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Abstract

Stray voltage (< 10 V) may impair animal welfare. Our objectives were to: i) determine the threshold at which heifers react to voltage and ii) investigate effects of past experience and random applications of voltage. Firstly, forty heifers were trained to eat from two metallic feeders at the end of a test corridor. For 20 heifers, voltage was applied for 2 min (every day in steps of 0.33 V, 0 to 5 V) to the feeder (F1) in which the heifer started to eat (VOLT). Heifers could change to the non-electrified feeder (F2) if they wished. Twenty heifers (CONT) followed the same procedure without voltage exposure. For voltages ≥ 2.3 V, percentage of feed eaten from F1 (%FeedF1) was lower, time spent eating in F1 and latency to change to F2 were shorter compared to 0 V. At 2 V and above, more VOLT than CONT heifers performed muzzle-licking and abrupt head movements. Secondly, after four weeks, the same heifers were exposed to 3.3 V for either 11 consecutive days (DAILY, n = 20) or randomly on 4/11 days (RAND, n = 20). CONT heifers had higher cortisol concentrations than VOLT heifers on the first day of test. %FeedF1 was higher for RAND than DAILY heifers. The threshold at which avoidance behaviour started appeared to be 2.3 V in our experimental conditions. Adaptation was more difficult with unpredictable rather than predictable voltage and past experience seemed to reduce the effects of subsequent exposure.

Keywords: *animal welfare, cattle, past experience, stray voltage, stress, unpredictability*

Introduction

Electricity is essential to modern farming techniques and several electrically powered machines are used, such as milking machines, heated water bowls, etc. Leakage of current from this type of equipment, electric and magnetic induction, faulty connections between the electrical circuit and the earth can lead to the undesirable electrical phenomenon called stray voltage (for a review, see Deschamps 2002). Stray voltage, usually less than 10 V, can produce a low current flowing through farm animals (for a review, see Hultgren 1990). According to Ohm's Law, the current depends on the voltage level and the electrical resistance of the animal. The electrical resistance is the sum of the resistance of body tissues and of the contact resistance of the animal to the ground and to the electrified element. Although some effects of stray voltage have been reported on health, behaviour and production parameters in dairy cattle field studies (Salisbury & Williams 1967; Wilson *et al* 1996), in controlled studies, only behavioural modifications were confirmed, but not health and production effects. Indeed, using either electrodes to apply a current in dairy cows, from the right front knee to the right rear hock (the current varied from 0.7 to 12.5mA rms — root mean square — which corresponded to a voltage between 0.2 to 3.9 V rms; Lefcourt 1982; Lefcourt *et al* 1986), or using a non-piercing ball-end nose clip and a metallic floor to apply a current from the muzzle to the four hooves (the current varied between 2 to19 mA peak; Reinemann *et al* 1999) behavioural responses, such as sudden withdrawal, hoof lifting, vocalisations, kicks or jumps were observed for currents starting at 0.7 up to 19 mA. In other controlled studies, physiological responses to current exposure were investigated. An increase in heart rate and in plasma cortisol concentration were observed for currents starting at 3.6 up to 12.5 mA rms (from 2.0 to 7.8 V rms) applied with electrodes on the lumbosacral region (Gorewit & Scott 1986) or from the udder to the four hooves (use of electrodes and metallic floor; Henke Drenkard *et al* 1985).

In addition, stray voltage threshold has often been studied on a relatively small number of animals (< 10 cows) and without allowing the animals to avoid the electric stimulus (for a review, see Hultgren 1990). This is important since it has been shown that when lambs had the opportunity to change from an electrified feeder to a non-electrified feeder, a lower threshold of response was observed compared to the lambs which were not able to change feeder (Duvaux-Ponter *et al* 2006).

The first objective of this work was to determine the threshold at which heifers react to voltage. The voltage was applied to a device similar to that which is found in farms (ie metallic feeder) in a voltage range encountered in field conditions. The animals were able to avoid the voltage if they wished. This work was a prerequisite to a field-size experiment which was designed to study the effect of chronic exposure to stray voltage on welfare in dairy cows under farm conditions.

Since stray voltage in a farm occurs mostly in an unpredictable manner (Deschamps 2002), the animals may not be aware of when and where they will come into contact with electricity. If the application of a voltage is foreseeable, ie if it is applied every day (which is the case in many experiments), it might be easier for the animal to habituate to the stressor (Gorewit *et al* 1985; Henke Drenkard *et al* 1985). If voltage is applied randomly and is therefore unpredictable for the animal, the same stressor could be perceived as more aversive and could delay animal adaptation. This unpredictability can be an important source of stress and could impair animal welfare (Brugère 2002; Sandem *et al* 2004).

The second objective of this work was to investigate how an animal's past experience to voltage and its random application could affect the animal's behavioural and physiological responses to subsequent exposure to voltage.

Materials and methods

The scientist in charge of the experiments was licensed to perform experiments on animals and the staff who applied the experimental procedures have attended a special experimentation course approved by the French Ministry of Agriculture.

Study animals

Forty Holstein heifers were tested (starting age of 216 [± 59.1] days and 235 [± 54.6] kg of bodyweight; mean [± SD]). They were housed in four pens, each pen containing 10 heifers of similar weight and age in order to avoid competition at feeding time. The animals were fed once a day at 1600h with a total mixed diet (DM basis: 38% sugar beet pulp, 35% straw, 16% rapeseed meal, 8% pulpless orange segments, 3% molasses, vitamins and minerals) covering the nutritional requirements for a 200 kg heifer (INRA 1988). Heavier animals received 2.5 kg of the total mixed diet as a supplement per 100 kg above 200 kg bodyweight. Water, straw and mineral fortified salt licks were available *ad libitum*. The pens were straw bedded.

Experimental apparatus

The experimental facility was adjacent to the home pens. It consisted of a control room, a test corridor (5.0 × 2.5 m; length × width), a starting cage (2.0 × 0.8 m) with a sliding gate, and two waiting pens (3.0 × 4.5 m) at the entrance and exit of the test corridor. The electrical exposure system, at the end of the corridor, consisted of two metallic feeders where voltage was applied and electrical characteristics were recorded by the control-command system installed in the adjacent control room. The metallic feeders were electrically insulated from all the metallic parts of the experimental facility. Each feeder was filled with 700 g of a mixture of concentrate (50%) and sugar beet pulp (50%) so that the heifer was able to eat throughout the test in the same feeder or to change feeder. An aluminium plate (2.5 × 2.5 m), also insulated from the other metallic parts, was placed on the floor beneath the metallic feeders. The walls of the test corridor were covered with plain wood to visually isolate the heifer being tested from the others.

