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Learned control of urinary reflexes in cattle to help reduce greenhouse gas emissions

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Indiscriminate voiding of excreta by cattle contributes to greenhouse gas (GHG) emissions and soil and water contamination[1,2]. Emissions are higher in animal-friendly husbandry offering cattle more space[2] — a trade-off we call the 'climate killer conundrum'. Voiding in a specific location (latrine) would help resolve this dilemma by allowing ready capture and treatment of excreta under more spacious farming conditions. For urination, toileting requires self-control and coordination of a complex chain of behaviors including awareness of bladder fullness, overriding of excretory reflexes, selection of a latrine and intentional relaxation of the external urethral sphincter[3]. Attempts to train toileting in cattle have so far been only partly successful[4–6], even though their excretion and associated neurophysiological control are similar to those in species capable of toileting[3]. Similarly, very young infants have been considered incapable of self-initiated voiding, but they can be taught with extensive training[7]. Using a backward chaining, reward-based training procedure, we here show that cattle can control their micturition reflex and use a latrine for urination. Such self-control provides evidence that animals can learn to respond to and reveal internal experiences via appropriately trained operant behaviors, thereby providing another way to explore their subjective states.

In our study, 16 calves (across two cohorts, n = 8) underwent individual toilet training in a three-step backward chaining procedure (Supplemental information). In the first phase (*in-latrine training*), the calves were confined to a distinctive area (latrine; Data S1C) and every urination event was rewarded with food. Increasing frequency of orientation to the reward as training progressed would demonstrate success in bringing micturition under control of the rewards. *In-latrine training* was also designed to establish the latrine as the correct voiding location.

Reliable and rapid orientation to the reward (learning) was seen in 10 of 16 calves (4/8 calves in cohort 1 vs. 6/8 in cohort 2; p = 0.608). The mean slope of the learning curves (Figure 1A) calculated from the first urination accompanied by reward orientation to the last urination) was 0.73 ± 0.08 (± SE) for the calves that orientated to the reward, which was significantly steeper than that for the calves that did not reliably orientate to the reward (0.27 ± 0.03; W = 60, p = 0.001). These results support published evidence that urination behavior in cattle can be modified by rewards[5,6] and demonstrated the foundation step for backward chaining of toileting in a majority of the calves.

In the next phase (*toileting training*), self-control of the entire toileting sequence and the degree to which the latrine had been established as the correct voiding location were evaluated. Importantly, self-initiation and self-control of voiding would demonstrate that calves have the capacity to attend and respond to cues arising from internal experiences, thus showing interoceptive awareness[8]. All calves continued to this phase, as even the poorer learners orientated to the reward during *in-latrine training*. Calves accessed the latrine from an alley through an animal-activated gate and exited the latrine after each urination (Data S1C). Urinations initiated in the latrine were rewarded, as for *in-latrine training*, but urinations initiated in the alley were followed immediately by an unpleasant stimulus (three-second water spray). The behavior associated with each urination event in *toileting training* was assigned to one of four possible sequences (Figure 1D).

The development of toileting is shown by the record of cumulative rewards for those sequences that ended with voiding in the latrine (Seq++ and Seq+) across successive urinations (Figure 1B) and can be quantified from the slopes of the curves (calculated from the first rewarded urination to the last urination). Control of urinary reflexes was learned quickly by 11/16 calves (4/8 calves in cohort 1 vs. 7/8 in cohort 2; p = 0.282; Video S1). They reliably entered the latrine to urinate as shown by a mean curve slope of 0.77 ± 0.05, i.e. 77%

