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Assessing cognitive performance in dairy calves with a modified hole-board test

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Abstract

The hole-board test has been used to assess working and reference memory in a variety of species, but not in cattle. We developed and applied a modified hole-board test for dairy calves. Fifteen bottles were evenly spaced along three walls of a test arena; 11 of these were empty and 4 were ‘baited’ with milk. Calves were exposed daily (over an 11-day learning period) to the test arena with the location of the baited bottles held constant; the location of the 4 baited bottles was then changed and calves were re-trained on these new locations (over a 7-day re-learning period). Working memory (no. of revisits to the baited set), general working memory (no. of revisits to the whole set), and reference memory (avoidance of non-baited bottles) were assessed daily. Performance significantly improved during the learning period for reference and general working memory. Working memory tended to improve, albeit non-significantly. The change in bottle location initially reduced all performance measures, but these subsequently improved during the 7-day re-learning period. These results indicate that a modified hole-board test can be used to assess calf cognition, and thus may be helpful in future research designed to investigate the effects of housing and rearing conditions on cognition.

Keywords: short-term memory, long-term memory, animal welfare, dairy cattle
Introduction

There is a growing interest in farm animal cognition. Research on cognitive abilities of animals used for food production follows two main objectives. First, an improved understanding of how these animals perceive their world can help provide animals with an environment that matches their needs (Nawroth et al. 2019). For instance, Singhal et al. (2019) postulated that diverse and frequent cognitive stimulation, missing in many farm environments, promotes better engagement with the environment and better learning capacities. In addition, comparing the cognitive abilities of animals raised in different conditions can help assess the effects of these rearing conditions on the animals (Nawroth et al. 2019). For instance, social housing is known to improve the cognitive abilities of dairy calves (Gaillard et al. 2014; Meagher et al. 2015).

Previous studies have shown the capacity of young dairy calves to navigate spatial learning tasks (Lecorps et al. 2018, 2019; Ede et al. 2019; Bučková et al. 2020), as well as other operant discrimination tasks used for judgment bias testing (Neave et al. 2013). Calves can even learn to discriminate locations based on probabilities of being rewarded and punished (Lecorps et al. 2019). The capacity of young dairy calves to navigate spatial mazes is consistent with studies showing that adult cattle can remember the location of food for up to 40 days (Ksiksi and Laca 2002) and the configuration of mazes for up to 6 weeks (Hirata et al. 2016). Although previous studies highlighted calves’ cognitive capacities and the positive impact of improving housing conditions on their cognition, much remains to be learned regarding their cognitive abilities and how these are affected by rearing methods.

Various tests have been designed to assess animal cognition, including the hole-board test that allows an assessment of several behavioral and cognitive domains (van der Staay et al. 2012). This spatial discrimination task assesses how animals learn and access memories regarding the location of treats (typically provided at 4 of 16 possible locations), allowing the
assessment of both working memory (i.e., the capacity to memorize which locations have already been visited within a trial) and reference memory (i.e., the capacity to memorize where locations are rewarded across successive trials). In addition, the test can also be used to assess behavioral flexibility by exploring how animals respond to changes in the baited locations (e.g., Grimberg-Henrici et al., 2016).

The hole-board test is well-suited for assessing the effects of housing and husbandry on cognition (van der Staay et al. 2012). For instance, early-life and chronic stress typically reduce performance of some or all measures described above (see a review by Conrad 2010), and environmental enrichment typically improves performance (Peña et al. 2009; Bolhuis et al. 2013; Grimberg-Henrici et al. 2016). Deficits in these cognitive skills can impair an animals’ ability to cope with their environment given that short-term and long-term memory are essential in many situations. For instance, low working memory individuals are more sensitive to the negative effects of stress (Otto et al. 2013).

The hole-board test has been used not only in laboratory rodents, but also in other species including laying hens (Gallus gallus domesticus, Tahamtani et al. 2015), pigs (Sus scrofa; Bolhuis et al. 2013) and goldfish (Carassius auratus; Durán et al. 2008). To our knowledge, no studies have attempted to apply this test in cattle. The aim of this study was to develop and assess an adapted version of the hole-board test in milk-fed dairy calves, using bottles presented in a U-shaped arena.

