



Review article

Conditionability of ‘voluntary’ and ‘reflexive-like’ behaviors, with special reference to elimination behavior in cattle

Neele Dirksen^{a,1}, Jan Langbein^{a,*,1}, Lindsay Matthews^{b,1}, Birger Puppe^{a,c}, Douglas Elliffe^b, Lars Schrader^{d,1}^a Leibniz Institute for Farm Animal Biology, Institute of Behavioral Physiology, Dummerstorf, Germany^b The University of Auckland, School of Psychology, Auckland, New Zealand^c University of Rostock, Faculty of Agricultural and Environmental Science, Behavioral Sciences, Rostock, Germany^d Friedrich-Loeffler-Institut, Institute of Animal Welfare and Animal Husbandry, Celle, Germany

ARTICLE INFO

Keywords:

Neurophysiological control of elimination
Toilet training
Cattle
Classical conditioning
Operant conditioning

ABSTRACT

Typically, cattle urinate and defecate with little or no control over time and place. The resulting excreta contributes to a range of adverse effects on the environment and the animals themselves. These adverse effects could be substantially ameliorated if livestock could be toilet trained. Toilet training requires an animal to suppress impending voiding (a reflexive-like behavior), move to a latrine (voluntary behavior) and reinitiate voiding. Here, we review the neurophysiological processes and learning mechanisms regulating toileting. The suppression and initiation of voiding occur primarily via the coordinated activity of smooth and striated anal and urinary sphincter muscles. The autonomic and somatic nervous systems, along with central processes, regulate these muscles. In several mammalian species, voluntary control of the sphincters has been demonstrated using classical and/or operant conditioning. In this review, we demonstrate that the neurophysiological and behavioral regulation of voiding in cattle is likely to be similarly conditionable. The management of excreta deposition in cattle could have major benefits for reducing livestock greenhouse gas emissions and improving animal health/welfare.

1. Introduction

The focus of this review is to characterize the neurophysiological regulation and possible learning mechanisms that could be utilized to control elimination behavior and to determine the potential to successfully toilet train cattle. The smooth and striated muscles of the bladder and anal sphincters and pelvic floor are involved in controlling eliminative behavior in mammals (Arya and Weissbart, 2017; Salomon et al., 2008). These muscles are controlled by both the autonomic and somatic nervous systems. In the brain, more specifically in the periaqueductal gray (PAG), the signals from the bladder/rectum are combined with information from higher brain centers to influence the ongoing retention or voiding of excreta (Arya and Weissbart, 2017; Blok, 2002; Salomon et al., 2008). To train voluntary elimination in a latrine in cattle, both non-associative and associative learning procedures would likely be relevant (Fig. 1). Non-associative learning processes would be involved during familiarization with a latrine and any associated rewards (Abramson and Kieson, 2016). In addition, voluntary

movement to a latrine and the control of bladder and anal sphincters would need to be trained (Fig. 1). Typically, voluntary behaviors are trained with operant conditioning procedures and reflexive behaviors using classical conditioning techniques (Abramson and Kieson, 2016; Willis and Mein, 1983). Both are based on associative learning (Abramson and Kieson, 2016).

In addition to humans (Fowler et al., 2008), other mammals such as dogs and cats (Lord et al., 2008) show learned control over eliminative behavior. A trainability of elimination in cattle has been attempted, but so far, it has not been completely successful (Vaughan et al., 2014a; Whistance et al., 2009). Cattle urinate and defecate several times a day with little control regarding time or place (Hafez and Bouissou, 1975; Vaughan et al., 2014a; Whistance et al., 2011), which leads to excreta being discharged that is harmful to the environment, both at pasture and in indoor housing (Bouwman et al., 2002; Braam and Swierstra, 1999; Chadwick et al., 2018). The mixing of urine and feces is particularly harmful in exacerbating the emission of ammonia from indoor cattle production systems (Braam and Swierstra, 1999; van Dixhoorn

* Corresponding author at: Wilhelm-Stahl-Allee 2, 18196, Dummerstorf, Germany.

E-mail address: langbein@fbn-dummerstorf.de (J. Langbein).¹ These authors contributed equally to the writing of this manuscript.

