
Peer reviewed version
License (if available): CC BY-NC-ND
Link to published version (if available): 10.1016/j.applanim.2020.105179
Link to publication record in Explore Bristol Research
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Elsevier at https://doi.org/10.1016/j.applanim.2020.105179. Please refer to any applicable terms of use of the publisher.

**University of Bristol - Explore Bristol Research**

**General rights**

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/
Two assays of working memory in companion dogs: the holeboard and disappearing object tasks

Melissa Smith, Joanna C. Murrell¹, Michael Mendl*

Bristol Veterinary School, University of Bristol, Langford House, Langford, BS40 5DU, UK

¹ Present address: Highcroft Veterinary Referrals, 615 Wells Rd, Whitchurch, Bristol, BS14 9BE, UK

* Corresponding author: mike.mendl@bris.ac.uk
ABSTRACT

Variation in executive function and age-related cognitive decline may underlie the emergence of behaviour and welfare problems in dogs. A better understanding of such links, and of dog cognition in general, will be facilitated by the development of cognitive tasks that can be readily implemented, including with publicly-owned dogs that are available for relatively short testing periods. Working memory is a key component of cognitive and executive function that is often measured using tests such as delayed-non-match-to-sample or radial-arm maze which require extensive training and testing. Here we successfully adapt the Holeboard Task to measure working and reference memory in dogs, and show that another test of working memory, the Disappearing Object Task, can be performed in a single day. Working memory (p=0.002) and reference memory (p<0.001) scores in a 16-hole Holeboard Task (four holes baited) increased across sessions, with reference memory scores falling steeply as expected when the configuration of baited buckets changed. In the Disappearing Object Task dogs were able to successfully locate an object displaced behind one of four visual barriers, and their ability to do this fell as the memory retention interval (0s, 30s, 60s, 120s, 240s) between hiding and locating the object increased (p<0.001). Holeboard and Disappearing Object working memory measures were not correlated, possibly due to differences in the motivational context and exact learning demands of the tasks. In summary we show that the Holeboard Task can be adapted for use in dogs and that the Disappearing Object Task can be implemented in a single day. The latter task may be particularly useful for working memory studies of dogs owned by the public where prolonged access is often infeasible, and the three-day Holeboard Task is faster to implement than other commonly used laboratory-based tasks.

Keywords

Working memory, Cognition, Dog, Holeboard task, Disappearing object task
1. Introduction

Many recent studies have investigated the working memory capacity of domestic dogs (Adams et al., 2000; Arts et al., 2009; Macpherson and Roberts, 2010; Snigdha et al., 2012; Zanghi et al., 2015), in part because of their use as a model of age-related cognitive decline (Adams et al., 2000; Tapp et al., 2003). Working memory is also an important component of executive function, a term referring to a range of abilities that allows an animal to respond in a controlled and context-appropriate manner to situations, for example by withholding certain responses, retaining and manipulating information, and responding flexibly (Olsen, 2018). Individual differences, declines and dysfunctions in executive function may have important implications for the occurrence of behaviour and welfare problems (Olsen, 2018), so it is important to be able to readily measure components such as working memory, including in publicly-owned dogs who may only be available for short periods of testing. However, the tasks frequently used to assess working memory such as delayed non-match to sample and radial maze, often require prolonged training (Adams et al., 2000; Tapp et al., 2003; Craig et al., 2012; Zanghi et al., 2015) and dogs do not always perform well (Macpherson and Roberts, 2010). Therefore, despite their popularity in laboratory dogs, they are largely unsuitable for assessing working memory in companion animals owned by members of the public.

Therefore, there is a need for more rapid assessments of working memory that can be performed in relatively short periods of time in dogs owned by members of the public and laboratory dogs alike.

The appetitive holeboard task has been used to assess spatial working and reference memory in many species (van der Staay et al., 2012) but it has not previously been adapted for use in domestic dogs. It is advantageous in that it does not require extensive training and relies instead on animals' natural appetitive and exploratory behaviours (van der Staay et al., 2012). The task involves releasing the animal into an arena containing several evenly-spaced holes or buckets, each of which contains a small amount of inaccessible food beneath a mesh screen for scent-masking, and a proportion of which are consistently baited with accessible food rewards. After several trials, the animal learns which holes/buckets are baited with food and preferentially visits these. The animal’s ‘reference memory score’ is calculated from the proportion of total hole/bucket visits that were to these baited buckets, and the ‘working memory score’ is calculated from the proportion of visits to baited buckets that were to buckets that had not been previously visited that trial, and as such measures the animal's tendency to recall locations it has already visited during a trial and avoid revisiting these.

