, as measured by high LA levels and short durations of immobility. Regardless of the testing environment, these severely impaired animals often engaged in non-goal-directed activities, such as repetitive corner exploration, which we considered a stereotyped activity pattern. Moreover, this behavioural pattern was linked to a low (vocal and motor) response to both social isolation and human interaction. Dogs suffering from mCI were characterised by an intermediate profile in the behavioural continuum between the intact dogs and the severely impaired ones. Thus, mCI dogs displayed a similar locomotor and exploratory pattern to that exhibited by the CU dogs, but, similar to the sCI dogs, they engaged in a high number of corner-DB. Furthermore, mCI dogs tended to be less reactive to any stimulus introduced in the testing environment than their healthy intact counterparts.

Considering the previous behavioural patterns, testing aged pet dogs in spontaneous behaviour, as well as countersign data obtained from owner-based questionnaires, may help to characterise the severity of canine CDS. In addition, some behavioural measures analysed during testing may help the monitoring of disease considering the changes experimented by impaired animals during the follow-up period with respect to baseline (e.g. increase in the frequency of corner-DB). This temporal evolution may suggest a possible worsening of clinical symptoms but this need to be investigated further.

The behavioural profiles observed in this study consistently agree with the classification of cognitive status obtained by our questionnaire in the same cohort of studied dogs. These questionnaires that include psychometric scales have been successfully used for measuring and phenotyping behaviour in dogs (van den Berg et al., 2006). Furthermore, this questionnaire-based classification of cognitive status was related to plasma Aβ levels (González-Martínez et al., 2011). Nevertheless, it is possible that a CDS diagnosis included dementias other than AD-like dementia (e.g., vascular dementia). It is important to note that tools and criteria for CDS diagnosis in veterinary medicine need to be still considerably developed and validated. We hope this study contributes to this aim.

Conclusions

Social responsiveness was primarily affected by age but also influenced by the severity of cognitive impairment. Thus, we observed young dogs displayed more interactions involving physical contact with humans and more vocalisations in response to social isolation. On the other hand, aged animals spent more time in the mirror area suggesting a deficit in habituation to the reflection of a fake dog image. Suffering CDS, especially if severe, influenced social responsiveness so that it decreased the response to social isolation and increased the response to the mirror reflection.

Since the present results (and those reported in the companion paper) are congruent with the classification of cognitive status obtained by our questionnaire, they might serve as an indirect validation of such questionnaire for the diagnosis of CDS. Further research should be carried out to validate psychometric owner-based questionnaires for CDS diagnosis in dogs.

Conflict of interest statement

Disclosure statements for the authors: PP and MS are employees at Araclon Biotech Ltd. BR and AG-M are supported by grants from Araclon Biotech to the Universidad de Zaragoza and Universidad de Santiago de Compostela, respectively. GS, M-LS, SG-B, JP and AV have no actual or potential conflict of interest.

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Table 1

Behavioural measures in the human interaction and the mirror tests

Behavioural measure	Description
Immobility-cohesion[a]	Total number of episodes in which the dog remains sitting or lying in virtually physical contact with the person.
Active interactions[a]	Total number of episodes in which the dog initiates physical contact with the person by climbing, pushing with the head and/or displays face-licking.
Total time in the mirror area[b]	Total time (s) spent in front of the mirror.
LA into the mirror area[b]	Proportion (%) of LA displayed at the mirror area.

[a]Specific measures in the human interaction test; [b]Specific measures in the mirror test.

