Effect of age and severity of cognitive dysfunction on two simple tasks in pet dogs

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Abstract

Dogs exhibit age-dependent losses in learning and memory as well as a progressive accumulation of neuropathology that parallels that which has been observed in normal human aging and early Alzheimer's disease. These deficits have been extensively studied using a number of standard cognitive tasks in the laboratory; however, appropriate tools for their assessment in veterinary clinics are still lacking. The aim of this study was to evaluate the effect of age and the severity of cognitive dysfunction syndrome (CDS) on two simple tests conducted in a clinical setting. Thus, a food searching (FS) task and a problem-solving (PS) task were administered to young (1-4 years, $n = 9$), middle-aged (5-8 years, $n = 10$), cognitively unimpaired aged (≥ 9 years, $n = 31$), and cognitively impaired aged (≥ 9 years, $n = 37$) animals. Classification of cognitive status was carried out using an owner-based questionnaire, and in the impaired group, dogs were categorized as having either mild or severe CDS.

During the FS task, younger dogs (< 9 years) were able to locate the food quicker and with more success than the aged groups (≥ 9 years). Moreover, dogs with severe CDS exhibited the poorest performance compared to those suffering from mild CDS or their healthy counterparts. In the PS task, the younger dogs performed better than the aged dogs in getting the food, but there were no differences as a function of CDS severity. The FS task may help to better characterize cognitively affected dogs in the clinical setting. The use of this or similar tasks will require further investigations in the field.

Keywords: Dog; Aging; Cognitive dysfunction; Memory; Learning

Introduction

Age-related cognitive deficits in learning and memory have been extensively studied in a systematic and controlled manner in laboratory beagles utilizing a number of standard cognitive tasks (Adams et al., 2000; Tapp and Siwak, 2006). Different categories of aging beagles have been identified on the basis of neuropsychological test performance, and these categories have been proposed to model different subgroups of aged humans, including normal aging, mild cognitive impairment and early Alzheimer's disease (AD) (Adams et al., 2000; Cotman and Head, 2008). This cognitive decline in aged canines may have links to several types of progressive accumulation of neuropathology that also parallels that which has been observed in normal human aging and dementia. For instance, there is evidence of β-amyloid (Aβ) deposits and amyloid angiopathy in the brains of aged dogs (Borras et al., 1999; Uchida et al., 1992). Interestingly, we have recently found changes in plasma Aβ levels during pathological aging in companion dogs that exhibit patterns similar to those previously reported for human mild cognitive impairment and AD (González-Martínez et al., 2011). Other features of AD, such as neurodegeneration and oxidative damage, have also been reported in aged dogs (Bernedo et al., 2009; Colle et al., 2000; Head et al., 2002; Insua et al., 2010; Pugliese et al., 2006; Rofina et al., 2006).

Clinically, 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). This syndrome includes a number of behavioural signs such as changes in sleep-wake cycle, decreased social interaction, loss of prior housetraining, disorientation and changes in the level of activity (Ruehl and Hart, 1998; Landsberg et al., 2003). To determine whether a dog might be showing

CDS signs, veterinarians must rely almost entirely on owner-reported history (Landsberg and Araujo, 2005). The abovementioned neuropsychological tests are labour intensive, requiring previous training for the dog and the tester, and are therefore nearly impossible to perform outside of a laboratory setting. Although owner-based observational questionnaires are very useful for checking behavioural deficits, the need for more objective diagnostic procedures in clinical settings has been already stressed (Head et al., 2008; Salvin et al., 2011a).

The aim of this study was to assess the effect of age and the severity of cognitive dysfunction on two simple tasks administered in the clinical setting in pet dogs. We hypothesized that test performance would be related to age as well as to the severity of cognitive dysfunction in our cohort of dogs.

