Effect of age and severity of cognitive dysfunction on spontaneous activity in pet dogs. Part 1: Locomotor and exploratory behaviour

B. Rosado[a,1], Á. González-Martínez[b,1], P. Pesini[c,*], S. García-Belenguer[a], J. Palacio[a], A. Villegas[a], M-L. Suárez[b], G. Santamarina[b], M. Sarasa[c].

[a] *Departamento de Patología Animal, Facultad de Veterinaria, Universidad de Zaragoza, 50013 Zaragoza, Spain*

[b] *Departamento de Ciencias Clínicas Veterinarias, Facultad de Veterinaria de Lugo, Universidad de Santiago de Compostela, 27002 Lugo, Spain*

[c] *Araclon Biotech Ltd. Zaragoza, 50012 Zaragoza, Spain*

* Corresponding author. Tel.: +34 876 241 666.

E-mail address: pedropesini@araclon.com (P. Pesini).

[1] These authors contributed equally to the work.

Abstract

Age-related cognitive dysfunction syndrome (CDS) has been reported in dogs and it is considered a natural model of Alzheimer's disease. Changes in spontaneous activity, including locomotor and exploratory behaviour, and social responsiveness, have been related to the age and cognitive status of kennel-reared beagle dogs. The aim of this study was to assess the influence of age and severity of CDS on locomotor and exploratory behaviour of privately-owned dogs. This is the first part of a two-part report on spontaneous activity in pet dogs. An open-field (OF) test and a curiosity test were administered at baseline and 6 months later to young (1-4 years, $n = 9$), middle-aged (5-8 years, $n = 9$), cognitively unimpaired aged (≥ 9 years, $n = 31$), and cognitively impaired aged (≥ 9 years, $n = 36$) animals. Classification of cognitive status was carried out using an owner-based observational questionnaire, and in the cognitively impaired group, dogs were categorised as having either mild or severe cognitive impairment. Dogs were recorded during sessions in the testing room and the video-recordings were subsequently analysed.

The severity of CDS but not age influenced locomotion and exploratory behaviour so that the more severe the impairment, the higher the locomotor activity and frequency of corner-directed (aimless) behaviours, and the lower the frequency of door-aimed activities. Curiosity directed toward novel stimuli exhibited an age-dependent decline although severely affected animals displayed more sniffing episodes directed towards the objects than the rest of aged animals. OF activity did not change after 6 months. Testing aged pet dogs on spontaneous behaviour may help to better characterise cognitively affected individuals.

Keywords: Canine; Aging; Cognitive dysfunction; Spontaneous activity; Open-field

Introduction

Dogs may naturally develop neuropathological and cognitive signs that parallel those seen in normal human aging and early Alzheimer's disease (AD) (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). Age-related cognitive deficits on learning, memory, executive and visuospatial function have been extensively studied in a systematic and controlled manner in laboratory beagles through a number of standard cognitive tasks (Adams et al., 2000; Tapp and Siwak, 2006; Cotman and Head, 2008). Interestingly, other aspects of behaviour seem to be affected by age. Such behaviours are referred to as spontaneous activity, including locomotion, exploratory behaviour, and social responsiveness (Tapp and Siwak, 2006). Open-field (OF) activity investigation has been used to study these spontaneous behaviours during aging in kennel-housed dogs, mostly beagles (Head et al., 1997; Siwak et al., 2001).

In Veterinary medicine, the age-related cognitive decline in pets is referred to as cognitive dysfunction syndrome (CDS), and could affect more than 22% of the canine geriatric population (Neilson et al., 2001; Azkona et al., 2009). Diagnosis of CDS is made by pathological assessment once other medical and behavioural causes are ruled out (Landsberg and Araujo, 2005; Landsberg et al., 2011). Owner-based observational questionnaires are very useful to check behavioural and cognitive deficits, but the need for more accurate and objective diagnostic procedures in clinical settings has been recognised (Head et al., 2008). Testing dogs may help to better characterise affected individuals, and also help in the monitoring of the disease. To date there are no published data on spontaneous activity tests in client-owned dogs.