Procedure

During the first experiment, heifers were submitted once a day to a 2-min individual test with a progressive increase in the applied voltage. This was done to study the threshold at which a large and durable modification in behaviour was observed. The second experiment was performed to study the effects of the type of electrical stress (daily vs random) and the effect of past experience to voltage on physiology and behaviour. Each experiment was preceded by a habituation period. Heifers were never in a fasted state during both experiments. The handling order of the heifers from the four pens was alternated each day. The heifers which came spontaneously to the entrance of the starting cage were tested first in order to limit stress due to handling. The tests took place between 0800 and 1300h.

Experiment 1

During a 6-week habituation period (once a day, 5 days a week), heifers were trained progressively to become accustomed to the test procedure without the application of voltage. In addition, the heifers were habituated to be restrained in a neck-lock in the exit pen for at least 60 min and for the tail to be handled to facilitate caudal venipuncture blood sampling during the test period.

During the test period, which lasted 19 consecutive days, the 40 heifers were divided into two balanced groups according to their weight, age and home pen: 20 heifers were submitted to a daily progressive increase in voltage, in steps of 0.33 V, starting at 0 V up to 5 V (VOLT group, 259 [± 57.5] days old, 270 [± 53.8] kg bodyweight; mean [± SD]) while the 20 remaining heifers followed the same procedure but without voltage (CONT group, 259 [± 62.1] days old, 273 [± 55.3] kg bodyweight).

In order to standardise the beginning of the test, the heifer was left for 15 s in the starting cage before the sliding gate was opened to give access to the test corridor. The sliding gate was closed after the passage of the animal. The test started (t0) once the heifer had eaten for at least 5 s in one feeder and lasted 2 min. For the VOLT heifers only, the voltage was applied for 2 min to the first feeder (called the electrified feeder thereafter) in which the heifer had started to eat for at least 5 s. This allowed the heifer to change to the second (non-electrified) feeder if it wished. After 2 min, the feeders were closed with a lid and the heifer was driven to the exit door. Heifers were restrained in the neck-lock in the exit pen until blood collection.

No voltage was applied during the first 4 days of the test period to obtain basal measurements for each animal. The quantity of feed left in each feeder was measured at the end of each 2-min test.

Experiment 2

After a break of 2 weeks without any disturbance, a re-habituation period of 4 weeks was performed using the same procedure as in the first experiment and without voltage exposure. The forty heifers used previously were allocated to one of 4 groups: previous VOLT heifers with daily voltage exposure (VOLT-DAILY, n = 10), previous VOLT heifers with random voltage exposure (VOLT-RAND, n = 10), previous CONT heifers with daily voltage exposure (CONT-DAILY, n = 10) and previous CONT heifers with random voltage exposure (CONT-RAND, n = 10). During the test period, heifers were exposed to a voltage of 3.3 V applied during 2 min to the feeder in which the heifer had initially started to eat: either, every day over 11 consecutive days (DAILY) or randomly on 4 out of 11 days (on the 1st, 4th, 6th and 10th day, RAND). The four groups were balanced according to weight and age.

Behavioural observations

Four digital camcorders (MSH-255, AXM, Paris, France) were placed around the test corridor to continuously record the heifers' behaviour during the test. The video files were saved on a DVD. A Quad 30® allowed the simultaneous visualisation of the images recorded by the four cameras. All films were encoded and analysed using The Observer® Software System for Behavioural Research (Noldus Information Technology, Wageningen, The Netherlands).

During the first and second test periods, the total feed intake and the electrified feeder intake as a percentage of total feed intake (percentage of feed eaten from the electrified feeder) were calculated for each animal and each day. The time spent eating in the electrified feeder and the latency to change to the non-electrified feeder after the application of the voltage were measured. The number of heifers performing abrupt head movements (ie side-to-side head shaking or an abrupt backward movement of the head) and muzzle-licking were also recorded.

The use of two feeders allowed the heifers to choose which feeder to eat in first. Heifers were characterised according to the strength of their laterality (called side-preference thereafter) by their first preference for one of the feeders. The preference was noted, irrespective of its side, according to three modalities: strong, mild or no first feeder preference. Strong side-preference was defined as the situation when the heifer ate in the same feeder for the first time for more than 90% of the tests, a mild side-preference between 60 and 90%, and no side-preference for less than 60% of the tests.

Cortisol concentration measurements

Blood samples were collected 15 min after t0. This sampling time allows the measurement of an elevation in cortisol following a mild stressor (routine veterinary procedures) as shown by Alam and Dobson (1986). Moreover, the hypothalamic-pituitary-adrenal (HPA) response to an aversive situation is generally assessed by measuring corticosteroid levels in blood at least 10 min after the animal has been first exposed to it (for a review, see Mormède *et al* 2007). Blood was collected by caudal venipuncture. Moreover, samples were taken within 2 min of raising the tail of the heifer by the experimenter. This time interval is likely to be insufficient for plasma cortisol concentrations to have been affected by the handling associated with blood collection (Broom & Johnson 1993).

During the test period of Experiment 1, blood samples were taken when voltage application was 1, 3 and 5 V. During the test period of Experiment 2, blood samples were taken on the 1st and the 10th day of voltage application.

Blood samples were centrifuged at 3,000 g for 10 min at 4ºC. Plasma was stored at –20ºC until analysis. Plasma cortisol was measured by ELISA using an automated method (Elecsys, Roche Diagnostics, Meylan, France). The sensitivity of the cortisol assay was 0.36 ng ml^{-1}. The inter-assay coefficient of variation was 4.5% at 124.69 ng ml^{-1}.