Another task that does not require prolonged training is the disappearing object task devised by Fiset et al. (2003). This has been previously used in companion dogs in a single study with testing requiring only two days per dog (Fiset et al., 2003). This task involves three phases: a shaping
phase in which a dog is conditioned to associate touching a particular object with obtaining a food reward; a training phase in which the dog watches the object being hidden behind one of four identical boxes and is rewarded for successfully visiting the correct box; a testing phase in which a barrier is erected between the dog and the boxes and the dog is gently restrained after the object is hidden such that it can’t maintain eye-contact or posture towards the object location and, following a variable interval, is released and allowed to visit a box (Fiset et al., 2003).

Fiset et al. (2003) demonstrated that the proportion of successful visits to the correct box significantly decreased with increasing intervals between observing the object being hidden and being allowed to retrieve it suggesting dogs were using their working memory rather than other cues such as scent to locate the object. Additionally, dogs were more likely to search for the object in locations closest to the true location (Fiset et al., 2003), suggesting they retained a memory of its approximate location and were therefore using spatial working memory rather than other strategies (Fiset et al., 2003), as found in human infants (Bjork and Cummings, 1984).

In the present study we aimed to develop the holeboard task as a time-efficient method for studying working and reference memory in dogs and sought to implement a version of the disappearing object task in only one day per dog. We also investigated correlations between the dogs’ performance in both tasks to determine whether the tasks measure a common unitary construct of spatial working memory.

2. Methods

Ethical approval was obtained from the University of Bristol Animal Welfare and Ethical Review Body (VIN/15/042).

2.1. Animals

Ten dogs owned by University of Bristol staff were recruited. Dogs were 1-13 years old (mean ± 95% confidence interval = 7.4 ± 3.0 years) with a mean weight of 22.5 ± 5.5kg, and included six females (three neutered, three entire) and four males (three neutered, one entire) of nine different breeds. Some of the dogs had participated in previous unrelated behavioural studies but none had undergone any training related to the current tasks or been exposed to the holeboard or disappearing object apparatus. Six dogs were gundog-type, two terrier-type, and one each of pastoral- and hound-type.
2.2. Holeboard task

Sixteen buckets were each constructed from a 5L plant pot (17.6x23cm) with perforated base, inside a 5L bucket (20x21cm) weighed down with a plastic bag containing ~800g gravel, held together by clamps over the pot rim and bucket handle, as shown in Fig. 1A,B. Food rewards used in the task were individual pieces of Hill’s Vet Essentials Adult Medium Dog kibble (Hill’s Pet Nutrition Inc., Topeka, Kansas, USA). Six food rewards were placed inside the inaccessible compartment below the plant pot and an additional food reward was placed within the accessible compartment of four baited buckets. The buckets were then placed as shown in Fig. 1C on the coordinates of a 4x4 grid within a 3.75x3.75m arena.

During each trial, dogs were allowed to enter the arena once the gate was open and remained in the arena until all four baited buckets had been visited or three minutes had elapsed, whichever occurred first. Dogs were then leashed within an adjacent holding area containing a bed and water bowl from which they were unable to see the arena for five minutes, and the same buckets were rebaited before removing the leash and opening the gate to begin the next trial. Each dog performed 10 trials per session with two sessions per day for three days. On days one and two an initial configuration was used, whereas on day three a different reversal configuration was used, as shown in Fig. 1C.

For each trial, the reference memory score (proportion of visits to all buckets that were to baited buckets) and working memory score (proportion of visits to baited buckets that were to previously unvisited buckets), were calculated from observation of video footage of the task performed using BORIS software (Friard and Gamba, 2016) by a single trained person who was blind to dog signalment and study hypotheses.