This is the first part of a two-part report on spontaneous activity in pet dogs. The aim of this study (Part 1) was to assess the effect of age and severity of cognitive dysfunction on the locomotor and exploratory behaviour in dogs. To this end, an OF and a curiosity test were administered at baseline and 6 months later to privately-owned dogs varying in age and cognitive status. We hypothesised that locomotor and exploratory behaviour would be related to age as well as to the severity of cognitive dysfunction in our dog population.

Materials and methods

Subjects

Two veterinary teaching hospitals (Universidad de Zaragoza and Universidad de Santiago de Compostela, Spain) contributed to the collection of cases used in this study. Dogs were all small to medium-sized, living with their owners (i.e., pets) and not referred to behavioural consultants at the time of admission. Prior to inclusion in the study, all dogs were screened by a routine physical and neurological examination, complete blood count, serum biochemistry, thyroid hormone measurement, and urinalysis when needed. Animals with primary organ system failure (other than brain degeneration), hypothyroidism, untreated Cushing's syndrome and seriously affected mobility were excluded from the study. Animals with severe loss of visual capacity were also excluded.

Eighty-five animals were initially enrolled in the study (baseline period) and followed up after 6 months. The classification of cognitive status was carried out using an owner-based observational questionnaire (Table 1). The owners of the aged dogs were asked to compare the dog's present behavior to its behavior prior to 9 years of age, when the dog was a younger adult. After matching the affected items (Yes or No answer), the owner was asked to grade the severity of the impairment for each category using a five-point scale (0=non-impaired; 4=severely impaired). A dog was considered cognitively impaired when two or more categories were impaired and the total dysfunction score (TDS, the sum of scores attributed by the owner to each of the four categories) was ≥2 points. A score of 2 to 5 points was described as mild cognitive impairment (mCI) and ≥6 points as severe cognitive impairment (sCI).

A study on the plasma Aβ levels in this cohort of dogs has been recently published (González-Martínez et al., 2011). The subjects were sorted into (1) young (YG, 1-4 years, n = 9), (2) middle-aged (MA, 5-8 years, n = 9), (3) cognitively unimpaired aged (CU, ≥9 years, n = 31), and (4) cognitively impaired aged (CI, ≥ 9 years, n = 36). This last group was further subdivided into mild cognitively impaired (mCI, n = 20) and severe cognitively impaired (sCI, n = 16) animals (see above). 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 rooms in both participating universities were 2.07 x 2.76 m in size. The floor was marked into 12 squares 69 x 69 cm with black electrical tape to assist in localising the animal's position. Prior to each test session, the floor was thoroughly cleaned with a commercial enzymatic detergent solution for hygienic purposes and to prevent a behavioural response to the odor of other dogs.

Four spontaneous activity tests were conducted at both baseline and follow-up periods in the following sequence: OF test, human interaction test, curiosity test, and mirror test. All tests were 3 min in duration and there was a 5-min interval between tests, during which the dog was returned to the owner. In this first part (Part 1) of the work only the OF test and the curiosity test are described. The remaining tests are described in a companion paper (Part 2). The same person at each university conducted both tests. A modified version of testing procedures conducted by Siwak et al. (2001) in beagle dogs was used. A description of testing procedures is stated below.

OF test - The animal was gently pushed to enter the testing room and the locomotor and exploratory behaviour in the absence of any stimuli was examined.

Curiosity test - Three distinct objects (a red Kong®, a yellow rubber ice tray and a plush-rattle ostrich) were placed in fixed positions in the central area of the testing room. The objects were cleaned with a detergent solution before each session. This test was conducted to assess the reaction of each dog to novel objects.