Electrical measurements

EDF R&D (Electricité de France Research & Development, France) provided the electrical circuit allowing exposure to the chosen alternating (50 Hz) voltage (from 0 to 5 volts [± 0.01] volt). In order to measure the voltage (rms) applied to the feeder and the current (rms) flowing through the circuit (feeder-heifer-metallic plate), a multichannel transient recorder (Nicolet 2580 Data Acquisition System, Nicolet Technologies, Madison, WI, USA) associated with an analyser software was configured to start automatically the recording of the current and voltage. Current measurements (rms) were performed by a current probe with effect Hall Tektronix, placed on the circuit at the exit of the power supply box. This current probe was connected to the Nicolet 2580 through a 50Ω coaxial link and was calibrated to record current from 0.6 mA (which meant a voltage above 1 V for a resistance of 1,700 ohms). For each test, the current (rms) crossing the animal was calculated using the software Team Pro (Thermo Fisher Scientific Inc, Waltham, USA). Five measurements of current were averaged per heifer and per day to obtain the mean current. The resistance of the set-up feeder-heifer-metallic plate was calculated by averaging all the data (ie 5 measurements per heifer and per voltage level). The maximal current flowing through the heifer during the test was also recorded (called maximal current thereafter).

Statistical analysis

Statistical analyses were performed using the Statistical Analysis System software (SAS®, version 9.1.3).

The behavioural observations of Experiment 1 were analysed by the MIXED procedure with the model:

$Y_{ij} = \mu + U_i + W_j + L_j + r_j + p_j \times D + e_{ij}$

where μ represents the overall mean; U_i the fixed effect of Voltage $_i$ (from 0 to 5 V in steps of 0.33 V for the VOLT group and set to 0 V for the CONT group); W_j the body weight of the animal $_j$ as a co-variable; L_j the fixed effect of the side-preference of the animal $_j$ (3 modalities: strong, mild and no-side preference); r_j the random effect of the animal $_j$; p_j the random regression coefficient of Y on day D for animal $_j$ and e_{ij} the residual error. This model allowed the day effect to be taken into account with the 20 control heifers. Bodyweight of the animal was used as a co-variable, because it may explain part of the variability observed in the resistance in farm animals.

Behavioural data from Experiment 2 were only analysed on the days when voltage was applied to all the heifers, ie 1st, 4th, 6th and 10th day. The MIXED procedure was used with the model:

$Y_{xyi} = \mu + T1_x + T2_y + C_i + T1_x \times T2_y + \text{day} + \text{day} \times T1 + \text{day} \times T2_y + W_i + r_i + e_{xyi}$; where μ represents the overall mean; $T1_x$ the fixed effect of the past experience during the Experiment 1 (CONT vs VOLT: voltage-naïve vs voltage-experienced heifers); $T2_y$ the fixed effect for the manner in which voltage was applied (RAND vs DAILY: random vs every day application of voltage); C_i the maximal current flowing through the heifer as a co-variable; $T1_x \times T2_y$ the interaction between the past experience and the manner in which voltage was applied; day the fixed effect of day (1st, 4th, 6th and 10th day); day × $T1_x$ the interaction between the day and past experience; day × $T2_y$ the interaction between day and the manner in which voltage was applied; W_i the bodyweight of the animal $_i$ as a co-variable; r_i the repeated effect of the animal $_i$ and e_{xyi} the residual error.

Cortisol during Experiment 1 (1, 3 and 5 V) and the 1st day of Experiment 2 were analysed separately with a MIXED procedure with the treatment (CONT vs VOLT) as a fixed effect and bodyweight as a co-variable. The same model was used to study plasma cortisol on the 10th day of Experiment 2, with, in addition, the manner in which voltage was applied included in the model (RAND vs DAILY). Due to technical problems, 10 tests out of 760 could not be recorded during Experiment 1 and one test out of 160 could not be recorded during Experiment 2.

Finally, qualitative data collected during the two experiments were analysed with a Chi-square test. During Experiment 1, due to the low number of animals performing abrupt head movements and muzzle-licking, the data were compiled for three periods: from 0.3 to 1.6 V, from 2 to 3.3 V and from 3.6 to 5 V.

All data are presented as least square means (LSMeans [± SEM]), except when otherwise stated.

Results

Experiment 1

An effect of day and of the co-variable 'bodyweight' were observed for total feed intake ($P < 0.001$), for electrified feeder intake as a percentage of total feed intake ($P < 0.05$), for time spent eating in electrified feeder ($P < 0.05$) and for latency to change to the non-electrified feeder ($P < 0.01$). Heavier heifers ate more feed, had a smaller electrified feeder intake as a percentage of total feed intake, spent less time eating in the electrified feeder, and had a shorter latency to change to the non-electrified feeder than lighter heifers.

A voltage effect was found for total feed intake ($P < 0.001$), for electrified feeder intake as a percentage of total feed intake ($P < 0.001$), for time spent eating in the electrified feeder ($P < 0.001$) and for latency to change to the non-electrified feeder ($P < 0.001$).

Lower total feed intake was observed at 2 V and for voltages from 3 to 4 V ($P < 0.05$) compared to feed intake at 0 V. Electrified feeder intake as a percentage of total feed intake (Figure 1), time spent eating in the electrified feeder and latency to change to the non-electrified feeder (Figure 2), were lower at ≥ 2.3 V ($P \leq 0.01$) compared to 0 V.

The number of heifers performing abrupt head movements and muzzle-licking according to the three periods of voltage exposure (from 0.3 to 1.6 V, from 2 to 3.3 V and from 3.6 to 5 V) is presented in Table 1. From 0.3 to 1.6 V, no differences were observed for the number of heifers performing abrupt head movements and muzzle-licking. From 2 to 3.3 V, more VOLT heifers performed abrupt head movements ($P < 0.05$) and muzzle-licking ($P < 0.01$) compared to CONT heifers. The same result was observed for voltages from 3.6 to 5 V: more VOLT heifers performed abrupt head movements ($P < 0.05$) and muzzle-licking ($P < 0.01$) compared to CONT heifers (Table 1).

An effect of the co-variable 'bodyweight' ($P < 0.05$) was observed for plasma cortisol concentrations at 1, 3 and 5 V. At 1 V, plasma cortisol concentrations were higher in VOLT heifers than in CONT heifers ($P < 0.05$). No differences were observed at 3 and at 5 V (Table 2).