Material and methods

Subjects

Two veterinary teaching hospitals (Universidad de Zaragoza and Universidad de Santiago de Compostela, Spain) contributed to the recruitment of cases used in this study. Dogs were all small to medium-sized, living with their owners (i.e., pets) and had not been 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-seven animals were enrolled in the study. These animals had been previously tested in four 3-min spontaneous activity tests (Rosado et al., 2012a and 2012b). Furthermore, a study on the plasma Aβ levels in this cohort of dogs has been recently published (González-Martínez et al., 2011). The classification of cognitive status was based on a "dysfunction score" obtained from an owner-based observational questionnaire (González-Martínez et al., 2011; Rosado et al., 2012a). The subjects were sorted into - young (YG, 1-4 years, *n*=9); middle-aged (MA, 5-8 years, *n*=10); cognitively unimpaired aged (CU, ≥ 9 years, *n*=31); and cognitively impaired aged (CI, ≥ 9 years, *n*=37). The last group was further subdivided into mild cognitively impaired (mCI, *n*=20) and severe cognitively impaired (sCI, *n*=17) animals. The abbreviation YM was used to refer jointly to YG and MA individuals.

Distribution of breeds within each group was as follows: American Cocker Spaniel (YM *n*=1), Beagle (YM *n*=1), Bloodhound (mCI *n*=1), Bobtail (sCI *n*=1), Border Collie (YM *n*=1), Boston Terrier (YM *n*=1), Dalmatian (CU *n*=1), English Bulldog (YM *n*=1, sCI *n*=1), English Setter (mCI *n*=1), English Cocker Spaniel (YM *n*=1, CU *n*=3, mCI *n*=3, sCI *n*=3), Chihuahua (CU *n*=1, sCI *n*=1), Fox Terrier (sCI *n*=1), French Bulldog (YM *n*=2), German Shepherd (mCI *n*=1), Labrador Retriever (sCI *n*=1), mixed (small-medium) (YM *n*=8, CU *n*=13, mCI *n*=8, sCI *n*=2), Pomeranian (YM *n*=1), Poodle (sCI *n*=1), Samoyed (CU *n*=1, sCI *n*=1), Scottish Terrier (mCI *n*=1), Schnauzer (CU *n*=2, mCI *n*=2), Shih-Tzu (sCI *n*=1), Siberian Husky (CU *n*=2), Spaniel Breton (sCI *n*=1), Spitz (CU *n*=4), Teckel (CU *n*=1), West Highland White Terrier (YM *n*=3, CU *n*=1, sCI *n*=1), Yorkshire Terrier (CU *n*=4, sCI *n*=2).

Animals were treated according to 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

Tests were carried out in a regular room (approximately 6.5 x 4.5 m) at each veterinary teaching hospital. Both testing rooms contained furniture that did not hinder the movement of the animals. The same person at each university conducted both tests. Animals were tested after a fasting period of 10 h. The design of these tests was based on previously published although not peer-reviewed and non-validated tests (Coren, 1994). A description of testing procedures is stated below.

Food searching (FS) task

The aim of this task was to test the dog's ability to search and locate food.

The owner was instructed to have the dog sit in the centre of the room while on a leash. The tester was placed in front of the dog (approximately 60 cm), and the tester showed the dog three small cube-like pieces of food (ham). The tester moved backward, shaking the hand containing the food while communicating and maintaining visual contact with the dog, and placed the food in a corner of the room. Once there, the tester stared and pointed at the food for a few seconds (2-3 s) to increase the visual processing time for the dog. Then, the owner was asked to leave the room with the dog on a leash and to wait outside for 15 s. Afterward, they returned to the room, and the dog was placed without a leash in the centre of the room, allowing him/her to freely explore the room for 1 min. Neither verbal cues nor gestures (e.g., pointing at) noting the location of food were intentionally displayed by the owner or the tester. In addition, once in the centre of the room, the dog was placed sitting with his/her back to the owner and the tester, minimizing the possible influence of human cues.

The previous procedure was conducted a maximum of three times. When a dog successfully completed one trial (i.e., found the food), then the test was concluded. Otherwise the test was subsequently repeated. In each trial, the behaviour of the dog was classified and scored as follows - the dog goes directly towards the food (1 point); the dog searches the food and finds it within a maximum of 1 min (2 points); the dog searches the food but does not find it within a maximum of 1 min (3 points); the dog does not make any attempt to search the food (4 points). The FS total score was the sum of the points obtained during the trials and ranged from 1-12 points.

Problem-solving (PS) task

The aim of this test was to assess the ability of an animal to obtain food by manipulating an object.

The tester showed the dog three pieces of food (ham) and allowed the dog to sniff and lick the hand that held the food. Then, the food was placed on the floor and covered with a lidless, translucent plastic box turned upside down. The dog was given a 2 min period to remove the box so the food could be eaten. During the task, the owner was urged to encourage the dog to search for the food and to point to the box.