Dogs were continuously recorded during sessions with a lightweight video-camera mounted above the testing room that enabled a clear view of the dog's behaviour during the tests. Video recordings were subsequently analysed by two observers (BR and AG-M). Inter-observer reliabilities for the analysed measures expressed as an intra-class correlation coefficient ranged between 0.8 and 1.0 for both consistency and agreement assessment. 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 for duration and frequency of occurrence are described in Table 2. These measures were a selection of a larger number of analysed behavioural measures. Thus, a broad list of behavioural measures was initially depicted based on preliminary observations (ad libitum sampling) and previous studies in the field (Head and Milgran, 1992; Head et al., 1997; Siwak et al., 2001). Data reduction was based on distinct criteria and was performed in consecutive steps. First, we rejected those variables for which the frequency of occurrence was too low to be statistically analysed (e.g., elimination, stereotypical and other-scratching behaviours). Second, we explored data to detect those measures with more potential as explanatory variables, as noted by a higher number of correlations with the rest of variables across tests and groups (Spearman's rank correlation test). When two variables were consistently correlated, we selected the more easily measurable data. We summed simple structural measures not consistently correlated to create functional measures. This was the case for vocalisations, door-directed behaviours (door-DB) and corner-directed behaviours (corner-DB).

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

Demographic information for each group at baseline is shown in Table 3. Non-significant differences were found for sex, reproductive status, and weight or body condition among groups. Aged groups (i.e., CU and CI) did not differ significantly in age.

The frequency of behavioural measures in each test within the general study groups as well as in the aged groups is summarised in Tables 4a and 4b, respectively, whereas duration results are shown in Tables 5a and 5b. With a few exceptions, which are noted for the curiosity test, we found no significant differences between results at baseline and the follow-up across groups and tests. Considering this, data from both periods were analysed jointly. A description of the main features follows below.

OF test

Younger dogs (YG and MA) vocalised more than aged animals (CU and CI) (Table 4a). The total time spent in the door area did not significantly vary as a function of age in cognitively intact dogs (YG, MA and CU), but the average time in this area was higher in YG than in CU dogs (Table 5a). However, among the aged dogs, those that were cognitively-impaired (CI) spent less time (total and average) at the door area than the younger groups, and showed less door-DB and more corner-DB than the rest of the groups (Table 4a, 5a). When the severity of cognitive impairment was taken into account, it was observed that sCI animals showed less door-DB and more corner-DB than the CU group (Fig. 1). In addition, they showed less vocalisation than their healthy counterparts. Furthermore, each time that sCI dogs entered the door area they spent less time in this area than the rest of groups and displayed the highest LA, in parallel with a shorter amount of time spent in immobility (Tables 4b, 5b; Fig. 3).

Curiosity test

Despite introducing objects in the testing room, dogs suffering from sCI still showed more LA than the mCI and CU animals, as occurred in the OF test (Table 4b and Fig. 3). In addition, the sCI dogsspent less average time in the door area as well as less time in immobility than the mCI and CU groups (Table 5b). With regard to specific measures for this test, YG animals showed more playing episodes, more sniffing episodes directed towards the objects (Fig. 3), and consequently a higher LA into the central area where the objects were placed, compared to the rest of the study groups (Table 4a). Interestingly, sCI dogs displayed more sniffing episodes directed towards the objects than the rest of geriatric groups (Table 4b). In this test, the frequency of corner-DB in sCI dogs was higher than that seen in mCI and CU groups, whereas the frequency of door-DB was lower than that seen in the CU group.

With respect to the OF test, the curiosity test demonstrated a decrease in the frequency of vocalisations ($P < 0.05$) and door-DB ($P < 0.01$) only in YG animals. The

MA group increased the amount of time spent in immobility ($P < 0.05$), and the aged groups even increased the average time spent in the door area (CU, $P < 0.05$; CI, $P < 0.001$), yet decreased their general LA ($P < 0.001$). Further, the time spent in the door area increased in both mCI and sCI animals ($P < 0.001$). With respect to the OF condition, the LA into the central area of the testing room, where the objects were placed, increased significantly in all cognitively-intact groups (YG, $P < 0.001$; MA and CU, $P < 0.05$) but not in the CI groups (mCI and sCI) with respect to the OF test conditions. In addition, the frequency of corner-DB decreased in all groups (YG, CU and mCI, $P < 0.001$; MA, $P < 0.05$), except for the sCI animals.