**Methods**

The study was approved by The University of British Columbia’s Animal Care Committee (# A19-0128) and cared for according to the guidelines outlined by the Canadian Council of Animal Care (2009).

**Animals and housing**
Ten Holstein calves (3 males and 7 females) were enrolled in this study (BW: 39.4 ± 6.3 kg). Within 6 h after birth, calves were separated from their dam, fed 4 L of > 50 g/L IgG colostrum and then kept in individual housing for 5 days. Calves were then housed in pairs in a double pen (2.4 x 2.0 m) for the duration of the experiment and were fed 4 L of whole pasteurized milk twice per day (at 08:00 and 16:00 h) using a nipple bottle. Testing always occurred in the morning and replaced the first meal (2 L were provided during testing and 2 L were provided at the end of the test when the calf was back to its home-pen. All calves had ad libitum access to water, hay, and grain.

**Apparatus**

The hole-board test typically uses holes on a platform when tested in rodents or buckets placed on the ground when tested in other species such as pigs (Bolhuis et al. 2013) and laying hens (Tahamtani et al. 2015). Pre-weaned calves drink milk, so we used bottles (like the ones used for calf feeding) placed along three sides of an arena (see Figure 1). Calves were tested daily over 11 days (the learning phase) to remember the locations of 4 baited bottles (containing milk) placed among 11 unbaited (i.e., empty) bottles. Locations of the baited bottles were changed for the re-learning phase of the study and kept constant for the remaining 7 days. We assessed cognitive performance daily throughout the 18 days period, predicting that calves’ performance would improve from day 1 to 11, decline on day 12 (the day of location change) and then again improve until day 18.
Training phase

To allow calves to become familiar with the experimental arena, each pair was moved to the arena (with no bottles presented) for 5 min. On the following day, calves were brought to the arena individually and allowed to drink milk from a single bottle containing 0.5 L of milk (this reward was constant throughout the phases described below) placed randomly in one of the 15 positions available. All calves found the bottle and drank from it. The following day, 6 baited bottles were displayed (pseudo-randomly located; 2 per side), with the remaining slots left empty. If calves drank from all bottles, they proceeded to the next training step where only three of the six bottles contained milk and the remaining three bottles were left empty. This step was included to habituate calves to encountering empty bottles and keep searching for baited ones. Calves proceeded to the testing phase (i.e., with 4-baited and 11-unbaited bottles) if they drank milk from the 3 baited bottles and visited at least 2 unbaited ones.

Testing phase

For the testing phase, each of the 15 locations contained a bottle but just 4 of these were baited. Locations were determined pseudo-randomly using a random number generator with
the constraint that all three sides had at least one baited bottle. Calves were tested individually, once daily in the morning between 8 and 10 am (i.e., 16 to 18 h after last milk meal, following the farm standard feeding routine for hand-fed calves). A ‘visit’ was scored when calves touched the nipple of a bottle with their nose and not with any other parts of their body. If calves touched an unbaited bottle, no extra punishment was applied. Trials ended when calves found and drank from the four baited bottles, or when calves stopped interacting with bottles for more than 30 s, or after 5 min elapsed, whichever occurred first. These time limits were designed to limit frustration animals would experience if they struggled to find baited bottles. Visits were scored live by an observer who recorded which bottles were touched and whether calves drank milk from and emptied the baited bottles. In addition, a camera (WV-CW504SP, Panasonic, Osaka, Japan) was positioned above to record the behavior of the calf and confirm which bottles were visited by each calf.

Calves were tested for 11 days with baited bottles placed in the same location (each calf had a different set of baited locations). On day 12, locations were changed (i.e., re-learning phase) and remained constant for the following 7 days when testing was stopped (day 18). Baited locations were determined using the same procedure described above, with the exception that a bottle could not be placed at the same position as before the change.

To assess if calves were relying upon olfactory cues to detect bottles containing milk, we performed an additional choice test with a bottle containing milk vs. an empty bottle, using 6 additional calves (all female), repeated 12 times over 5 sessions; these calves performed at chance when repeatedly tested (Supplementary materials 1).