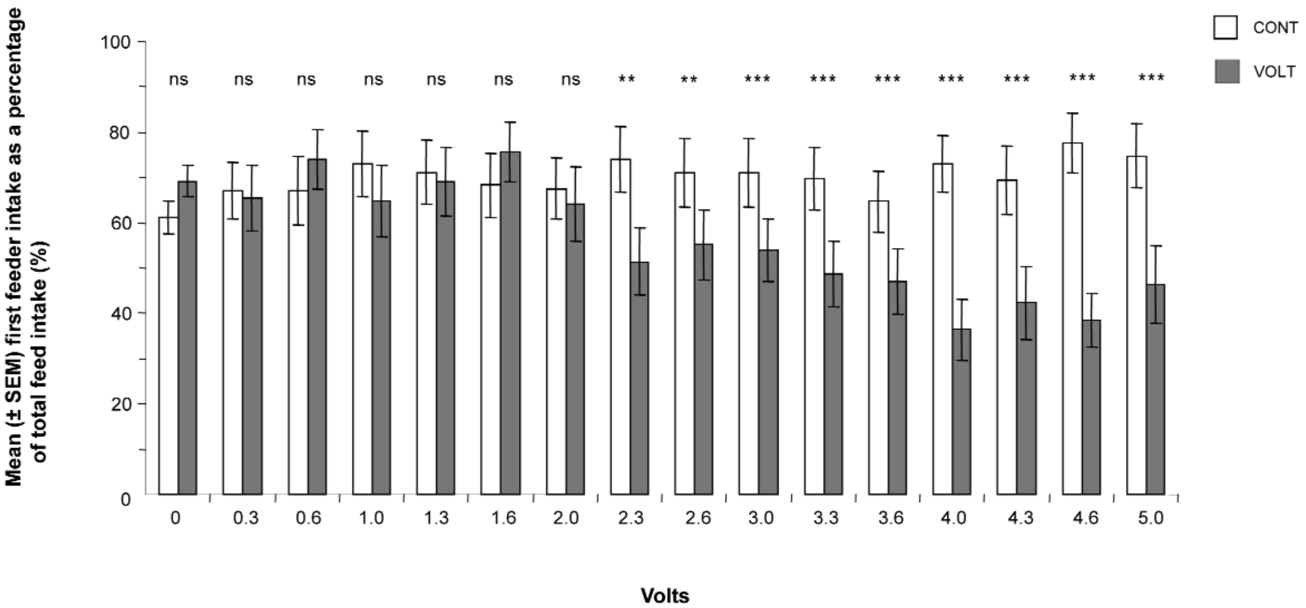
Twenty-two heifers had a strong side-preference, 11 had a mild side-preference and 7 had no first feeder side-preference. An effect of the side-preference was observed on the electrified feeder intake as a percentage of total feed intake ($P < 0.01$): heifers showing a strong side-preference had a greater electrified feeder intake as a percentage of total feed intake than heifers showing a mild or no side-preference. An effect of the side-preference was observed on the time spent eating in the electrified feeder ($P < 0.01$): heifers showing a strong side-preference spent more time eating in the electrified feeder than heifers showing a mild or no side-preference. An effect of the side-preference was observed on the latency to change to the non-electrified feeder ($P < 0.001$): heifers showing a strong side-preference had a greater latency to change to the non-electrified feeder than heifers showing a mild or no side-preference. No effect of the side-preference was observed on total feed intake ($P > 0.10$). Results are given in Table 3.

The resistance of the feeder-heifer-metallic plate averaged 1,704 (± 103.7) ohms (mean [± SEM]) over the experiment. The maximal current (rms) and mean current (rms) flowing through the set-up feeder-heifer-metallic plate for each day of test are presented in Figure 3. When 2.3 V were applied to the feeder, the maximal current through the heifers' bodies was 3.5 [± 0.24] mA and the mean current 2.5 [± 0.13] mA.

Experiment 2

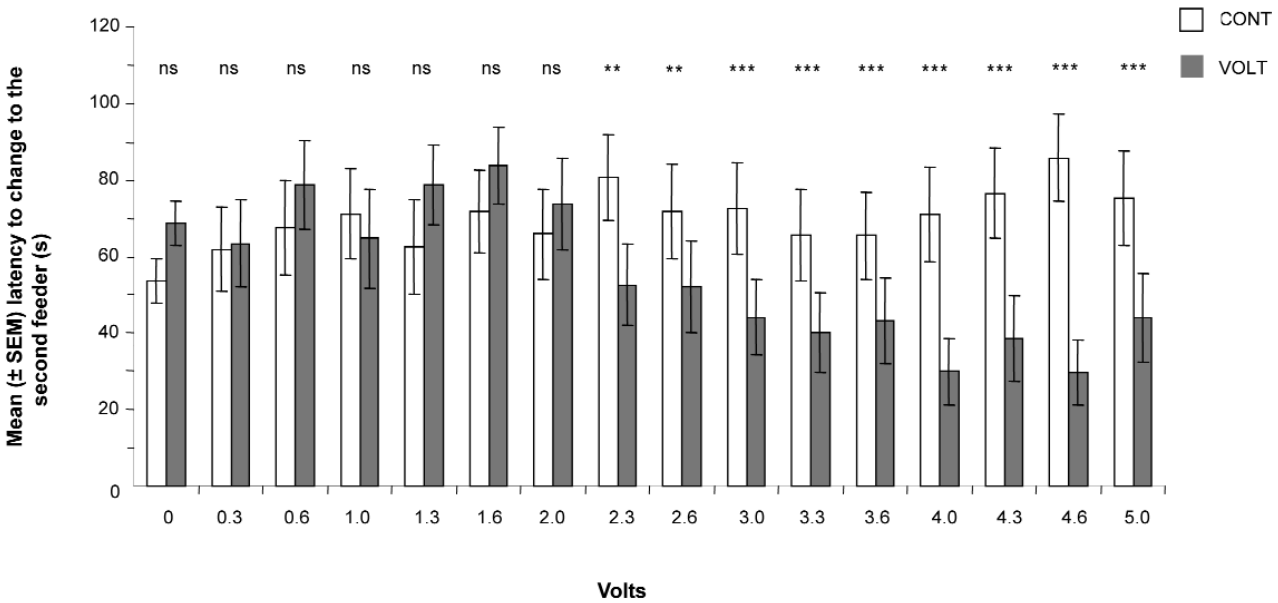
An effect of day was found for total feed intake ($P < 0.05$), which was lower on the 1st day in comparison with the other

