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Pre-slaughter factors linked to variation in responses to carbon dioxide gas stunning in pig abattoirs

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ABSTRACT

The stunning process is an important component of slaughter with implications for animal welfare due to the potential distress and pain in the case of a sub-effective or lengthy stun. This study examined the factors correlated with variation in responses to carbon dioxide (CO_2) stunning of pigs in five Australian commercial abattoirs. A total of 1 769 pigs (199–492 focal pigs per abattoir) were individually followed from lairage to post-stunning. A standardised observation protocol was used based on a literature review of the pre-slaughter factors that may influence the reaction to CO_2 stunning, such as animal background, lairage conditions, handling, stunning system and conditions. Pigs lost posture 22.5 ± 0.2 s after commencement of descent of the gondola into the CO_2 chamber. Latency to loss of posture was associated with farm of origin and time of day, which could be linked to various factors. Pigs that crawled or attempted to escape while in the gondola within the CO_2 chamber took longer to lose posture. Crawl and escape attempts differed between abattoirs (0.6–46.2% of the pigs observed) as well as mounting other pigs (1.0–24.3%). Greater amounts of forceful contacts during handling in the race were related to more mounting in the gondola, but to less pigs crawling or attempting to escape. Mounting in the gondola was more frequent for pigs from lairage pens of mixed sexes, followed by pens of entire males and finally pens of females. Males were also twice as likely to show crawl and escape attempts than females. Gasping in the gondola was relatively frequent (63.1–81.8%) and was associated with higher activity in the lairage pen and higher skin injuries. Convulsions (60.1–69.6%) were generally observed after loss of posture. The type of CO_2 system (group-wise vs single-file loading) had no significant effect on behaviour in the gondola. Nevertheless, pigs slaughtered in abattoirs with group-wise loading systems and automatic gates had lower cortisol concentrations post-stunning, which may be linked to minimal handling by stockpeople, other factors related to the systems, or differences in timing of when blood samples were taken. In conclusion, substantial variation in the reaction of pigs to CO_2 stunning was observed between and within abattoirs using a uniform protocol for data collection. This variation in outcomes between abattoirs and stunning systems and the relationships between handling and behavioural outcomes indicates that improvements can be made to reduce aversive responses to CO_2 stunning. In particular, avoiding mixing pigs of different sexes in lairage and aversive handling in the race may reduce aversive response to CO_2 stunning.

Implications

Behavioural responses in the gondola during exposure to CO_2 stunning such as crawling, escape attempts and mounting appear to be highly variable and possibly multi-factorial, with no simple relationships to the range of measures collected within this study. Nevertheless, the variation in outcomes between abattoirs, and in particular the very low levels of crawling and escape and mounting in the gondola in one abattoir, suggesting that it is possible to stun pigs with CO_2 while minimizing aversive reaction. In particular, avoiding mixing pigs of different sexes in lairage and aversive handling in the race may reduce aversive response to CO_2.

Introduction

Carbon dioxide (CO_2) gas stunning is currently the most common method to stun pigs for meat consumption in Australia and Europe (European Food Safety Authority Panel on Animal Health and Welfare [EFSA], 2004). Its main welfare advantage over electrical and mechanical stunning methods is that, at least in the more recent CO_2 systems, pigs can be moved in groups, which is closer to their natural behaviour (EFSA, 2004). This can reduce pre-slaughter handling stress in comparison to moving pigs in a single file and possibly using a restraining device (EFSA, 2004). The main disadvantage of CO_2 stunning is that it

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does not induce instantaneous insensibility, and exposure to CO_2 at concentrations high enough to induce insensibility has been shown to be aversive to pigs (Raj and Gregory, 1995; Velarde et al., 2007). There is also variation between individual pigs in the reaction to CO_2, from no observable reaction to vocalisations and violent attempts to escape (EFSA, 2020).

Various factors can influence pain, fear and distress in abattoir settings, including previous experience and breed of the animal, facility design, handling techniques, and the stunning and killing methods (Terlouw, 2005; Grandin, 2013). However, the extent of the influence of handling, breed, previous experience (e.g. type of housing systems) and the stunning system design on the response of pigs to CO_2 stunning is unclear.