Statistical analysis

Cognitive performance was assessed for every trial based on definitions provided by van der Staay et al. (2012). Two scores were used to assess working memory. First, working memory (WM) was assessed as the number of rewarded visits to the baited bottles divided by
the total number of visits to the baited set, with a score of 1 indicating that the calf never revisited baited bottles; this score was used to assess whether calves remembered which baited bottles they had visited (from the baited set) and avoided revisiting these bottles. Revisits to baited bottles that were not emptied during a previous visit were counted as rewarded if the calf drank from the bottle. Second, general working memory (GWM) was calculated as the number of different bottles visited (baited and unbaited) divided by the total number of bottles visited. This score assesses the proportion of revisits, with a score of 1 indicating that the calf never visited the same bottle more than once within a trial; this score was used to assess whether calves remembered which bottles they visited (baited and unbaited) and avoided revisiting these bottles. Finally, reference memory (RM) was measured as the total number of visits to baited bottles divided by the total number of bottles visited. This score gives a good sense of how well calves remembered the locations of the baited bottles and avoided visiting unbaited locations. We also recorded the total number of bottles visited and trial duration.

Statistical analyses were run using SAS (version 9.4; SAS Institute Inc., Cary, NC). Of the 10 animals enrolled in this study one was excluded because of illness during the testing period, resulting in a final sample size of nine calves. Linear mixed models were used to assess changes in calf performance with time. Four models were built (one per outcome: working memory, general working memory, reference memory and number of visits) including testing days (from 1 to 11) as a continuous factor and calf identity as a random factor.

To explore the effects of change in location (i.e., re-learning) on calf performance, we ran similar models using only day 11 and 12 (change day). Data were missing for one calf on day 12, resulting in a sample of 8 calves for this analysis. To confirm that calves re-learnt the new locations, we compared performance on days 12 to 18 using similar models. Normality of residuals were confirmed graphically. When assessing working memory, the residuals were not normally distributed and this could not be resolved with transformation, so we used a non-
parametric test (Fisher-Pitman permutation test) for this comparison. More details on the statistical analysis are provided in Supplementary materials 2.

178 Results

Over the 11-day training period, general working memory and reference memory scores increased (GWM: $F_{1,89} = 5.78$, $P = 0.018$, RM: $F_{1,89} = 21.58$, $P < 0.001$), working memory scores increased albeit non-significantly (WM: $F_{1,89} = 3.18$, $P = 0.08$), and the total number of visits decreased ($F_{1,89} = 7.51$, $P = 0.007$) (Fig 2). When the locations of baited bottles were changed on day 12, working memory, general working memory and reference memory scores all decreased (WM: $Z = 2.29$, $P = 0.008$, GWM: $F_{1,7} = 11.58$, $P = 0.011$, RM: $F_{1,7} = 44.95$, $P < 0.001$), and the number of visits to bottles increased ($F_{1,7} = 20.33$, $P = 0.003$). Over the next 6 days, general working memory and reference memory scores again increased (GWM: $F_{1,52} = 11.07$, $P = 0.0016$; RM: $F_{1,52} = 19.29$, $P < 0.001$), working memory scores increased non-significantly (WM: $F_{1,52} = 3.74$, $P = 0.059$), and the total number of visits decreased ($F_{1,52} = 13.04$, $P < 0.001$). Throughout the study we noted considerable individual variation in performance (as illustrated in Supplementary materials 3).
Figure 2. Changes in calf (n = 9) cognitive performance over 18 days of testing in a modified hole-board test. Working memory (a), general working memory (b), reference memory (c) and number of bottles visited (d) were assessed daily for 18 days. Calves were trained to find 4 baited bottles pseudo-randomly located among 11 unbaited bottles during the first 11 days of testing. On day 12 the locations of the baited bottles were re-randomized and testing ended on day 18. A visit was recorded each time a calf touched a bottle’s nipple with its nose.

Discussion

We developed an adapted version of the hole-board test for milk-fed dairy calves. Calves learnt the locations of baited bottles over time; performance declined when the locations of the baited bottles were changed but then improved with continued training indicating some degree of behavioral flexibility. These results demonstrate the potential of this modified hole-board test to assess working memory, reference memory and behavioral flexibility in dairy calves, and potentially in other milk-fed mammals.