2.3. Disappearing object task

This task was performed in the same arena as the holeboard task, and either one or two days after the reversal phase of the holeboard study, with apparatus set up as shown in Fig. 2. Four open-backed wooden boxes (30cm high, 20cm wide, 15cm deep, designated as boxes 1-4 from left to right when viewed front-on) weighed down with identical plastic bags containing ~800g gravel were used as hiding places for the disappearing object; a tennis ball or squeaky rubber ring (depending on the dog’s apparent preference) attached to a 125cm long, 1mm thick length of transparent nylon string. A barrier consisting of a 120cm high, 160cm long and 3mm thick wooden board was placed in front of the dog and the experimenter who held the dog on a leash during the interval period such that the dog was unable to see any of the boxes.
The task proceeded largely as previously described by Fiset et al. (2003), except that all phases were carried out in one day. On arrival, the dog was allowed to explore the holding area for at least 5 minutes whilst the apparatus was set up. One experimenter (E1) showed the dog a tennis ball and rubber squeaky toy and observed their interaction with the objects. E1 subjectively decided which object the dog appeared to prefer based on which object they initially approached, which object they interacted with for longer, and which object they showed more tail-wagging or jumping behaviours towards following presentation.

In the shaping phase, the second experimenter (E2) held the dog’s collar to restrain them whilst E1 placed the object on the ground near the dog and encouraged them to approach it using verbal cues and pointing. The dog received a food reward (one piece of Hill’s Vet Essentials Adult Medium Dog kibble (Hill’s Pet Nutrition Inc., Topeka, Kansas, USA)) after touching the object with their nose, mouth or forepaw. E2 then called the dog back to the start point to begin the next trial, which proceeded as before. Once the dog had successfully touched the object five times, it was gradually moved closer to the arc of boxes with each successful trial. Once the object reached the boxes, further trials were performed placing the object between two of the four boxes (randomly selected and placed such that the object was still visible). After ten successful consecutive trials, the training phase began.

In the training phase, E2 restrained the dog at the designated start point as previously. E1 called the dog to get their attention, then lifted the object using the nylon cord to suspend it in mid-air, and then moved the object in front of all four boxes and then behind the target box (pseudorandomly generated using the RAND() function in Microsoft Excel such that each box would be selected six times, but never after a previous trial with the same target box), observing the dog’s eyes to ensure they were still following the object and repeating the process if not. As in the study by Fiset et al. (2003), if the target box was box 1 or 2, the object was moved in front of boxes 4, 3, 2, 1 and then behind the target box, and if the target box was box 3 or 4, the object was moved in front of boxes 1, 2, 3, 4 and then behind the target box. The wooden barrier was placed in front of the dog (to habituate them before the testing phase) and immediately removed. The dog was then released whilst E1 looked towards the back wall of the arena and E2 looked towards E1, to prevent offering visual cues regarding the object location. Once the dog had visited (looked behind) one of the four boxes, or after 60 seconds if the dog had not visited any boxes, E1 led them out of the arena by the collar. If the box visited was the target box, the dog received verbal praise, petting and a food reward, otherwise they received no reward.

In order to complete the task in just one day, the testing phase commenced after 24 training trials regardless of the dog’s success. Binomial tests (p ≤ 0.00052) showed that during the training...
session 7 out of 9 dogs (training data for one dog was lost) had probabilities of locating the correct
box on each training trial (0.58, 0.65, 0.67, 0.71, 0.71, 0.75, 1.0) that were significantly greater than
expected by chance (0.25). Two dogs did not achieve this level of performance during training
(0.375, p=0.121; 0.33, p=0.234). Interestingly, there was a positive correlation between the dogs’
proportion of correct choices during training and during the three subsequent test sessions that was
statistically significant when parametric, but not non-parametric, analyses were performed
(Pearson’s r=0.821, N=9, p=0.007; Spearman’s ρ=0.552, N=9, p=0.123). We controlled for dog ID in
our analyses of the disappearing object task to account for any differences between dogs.

In the testing phase, trials proceeded largely as described for the training phase, however
once the object was hidden the wooden barrier was placed between the dog and the gate aperture
for a specific interval (0, 30, 60, 120 or 240 seconds). There were three trial-blocks of 20 trials each,
with the target box and interval pseudorandomly generated using the RAND() function in Microsoft
Excel, such that each combination of box and interval would occur once per trial-block and the same
target box or interval did not occur in two consecutive trials. Dogs were walked outside for five
minutes and offered drinking water between each of the three phases and between each trial-block
of the testing phase. The box visited on each trial was recorded by E1 using pencil and paper.