Figure 1



Mean (± SEM) first feeder intake as a percentage of total feed intake (%) in 20 Holstein heifers exposed to stray voltage (VOLT, grey columns) and 20 control heifers with no voltage exposure (CONT, white columns). The voltage was applied for 2 min (every day in steps of 0.33 V, between 0 to 5 V) to the first feeder (out of two) in which the heifer initially started to eat. CONT heifers followed the same procedure without voltage exposure. ns: non significant; $P > 0.01$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Figure 2



Mean (± SEM) latency (s) to change to the 2nd feeder in 20 Holstein heifers exposed to stray voltage (VOLT, grey columns) and 20 control heifers with no voltage exposure (CONT, white columns). The voltage was applied for 2 min (every day in steps of 0.33 V, between 0 to 5 V) to the first feeder (out of two) in which the heifer initially started to eat. CONT heifers followed the same procedure without voltage exposure. ns: non significant; $P > 0.1$; ** $P \leq 0.01$; *** $P \leq 0.001$.

days (4th, 6th and 10th, $P < 0.05$). No effect of day was found for the other variables. An effect of the co-variable 'bodyweight' was found for total feed intake, for electrified feeder intake as a percentage of total feed intake, for time spent eating in the electrified feeder and for latency to change to the non-electrified feeder ($P < 0.05$). Heavier heifers ate more feed, had a smaller electrified feeder intake as a percentage of total feed intake, spent less time eating in the electrified feeder, and had a shorter latency to change to the non-electrified feeder than lighter heifers.

Table 1 Number of Holstein heifers performing abrupt head movements and muzzle-licking. Twenty heifers were exposed to stray voltage (VOLT) and 20 heifers were used as control with no voltage exposure (CONT). The voltage was applied for 2 min (every day in steps of 0.33 V, between 0 to 5 V) to the first feeder (out of two) in which the heifer initially started to eat. CONT heifers followed the same procedure without voltage exposure.

	VOLT	CONT	*P*-value
	Number of heifers performing abrupt head movements		
From 0.3 to 1.6 V	10/97	9/100	ns
From 2.0 to 3.3 V	19/98	8/98	$P < 0.05$
From 3.6 to 5.0 V	26/98	12/99	$P < 0.05$
	Number of heifers performing muzzle-licking		
From 0.3 to 1.6 V	11/97	12/100	ns
From 2.0 to 3.3 V	27/98	12/98	$P < 0.01$
From 3.6 to 5.0 V	34/98	17/99	$P < 0.01$

Table 2 Mean (± SEM) plasma cortisol concentrations (ng ml^{-1}) in 20 Holstein heifers exposed to stray voltage (VOLT) and 20 heifers used as control with no voltage exposure (CONT). The voltage was applied for 2 min (every day in steps of 0.33 V, between 0 to 5 V) to the first feeder (out of two) in which the heifer initially started to eat. CONT heifers followed the same procedure without voltage exposure. Blood samples were collected 15 min after the beginning of the test.

	VOLT	CONT	*P*-value
	Plasma cortisol concentrations (ng ml^{-1})		
1 V	5.2 (± 0.78)	2.4 (± 0.80)	$P < 0.05$
3 V	4.8 (± 1.17)	5.2 (± 1.24)	ns
5 V	2.6 (± 0.37)	2.4 (± 0.42)	ns

Table 3 Mean (± SEM) effect of side-preference on behavioural responses of Holstein heifers. Twenty heifers were exposed to stray voltage (VOLT) and 20 heifers were used as control with no voltage exposure (CONT). The voltage was applied for 2 min (every day in steps of 0.33 V, between 0 to 5 V) to the first feeder (out of two) in which the heifer initially started to eat. CONT heifers followed the same procedure without voltage exposure. Strong side-preference was defined as the choice of the same feeder as first feeder for more than 90% of the tests, a mild side-preference between 60% and 90%, and a no side-preference for less than 60% of the tests.

	Side-preference			*P*-value
	No (n = 7)	Mild (n = 11)	Strong (n = 22)	
Total feed intake (g)	481 (± 23.1)	463 (± 18.3)	482 (± 14.1)	ns
Electrified feeder intake as a percentage of total feed intake (%)	41.7 (± 7.57)^a	42.7 (± 6.21)^a	62.1 (± 4.97)^b	$P < 0.01$
Time spent eating in electrified feeder (s)	43.2 (± 10.32)^a	44.9 (± 8.44)^a	71.2 (± 6.80)^b	$P < 0.01$
Latency to change to the non-electrified feeder (s)	21.2 (± 12.53)^a	29.1 (± 10.3)^a	66.5 (± 8.07)^b	$P < 0.001$

Within a row, means without a common superscript differ ($P < 0.05$).

An effect of the co-variable 'maximal current flowing through the heifer' was observed on the total feed intake ($P < 0.001$). No effect of the co-variable, 'maximal current flowing through the heifer' was observed on the other variables.

No effect of past experience to voltage (VOLT vs CONT) and interaction between past experience and day were observed for any of the behaviour variables.

An effect of the manner in which voltage was applied (RAND vs DAILY) was observed on electrified feeder intake as a percentage of total feed intake, which was greater for RAND heifers than for DAILY heifers (45.9 [± 3.35]% vs 36.3 [± 3.35]%, respectively, $P = 0.050$). No differences were observed between DAILY and RAND heifers concerning total feed intake, the latency to change to the non-electrified feeder and the time spent eating in the electrified feeder. More RAND heifers tended to perform muzzle-licking than DAILY heifers (28 out of 80 tests vs 18 out of 79 tests, respectively, $P = 0.089$). No interaction between the manner in which voltage was applied and day was observed.

A trend for an interaction between past experience to voltage (VOLT vs CONT) and the manner in which voltage was applied (RAND vs DAILY) was observed for total feed

Figure 3

Maximal current (mA, rms, in black) and mean current (mA, rms, average of 5 measurements for each heifer, for each voltage, in grey) flowing through the set-up feeder-heifer-metallic plate in 20 Holstein heifers (VOLT) exposed to voltage applied for 2 min (every day in steps of 0.33 V, from 1 to 5 V) to the first feeder (out of two) in which the heifer initially started to eat.