This test was performed only once, and the behaviour of the dog was classified and scored as follows - the dog tries to get the food and obtains it within a maximum of

2 min (1 point); the dog tries to get the food but does not obtain it within a maximum of 2 min (2 points); the dog sniffs the box but does not try to get the food (3 points); and the dog does not make any attempt to get the food (4 points).

Statistical analysis

Distribution of qualitative variables was assessed by Chi-square test or Fisher's exact test when the expected frequencies were less than or equal to 5, respectively. Average differences in the FS total score (quantitative variable) were assessed by the Mann-Whitney *U* test. Correlations were calculated by the Spearman's rank correlation test. Calculations were carried out using the statistical program SPSS 17.0 for Windows (SPSS, Inc.), and $P < 0.05$ denoted statistical significance.

Results

We found no significant differences for any studied variable between the YG and MA groups, and therefore, they were analysed jointly. Table 1 shows demographic information for each group at baseline. No significant differences were found for sex, reproductive status, and weight or body condition among groups. Aged groups (CU and CI) did not differ significantly in age.

The percentages for the studied variables within the general study groups (YM, CU and CI) as well as in the aged groups (CU, mCI, sCI) are summarized in Figures 1-6. A description of main features for each test follows below.

Food searching task

In this test, YM differed from CU and CI animals in all of the studied variables (*P*<0.01). Thus, during the first trial, whereas all the YM dogs (*n* =19) searched the dropped food, approximately 23% of the CU dogs (*n*=7) and approximately 49% of the CI dogs (*n*=18) did not make any attempt to search for the dropped food. Furthermore, all but one YM animal (*n*=18, approximately 95%) found the food during the first trial, whereas less than 65% of CU (*n*=20) and 44% of CI dogs (*n*=16) found the food. Interestingly, most YM animals (*n*=16, approximately 84%) went directly towards the corner where the food was located once they were taken off of their leash, whereas this occurred in less than 32% of the CU dogs (*n*=10) and 22% of the CI dogs (*n*=8). The CU and CI groups exhibited the worst performance in this test as compared with the younger animals. This difference was reflected in the FS total score, which was significantly higher in the CU (4.29±4.25) and the CI (6.61±5.01) groups than in the YM group (1.32±0.95) ($P < 0.0001$). In general, the CI group displayed a poorer performance than the CU group. However, significant differences were only found between the sCI and the CU animals ($P<0.05$), while CU and mCI animals did not significantly differ for any tested variable. In particular, during the first trial, almost two-thirds of sCI dogs (*n*=11, approximately 65%) did not attempt to search for the food. In fact, only seven sCI dogs (approximately 41%) searched for the food across the three trials and only six dogs (approximately 35%) finally found it. This result contrasts with the rest of the aged dogs, where 70% of the mCI (*n*= 14) animals and approximately 81% of the CU (*n*= 25) animals successfully completed the test (i.e., found the food). The FS total score was significantly higher in sCI dogs (8.65±4.65) than in CU dogs (4.29±4.25; $P < 0.001$) and in mCI dogs (4.95±4.77; $P < 0.05$).

The FS total score showed a significant correlation with the total number of items affected in the questionnaire ($r = 0.443$; $P < 0.001$) as well as with the dysfunction score ($r = 0.427$; $P < 0.001$) obtained from the questionnaire across all dogs. Figures 7 and 8 represent scatter plots for these correlations. In the aged groups, only a weak correlation was found between the FS score and the ratio Aβ42/40 ($r=-0.25$; $P < 0.05$).

Problem-solving task

Again, during this test YM differed from CU and CI animals in all of the studied variables ($P<0.01$). All the YM animals searched for the food by manipulating the box, and most of them ($n=17$, approximately 89%) finally obtained it within a 2 min period. In contrast, slightly more than a half ($n=16$, approximately 52%) of CU dogs were able to locate the food within a 2 min period. These differences were even larger when compared to the CI group, where only approximately 35% of dogs found the food (mCI $n=7$ and sCI $n=6$). We found no differences among the aged groups for any studied variable.