2.4. Statistical analysis

Holeboard results were analysed using the repeated measures general linear model function
in SPSS, with session and trial as within-subject factors and age group (1-5 years (N=3); 6-10 years
(N=4); 11-15 years (N=3)) as a between-subjects factor. Disappearing object results were analysed
using mixed effects logistic regression in R using the glmer() function from the lme4 package,
including the fixed effects of interval, trial-block, box number, trial number, weight, age group, sex,
neutering and the random effect of dog ID. Model estimates were based on an adaptive Gaussian
Hermite approximation with 100 integration points. Odds ratios with upper and lower 95%
confidence intervals were calculated from model estimates. The predict() function was used to plot
predicted probabilities of success at each interval between 0 and 240s.

The correlation between the mean working memory score during the final pre-reversal
session (session 4) of the holeboard task when the dogs should have been performing most
effectively, and the overall proportion of successful visits during the disappearing object task for
each dog were assessed using a Spearman’s rank correlation test in R.

3. Results
3.1. Holeboard task

A significant effect of session was found on both working memory score ($F_{9,35}=4.88$, $p=0.002$) and reference memory score ($F_{9,35}=10.55$, $p=0.00003$). Working memory score increased across sessions 1-4 and then plateaued with only a small dip on session 5 when the configuration of baited buckets changed, indicating that within-test searching was still informed by working memory at this stage (Fig. 3A). Reference memory score also increased across sessions 1-4 but then, as expected, fell sharply on session 5 when baited bucket configuration was altered (Fig. 3B). A significant effect of trial was found on reference memory score ($F_{9,35}=2.718$, $p=0.01$) but not working memory score ($F_{9,35}=1.169$, $p=0.33$), with reference memory score slightly increasing with increasing trials, as shown in Fig. 3C. There was no significant effect of age group on reference memory ($F_{2,7}=0.598$, $p=0.576$) or working memory ($F_{2,7}=0.550$, $p=0.6$) scores and no significant interactions between any factors.

3.2. Disappearing object task

Variables that had a significant effect on the probability of successfully locating the object included memory retention interval, with dogs being less likely to successfully find the object as interval increased (odds ratio=$0.992$, 95% CI=$0.990$-$0.994$; $z=-7.17$, $p=7.52 \times 10^{-13}$; Fig. 4A), trial, with dogs being less likely to find the object with progressive trials within a trial-block (odds ratio=$0.962$, 95% CI=$0.933$-$0.991$; $z=-2.528$, $p=0.0115$), and box, with dogs being less likely to successfully find the object when it was hidden behind box 2 (but not when it was hidden behind boxes 3 or 4) compared with box 1 (odds ratio=$0.593$ (95% CI=$0.362$-$0.972$), $z=-2.071$, $p=0.0383$; Fig. 4B). Whilst dogs were also slightly less likely to find the object when it was hidden behind box 3 compared with box 1, this effect was not significant (odds ratio=$0.789$ (95% CI=$0.485$-$1.286$), $z=-0.951$, $p=0.342$).

We investigated whether using data from just the first trial-block would also reveal the critical effect of memory retention interval, since this would decrease the time required for testing and hence make use of the task with publicly-owned dogs even more feasible. Data from just the first trial-block did indeed show that, as retention interval increased, performance dropped (odds ratio=$0.993$, 95% CI=$0.990$-$0.997$; $z=-3.249$, $p=0.0012$) and, as in the full dataset, dogs were less likely to find the object as trial-block progressed (trial effect: odds ratio=$0.933$, 95% CI=$0.881$-$0.987$; $z=-2.39$, $p=0.017$). However, dogs were less likely to find the object when it was hidden behind box 3 (not box 2 as in the full dataset analysis), compared to box 1 (odds ratio=$0.326$ (95% CI=$0.129$-$0.827$), $z=-0.236$, $p=0.018$).
3.3. Correlation between task performance

There was no significant correlation between the working memory score of individual dogs during session 4 of the holeboard task and their proportion of correct visits in the disappearing object task ($p = -0.073$, $p=0.841$).