To our knowledge, ours is the first study to develop and use this type of spatial learning task in cattle. Our results are consistent with the idea that cattle can use spatial memory to find
food (Rørvang and Nawroth 2021). We see two advantages of using this type of complex spatial learning task. First, given the good spatial learning skills of some farm animals (e.g., pigs, Mendl et al. 1997; cattle, Rørvang and Nawroth 2021), complex spatial learning tasks are more likely to detect differences between animals reared in different ways (Jansen et al. 2009). In addition, compared to other simpler cognitive tests such as a T-maze or a discrimination task, the hole-board test measures several aspects of animal cognition, providing opportunities to distinguish what aspects of cognitive processes are affected by rearing conditions. For instance, a study in pigs found that environmental enrichment improves working memory but not reference memory, suggesting that enrichment affects self-inhibition capacities rather than memory per se (Bolhuis et al. 2013). Given these attributes, we believe that the hole-board test is a useful addition to other behavioral tests available to assess calf cognition and welfare.

We encourage future work to use this test to explore the effects of housing and living conditions on calf cognition. For instance, previous work on calves has shown that individual housing has negative effects on a calf cognition (Gaillard et al. 2014; Meagher et al. 2015); new work may wish to consider aspects of physical enrichment that are known to influence cognition in other species (e.g., Bolhuis et al. 2013). This type of test may also be useful for understanding the severity of negative affective states. Only more severe or long-lasting stressors are expected to affect cognitive performances of animals (Sandi and Pinelo-Nava 2007). For instance, social mixing had no effect on how well pigs performed in the hole-board test (Arts et al. 2009), but environmental enrichment did affect working memory (Grimberg-Henrici et al. 2016). Thus, these measures provide a way of assessing the biological effects of rearing conditions for farm animals. We call special attention to variability in calf performance (both within and between individual variation were high); this variability may explain why we did not detect significant differences for working memory in both the learning and re-learning phases. Future work should investigate the reasons for individual variation in this type of test.
Our study used milk-fed calves, but the basic task may be translatable to older cattle, for example, by using buckets containing solid feed as used in previous work with a radial-arm maze (Bailey et al. 1989). A limitation of the method used in the current study is that calves may have used olfactory (or other sensory cues) to identify baited bottles. We consider this possibility unlikely for two reasons: 1) we found no evidence that calves were able to discriminate empty versus baited bottles in preliminary testing (see Supplementary material), and 2) calves continued to visit the previously baited locations when baited locations were changed on day 12. Anecdotally, we observed calves passing by baited bottles but failing to drink on many occasions, indicating that they do not rely upon olfactory cues.

Another limitation is the U-shaped design we used (bottles were placed on the three sides of a squared arena). Although cues are often placed in a square fashion using 9 or 16 cues, many alternative shapes and cue presentations have been used (see van der Staay et al. 2012). In our case, the U-shaped design (also used with pigs; Mendl et al. 1997), may have initially pushed animals to explore cues one at the time in a clockwise or anti-clockwise fashion. Such a strategy could explain why some individuals had relatively high performance on working and general working memory in the first days of testing, as systematic exploration prevents revisits. Our design also limits the number of cues presented in the immediate vicinity of a location just visited (i.e., either 1 or 2) versus with a typical square design (from 3 to 8). This difference did not prevent the assessment of working and reference memory as calves obviously improved performance with repeated testing, but caution is needed when comparing studies that use different designs.

Conclusion

We modified and applied the hole-board test to milk-fed dairy calves. Calves were able to learn the locations of bottles that contained milk, and seemed to rely on location rather than
other cues. We concluded that this test has potential to better assess calf cognition and the relationship between cognition and welfare.

**Declarations**

**Ethics approval.** The study was approved by The University of British Columbia’s Animal Care Committee (# A19-0128) and cared for according to the guidelines outlined by the Canadian Council of Animal Care (2009).

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**Conflicts of interest.** The authors have no conflict of interests to declare.

**Availability of data and material.** Data is available as supplementary material.

**Code availability**
Statistical codes used for this study is available as supplementary material.

**Authors’ contributions.**

B.L. M.v.K. and D.W. conceived the experiment, B.L. and R.W. collected the data. B.L., R.W. and D.W. analyzed and interpreted the data. B.L., R.W., M.v.K. and D.W. drafted and reviewed the article.

**References**