Discussion

In this study, we tested 87 pet dogs, varying in age and cognitive status, on two simple tasks. Classification of cognitive status was carried out by means of an owner-based questionnaire, once ruled out other medical causes. This questionnaire is not validated, but its score has been previously shown to correlate with plasma Aß42 levels and Aß42/40 ratio values in the unimpaired and mildly-impaired aged groups (González-Martínez et al., 2011). Consistent behavioural profiles in spontaneous activity have been also reported in the same cohort of dogs considering the classification obtained from this questionnaire (Rosado et al., 2012a and 2012b).

It is important to stress that the two tests performed in the present study were conducted in a clinical setting unlike previously published studies on cognitive testing in beagle dogs, which were performed in a laboratory environment. These previous studies have shown age-related impairments in specific learning and memory measures, as well as in visuospatial and executive functions, through a number of standard cognitive tasks, which are systematically performed in a testing box (Adams et al., 2000; Tapp and Siwak, 2006; Cotman and Head, 2008). Considering the methodological differences in assessing cognitive function, the comparison of our results with those previously obtained in the laboratory should be carried out with caution.

The first test we administered involved a FS task used to measure the ability of dogs to remember the location of food placed in a corner of the room for a short period of time (approximately 15 s) across a maximum of three trials. We found clear age-related differences in the FS task performance, with younger dogs (<9 years) performing better than aged dogs (≥9 years) in terms of speed and success in locating the food after a short delay outside the room. Thus, all younger dogs found the food across trials (all but one during the first trial), and in most cases, this goal was attained by directly heading towards the corner where the food was placed. However, the higher FS scores in the aged groups indicate that this ability is compromised in the aged dogs, especially in those showing CDS signs. In particular, the sCI dogs achieved the highest

FS scores, denoting the great difficulty of these cognitively affected dogs in finding the food.

The previous results suggest that retention of spatial information regarding the food location (i.e., spatial episodic memory) was affected by age and cognitive status in the studied dogs. However, the FS task was not previously tested without the 15 s delay and, therefore, we cannot assure that dogs displayed the same behaviour without the delay. Considering this, it is possible that the difficulty in finding dropped food showed by the aged dogs during the FS task was a consequence of a more general age-related effect. Anyway, studies conducted in kennel-reared beagles have shown that deterioration in spatial ability occurs early in the aging process, between 6 and 7 years of age. Impairments in spatial ability are also a common feature of human aging and are more severe in neurodegenerative disorders (Tapp and Siwak, 2006; Cotman and Head, 2008). Clinically, however, the cognitive decline in pet dogs might not become apparent until the age of 11 years or older (Landsberg and Araujo, 2005). Nevertheless, the FS task administered in this study was capable of showing differences between younger dogs and those clinically categorized as "successful agers" (i.e., cognitively unimpaired older dogs, according to an owner-based observational questionnaire) regarding their ability to find some dropped food in the room. Notably, according to a recent survey on senior dogs, "difficulty in finding dropped food" might be a frequent behavioural change that accompanies "successful aging" (Salvin et al., 2011b).

Furthermore, this simple task was able to differentiate animals as a function of the severity of CDS. The results of this task, besides complementing data obtained from the owner-based questionnaire, may help to characterize the severity of canine CDS in aged pets. Unfortunately, this test was not sensitive enough to differentiate between successful agers and those suffering from mild CDS, which can be delineated in laboratory dogs that undergo more sophisticated spatial learning and memory tests (Tapp and Siwak, 2006; Cotman and Head, 2008). Furthermore, it is possible that the absence of differences between aged animals relates to the small number of animals in each group.

The second test we administered was a PS task in which the animal must manipulate (with the mouth or paws) a lid-free box covering pieces of food dropped on the floor. Thus, the PS task may reflect prior procedural learning ability (i.e., the process of acquisition of sensorimotor and perceptual skills) in obtaining food by manipulating objects. Performance on procedural learning tasks in beagle dogs has been shown to be profoundly affected by previous life experience, which suggests a complex relationship between age and this type of learning (Tapp and Siwak, 2006; Cotman and Head, 2008). In our study, all dogs were raised as pets, and they were therefore all expected to have had varied life experiences, including those that involved manipulating objects. We found that the PS task was age-sensitive, with younger dogs again performing better than the aged groups. Almost 90% of the younger dogs obtained the food within a 2 min period, while (approximately) only half of the unimpaired and one-third of the impaired aged animals were able to do the same.