4. Discussion

Our findings demonstrate that both the holeboard and disappearing object tasks can be performed in dogs in relatively short timespans of only three days for the holeboard task and a single day for the disappearing object task. Additionally, this is the first published study showing that the holeboard task can be successfully adapted for use in dogs. The pattern of increasing working and reference memory scores with each session followed by a decrease in reference memory after configuration change is similar to that seen in studies in other species (van der Staay, 1999; Gieling et al., 2012; Haagensen et al., 2013b; Antonides et al., 2015). The mean working memory score reached in the final pre-reversal session ($0.834 \pm 0.0366$) was similar to those previously recorded in rats (van der Staay, 1999) and pigs (Arts et al., 2009; Gieling et al., 2012; Bolhuis et al., 2013), indicating greater similarity across species in performance in the holeboard task than in the radial maze task in which dogs were previously found to have “surprisingly low” performance compared to rats (Macpherson and Roberts, 2010). This could suggest that the holeboard task engages similar learning and memory processes in rats, pigs and dogs, hence facilitating comparative studies. The mean reference memory score ($0.353 \pm 0.0157$) observed in the final pre-reversal session within this study were somewhat lower than those seen in most studies in rats and pigs (van der Staay, 1999; Arts et al., 2009; Gieling et al., 2012), however some studies in pigs found similar reference memory scores (Haagensen et al., 2013a; Haagensen et al., 2013b). This difference may be due to the close spacing of trials in this study as decreased temporal spacing between trials has been shown to adversely affect reference but not working memory in rodents (Spreng et al., 2002; Commins et al., 2003).

The holeboard task thus appears to offer advantages over other tests of working memory that require longer periods of training and/or generate relatively poor performance in dogs. Because it does not require the presence of a researcher or handler whilst the animal is performing a trial, it may be useful for assessing the working and reference memory of non-domestic canids housed in zoos or wildlife parks.

The results of the disappearing object task were similar to those found by Fiset et al. (2003) in the original two-day version of the task, with dogs' probability of successfully finding the object...
decreasing with increasing retention interval duration as expected. In our study, dogs were also less likely to find the object when it was hidden behind box 2 compared to box 1. They also showed a non-significant trend to be less likely to find the object when it was hidden behind box 3 compared to box 1 (which became significant in the analysis of trial-block 1 only). Dogs have previously been shown to use ‘external’ environmental cues when solving problems (Milgram et al., 1999). It is therefore possible that the walls close to the outer boxes (1 and 4) acted as environmental cues allowing easier differentiation of these boxes compared to the inner boxes (2 and 3). Because each dog had the same number of trials with each target box at each interval, this cue salience effect should not have affected other results. Dogs were also less likely to successfully find the object with increasing trials within a trial-block. This may be due to inattentiveness as the task progressed, as if it was due to satiety we would expect that dogs would also have shown a decreased probability of finding the object in subsequent trial-blocks, but this was not the case.

The task, which took between 5-6h, including breaks, could be shortened to further increase feasibility. Since there was no effect of trial-block, and a further analysis showed that the effect of memory retention interval was still statistically significant when only data from trial-block 1 were included in the model, it would be feasible to use only one trial-block rather than three. Additionally, since the dogs’ performance reached chance levels at around 207 seconds (Fig. 4A), it would be reasonable to omit the 240s trial-block from future studies as dogs would be expected to be performing no better than expected by chance at this point.

There was no significant effect of age on working memory outcome measures in either task, although other studies have found that working memory decreases with increasing age in dogs (Adams et al., 2000; Tapp et al., 2003; Snigdha et al., 2012). However, it is probable that the small numbers of dogs of each age category recruited within this study were insufficient to detect such an effect.