Most experimental studies that investigated CO_2 stunning in pigs either omitted to report on components of the slaughter process such as the handling techniques used or were performed in research settings which may not encompass or replicate the conditions seen in commercial abattoirs (Atkinson et al., 2012). Therefore, this study focused on assessing the factors that affect the response of individual pigs to CO_2 stunning in commercial settings and in various abattoirs, in the Australian context.

Material and methods

Abattoirs

The study was conducted across five pork abattoirs (referred to from here on as abattoirs A1–A5) in four Australian states. The facility layout of the abattoirs varied, but all included a lairage area. Pigs were immediately placed into lairage pens upon unloading following truck arrival (some pigs were moved in the same group from one lairage pen to another in some abattoirs), and then into a forcing pen and a race leading to the CO_2 stunning chamber. Pigs were moved in groups of 10 to 15 pigs in the forcing pens in all abattoirs, and all the way to the gondola in group-wise system abattoirs (also referred to as 'backloader' or 'side-loading'; Abattoirs A4–A5) but in a single file in the race of abattoirs with single-file loading system (Abattoirs A1–A3). This difference was because three abattoirs used a single-file entry to lead pigs to the gondola within the CO_2 stunning chamber (Abattoirs A1–A3), whereas two abattoirs used a group-wise system with automated push/hoist gates in the forcing pen and race that minimises the need for stockpeople handling (Abattoirs A4–A5). All abattoirs used the paternoster system to pass pigs through the CO_2 stunning chamber. Abattoirs with single-file entry had two adjacent race files leading to the entry of the gondola, from which pigs were loaded in the gondola usually alternatively. Gondolas in the single-file entry systems had the capacity to hold two to three slaughter-weight pigs within each gondola (average A1: 2.0, A2: 2.4, A3: 2.1 pigs per gondola), whereas gondolas in the group-wise systems had the capacity to hold five to eight slaughter-weight pigs within each gondola (average A4: 3.1, A5: 5.0 pigs per gondola). This provided between 0.57 and 0.75 m^2 of floor space per pig, with the average pig size in Australia being slaughtered around 75–85 kg live weight. Carbon dioxide concentration within the chamber was set from 90 to 92% between abattoirs, with sensor alarms set when CO_2 concentrations reached 5 to 14% below those thresholds. The measured CO_2 concentrations throughout the course of the study was always superior to 80% at the stunning position. The average line speed over a day varied from 255 pigs per hour to 400 pigs per hour between abattoirs, and the intended stun-stick interval varied from 30 to 60 s between abattoirs.

Subjects and data collection

Between 199 and 492 focal pigs destined for slaughter for human consumption were individually followed at each abattoir ($n = 1\ 769$ pigs in total) from lairage to post-stunning, over two consecutive days per abattoir, with an attempt to get a representative sample of the variety of pigs killed at each abattoir (e.g. different farms, housing systems and genetic background). Pigs were handled according to individual abattoir standard processes in order to capture the routine variation in practices within and between abattoirs without interference from the researchers apart from marking focal pigs for data collection purposes.

Focal pigs were selected in the lairage pens throughout the day choosing in each pen one pig closest to the researcher, one pig farthest from the researcher and one pig in between, with on average 18 focal pigs per 100. Focal pigs were marked using marking spray (Stockmarker, Leader Products, Craigieburn, Australia), without restraint, to allow for individual identification during behavioural observations and blood sampling. The sex composition of the groups of pigs in the lairage pen and the sex of the individual focal pigs were also recorded. The observer was outside the pen while conducting the observations. Prior to marking, a single observer, using a 3-point scale, recorded at pen level:

- the level of activity of pigs in the lairage pen assessed during a 1-min observation period: 1 = More than 50% pigs lying or inactive, 2 = More than 50% pigs active, or 3 = More than 50% active and aggression and/or mounting observed;
- lairage pen stocking density: 1 = pigs occupy <25% of floor space, 2 = pigs occupy 25–75% of floor space, 3 = pigs occupy >75% of floor space;
- skin injuries of the focal pig: 1 = only minor scratches, 2 = 50% coverage of scratches or some cuts, 3 = several serious wounds with the majority of the body covered in scratches or cuts.