Table 2a

Behavioural measures scored for frequency of occurrence in the general groups

Measure	Test	YG (*n* = 18)	MA (*n* = 18)	CU (*n* = 56)	CI (*n* = 59)
		Mean ± SD			
Vocalisations (times)	Human	$6.9 \pm 6.2^{\text{CI}}$	$12.2 \pm 19.0^{\text{ci}}$	$6.5 \pm 8.8^{\text{ci}}$	$6.9 \pm 20.0^{\text{YG,ma,cu}}$
	Mirror	$42.4 \pm 43.6^{\text{cu,CI}}$	$23.1 \pm 27.8^{\text{ci}}$	$18.1 \pm 21.0^{\text{yg,CI}}$	$16.4 \pm 34.2^{\text{YG,ma,CU}}$
LA (number of squares crossed)	Human	23.3 ± 18.5	17.9 ± 16.5	30.1 ± 35.1	37.3 ± 33.1
	Mirror	19.7 ± 14.6	17.0 ±17.1	21.2 ± 21.8	32.7 ± 31.8
LA into the central area (% of LA)	Human	$34.0 \pm 17.0^{\text{CI}}$	28.2 ± 15.8	$25.9 \pm 17.0^{\text{ci}}$	$17.8 \pm 16.8^{\text{YG,cu}}$
	Mirror	23.1 ± 12.2	15.8 ± 16.0	15.7 ± 13.4	17.5 ± 13.4
Door-DB (times)	Human	3.0 ± 3.0	2.5 ± 2.7	$4.8 \pm 5.1^{\text{ci}}$	$3.1 \pm 4.5^{\text{cu}}$
	Mirror	$6.3 \pm 6.0^{\text{ci}}$	7.9 ± 8.5	$5.9 \pm 7.0^{\text{CI}}$	$5.6 \pm 15.6^{\text{yg,CU}}$
Corner-DB (times)	Human	0.4 ± 0.8	$0.1 \pm 0.5^{\text{ci}}$	$0.2 \pm 0.7^{\text{CI}}$	$2.0 \pm 5.1^{\text{ma,CU}}$
	Mirror	0.3 ± 0.5	0.4 ± 0.8	$0.2 \pm 0.6^{\text{CI}}$	$1.3 \pm 2.5^{\text{CU}}$
Immobility-cohesion (times)	Human	$1.2 \pm 0.9^{\text{ma,CU,CI}}$	$0.5 \pm 1.0^{\text{yg}}$	$0.7 \pm 1.2^{\text{YG,ci}}$	$0.3 \pm 0.7^{\text{YG,cu}}$
LA into the mirror area (% of LA)	Mirror	$18.1 \pm 12.3^{\text{CU}}$	$18.0 \pm 12.3^{\text{CU}}$	$33.1 \pm 16.0^{\text{YG,MA}}$	27.8 ± 15.8

YG: young; MA: middle-aged; CU: cognitively unimpaired aged; CI: cognitively impaired aged. Human indicates the human interaction test.

Different letters in each line indicate significant differences between groups (capital letters: $P < 0.01$; lower case letters: $P < 0.05$).

Table 2b

Behavioural measures scored for frequency of occurrence in the aged groups.

Measure	Test	CU (n = 56)	mCI (n = 34)	sCI (n = 25)
			Mean ± SD	
Vocalisations (times)	Human	6.5 ± 8.8^{s}	10.1 ± 25.4^{s}	2.6 ± 5.8^{s,m}
	Mirror	18.1 ± 21.0^{S}	24.0 ± 42.4^{s}	6.4 ± 14.2^{CU,m}
LA (number of squares crossed)	Human	30.1 ± 35.1	29.5 ± 29.9	47.9 ± 34.9
	Mirror	21.2 ± 21.8^{S}	22.0 ± 28.5^{S}	46.7 ± 30.9^{CU,M}
LA into the central area (% of LA)	Human	25.9 ± 17.0	17.8 ± 15.7	17.8 ± 18.5
	Mirror	15.7 ± 13.4	18.2 ± 14.9	16.6 ± 11.4
Door-DB (times)	Human	4.8 ± 5.1^{S}	4.3 ± 5.4^{s}	1.4 ± 1.7^{CU,m}
	Mirror	5.9 ± 7.0^{S}	8.5 ± 20.3^{s}	1.7 ± 2.3^{CU,m}
Corner-DB (times)	Human	0.2 ± 0.7^{m,S}	2.1 ± 6.3^{cu}	1.8 ± 2.8^{CU}
	Mirror	0.2 ± 0.6^{m,S}	0.9 ± 1.7^{cu}	1.9 ± 3.2^{CU}
Immobility-cohesion (times)	Human	0.7 ± 1.2^{s}	0.3 ± 0.8	0.2 ± 0.4^{cu}
LA into the mirror area (% of LA)	Mirror	33.1 ± 16.0^{m}	24.5 ± 17.0^{cu}	32.0 ± 13.3

CU: cognitively unimpaired; mCI: mild cognitive impairment; sCI: severe cognitive impairment. Human indicates the human interaction test.

Different letters in each line indicate significant differences between groups (capital letters: P<0.01; lower case letters: P < 0.05); m/M and s/S letters refer, respectively, to mCI and sCI.