The poorer performance of aged dogs may be due to a reduced interest in exploring novel objects. Interestingly, we have recently found an age-dependent decline in curiosity in the same cohort of dogs when using non-cognitive testing procedures (Rosado et al., 2012a). However, we did not find any significant difference among the aged groups on PS performance as a function of the severity of CDS. Similarly, a number of studies have found intact procedural motor-learning capacities in AD patients although their overall performance levels in terms of reaction and movement time never reached the levels of the healthy controls (van Halteren-van Tilborg et al., 2007).

All animals were medically assessed and the questionnaire included questions related to sensory and physical impairment in order to rule out severely affected animals (González-Martínez et al., 2011). However, we cannot rule out that some differences during the performance of both tasks among the aged animals could be related to mild sensory impairments. In addition, a simple lack of motivation on food could also have affected responses in aged animals.

Conclusions

This study shows that a simple food searching task performed in a clinical setting was sensitive to age and the severity of cognitive dysfunction in dogs. Younger dogs (< 9 years) were quicker and more successful in locating food than the aged groups (> 9 years). In particular, dogs exhibiting severe CDS displayed the poorest performance as compared with those suffering from mild CDS or their healthy counterparts. Furthermore, this work also shows an effect of age (but not of severity of cognitive dysfunction) on test performance in a problem-solving task, as younger animals were better able to obtain the food. We hope this study contributes to the development of new tools for CDS diagnosis in veterinary medicine. The use of these or similar tasks will require further investigation in the field. These investigations should be moreover performed due to the relevance of canine CDS as a model of AD.

Conflict of interest statement

P.P. and M.S. are employees at Araclon Biotech Ltd. B.R. and A.G.-M. are supported by grants from Araclon Biotech to the Universidad de Zaragoza and Universidad de Santiago de Compostela, respectively. G.S., M.-L.S., S.G.-B., J.P. and A.V. have no actual or potential conflict of interest.

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Table 1

Demographic data in the studied canine population

Group		Males (%)	Females (%)	Neutered (%)	Age (months; mean ± SD)	Weight (Kg; mean ± SD)	Body condition (1-5; mean ± SD)[†]
YM	(*n* = 19)	42.1	57.9	36.8	58.5 ± 31.6	12.3 ± 8.4	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
mCI	(*n* = 20)	55.0	45.0	45.0	148.7 ± 26.8	15.4 ± 9.4	3.6 ± 0.7
sCI	(*n* = 17)	64.7	35.3	41.2	157.24 ± 23.6	13.9 ± 9.4	3.2 ± 0.4
		$P = 0.57$[a]		$P = 0.98$[a]	$P = 0.19$[b]	$P = 0.58$[c]	$P = 0.15$[a]

YM, young and middle-aged; CU, cognitively unimpaired aged; mCI, mild cognitive impairment; sCI, severe cognitive impairment.

[†]Body condition scale (1-5) – 1, too thin; 2, thin; 3, ideal; 4, heavy; 5, too heavy

[a] No significant difference among groups (Chi-square test)

[b] No significant difference between CU, mCI and sCI groups (Kruskal-Wallis test)

[c] No significant difference among groups (Kruskal-Wallis test)

Figures legend

Figure 1

Distribution (percentage) of animals according to the Food searching (FS) task classification (first trial).

YM, young and middle-aged; CU, cognitively unimpaired aged; CI, cognitively impaired aged.

Figure 2

Distribution (percentage) of aged animals according to the Food searching (FS) task classification (first trial).

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

Figure 3

Distribution (percentage) of animals according to the number of trials to success (finding the food) during the Food searching (FS) task.

YM, young and middle-aged; CU, cognitively unimpaired aged; CI, cognitively impaired aged.

Figure 4

Distribution (percentage) of aged animals according to the number of trials to success (finding the food) during the Food searching (FS) task.

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

Figure 5

Distribution (percentage) of animals according to the Problem solving (PS) task classification.

YM, young and middle-aged; CU, cognitively unimpaired aged; CI, cognitively impaired aged.

Figure 6

Distribution (percentage) of aged animals according to the Problem solving (PS) task classification.

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

Figure 7

Scatter plot for correlation of the Food searching (FS) total score and the total number of items affected in the questionnaire.

Figure 8

Scatter plot for correlation of the Food searching (FS) total score and the questionnaire´s dysfunction score.