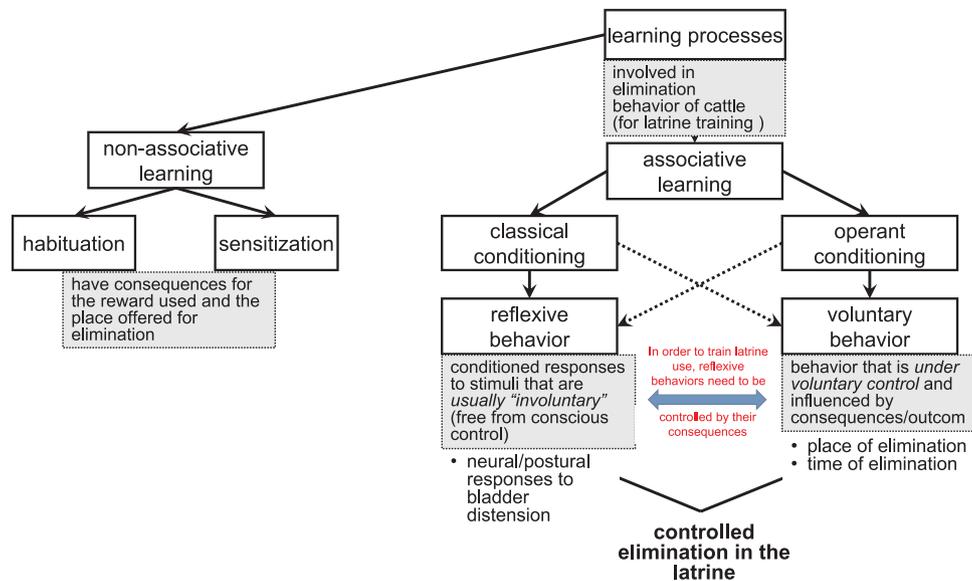


Fig. 1. Schematic overview of learning processes likely to be involved in the training of latrine use for urination in cattle.

et al., 2017). Further, high-usage areas in barns become contaminated with excreta and must be cleaned regularly to avoid infections of the claws and udders (Chapinal et al., 2013; Magnusson et al., 2008; Somers et al., 2005). Latrine use by cattle kept outdoors or indoors would provide extensive opportunities to mitigate many of the adverse environmental effects arising from greenhouse gases derived from bovine livestock farming. For example, urine and feces could be more easily separated, thereby reducing the amount of ammonia formed (van Dixhoorn et al., 2017). It has been suggested recently that ammonia emissions could be reduced by 56 % if only 8 out of 10 urine excretions could be collected (Verdoes and Bokma, 2017).

The review begins with a brief overview of the neurophysiological mechanisms regulating elimination in mammals and the natural behavior of elimination in cattle. The paper presents and discusses the potential roles of non-associative and associative learning processes that would likely be involved in toilet training cattle (as shown in Fig. 1).

2. Survey methodology

We started by reviewing the published research on the neurophysiology of voiding behavior and toilet training in domestic animals as well as in other nonhuman animals and humans. We surveyed both books and the scientific literature in PubMed, the Web of Science database and Google Scholar websites using the terms “learning”, “reflex”, “voluntary behavior”, “training”, “conditioning”, “neurophysiological control”, “urination”, “defecation” and “cattle” and screened all resulting articles published up to January 2020 relating to our topic area. Publications in English, German and Dutch were included.

3. Neurophysiological regulation of elimination

Before addressing the possibility of training elimination behavior in cattle, we briefly describe the neurophysiological mechanisms controlling urination and defecation in mammals in this chapter.

As for the neurophysiological mechanisms and musculature involved in the control of the anal and urinary sphincters, the best information available is for humans, and this will also be utilized for our review. Fig. 2 summarizes the innervation of the different parts of the voiding system. Both the vegetative/autonomic nervous system (ANS) and the somatic nervous system (SNS) are involved in the control of eliminative behavior (Fig. 2) (de Groat, 2006; Fowler et al., 2008). The detrusor muscle surrounding the bladder and the internal sphincter

(anal and urinary) are smooth muscles (Salomon et al., 2008). These are under autonomic (involuntary) control (Arya and Weissbart, 2017). In contrast, the external sphincters (anal and urinary) are striated muscles and can be voluntarily controlled by the somatic nervous system (Blok, 2002).

As the bladder/rectum fill, stretch sensors on the bladder wall and in the rectum transmit information via afferent nerve fibers to the spinal cord (Fowler et al., 2008; Holstege, 2016); these signals are then transferred to the PAG in the brain (Blok, 2002; Holstege, 2016). In the PAG, information from the stretch receptors is brought together with descending impulses (perceptive, emotional, vegetative and motor) from higher brain centers so that the PAG regulates elimination (Blok, 2002; Holstege, 2005, 2016). The involvement of the PAG is very important to make possible the suppression of voiding in a life-threatening situation (Holstege, 2005). When voiding is inhibited, the PAG transmits an inhibitory signal to the spinal cord via the pelvic organ stimulating center (POSC) (known as Barrington’s nucleus, M-region or pontine micturition center) in the pons (as reported for humans, cats and rats) (Blok and Holstege, 1994; Holstege, 2014, 2016). Therefore, the sympathetic fibers of the *Nervus hypogastricus* carry the signal to inhibit the detrusor or rectal muscles and to activate the internal sphincter muscles. The external sphincter muscles are activated by the somatic fibers of *N. pudendus* and are therefore likely to be under voluntary control (Fowler et al., 2008; Holstege, 2016; Katsui et al., 2009).

When the PAG gives the signal for elimination, sympathetic activity is switched over to parasympathetic (ANS/involuntary) activity, and the parasympathetic nerve fibers of the *Nn. pelvini* activate the contraction of the detrusor or rectal muscles and to activate the internal sphincter muscles (Fowler et al., 2008; Holstege, 2016; Katsui et al., 2009). Furthermore, the PAG signals lead to the relaxation of the external and internal sphincter muscles (Fig. 2) (Arya and Weissbart, 2017).