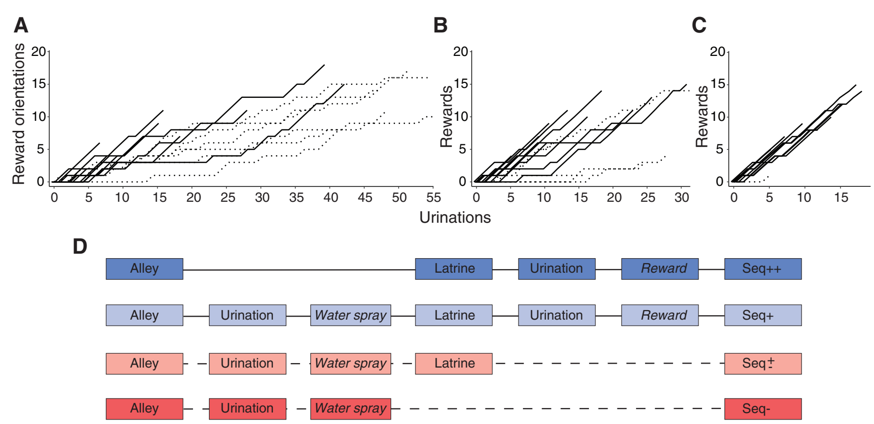

Figure 1. Cumulative learning curves and possible behavior sequences.
Cumulative learning curves for individual calves for (A) *in-latrine training* (n = 16), (B) for *toileting training* (n = 16) and (C) for *toileting+training* (n = 11). Calves meeting the learning criteria (Supplemental information) are shown with solid lines and those not meeting the criteria with dotted lines. (A) Reward orientation was defined as orientation to the reward bowl during or immediately after urination and prior to reward delivery. (B,C) Rewards were delivered after entry to and urination in the latrine. (D) The four possible behavior sequences. Urinations were either correct and rewarded (blue, Seq++ and Seq+, voiding in the latrine) or incorrect and not rewarded (red, Seq±, Seq-, voiding in the alley). Seq++ was the ideal behavior as entry to the latrine is completely self-initiated, whereas in Seq+ a corrective unpleasant stimulus was applied for urinations initiated outside the latrine. In Seq±, the calf entered the latrine after a corrective stimulus but did not reinitiate urination, whereas in Seq- the calf did not enter the latrine.

of sequences included urinations in the latrine, which was numerically much greater than that for the non-learners (0.41 ± 0.06). The better performance of calves in cohort 2 was probably due to individual differences in learning performance or more effective correction of urinations in the alley after adjustment of the sprinkler position (Supplemental information).

The number of urinations did not differ between learners and non-learners (W = 30, p = 0.820). Thus, failure by non-learners to toilet consistently cannot be attributed to fewer opportunities to learn. Rather, further training is likely required. Calves that had shown reward orientation in *in-latrine training* were more likely to learn in *toileting training* compared to calves that did not show this behavior (p = 0.036). This and the steep curves for the learners suggest that learning the correct voiding location occurred at the same time as urination came under the control of reward (during *in-latrine training*).

In many toileting situations, self-control of voiding reflexes over extended distances is required. Thus, in the last phase (*toileting+training*) we evaluated toilet use when the area outside the latrine was increased by extending the lead-up alley. The cumulative rewards for toileting (Seq++ and Seq+ combined) across urinations for each calf in this phase are shown in Figure 1C.

Ten of the 11 calves that met the *toileting training* learning criterion continued to use the latrine consistently in *toileting+training.* For these 10, the mean learning slope was 0.83 ± 0.04, i.e. 83% of sequences included urinations in the latrine. In addition, the slopes for these calves did not differ between the *toileting* and *toileting+training* phases (V = 10; p = 0.155). Thus, toileting was maintained with the increase in alley length. Further research is needed to determine the greatest distances over which continence can be maintained in cattle.

Learning to voluntarily suppress or interrupt and reinitiate contraction of the external urethral sphincter is a key component in the pathway to learn latrine use. Calves were able to reinitiate voiding after interruption as indicated by similar durations of urination in both Seq++ and Seq+ in the latrine (16.2 ± 0.9 s).

Interoceptive awareness during toileting is evidenced by self-initiated latrine use, i.e. Seq++, and developed quickly. In the first half of *toileting training*, 29 ± 6% of urinations were self-initiated in the latrine, increasing to 73 ± 5% in the second half (V = 0, p = 0.004), a level that was maintained in *toileting+training* (72 ± 5%). Thus, our study demonstrates the ability of cattle to attend to and voluntarily control internal voiding reflexes, and adds to a growing body of evidence that involuntary as well as voluntary behaviors of animals are amenable to modification by rewards[3]. The demonstration of interoceptive awareness in a non-human animal could further indicate that other subjective experiences or affective states that are not readily accessible but important to understanding animal well-being[9] can be measured using appropriate operant conditioning procedures.

Remarkably, the calves showed a level of performance comparable to that of children and superior to that of very young children[7]. The success of our procedure is likely attributable to two key factors: the establishment of strong reward-based control over the reflex at the beginning of training, and the rapid development of responsiveness to internal reflex cues. The use of an unpleasant stimulus following ‘mistakes’ outside of the latrine likely also played a significant role, resulting in inhibition of voiding in the alley compared to urinations in the latrine (urination durations: Seq+ = 3.0 ± 0.3 s; Seq± = 7.7 ± 2.1 s; Seq- = 6.7 ± 1.1 s; χ^2 = 23.2, df = 4, p < 0.001).

Our findings are original and reveal a hitherto unrealized opportunity to harness the cognitive capacities of animals to help resolve pressing environmental issues without compromising animal welfare. We have shown that a majority of cattle can be trained to deposit most of their urine in a defined location, enabling the development of more effective methods to collect, treat and dispose of pure urine than is currently possible with technical solutions alone[2]. Modelling exercises have calculated that capture of about 80% of cattle urine in latrines could lead to a 56% reduction in ammonia emissions[10]. Further, by reducing contamination of the living areas, the cleanliness, hygiene and welfare of livestock can be improved whilst simultaneously reducing environmental pollution[3]. Hence, clever cattle can help in resolving the climate killer conundrum.

SUPPLEMENTAL INFORMATION

Supplemental information including acknowledgements, inclusion and diversity statement, methods, analysis, results, one video and one data file can be found with this article online at https://doi.org/10.1016/j.cub.2021.07.011.

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