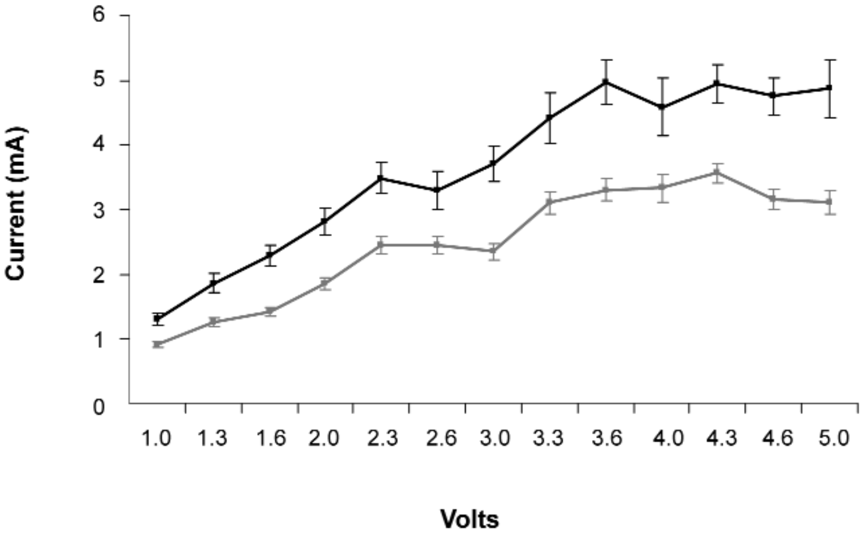


Table 4 LSMean (± SEM) behavioural responses of Holstein heifers exposed to stray voltage. Voltage (3.3 V) was applied during a 2-min test to the feeder in which the heifer initially started to eat, either randomly (4 out of 11 days, VOLT-RAND), or daily (on the 11 test days, VOLT-DAILY) using 20 heifers which had previously experienced voltage (VOLT). The same procedure was applied to 20 voltage-naïve heifers (CONT-RAND and CONT-DAILY). (LSMeans were calculated with the data from 4 days).

	Interaction between past-experience and method to apply voltage*				*P*-value
	VOLT-RAND	VOLT-DAILY	CONT-RAND	CONT-DAILY	
Total feed intake (g)	624 (± 18.1)[ab]	606 (± 17.7)[ab]	586 (± 17.9)[b]	639 (± 17.7)[a]	$P = 0.060$
Electrified feeder intake as a percentage of total feed intake (%)	43.3 (± 4.83)	40.6 (± 4.73)	48.6 (± 4.77)	32.1 (± 4.73)	ns
Time spent eating in the electrified feeder (s)	46.2 (± 6.72)	44.5 (± 6.58)	51.9 (± 6.64)	33.01 (± 6.67)	ns
Latency to change to the non-electrified feeder (s)	30.2 (± 6.92)[ab]	33.9 (± 6.78)[ab]	42.6 (± 6.83)[a]	19.1 (± 6.87)[b]	$P = 0.057$
Number of heifers performing abrupt head movements/number of tests	13/40[a]	18/40[ab]	24/40[b]	14/39[a]	$P = 0.061$
Number of heifers performing muzzle-licking/number of tests	11/40	8/40	17/40	10/39	ns

* n = 10 per group. Within a line, means without common superscripts (a, b) differ ($P < 0.05$).

intake ($P = 0.060$): CONT-DAILY heifers ate more than CONT-RAND heifers while VOLT-DAILY and VOLT-RAND heifers were intermediate (Table 4). A trend was observed for latency to change to the non-electrified feeder ($P = 0.057$): CONT-RAND heifers showed a greater latency to change to the non-electrified feeder than CONT-DAILY heifers; VOLT-RAND and VOLT-DAILY were not different from the other groups (Table 4). In addition, a trend was observed for the number of heifers performing abrupt head movements ($P = 0.061$): more CONT-RAND heifers performed abrupt head movements than CONT-DAILY and VOLT-RAND heifers (Table 4).

On the first day when voltage (3.3 V) was applied to all heifers, CONT heifers had higher plasma cortisol concentrations than VOLT heifers (5.5 [± 0.73] ng ml^{-1} vs 3.2 [± 0.69] ng ml^{-1}, respectively, $P < 0.05$). On the 10th day, there was no effect of past experience to voltage (CONT vs VOLT) or the manner in which voltage was applied (RAND vs DAILY) on plasma cortisol concentrations.

Discussion

Voltage threshold

At 2.3 V (rms) and above, electrified feeder intake as a percentage of the total feed intake decreased, heifers spent less time eating in this feeder and they changed more quickly to the non-electrified feeder. In addition, more heifers receiving voltage made abrupt head movements and performed muzzle-licking at 2 V and above than control heifers. These observations clearly suggest that 2.3 V is the voltage threshold at which important and persistent changes in feeding behaviour are observed in heifers under our experimental conditions.

In the present experiment, an alternating (50 Hz) voltage threshold of 2.3 V (rms) corresponded to a maximal current (crossing the circuit feeder-heifer-metallic plate) of 3.5 mA (rms) from which persistent changes in the behaviour of heifers were observed. These results are consistent with the literature. Indeed, in a recently published paper, Erdreich *et al* (2009) have shown, using a meta-analysis, that cows exhibited their first significant behavioural responses at 3.0 mA (rms).

In 6 dairy cows, Norell *et al* (1983) observed a specific avoidance response (ie mouth opening) to a mouth-all hooves shock during a learned behavioural test (pushing with the muzzle on a metal plate) exhibited 14% of the time at 1.0 mA and 92% at 4.0 mA. Lefcourt and Akers (1982) reported that only mild behavioural responses (defined as a small sudden movement or vocalisation) occurred in most Holstein cows (n = 5) exposed to a voltage of 0.7 V (3 mA) between the right front knee and the right rear hock. However, more violent discomfort behaviours were observed after exposure to higher voltages from 1 to 1.5 V (from 3.4 to 5.1 mA). Indeed, Henke Drenkard *et al* (1985) observed that all of the 6 tested cows jumped, kicked, arched their backs and made a backward movement after the application of a 4 or a 8 mA current between the udder and the hooves (which corresponded to a voltage from 2.0 to 7.8 V). In addition, apart from the experiment of Norell *et al* (1983), the current was applied using electrodes and the cows were unable to avoid it which could explain the violent discomfort behaviour. The currents recorded in our experiment were lower than those applied in these experiments which can explain why no violent discomfort behaviours (ie arching of the back, jumping, and kicking) were observed. Another explanation is that our heifers had the choice, after being exposed to voltage, to eat in the non-electrified feeder and so avoid prolonged exposure to voltage.