In young humans, the neuronal connections between the POSC and the brainstem are not yet fully developed (Foehlinger, 2013; Fowler et al., 2008); therefore, urination and defecation are controlled by primitive reflex pathways organized in the spinal cord (Fowler et al., 2008). The offspring of many species, such as cats and rats, have an exteroceptive somato-bladder reflex that is triggered when the mother licks the perineal region (de Groat, 2002; Fowler et al., 2008; Sugaya et al., 2005). A similar reflex has been detected in human infants (Fowler et al., 2008) and calves (Metz and Metz, 1986). During development the reflex disappears and alternative neuronal connections

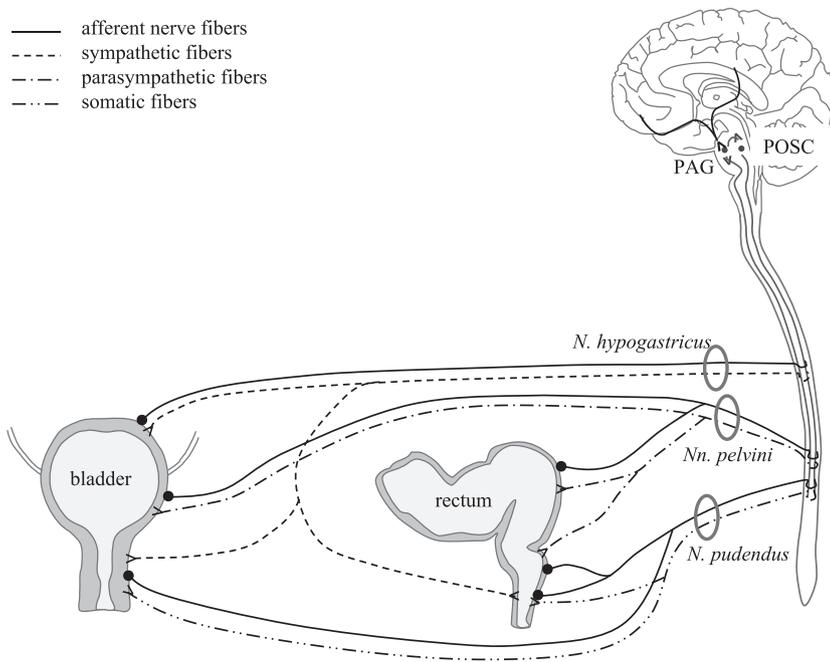


Fig. 2. Neural control of the bladder and the rectum in humans. Information on the filling level of the bladder and rectum is transmitted directly to the PAG via afferent nerve fibers. There, this information is linked to descending information from higher brain centers, which decide when and where voiding takes place. In the case of voiding, the PAG transmits these signals via the POSC to the parasympathetic nerve fibers of the *Nn. pelvini* (involuntarily), causing the detrusor or rectal muscles to contract. In addition, the voiding signal leads to the relaxation of the internal and external sphincter muscles. In the case of storage, inhibitory signals from the PAG via the POSC and the sympathetic fibers of the *N. hypogastricus* lead to the inhibition of the detrusor or rectal muscles and the activation of the internal sphincter muscles (involuntary). In addition, the external sphincter muscle is also activated (voluntarily) by somatic fibers of *N. pudendus*. Modified from Salomon et al. (2008); Holstege (2016) and Arya and Weissbart (2017).

that link with the PAG are favored (de Groat, 2002), which provide the potential for greater voluntary control of voiding.

In cattle, the somato-bladder reflex wanes particularly quickly (Metz and Metz, 1986). Metz and Metz (1986) observed that calves urinate and defecate readily in response to anogenital licking by the dam at 1 and 2 d of age but not on the third day postpartum. In contrast, in species such as cats and rats that are less mature developmentally, the reflexive responsiveness to maternal licking lasts for two to four weeks (de Groat, 2002).

In summary:

- The rapid waning of the somato-bladder reflex in cattle suggests that cattle may be able to learn to control elimination very early in life.
- During toilet training, the main aim is to influence the higher brain centers, thereby gaining control of the striated muscles of the pelvic floor (Blok, 2002; Holstege, 2016) so that individuals can control when and where elimination is appropriate (Holstege, 2016).

4. Natural behavior and elimination

The natural behavior of a species is likely to be relevant to the likelihood that individual animals can learn or be trained to show latrine use behavior (Kilgour and Albright, 1971). Species such as pigs (Buchenauer et al., 1982), horses (Lamoot et al., 2004), dogs and cats (Lord et al., 2008) tend to void in specific locations in their normal living environment without specific training; this is used as a social signal for space/dominance or for mating (Holstege, 2005; Kilgour and Albright, 1971). Such behavior implies some degree of voluntary control over urination and defecation reflexes. Most domestic cattle do not show a similar behavior regarding the deposition of their excreta, but some breeds, e.g., Chillingham cattle, are thought to mark a familiar area with feces (Whitehead, 1953 cited in Kilgour and Albright, 1971), indicating that cattle may have a tendency to defecate in defined locations, which could facilitate latrine training.

Before elimination, cattle occupy a characteristic position: at first, the body is slightly bent backwards, then the tail is raised, and the back is either slightly arched (defecation) or strongly arched (urination). Sometimes the hind legs are slightly spread before defecating, whereas before urination, they are placed forwards and spread further (Aland et al., 2002; Villettaz Robichaud et al., 2011).