Abattoirs had between two and five stockpeople handling the pigs from lairage to the CO_2 stunning chamber. A trained observer recorded handling through the frequency of tactile, auditory and visual interactions used by stockpeople, including the possible use of electric prodders, on focal pigs. Stockpeople behaviour was classified based on previous studies (Coleman et al., 1998) as P3 (slap, push, shout, piercing whistle, artificial noise, stomp; 'moderate aversive' interactions), P4 (hit, caught, hard push; 'highly aversive' interactions with stockpeople or gates) or P5 (use of electric prodder), while the focal pig was in the forcing pen and in the race, with these two locations recorded separately. The observer also recorded the behavioural reaction of the focal pig to handling, through the occurrence of mount, slip and fall, vocalisations, and crawl and escape attempt, in the forcing pens and the race, with these two locations recorded separately. All behaviours were recorded using a 1–0 recording method (i.e. either seen or not seen) during 5 s bouts. The focal pig's speed of movement was measured with a time watch based on the time to move from the start to the end of the forcing pens and from the start of the race to the entry of the gondola, with the forcing pen and race recorded separately. Behaviour was recorded using video cameras, although live observations were used (A3 in the forcing pen and race and A2 in the race) where there were limitations in the design features of the facility that were not always conducive to viable video recordings of behaviour through the overhead CCTV camera system, or due to restrictions on collecting video footage in these areas put in place by the participating abattoir. Both types of observations were conducted by trained observers using the same ethogram, ensuring high inter-observer reliability. Inter-observer reliability was conducted before starting the observations and superior to 90%.

Once the focal pig entered the gondola, pig behaviour was recorded using video cameras attached inside the CO_2 chamber, covering a field of view from the entry to the gondola to until just a few seconds after the gondola moved beyond the first stop. Following pigs beyond the first stop was not possible because the next gondola started blocking the view. From the video recording, the following measures were recorded: total number of pigs in the gondola, latency to CO_2 exposure, and for the focal pig: latency to loss of posture (LoP), gasping, crawl and escape attempt, mounting of other pigs, and latency to disappear from the camera field of view (see ethogram in Table 1). Righting response was not scored, but it was indirectly accounted for with loss of posture, which was only scored when the righting response stopped. Behaviour of the focal pig was recorded using a 1–0 recording method. Once the focal pig exited the gondola, it could be systematically or randomly tested for corneal eye reflex by a stockperson and the occurrence of any potential indicator of consciousness (e.g. rhythmic breathing, spontaneous eye blinking, or attempts to raise the head or vocalisations) was recorded as an ineffective stun by the researchers collecting post-slaughter blood. In most locations, it was not possible to get an unobstructed visual on the stockperson checking the effectiveness of the stun, and anecdotally, there appeared to be a lot of variation between stockpeople and abattoirs in the level and consistency of the checks; therefore, we did not record effective stuns, rather we recorded ineffective stuns identified by the requirement of a re-stun based on the observation of a possible eye blink, breathing or leg movement; all others were assumed effective. Re-stun due to signs of return to consciousness, tested in each abattoir through corneal reflex, occurred rarely (0.45% overall, 1 case each in A1, A2, A3, A5 and 4 cases in A4 including two cases in the same gondola). Finally, 10 ml of blood was collected from focal pigs in heparin tubes immediately after the ventral-neck incision, kept on ice, centrifuged, and the plasma fraction aliquoted to microtubes and stored at −20 °C. Plasma samples were later analyzed for cortisol concentrations using a radioimmunoassay kit (ImmuChem™ Coated Tube Cortisol ^{125}I RIA Kit, MP Biomedicals LLC, Orangeburg, NY). Sample results with CV more than 5% between duplicates were re-analyzed.