Compared to the baseline period, at follow-up, we found that mCI animals showed an increase in the average time spent in the door area and a decrease in the immobility position ($P < 0.05$), whereas sCI animals showed an increase in the total time spent in the door area ($P < 0.01$) and in the frequency of corner-DB ($P < 0.05$).

Discussion

An OF test and a curiosity test were administered to 85 dogs varying in age and cognitive status in order to explore their locomotor and exploratory behaviour (results on vocalisations are discussed in the companion paper, Part 2). Each test was 3 min in duration, whereas previous OF studies in dogs used sessions lasting 10 min (Head and Milgran, 1992; Head et al., 1997; Siwak et al., 2001). The present results suggest that short sessions would better yield valid behavioural measures and would probably reduce biased results related to individual variations in temporal activity patterns, as previously shown in some species of rodents (Montiglio et al., 2010).

Independent of the test administered, the cognitively intact animals (YG, MA and CU), showed no differences in LA. However, CI dogs showed higher LA in the OF test than the rest of the groups. Interestingly, when the severity of cognitive impairment was considered, sCI showed the highest LA in both tests. These results are consistent with previous studies in kennel-reared beagles in which their locomotion was affected by cognitive status, not age (Head et al., 1997; Siwak et al., 2001; Siwak et al., 2003). Similarly, increased activity in the OF test has also been reported in mouse models of AD (Pietropaolo et al., 2008; Filali et al., 2011). Furthermore, an increase in aimless walking is frequently observed in human dementia. This apparent non-goal-directed locomotion is referred to as wandering, and its prevalence is thought to be greater in AD than in vascular dementia (Lai and Arthur, 2003). Increased walking has been suggested to be related to a dysfunction in the behavioural control mechanisms in the prefrontal cortical-striatal-pallidal circuitry (Siwak et al., 2001).

Exploratory behaviour partially depends on motor and spatial capabilities, and on the motivation to explore (Caston et al., 1998). In this study, the behavioural activities displayed in certain areas of the testing room and the time spent in them were studied as measures of locomotor or exploratory behaviour. The door area was considered relevant since it was the key spatial location in the room to study the animal's response to social isolation forced by the test conditions. On the other hand, corner-DB (i.e., sniffing or standing against corner zones) may be an aimless activity. In this study, we found that in cognitively intact dogs, the age of the animal did not affect the time it spent in the door area, independent of the test performed. This suggests that CU dogs were as spatially oriented as younger dogs. However, in the OF test, dogs suffering from sCI spent less time in the door area than their healthy counterparts. In addition, regardless the test, each time these severely affected dogs visited this area, they spent less time there than the CU and the mCI animals. Moreover, sCI animals showed the lowest frequency of door-DB (i.e., jumping, rearing, door scratching or sniffing) and the highest frequency of corner-DB. Taken together, these findings suggest a degree of disorientation in CI dogs, especially in those severely impaired. Furthermore, the scarcity of door-DB in CI dogs could also be accounted for, at least partially, by an influence of the cognitive dysfunction on the attachment behavioural system (Topál et al., 1998).

Tests involving novel discrete stimuli, such as in the curiosity test, may provide more sensitive measures of exploratory behaviour (Siwak et al., 2001). This test showed that YG animals displayed the highest frequency of playing and sniffing episodes directed towards the objects, which was subsequently accompanied with a higher number of times entering the central area than the rest of the groups. Similar to our results, Siwak et al. (2001) observed that younger beagles spent a longer time in contact with novel toys than impaired aged beagles, but they did not find inter-group differences in the sniffing of the toys. A decline with age in exploratory behaviour toward novel stimuli has also been found in rodents (Furchtgott et al., 1961; Brennan et al., 1981; Willig et al., 1987; Soffie et al., 1992).