In general, cattle urinate and defecate at higher frequencies during daylight when they are active than during the night (Hirata et al., 2011; Vaughan et al., 2014b), but the fecal output per defecation is higher during night, after longer resting periods (Hirata et al., 2011). Adult cattle have a higher frequency of elimination than juveniles (Aland et al., 2002; Vaughan et al., 2014b). Defecations are more frequent than urinations in cattle (Aland et al., 2002; Villettaz Robichaud et al., 2011), except in preweaned calves, in which urination is more frequent (Vaughan et al., 2014b). Substantial differences exist between individual animals in the frequency of elimination (Villettaz Robichaud et al., 2011), e.g., in cows, the number of defecations in a 24-h period varied between 8 and 29, and the number of urinations varied between 5 and 18 (Aland et al., 2002). These differences are consistent over time (Vaughan et al., 2014b). Vaughan et al. (2014b) observed correlations between elimination frequency and visits to a milk feeder or water drinker in calves, but Aland et al. (2002) found no correlation between elimination frequency and milk yield, the stage of lactation or feeding intensity in cows. Farmed cattle at pasture normally do not defecate in particular locations (Oudshoorn et al., 2008). Indoors, i.e., in loose housing systems, cattle defecate more often in the feed alley than in the resting areas. However, this was only partly related to the time they spend in these areas (Villettaz Robichaud et al., 2011).

Cows frequently defecate and urinate during standing and then walk forwards (Villettaz Robichaud et al., 2011; Whistance et al., 2011), and they often eliminate immediately after rising (Villettaz Robichaud et al., 2011) which suggests that cattle have some degree of voluntary control over urination and defecation. Further, Whistance et al. (2009) demonstrated that cattle can show an awareness of voiding behavior. If heifers were rewarded each time immediately after voiding, they started walking to the trainer shortly before or during voiding (Whistance et al., 2009).

Based on these observations of voiding behavior, it is perhaps not surprising that cattle can be trained to increase the frequency of urinations by operant conditioning (Vaughan et al., 2014a), but so far, there has been no conclusive demonstration of control of voiding reflexes.

5. Non-associative and associative learning procedures

Learning is defined as a change in behavior due to experience

(Barker, 1997) and includes two major categories: non-associative and associative learning (see Fig. 1).

In non-associative learning processes, e.g., habituation and its opposite, sensitization, the frequency of an existing behavior is modified in response to a stimulus, which is repeatedly presented (Abramson and Kieson, 2016). The defining characteristic of habituation is a behavioral response decrement (Harris, 1943), which is commonly exponential (Thompson and Spencer, 1966) but sometimes linear and is not due to sensory adaptation or motor fatigue (Rankin et al., 2009). Like all learning, habituation is a reversible process and shows spontaneous recovery. A stimulus similar to the habituated stimulus can elicit a similar response, a phenomenon called generalization. Habituation is very important in livestock farming because familiarization with (i.e., habituation to) new objects or procedures in living environments can reduce stress and improve animal performance (Abramson and Kieson, 2016; Doerfler et al., 2016; Wicks et al., 2004). In regard to latrine training of cattle, it would be important that animals are well habituated to the handling procedures and learning tasks.

In associative learning, an animal learns to perform a response (typically a novel one) based on an association between two or more stimuli or events (Abramson and Kieson, 2016). There are two main associative learning paradigms, i.e., classical (or Pavlovian) and operant conditioning. Classical conditioning is traditionally utilized in learning associated with involuntary, reflex-like responses (Willis and Mein, 1983), and operant conditioning typically concerns the training of voluntary, skeletal behaviors (Abramson and Kieson, 2016). In the section on neurophysiological regulation of voiding, we showed that toileting behavior involves the control of apparently reflexive-like bladder and rectal muscle movements and skeletal behaviors such as movement to a latrine, implying that a combination of classical and operant conditioning procedures may be required for successful training of latrine use in cattle.

For training latrine use in cattle, the following elements related to non-associative and associative learning (see Fig. 1) would be necessary:

- a Perceiving internal cues signaling imminent urination or defecation;
- b Withholding urination or defecation until arrival at a latrine and initiating urination/defecation after arrival at the latrine;
- c Habituating to the latrine area with all its components such as reward delivery or the reward itself;
- d Using environmental cues to identify specific locations in the housing environment as a latrine and reward area;
- e Transferring the learned behavior from one latrine to another – the cattle must be able to generalize;
- f Negotiating routes through the barn to the latrine.

Non-associative learning is likely to be required in familiarizing (habituating) subjects to features of the reward and latrine area. It would be helpful if the learning generalized to latrines in different locations. Associative learning may be required in training both voluntary (e.g., the inhibition of excretion, moving to the latrine) and reflexive (initiation of excretion) behaviors.