Table 3a

Behavioural measures scored for duration in the general groups

Measure	Test	YG (*n* = 18)	MA (*n* = 18)	CU (*n* = 56)	CI (*n* = 59)
			Mean ± SD		
Total time in the door area (s)	Human	69.1 ± 55.4	93.2 ± 66.5	72.1 ± 57.0	61.2 ± 52.2
	Mirror	112.8 ± 43.8^{CI}	105.3 ± 76.5^{ci}	85.2 ± 64.8^{ci}	$55.4 \pm 55.6^{YG,ma,cu}$
Average time in the door area (s/occurrence)	Human	14.6 ± 15.3	27.7 ± 41.2^{ci}	17.1 ± 26.1	13.2 ± 21.5^{ma}
	Mirror	25.8 ± 19.8^{CI}	28.8 ± 44.8	22.6 ± 31.7^{CI}	$11.9 \pm 18.8^{YG,CU}$
Immobility (s)	Human	54.8 ± 51.8	59.7 ± 68.0	49.7 ± 56.5	36.7 ± 55.7
	Mirror	$83.1 \pm 58.9^{cu,CI}$	63.1 ± 66.6	$55.9 \pm 53.8^{yg,ci}$	$40.9 \pm 58.5^{YG,cu}$
Total time in the mirror area (s)	Mirror	$22.8 \pm 30.6^{cu,ci}$	$17.8 \pm 25.3^{CU,CI}$	$55.1 \pm 56.2^{yg,MA}$	$52.7 \pm 49.4^{yg,MA}$

YG: young; MA: middle-aged; CU: cognitively unimpaired aged; CI: cognitively impaired aged. Human indicates the human interaction test.

Different letters in each line indicate significant differences between groups (capital letters: $P < 0.01$; lower case letters: $P < 0.05$).

Table 3b

Behavioural measures scored for duration in the aged groups.

Measure	Test	CU (*n* = 56)	mCI (*n* = 34)	sCI (*n* = 25)
			Mean ± SD	
Total time in the door area (s)	Human	72.1 ± 57.0	65.7 ± 53.5	55.1 ± 50.8
	Mirror	85.2 ± 64.8^{S}	69.3 ± 66.3	37.2 ± 29.4^{CU}
Average time in the door area (s/occurrence)	Human	17.1 ± 26.1^{s}	13.3 ± 15.8^{s}	13.0 ± 27.9^{cu,m}
	Mirror	22.6 ± 31.7^{S}	17.1 ± 23.0^{s}	5.0 ± 7.2^{CU,m}
Immobility (s)	Human	49.7 ± 56.5^{S}	48.8 ± 60.2^{s}	20.3 ± 45.0^{CU,m}
	Mirror	55.9 ± 53.8^{S}	53.6 ± 60.1^{s}	24.2 ± 52.8^{CU,m}
Total time in the mirror area (s)	Mirror	55.1 ± 56.2	36.3 ± 42.7^{S}	74.4 ± 50.1^{M}

CU: cognitively unimpaired; mCI: mild cognitive impairment; sCI: severe cognitive impairment. Human indicates the human interaction test.

Different letters in each line indicate significant differences between groups (capital letters: P<0.01; lower case letters: P < 0.05); m/M and s/S letters refer, respectively, to mCI and sCI.

Figure legends

Fig. 1. Testing room. The floor was marked into 12 squares 69 x 69 cm with black electrical tape to assist in localising the animal's position. Three main areas were distinguished, namely the door area, the mirror area and the central area.

Fig. 2a, 2b. Box and whisker plots (SE) for the frequency of vocalisations (a) and locomotor activity (i.e., number of squares crossed) (b) in the aged groups during the human interaction and the mirror tests. Circles represent outliers, defined as those cases that extend more than 1.5 box-lengths from the edge of the box. Asterisks represent significance compared to the sCI group. *, ** or *** represent $P < 0.05$, $P < 0.01$ or $P \leq 0.001$, respectively. Human indicates human interaction test.

Fig. 3. Box and whisker plots (SE) for the frequency of active interactions directed towards the person during the human interaction test. Circles represent outliers, defined as those cases that extend more than 1.5 box-lengths from the edge of the box. Asterisks represent significance compared to the YG group. *, ** or *** represent $P < 0.05$, $P < 0.01$ or $P \leq 0.001$, respectively.