Interestingly, we found that sCI dogs showed more sniffing episodes directed towards the objects than the rest of the geriatric groups. In addition, these sCI dogs maintained the frequency of corner-DB during the curiosity test compared to the OF test, while these behaviours decreased in all the other groups. This phenomenon, observed in the sCI dogs showing a high frequency of sniffing behaviour directed towards the room, the objects or the corners while maintaining a high LA, could be described as microstereotypies in the same sense used by O'Keefe and Nedal (1978), when they refer to repetitive nosing and sniffing behaviours in rodents with hippocampal lesions.

Conclusions

This work shows that locomotor and exploratory behaviour varies as a function of age and the animal’s cognitive status. In particular, we found an effect of the severity of CDS on the LA and exploratory behaviour directed to specific areas of the environment, so that the more severe the impairment, the higher the LA and frequency of corner-DB, and the lower the frequency of door-related activities. These changes in spontaneous activity resemble some aspects of human dementia reinforcing canine CDS as a natural model of AD. On the other hand, we observed an effect of age in exploratory behaviour directed to novel objects with curiosity exhibiting an age-dependent decline. Testing aged pet dogs on spontaneous behaviour may help to better characterise cognitively affected individuals.

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

SDC observational questionnaire

Category	**Items**
Sleep-wake cycle	
	Walking/Pacing at night
	Vocalizing (barking/whining) at night
	Sleeping less at night
	Sleeping noticeably more during the day
	Switching between insomnia and hypersomnia
Socio-environmental interactions	
	Decrease in greeting owners
	Decrease in soliciting attention from the owners
	Increase in following the owners around the house
	Decrease in playing with the owners
	Decrease in playing with other dogs
	Changes in personality (irritability, new fears, lack of interest on stimuli)
House-training and commands	
	Starting urinate/defecate in the house
	Decrease in signalling to go out for eliminating
	Decrease in urine marking (non-castrated males)
	Decrease in responding to prior learned commands
Disorientation	
	Staring into space (star gazing) or getting stuck
	Getting lost in the house or during routine walks
	Wandering (aimless walking) in the house
	Trying to pass through narrow places
	Standing at the wrong side of the door to go out
	Difficulty in navigating around or over obstacles
	Decrease in recognizing familiar people

Table 2

Behavioural measures in the OF and the curiosity tests

Behavioural measure	Description
Vocalisations[a]	Total number of episodes of barking, whining or yelping. Individual barks whereas bursts of whine/yelps were considered each as discrete episodes.
Total time in the door area[a]	Total time (s) spent in the door´s squares.
Average time in the door area[a]	Average time (s per occurrence) spent in the door´s squares each time the animal enters this area.
Locomotor activity (LA) [a]	Total number of squares entered. Entering a new square was considered when both forelimbs did it.
LA into the central area[a]	Proportion (%) of LA displayed at the central squares.
Immobility[a]	Total time (s) spent sitting or lying in the absence of all other measures except vocalisations.
Door-DB [a]	Total number of episodes of door scratching, door sniffing, jumping, rearing or freezing.
Corner-DB [a]	Total number of episodes of sniffing or standing against the corners of the room.
Playing[b]	Total number of play related behaviours directed towards the objects, including pushing, biting, mouthing, throwing, etc. Tripping over the objects while walking was not considered a play episode.
Sniffing the objects[b]	Total number of episodes in which the dog moves the nose or exhibits clear sniffing movements over the objects.

[a]OF measures; [b]additional measures in the curiosity test. DB: directed behaviours.

Table 3

Demographic data in the studied canine population.