5.1. Classical conditioning

5.1.1. Classical conditioning of reflexive behaviors

The milk ejection reflex is a well-known example of classical conditioning in mammals, including cattle. In the milk ejection reflex, suckling by the calf is the unconditioned stimulus (US), which elicits the unconditioned response (UR), in this case milk ejection (Ellendorff et al., 1982; Husv eth, 2011). Various neutral stimuli, such as the sight of the calf, if occurring in close proximity (time and place) to the US (suckling) on several occasions, may come to elicit milk ejection (Barker, 1997; Domjan, 1998; Husv eth, 2011). After successful conditioning, the sight of the calf becomes a conditioned stimulus (CS), and

the response (milk ejection) is the conditioned response (CR). However, highly selected dairy cattle breeds can be milked by machines without the presence of their calves. Here, the sound of the milking unit (Husv eth, 2011), the presence of the person who is milking the cows (Orihuela, 1990) or the application of the teat cups (Folley and Knaggs, 1966) have become the CS that elicits milk ejection (CR). The US is simulated suckling by the milking machine.

If the CS is presented on a number of occasions on its own (without the US), then the CR will gradually weaken and disappear, a process called extinction (Barker, 1997; Bouton and Moody, 2004). Extinction is important because it allows the animal to adapt to a changing environment, and the weakening of the CR to the CS shows that the CR was indeed a learned reflexive behavior (Bouton and Moody, 2004).

Even if a CR has been trained successfully to occur following the presentation of a CS, other stimuli such as loud noises occurring around the same time may inhibit the CR (Husv eth, 2011). The biological relevance of this inhibitory process is also important, as a cow needs to stop feeding its calf if there is a threat from a predator and switch to anti-predator responses (Brembs and Heisenberg, 2000;  hman and Mineka, 2001). Some evidence exists that reflexes can be subjected to voluntary control, which would be an important attribute in the training of voiding behavior (de Groat, 2006; Fowler et al., 2008). It has been shown in humans that voluntary inhibition (control) of the striated sphincter muscle reflex is possible when the person becomes aware that a toilet is not immediately available (Fowler et al., 2008).

Another feature of classical conditioning is that responses learned in one context can occur in other similar contexts, meaning generalization (Rankin et al., 2009; Thompson and Spencer, 1966). This generalization would be important in latrine training cattle, for instance, when animals move to different barns or areas of the farm, so that the animals would recognize distinctive cues for the appropriate voiding area and would not need to be retrained in each situation.

The generalizability of a classically conditioned response has been demonstrated with the milk ejection reflex in cows (Willis and Mein, 1983). One group of cows was presented with a blue disc concurrently with suckling by their calves. In a second group, the blue disc was presented when the calves were removed. This training occurred outside of the milking parlor. Subsequently, the blue disc was shown in the milking parlor, and the milk yield of the first group increased, whereas the milk yield of the second group decreased. This experiment shows two things: first, the CR can be generalized from the training location to another location. Second, milk ejection can be conditioned both to increase and to decrease (Willis and Mein, 1983). Both the inhibition and enhancement of sphincter reflexes are relevant to latrine training, as the excretory responses need to be withheld until a latrine has been entered and then activated once at the latrine.

As described above, the conditioning of reflexes by classical conditioning has been demonstrated in several species (Barker, 1997; Husv eth, 2011; Willis and Mein, 1983). Evidence exists that reflexes of cows can be inhibited involuntarily (e.g., stress effects on milk ejection) and that the reflexes can be enhanced and diminished via classical conditioning (Husv eth, 2011; Willis and Mein, 1983). However, has classical conditioning been demonstrated in toilet training procedures?

With humans, there appears to be only one published study in which classical conditioning has been postulated to account for toilet training. Mowrer and Mowrer (1938) developed a system in which an alarm sounded if a pad under a sleeping person became wet. Typically, before training, bladder distension during the night is an US for the relaxation of the urinary continence muscles (UR), and urine is released. After training with the Mowrer and Mowrer (1938) procedure, bladder distension was postulated to become a CS after repeated association with the alarm (US), which awakens the person and inhibits the activation of the urinary continence muscles (the UR and CR). After prolonged training, the CS may elicit muscle contraction without awakening (Henriksen and Peterson, 2013). The procedure is effective in preventing nocturnal incontinence; however, there is some doubt as to

whether it is an example of classical conditioning (Lovibond, 1963).

With calves, classical conditioning has been evaluated as a way to increase the frequency of urination by calves at a specific location (Vaughan et al., 2014a). During training, the calves were trained by being held in a latrine (pen) for 10 min twice a day until they had urinated on at least eight occasions in total. A diuretic (US) was used to increase the likelihood of urination (UR). During testing, the calves were placed in the latrine without diuretics. The frequency of urination of the experimental calves was compared with that of a control group that was administered saline solution during the training period. Successful classical conditioning would be revealed by a higher frequency of urination (CR) by the test calves when placed in the latrine (CS). There was no difference in urination between the experimental and control calves. However, while the results were interpreted as showing that it is not possible to classically condition the urination reflex (Vaughan et al., 2014a), the absence of conditioning may be attributable to methodological issues:

- There may have been an inadequate number of pairings of the CS and US in the training phase.
- The CS may not have been sufficiently distinctive as the training pen might have resembled other pens.

Although other types of reflexes have been classically conditioned in cows (Willis and Mein, 1983), there is no clear demonstration of successful conditioning of eliminative behavior (Vaughan et al., 2014a). However, based on our arguments presented above and below, it would be premature to conclude that cattle cannot learn control over their excretory behavior by classical conditioning.