Discomfort behaviour might be different according to the part of the body where the current is applied. Indeed, Gustafson *et al* (1985) observed that cows performed more hoof lifting than control cows when they were exposed to 3 mA from the front-to-rear hooves. However, when the cows were exposed to the same current from the mouth (metallic mouth bit) to all hooves, the response was an increase in mouth opening. Moreover, Norell *et al* (1983) observed that for current above 2 mA (rms) from the mouth to the four hooves, the plate pressing behaviour (to obtain food) was suppressed whereas this behaviour was not suppressed when the cows were exposed up to 6 mA (rms) from the front to the rear hooves. Therefore, the cow's reaction depends on the part of the body to which the current is applied which also means that the consequences for animal welfare will be different. Discomfort related to voltage exposure might be higher when voltage is applied to soft tissue like the mouth or the udder than on other parts of the body, such as the legs, since the electrical properties are different according to the tissue involved (Gabriel *et al* 1996). However, no information was found in the literature to corroborate this hypothesis.

In the present study, the application of voltage between the muzzle and the four hooves induced behavioural changes in the head of the heifers (abrupt backward movement of the head, side-to-side head movement and muzzle-licking). These results are in agreement with the conclusions of Reinemann (2003) who indicated that for studies in which current pulses were applied between the muzzle and the four hooves, facial activity was the most noticeable behavioural response. The abrupt backward movement could be interpreted as an indication of a startle response due to an unexpected event or discomfort and the side-to-side head movement as an indication of discomfort or frustration as proposed by Sandem *et al* (2002) in feed-deprived cows. Muzzle-licking, performed more often for voltages ≥ 2 V, might help heifers to reduce discomfort or pain provoked by the voltage. Indeed, licking, biting or looking at a painful area can be used as an indicator of discomfort or of a painful state (Sawyer 1998). These elements suggest that heifers receiving voltages ≥ 2 V, between the muzzle and the four hooves, exhibited behavioural patterns which could be linked to discomfort on the muzzle. However, it is not known whether this behaviour is due to the nociception induced by the current flowing through the muzzle or to the startle response induced by the occurrence of an unusual event.

The behavioural feeding variables indicated that at 2.3 V and above, the situation became uncomfortable enough to reduce eating in the electrified feeder. However, because the heifers were highly motivated to eat concentrate, they modified their feeding behaviour in order to cope with the application of voltage by changing more quickly to the non-electrified feeder but did not stop eating. However, it should be noted that, even if the heifers adapted their behaviour in order to cope with voltage, a general decrease in total feed intake was observed from 3 up to 4 V which could be partly explained by a decrease in feeding time due to an increase in the number of times heifers changed feeder and in the time spent making abrupt head movements or muzzle-licking. These observations suggest that voltages above 2 V are distressing. However, further investigation is needed before drawing any firm conclusions regarding nociception.

A few minutes after a stressful event, the level of corticosteroids increases in the blood (for a review, see Mormède *et al* 2007). The increase in cortisol (from 2 to 5 ng ml^{-1}) observed in VOLT heifers compared to CONT heifers 15 min after the application of 1 V to the feeder is similar to the increase observed in cows 13 min after the start of a routine veterinary procedure, such as rectal palpation or intramuscular injection (Alam & Dobson 1986). However, no differences were observed between control heifers and heifers exposed to higher voltages (3 and 5 V). These findings suggest that voltage produced a stress, or at least an arousal, at quite a low voltage (1 V). This voltage corresponds to a maximal current of 1.3 mA which is lower than the current of 8 mA at which an increase in cortisol concentrations was observed in cows by Henke Drenkard *et al* (1985). For higher voltages (3 and 5 V), no further physiological differences were observed, which could be explained by three hypotheses. Firstly, heifers may have quickly habituated to the application of voltage as previously observed in a case of mild stress by Andrade *et al* (2001) after repeated handling and Arnold *et al* (2007) after exposure to noise. Secondly, at 3 and 5 V, heifers have had much more experience of voltage, and the startling and novel components of the electrical stressor were therefore not present any more. Thirdly, at 2.3 V and above, heifers, by spending less time eating in the electrified feeder, reduced their exposure to the stressor.

On the basis of our results, it appears that two different thresholds can be defined. The first, at 1 V, corresponds to a transient increase in physiological stress responses. The second, at 2.3 V, leads to persistent behavioural reactions suggesting distress. Heifers perceiving an electricity stressor in their environment (as expressed by discomfort/painful state), modified their feeding behaviour in order to reduce the sensation. It is important to note that the detection of the thresholds could be quite different depending on the experimental design. The thresholds might have been higher if the animals did not have the possibility to escape voltage by eating in a non-electrified feeder (Duvaux-Ponter *et al* 2006). Moreover, the threshold would have been lower if it was applied to a water trough because of water's low resistance.

The persistent reaction threshold of 2.3 V observed in our experiment corresponds to the mean response of 20 heifers. Large variations were observed between individuals and some heifers changed their feeding behaviour and exhibited a discomfort/painful state before 2.3 V while others did not change feeder even at 5 V. The animals which did not change feeder could either have a greater resistance or the variability observed in response to voltage could be explained by a strong feeder side-preference. A strong feeder side-preference was observed for 22 heifers out of 40 when they started to eat. According to the task or to the experimental design, the percentage of animals with a strong side-preference can vary. Indeed, Kilgour *et al* (2006) observed in a specific side-preference test (crossing an obstacle in a corridor to the right or left side) that 80% of the animals (steers and heifers) showed a strong side-preference and crossed the obstacle systematically by the same side. In our experiment, heifers showing a strong feeder side-preference were more reluctant to change to the non-electrified feeder. This kind of response has already been reported in another experiment: Grandin *et al* (1994) observed a trend for heifers to resist modifying a choice once they were accustomed to a reinforcement associated with a specific side. In addition, the resistance to switching is likely to be greater when the choices are only mildly aversive. In our case, the reluctance to switch due to a strong side-preference could explain why some heifers persisted in eating in the electrified feeder at voltages up to 5 V. This side-preference perhaps led to an overestimation of the threshold at which heifers reacted to voltage.