Group		Males (%)	Females (%)	Neutered (%)	Age (months)	Weight (kg)	Body condition score (1-5)[†]
					Mean ± SD	Mean ± SD	Mean ± SD
YG	(*n*=9)	55.6	44.4	33.3	31.1 ± 17.7	11.8 ± 5.7	3.2 ± 0.4
MA	(*n*=9)	33.3	66.7	33.3	81.2 ± 16.7	13.2 ± 11.1	3.2 ± 0.4
CU	(*n*=31)	48.4	51.6	41.9	146.4 ± 35.2	12.2 ± 7.7	3.5 ± 0.8
CI	(*n*=36)	61.1	38.9	44.4	153.2 ± 25.5	15.0 ± 9.3	3.4 ± 0.6
		P= 0.45[a]		*P*= 0.89[a]	*P*= 0.14[b]	*P*= 0.59[c]	*P*= 0.52[a]

YG: young; MA: middle-aged; CU: cognitively unimpaired aged; CI: cognitively impaired aged.

[†]Body condition scale: 1(too thin) - 3(thin) - 3(ideal) - 4(heavy) - 5(too heavy).

[a] Non significant difference among groups (Chi-square test).

[b] Non significant difference between CU and CI groups (Mann Whitney U test).

[c] Non significant difference among groups (Kruskal Wallis test).

Table 4a

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)	OF	$35.4 \pm 38.0^{CU,CI}$	$18.4 \pm 13.5^{CU,CI}$	$10.8 \pm 14.7^{YG,MA,ci}$	$11.2 \pm 29.3^{YG,MA,cu}$
	Curiosity	19.7 ± 22.5^{CI}	24.3 ± 29.0^{CI}	16.2 ± 20.1^{CI}	$12.6 \pm 30.2^{YG,MA,CU}$
LA (number of squares crossed)	OF	25.4 ± 20.5^{ci}	21.8 ± 19.8^{ci}	28.0 ± 22.3^{CI}	$44.8 \pm 34.1^{yg,ma,CU}$
	Curiosity	19.3 ± 14.6	16.2 ± 15.6	20.1 ± 21.1	30.7 ± 31.7
LA into central area (% of LA)	OF	21.0 ± 10.7	19.5 ± 15.3	20.0 ± 14.1	18.3 ± 12.1
	Curiosity	$44.6 \pm 11.0^{ma,CU,CI}$	29.0 ± 14.4^{yg}	25.8 ± 20.1^{YG}	22.2 ± 16.1^{YG}
Door-DB (times)	OF	9.7 ± 7.7^{ci}	11.1 ± 8.0^{ci}	7.9 ± 6.5^{ci}	$5.4 \pm 5.5^{yg,ma,cu}$
	Curiosity	3.6 ± 3.0^{ma}	$9.5 \pm 8.3^{yg,ci}$	7.6 ± 8.1^{ci}	$6.3 \pm 10.2^{ma,cu}$
Corner-DB (times)	OF	1.2 ± 1.5^{ci}	0.7 ± 1.0^{CI}	1.2 ± 2.2^{CI}	$4.1 \pm 5.9^{yg,MA,CU}$
	Curiosity	0.1 ± 0.2^{CI}	0.2 ± 0.5^{CI}	0.5 ± 1.2^{CI}	$2.1 \pm 3.8^{YG,MA,CU}$
Sniffing the objects (times)	Curiosity	$4.3 \pm 3.0^{ma,CU,CI}$	2.2 ± 1.5^{yg}	1.9 ± 2.0^{YG}	2.0 ± 2.0^{YG}

YG: young; MA: middle-aged; CU: cognitively unimpaired aged; CI: cognitively impaired aged.