5.1.2. Classical conditioning of voluntary behaviors

In addition to reflexive behaviors, voluntary behaviors can also be conditioned classically. One example is autoshaping or sign-tracking, which describes an approach to a stimulus that predicts the availability of a positive event following repeated presentations of the stimulus and that positive event. Sign-tracking may function in nature to enhance interactions with reinforcers, such as food, and to send out signals, such as odors (Domjan, 1998).

Several mammals have been observed to approach a stimulus presented before a food reward. For example, rats licked and nibbled at a retractable lever while they were waiting for food, even though this did not affect the reinforcer availability (Peterson et al., 1972). In addition, monkeys reached for a lever in the same situation as they subsequently reached for their food (Schwam and Gamzu, 1975; Sidman and Fletcher, 1968). To our knowledge, there have been no studies on autoshaping in cattle. Regarding toilet training, autoshaping has been used in one study with humans (Siegel, 1977). The study investigated the effect of a floating target in the toilet on avoiding misdirected urinations in disabled persons. The results indicated rapid autoshaping of correct urination without the use of additional toilet training. Thus, operant responses can be shaped by classical conditioning contingencies and autoshaping may play a role in latrine training, e.g., by enhancing movement towards reinforcers.

5.2. Operant conditioning

5.2.1. Operant conditioning of voluntary behaviors

In this section, we present some examples of operant conditioning of voluntary behavior in cattle with potential relevance to latrine training. Kiley-Worthington and Savage (1978) used an operant conditioning procedure to determine if cattle could be encouraged to walk from pasture to a milking barn unassisted in response to an auditory stimulus. In the first week, an acoustic signal was coupled with a stockperson calling the cows to milking (visual and auditory cue). After one week, only the acoustic signal was activated at milking time, and 85 % of the cows went into the barn within five minutes after the signal.

Unfortunately, when called from pastures not used in training, the herd moved more slowly (Kiley-Worthington and Savage, 1978). Thus, the learned response failed to generalize to alternative locations. De Passillé et al. (1996) showed that calves do not transfer learned avoidance behavior for a specific person from one place to another. However, in another study, Munksgaard et al. (1997) found exactly the opposite. These results indicate that while cattle have the ability to generalize learned operants to new stimulus contexts, this generalization may not occur readily.

Wredle et al. (2004) demonstrated that heifers can be trained to approach a feeding station after an auditory signal when using a combination of classical and operant learning processes. They started with magazine training (Barker, 1997; Domjan, 1998) to build the association between a tone from the collar and the sound of the motor of the feeder. After this step, the feeder was activated when the heifer started walking towards the feeding station after receiving the tone from a collar around their neck. In the final step, the feeder was activated when the heifer entered the feeding station. All trained heifers went more often to the feeding station after the signal, and they could remember the task one month later. In a follow-up study, they investigated whether the heifers were able to generalize this behavior to a place other than where they were trained. One heifer was placed in its home pen, which was linked by a corridor to the training pen with the feeding station. After the heifer had moved freely between these pens for 30 min, it received a tone signal when it was in the home pen to go to the feeding station, but all heifers failed, meaning that they had problems with generalization. Wredle et al. (2006) trained cows individually to go to the automatic milking systems (AMS) after hearing a tone from a collar that they wore. During training, all cows learned to enter the AMS in reaction to the signal, but the behavior was not maintained during the test phase, when they were group-housed and called only 8 h after the previous milking. However, this was most likely only because in the test phase, the AMS was often not immediately available when the cow approached. This means that the cows had to wait too long for the reward (Wredle et al., 2006) and therefore the behavior extinguished. This result shows how important immediate reinforcement is to avoid extinguishing what has been learned (Abramson and Kieson, 2016; McLean, 2005). Wredle et al. (2004) trained cattle to approach a food source using both classical and operant conditioning and concluded that operant training led to faster acquisition and retention of the newly learned behavior for longer periods. However, they also found little evidence of generalization to novel locations.

Seo et al. (2002) used vibration on a neck collar as a discriminative stimulus to 'call' cattle to a feeding station. This use of a discriminative stimulus from a different sensory modality, compared with previous examples of auditory stimuli, provides evidence that a general learning mechanism is operating. Nevertheless, the nature of the stimuli used may modulate the effectiveness of conditioning procedures. Uetake and Kudo (1994) found that calves responded more to lights than to tone signals and that they preferred white and green lights to red lights.

These studies illustrate that cattle can be trained readily by operant procedures to walk to a specific place in response to external signals to obtain a reward (Seo et al., 2002; Wredle et al., 2004, 2006), even during normal farming operations. In addition, the literature contains many examples where cattle have been trained to learn routes through mazes (Bailey et al., 1989; Hirata et al., 2016) under experimental conditions. Cattle were able to distinguish between different amounts of food and where it was located in a five-arm parallel maze (Bailey et al., 1989). If they had formed an association with a reward, they could still remember it one year later (Hirata and Takeno, 2014). Thus, it does not seem to be a problem for cattle to move to a certain location according to a signal, but generalization to other locations is perhaps an issue (Wredle et al., 2004) which could be avoided with the use of conspicuous visual cues (Uetake and Kudo, 1994). Some general principles to aid learning include: that a potential reward should ideally be highly

valued; and successive steps in a training process should be mastered before progression (Hirata et al., 2016).