Perhaps surprisingly, there was no correlation between dogs’ working memory outcome measures in the two tasks. This may be because working memory is not a single unitary construct but several interconnected systems, as has been previously argued by some researchers (Cowan, 2008; Baddeley, 2010), and the tests were measuring different aspects of these systems. However, it may also be that the tasks differed in terms of the reinforcement provided and/or the learning required and that this resulted in different levels of performance. For example, some dogs may have been more motivated to obtain the object or the researcher’s attention in the disappearing object task, whereas others may have been more motivated to directly acquire food in the holeboard task compared to indirectly obtaining food as a reward for touching the object in the disappearing object task. Additionally, the holeboard task required dogs to learn and perform the task independently,
whereas the disappearing object task required observing the behaviour of a human (hiding the object) and receiving rewards directly from the researcher. Dogs are able to use cues given by humans to solve spatial tasks (Miklósi et al., 1998; Pongrácz et al., 2004). In one study, companion dogs spent more time watching their owners and were more impaired in the performance of an independent problem-solving task than working dogs (Topál et al., 1997). This could indicate that dogs that are more reliant on humans for provision of cues, which could be advantageous in the disappearing object task, may have impaired ability to perform independent problem-solving tasks such as the holeboard task.

Whilst this study successfully established the utility of the two tasks in assessing elements of dog spatial memory, it had limitations. These include the small sample size and associated difficulties in interpreting the lack of statistically significant effects of variables such as age and sex and the lack of correlation between the two measures of working memory. Moreover, the precise methods and test set-ups used here would likely need to be adjusted if dogs of very different sizes were to be used in future work. For example, in the holeboard task small breeds might have difficulty accessing the food buckets whilst large breeds might be constrained in their movements around the bucket array.

5. Conclusions

In summary, we demonstrated that elements of dog spatial working memory could be assessed in relatively short time periods in two tasks. By successfully adapting the holeboard task for use with dogs, spatial working and reference memory can be compared with that of other species in this task (van der Staay et al., 2012). The short time periods needed, especially for the disappearing object task, make these tasks suitable for use in companion animals owned by members of the public as well as providing alternatives that can be used in laboratory-housed dogs without requiring the large amount of training and testing time involved in, for example, delayed non-matching to position tasks (Adams et al., 2000; Tapp et al., 2003; Zanghi et al., 2015). Since dogs’ performance in the two tasks were not correlated, it is possible that they measured different features of the spatial working memory construct, although this lack of correlation could also be explained by differences between the two tasks in learning or motivation to obtain rewards.

Conflict of Interest

The authors acknowledge no conflicts of interest regarding this study.
Declarations of interest

None

Acknowledgements

This research was funded by the BBSRC South West Biosciences Doctoral Training Partnership (SWBio DTP) programme, grant number BB/J014400/1. We thank the dogs and their owners for taking part in the study.

Data availability

Data for this article are available on request from the authors.

References


Figure legends

Fig. 1. Photograph (A) and diagram (B) showing the structure of the buckets used for the holeboard task, with inaccessible and accessible food reward locations shown as grey circles. Buckets were placed as shown in (C), with black crosses showing baited and white crosses showing unbaited buckets. Thin black lines represent arena walls, thick black lines represent iron fencing, with a central gate location shown in white.

Fig. 2. Diagram showing the arena set up for the disappearing object task, at the point of releasing the dog to retrieve the object, which in this trial is hidden behind box 2. Identical boxes 1-4 were arranged in a semicircle around the entrance to the arena as shown. The wooden board was slid in front of the gate during the memory retention interval to block the dog’s view of the boxes. E1 shows the position of the researcher whose role was to move the object and reward the dog or remove them from the arena following an unsuccessful trial. E2 shows the position of the experimenter responsible for restraining the dog between trials. The arena fencing is shown in black with the gate shown in dashed black. Arena walls are shown in grey (full extent of walls not shown).

Fig. 3. Results of the holeboard task. The effect of session on (A) working memory score and (B) reference memory score, and (C) the effect of trial on reference memory score. Horizontal dotted lines show the reference memory score expected by chance if dogs were searching randomly. Vertical dotted lines show the point at which the configuration of baited buckets was changed. Values shown are means with 95% confidence interval error bars. For the effect of trial (C), the solid line represents a linear regression line with its 95% confidence interval shown in grey.

Fig. 4. The effect of (A) interval on predicted (black curve with grey 95% confidence intervals) and observed (points with 95% confidence interval error bars) probability of success and (B) target box (location of hidden object) on proportion of successful visits during the disappearing object task. Asterisks represent significant differences in probability of success compared to target box 1. Bars represent means with 95% confidence interval error bars.