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

Table 4b

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)	OF	10.8 ± 14.7^{S}	16.6 ± 37.2	3.9 ± 8.6^{CU}
	Curiosity	16.2 ± 20.1^{S}	20.0 ± 38.2^{s}	$2.5 \pm 4.7^{CU,m}$
LA (number of squares crossed)	OF	28.0 ± 22.3^{S}	36.1 ± 31.8^{s}	$56.7 \pm 34.2^{CU,m}$
	Curiosity	20.1 ± 21.1^{S}	23.4 ± 29.6^{s}	$40.6 \pm 32.4^{CU,m}$
LA into central area (% of LA)	OF	20.0 ± 14.1	17.2 ± 13.8	19.7 ± 9.5
	Curiosity	25.8 ± 20.1	21.3 ± 14.8	23.5 ± 18.1
Door-DB (times)	OF	7.9 ± 6.5^{s}	6.2 ± 5.9	4.4 ± 4.8^{cu}
	Curiosity	7.6 ± 8.1^{s}	7.3 ± 11.7	4.9 ± 8.0^{cu}
Corner-DB (times)	OF	$1.2 \pm 2.2^{M,S}$	3.9 ± 6.6^{CU}	4.3 ± 5.0^{CU}
	Curiosity	0.5 ± 1.2^{S}	1.3 ± 3.0^{s}	$3.2 \pm 4.5^{CU,m}$
Sniffing the objects (times)	Curiosity	1.9 ± 2.0^{s}	1.5 ± 1.7^{s}	$2.7 \pm 2.3^{cu,m}$

CU: cognitively unimpaired; mCI: mild cognitive impairment; sCI: severe cognitive impairment.

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 5a

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)	OF	115.6 ± 47.6$^{\text{CI}}$	110.2 ± 54.4$^{\text{CI}}$	82.8 ± 58.7	61.5 ± 47.0$^{\text{YG,MA}}$
	Curiosity	97.7 ± 63.9	118.3 ± 62.4	94.3 ± 61.5	88.5 ± 59.7
Average time in the door area (s/occurrence)	OF	33.0 ± 43.4$^{\text{cu,CI}}$	44.0 ± 62.0$^{\text{CI}}$	24.0 ± 40.6$^{\text{yg,ci}}$	11.9 ± 5.9$^{\text{YG,MA,cu}}$
	Curiosity	35.8 ± 44.9	33.9 ± 41.6	27.7 ± 36.3	31.0 ± 48.8
Immobility (s)	OF	75.7 ± 60.1$^{\text{ci}}$	58.4 ± 68.2	68.0 ± 62.7$^{\text{CI}}$	38.9 ± 50.7$^{\text{yg,CU}}$
	Curiosity	86.7 ± 71.0	82.3 ± 61.3	74.2 ± 61.9	54.1 ± 61.3

YG: young; MA: middle-aged; CU: cognitively unimpaired aged; CI: cognitively impaired aged.

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

Table 5b

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)	OF	82.8 ± 58.7^{s}	70.7 ± 51.7	49.0 ± 37.3^{cu}
	Curiosity	94.3 ± 61.5	97.0 ± 61.6	77.0 ± 56.3
Average time in the door area (s/occurrence)	OF	24.0 ± 40.6^{S}	16.1 ± 32.5^{s}	6.2 ± 10.0^{CU,m}
	Curiosity	27.7 ± 36.3^{s}	40.3 ± 54.8^{s}	18.2 ± 36.6^{cu,m}
Immobility (s)	OF	68.0 ± 62.7^{S}	50.0 ± 52.6^{s}	23.8 ± 44.8^{CU,m}
	Curiosity	74.2 ± 61.9^{s}	64.2 ± 59.3^{s}	40.3 ± 62.5^{cu,m}

CU: cognitively unimpaired; mCI: mild cognitive impairment; sCI: severe cognitive impairment.

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. Box and whisker plots (SE) for the frequency of door- and corner-DB in the aged groups during the OF 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 CU group. *, ** or *** represent $P < 0.05$, $P < 0.01$ or $P \leq 0.001$, respectively.

Fig. 2. Box and whisker plots (SE) for locomotor activity (i.e., number of squares crossed) in the aged groups during the OF and the curiosity 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.

Fig. 3. Box and whisker plots (SE) for the frequency of playing and sniffing episodes directed towards the objects during the curiosity 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 $P < 0.05$ or $P \leq 0.001$, respectively.