5.2.2. Operant conditioning of reflexive behaviors

Some evidence exists that reflexive behaviors (and, importantly, in the context of the present review, toileting) can be modified by operant conditioning procedures in several animal species. In humans, two early studies developed concepts that seem to have been the basis for many other studies in the context of operant conditioning of voiding reflexes. Van Wagenen et al. (1969) used an auditory alarm device attached to the genitals of nine subjects that emitted a tone when urination started. Tone activation resulted in a mild reprimand by the trainer causing a mild startle response and interruption of urination. The child was then taken to the toilet where urination was reinitiated and reinforced by the trainer. All subjects learned to control urination and generalize control to other environments. The second study, by Bernadet and Foxx (1971), combined positive reinforcement, positive punishment and scheduled toileting times. The subjects were reinforced every 5 min while dry and after correct elimination and they were punished after an accident. During the scheduled toileting times, the subject was placed on the toilet for 20 min or until voiding occurred. This procedure reduced the incontinence rate by 90 % (Bernadet and Foxx, 1971). Cicero and Pfadt (2002) developed a methodology from a combination of the Van Wagenen et al. (1969) and Bernadet and Foxx (1971) procedures using a combination of prompted schedules and immediate prompting in response to voiding outside of the toilet, and reward for urination in the correct location. The prompted schedule increased the likelihood of reinforcing correct behavior and, thus, promoted rapid success. Immediate prompting, on the other hand, created an appropriate behavior chain in response to the need to eliminate. All children were trained successfully with this method.

The normal discriminative stimuli for appropriate toileting behavior are bladder and rectum tension. With the Van Wagenen et al. (1969) and Cicero and Pfadt (2002) methodologies, the normal voiding stimuli are associated with additional external stimuli (e.g., prompts/alerts, mild startle), and this seems to facilitate successful toilet training. On the other hand, the normal voiding stimuli are not present in scheduled toileting procedures, which may slow the learning of appropriate toileting and the withholding of reflexive voiding responses (Osarchuk, 1973).

Taken together, these studies with humans demonstrate that operant conditioning procedures utilizing discriminative stimuli and reinforcers can be used very successfully to train appropriate toileting behavior. The addition of a mild stressor (or distractor) to interrupt inappropriate voiding and to provide a window of time for the person to move to a toilet can also be helpful.

So far, only two approaches have used operant conditioning procedures in experimental studies attempting to train latrine use in cattle (Vaughan et al., 2014a; Whistance et al., 2009). Whistance et al. (2009) used a group of indoor-housed heifers kept in their home pen comprising two distinct areas: a concrete floored section and an area bedded with straw. During training, the heifers were confined to the concrete area only. Immediately after urinating or defecating, the animal that had voided was presented with a food reinforcer held by a trainer. This procedure was continued until every heifer orientated to the trainer during voiding (or immediately after voiding). In the subsequent phase, both the straw-bedded and concreted areas were available, but the heifers were rewarded for voiding in the concrete area only. The heifers voided more in the concrete area than in the straw area. However, voiding occurred in both sections, and crucially, there was no increase in the frequency of moving from the straw area to the concrete floored section over time. Thus, there was no clear evidence of latrine use by the heifers (Whistance et al., 2009). One possible reason could be that not all elements necessary for latrine training (see Section above 5) were considered or trained appropriately. Success in withholding excretion (Element b) was not formally assessed in the

study, but interruption of some voiding episodes was observed when an animal moved to the reward location. This only occurred when the animals were confined to the concrete area (latrine). Movement to the reward location suggests that the animals could negotiate routes through the barn (Element f). However, there was no evidence that the cattle were able to distinguish between the latrine (concrete area) and non-latrine (straw area) (Element d). Possible reasons include: the latrine area was not sufficiently demarcated; and the animals could not distinguish between correct and incorrect voiding behavior. The use of a conditioned stimulus (Langbein et al., 2007) or punishment could be helpful by providing immediate feedback for incorrect behavior. Further, in the Whistance et al. (2009) experiment, the majority of voiding events in the training area would have gone unrewarded as training occurred for 6 h only each day, and this would have hindered learning. To conclude, the study was not well-designed with regard to the elements that need to be mastered in latrine training of cattle. However, one key prerequisite for successful toilet training (awareness of their elimination behavior) appears to have been demonstrated by the Whistance et al. (2009) study.

In the second reported evaluation of toileting in cattle, individual calves were trained to urinate when they were in a specific location (Vaughan et al., 2014a). As with the Whistance et al. (2009), a limited set of the requisite elements for successful training (see Section 5 above) were evaluated: Element b and d. On training days, single calves were kept in a test box and received a diuretic injection (to stimulate urination). After every urination event, a bell was sounded, and the calf was released from the box and received a milk reinforcer. On test days, a calf was kept for 15 min in the box but was not administered a diuretic. If the calf urinated, it was released and provided with a reward. If the calf did not urinate, it received a five-minute time out (absence of reward), and the procedure was repeated the following day. In comparison with yoked control (saline injection) calves, the experimental animals had a higher frequency of urination events on test days. The study showed the capability of calves to associate a specific location with urination (Element d) when trained using operant conditioning. This also means that calves are able to initiate urination voluntarily (a component of Element b). Unfortunately, there was no test of the remaining prerequisites for latrine training, therefore, the study does not provide a critical test of the ability of calves to learn to use a toilet.