The large variation observed between individuals for the threshold can result from a variation in the resistance of contact points. Several authors (Matte *et al* 1992; Reinemann *et al* 1999) have highlighted the fact that the resistance of contact points (entrance and exit) plays an important role in the amount of current flowing through the body. Matte *et al* (1992) exposed 10-week old pigs to 5 V from the muzzle to the four hooves on a wet or dry floor and the resistance varied from 2,786 [± 320] ohms for a dry floor to 954 [± 35] ohms for a wet floor. According to Figure 3, it may be possible that from 3.6 V heifers have modified the position of their muzzle to limit the current flowing through their body. Indeed, from 1 to 3.6 V the shape of the curve showed an increase in the current: the maximal current flowing through the body increased proportionally with the increase in voltage. However, from 3.6 to 5 V, the maximal current flowing through the body stopped increasing and reached a plateau of 5 mA. It would be interesting to study, more precisely, the resistance of different contact points (muzzle, tongue or lower jaw) in order to try to explain the variation in the maximal current flowing through the body.

An effect of bodyweight was observed on feeding behaviour: heavier heifers ate more feed than lighter heifers during the tests, which is in accordance with the literature (Agabriel *et al* 1987; D'Hour *et al* 1991).

Past experience and unpredictability

During the second experiment, a voltage of 3.3 V was chosen, higher than the threshold of 2.3 V to make sure that a large number of heifers perceived it.

No effect of previous voltage experience (experienced vs naïve) was observed on behaviour. However, higher cortisol concentrations were observed the first day of application of voltage in naïve heifers compared to voltage-experienced heifers.

The difference in cortisol between naïve heifers compared to voltage-experienced heifers was no longer observed at the end of Experiment 2. If an increase in cortisol concentration is interpreted as a stress indicator (Mormède *et al* 2007), these results showed that previous exposure to voltage probably helped the heifers to handle better the first subsequent application of voltage 6 weeks later. However, similar plasma cortisol concentrations between naïve and voltage-experienced heifers, at the end of the experiment, suggest that naïve heifers habituated to the application of voltage. These results are in accordance with the conclusions of Friend (1991) who put forward the idea that although animals may initially display signs of acute stress in the presence of a stressor, they often adapt and learn to cope with it. In calves, repeated exposure to a stressor (30-min transport) increased plasma cortisol concentrations during the first exposure but the increase became less marked in successive trials (Locatelli *et al* 1989). Another explanation for the lack of physiological response at the end of the second experiment could be the fact that the nociceptive threshold was increased following repeated exposures to voltage. Indeed, a change in nociception (hypoalgesia) has been reported in dairy cows exposed to an acute stressor (isolation) (Herskin *et al* 2004).

The heifers exposed to voltage in a random manner ate more from the electrified feeder and tended to perform more abrupt head movements than heifers whose feeder was electrified every day. Predictability of a stimulus is an important concept which helps in the understanding of the effect of an electrical stimulus on heifers. In addition, predictability is important in relation to animal welfare (Bassett & Buchanan-Smith 2007). Aneshansley and Gorewit (1991) described the predictability of an electrical exposure as a continuous function with two extremes: an electrical exposure is predictable if it is associated with some easily recognisable event (eg connection of the udder to the milking machine) whereas the opposite is a totally random exposure, occurring at any time or any place. In our experiment, the random application of voltage was not temporally predictable: no explicit signal associated with the application of voltage warned the heifers that voltage was going to be applied to the feeder. Heifers exposed every day to voltage were able to learn, predict and then adapt their behaviour in order to decrease the negative experience of voltage by changing more quickly to the non-electrified feeder, while the lack of predictability did not allow the heifers to learn to adapt their behaviour in order to avoid voltage.

Our findings confirm the interaction between the manner with which voltage was applied (random vs daily) and previous voltage experience (experienced vs naïve). It seems that naïve heifers receiving a daily application of voltage learned how to cope better with this uncomfortable situation (shortest latency to change feeder and highest total feed intake) compared to naïve heifers exposed to random applications. The latter heifers may have been more disturbed, less efficient and also had less days of exposure to adapt to the situation. The voltage-experienced heifers habituated more to voltage and showed intermediate behaviour between naïve heifers exposed to random applications of voltage and naïve heifers exposed to a daily application of voltage.

The fact that the co-variable 'maximal current flowing through the heifer' had no effect on the behavioural variables (except on total feed intake) indicates that the maximal current flow is not the only factor explaining the response of the animals to low-level stray voltage. Indeed, in farm conditions, the unpredictable component of stray voltage exposure could be a more important factor explaining part of the variability of the responses.

Animal welfare implications

Stray voltage (less than 10 V) can occur in an unpredictable manner on farms and may impair animal welfare. However, the involvement of stray voltage is difficult to diagnose and most studies have been performed under experimental conditions far removed from those encountered in farms and without giving any information on how the animals perceived stray voltage. The first objective of this work was therefore to determine the reaction threshold to stray voltage. Although we applied voltage to a metallic feeder, the animals could avoid the stressor at all times by changing feeder. Moreover, during the experiment, none of the animals exhibited dangerous behaviours (kicking, jumping or agonistic behaviours against the experimenter) that may have affected their health or the security of the experimenters and none of the animals stopped eating during the tests, whatever the voltage used. This work is the first in a series of experiments where, under farm conditions, the medium-term effect of stray voltage will be studied on animal welfare using behavioural, stress physiology and production parameters, based on the threshold defined in this experiment. Moreover, stray voltage occurring in an unpredictable manner may induce more negative effects than when it is experienced on a regular basis and therefore impair animal welfare. Another purpose of this work was to study the influence of unpredictability on heifers' reactions to stray voltage since it is an important factor which needs to be taken into account when studying stray voltage.

Conclusion

A voltage of 2.3 V appeared to be the average threshold at which avoidance and behavioural signs of discomfort/nociception started for a large number of heifers under our experimental conditions. Heifers exposed to unpredictable voltage had more difficulty in adapting to this stressor compared to heifers exposed to the same stressor in a predictable manner. Moreover, previous experience with voltage seemed to reduce the effects of subsequent exposures showing the importance of animal habituation to a stressor.

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