In conclusion, in humans, it is possible to train urination and defecation control, separately or simultaneously (Bernadet and Foxx, 1971; Hundziak et al., 1965; Cicero and Pfadt, 2002; Neale, 1963) using operant conditioning techniques. The necessary anatomical, neurological and physiological processes and learning abilities associated with voluntary control of voiding reflexes in humans are also found in cattle. Logically, then, it is likely that voiding behavior in cattle will also be conditionable, most probably using operant conditioning procedures (Vaughan et al., 2014a; Whistance et al., 2009).

6. Summarizing conclusion and practical outlook

We have described the neurophysiology of voiding reflexes and have shown that there are both voluntary and involuntary responses involved in initiation and control of voiding. Regarding the conditionability of voiding, we have described how operant methodologies are mainly applicable to modifying voluntary behavior and classical methodologies are mainly applicable to changing involuntary behavior although there is potential for these to crossover. Further, we have argued that because many voluntary behaviors need to be trained in successful toileting and because involuntary behavior is also susceptible to operant conditioning, operant conditioning is likely to be the main tool for training latrine use in cattle.

For successful latrine training, animals must learn to recognize the impending need to void (Element a), withhold urination/defecation until reaching the latrine, void (Element b) and then leave the toilet.

It is well known that cattle must learn many tasks in farming systems, and these are often self-taught (or by social learning) or trained with little input from the animal carers. For example, cattle learn autonomously the location of essential resources in the living environment, and how to operate equipment such as automatic feeders, water dispensers and entry to automatic milking systems (Wechsler and Lea, 2007). Furthermore, various studies have shown that it is possible to use operant conditioning to teach cows to move reliably to a certain place after prompting with visual (Kiley-Worthington and Savage, 1978), acoustic (Kiley-Worthington and Savage, 1978; Wredle et al., 2004, 2006) or vibrational signal (Seo et al., 2002) that serve as discriminative stimuli.

Therefore, cattle have a ready propensity to learn to move to specific locations, which is an important element in training latrine use. Cattle appear to be able to learn to associate urination with a specific location (Vaughan et al., 2014a), and another study found that cattle appear to have an awareness of an association between their elimination behavior and rewards (Whistance et al., 2009). These observations mean that the second part of latrine training (voiding in a specific location) should also be achievable. In practical situations, it would be important for an animal to leave the latrine area after voiding. Leaving the toileting area is an operant response, and, therefore, it should be readily trainable, as for movement into the latrine. Although reflexes are often classically conditioned, the results with other species suggest that latrine training with operant conditioning would seem to be more promising. As continence is controlled not only by the detrusor muscle and internal (smooth muscle) urethral sphincter of the urinary bladder (both under involuntary control) but also by the external urogenital sphincter (striated muscles), which is under voluntary control (Arya and Weissbart, 2017), it is likely that operant rather than classical conditioning procedures will be more useful for latrine training. Therefore, it seems promising to promote studies on latrine training in cattle, particularly using operant conditioning methodology.

This review is mainly concerned with the feasibility of latrine training of cattle from a neurophysiological and learning theory point of view. The next obvious steps are to test the ability of cattle to learn the full latrine training process and the potential for practical implementation. To ensure ready applicability of latrine training on farms, ideally, the training should be undertaken with minimal input from the animal caretakers. One possibility would be to link remotely-detected signs of voiding initiation (e.g., changes in tail position), movement toward a latrine and voiding in a latrine with remotely activated reward presentation. A number of technologies could be utilized for such purposes including thermal imaging, machine vision and machine learning which have been used, for example, in the automated detection of parturient and other behaviors in a range of species including cattle (Miller et al., 2020; Wurtz et al., 2019) and for the automated training of rodents (e.g., Poddar et al., 2013).

If feces and urine were voided into latrines, the alleys and cubicles in the barn would much less soiled, which means that the hooves and udder would have reduced exposure to high levels of bacteria on the floor surfaces, thereby contributing to improved animal health (Santman-Berends et al., 2016; Sarjokari et al., 2013). In addition, alleys would no longer need to be cleaned as much, providing labor and cost savings (Brantas, 1968; Chapinal et al., 2013; Somers et al., 2005). Furthermore, the environmental aspect of this approach should not be forgotten. The use of latrines would help to mitigate the emission of greenhouse gases such as N₂O and NH₃ by making it possible to collect and separate feces and urine (van Dixhoorn et al., 2017).

We have provided solid evidence that toilet training is within the learning capacities of cattle. Our motivation for writing the review is to provide a resource to inspire researchers to explore innovative methods to reduce some of the deleterious effects of cattle farming on climate change and animal health and welfare.

Funding

This work was supported by the Volkswagen Foundation [Az.: 92 221, May, 10th 2017]. The publication of this article was partly funded by the Open Access Fund of the Leibniz Institute for Farm Animal Biology (FBN). The funders had no role in the design, decision to publish, or preparation of the review manuscript.

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