Carbon dioxide concentration was measured through the internal chamber monitoring sensors of the abattoir and a single external CO_2 gas sensor (CM-0003 CO_2 Sampling Data Logger, CO_2 Meter, Inc., Ormond Beach, USA). This sensor was brought by the researchers to each abattoir and placed inside a gondola at pig's head level once daily during or at the end of the observations to assess CO_2 concentration. While in the chamber, the device made measurement every 15 s during the gondola rotation in the chamber, for a total of two rotations. None of the gondolas had pigs during the external CO_2 gas sensor measurements, and gondolas were sometimes run at a quicker pace than during the normal slaughter process, hence changing the timeline of a rotation but not the CO_2 concentration *per se*.

Table 1
Ethogram for the observation of pigs in the forcing pen and race and gondola during carbon dioxide (CO_2) exposure.

Pig behaviour in the forcing pens and race	
Slip and fall	Loss of balance, shoulder or haunches touch the ground
Mount	One or both front legs over the back of the animal in front
High Vocalizations	High pitch piercing vocalization or scream
Crawl and escape attempt	Scrabbling at the sides or corners trying to get out
Pig behaviour in the gondola in the CO_2 chamber (collected from the time the pig entered the gondola)	
Loss of posture	Pig is slumped down, making no more attempt to right itself
Gasping	Very deep breath through a wide open mouth, may involve stretching of the neck (Dalmau et al., 2010)
Convulsion	Muscle jerks or muscular contractions
Crawl and escape attempt	Scrabbling at the sides or corners trying to get out
Mounting	One or both front legs over the back of the animal in front
Latency to CO_2 exposure	Time for the gondola to lower a distance equal to the height of the pig; anecdotal observations (sniffing and raising head) show that pigs already respond to the gas in this area, hence assuming a detectable CO_2 concentration
Latency to disappear from the field of view	Time for the pig to disappear from the camera's field of view

Statistical analysis

Continuous data were checked visually for normality of the data and homogeneity of variance. Some continuous variables were log-transformed (for the variables: "Time of entry in gondola to CO_2 exposure" and "Time loss of posture to out of camera view (after first stop)"), after which they met these criteria. Continuous variables that did not meet the criteria for normality and homogeneity of variance were analyzed using a non-parametric test (Kruskall–Wallis test, for the Forcing pen and race pen variables "Total time from start to end"). Analysis of relationships between abattoirs and the recorded variables was conducted using χ^2 tests for binary or categorical data, where cell counts were all more than 5, or Fisher Exact test if some cell counts were <5, or ANOVA for continuous data (see Tables 2 and 3), and Spearman Rho correlation between the different behaviours observed in the gondola.

Latency to loss of posture was analyzed using Cox regression survival analysis with backward elimination, which allowed accounting for censored data (i.e. pigs that did not lose posture prior to disappearing from the camera's field of view) for which the latency to loss of posture was substituted by the latency to disappear from the field of view as the last point for which behavioural data could be collected. The full model included abattoir, system, day, farm of origin, lairage pen stocking density and level of activity, skin injuries, pen sex, pig sex, time of day, forcing pen and race variables, behaviours in the gondola, number of pigs in the gondola and latency to loss of posture.

Behaviours in the gondola (gasp, convulsion, mount, crawl and escape attempt) were recorded as binary variable (i.e. presence/absence) and consequently were analyzed individually using binary logistic regression models with a logit link function and using the backward elimination method set with a probability of 0.05 for variable entry and 0.1 for variable removal (SPSS statistical package, SPSS v.22, SPSS Inc., Chicago, Illinois, USA). The model included the same variables as for the latency to loss of posture. Because only 59.4% of the pigs had data recorded when passing through the forcing pens, due to logistic reasons, different binary regression models were run to test for the different forcing pen variables, to minimize the amount of missing observations due to list wise deletion by the model (i.e. not considering pig with one or more missing data point for a variable). Plasma cortisol concentrations were analyzed using a linear regression model with the backward elimination method. The model included the same variables as for the latency to loss of posture. *P* values <0.05 were considered significant.

Results

Data were obtained from 1 769 pigs across five abattoirs (A1: 492, A2: 199, A3: 291, A4: 481, A5: 306 pigs).

Latency to loss of posture in the gondola

The timelines of events in the CO_2 chamber differed between abattoirs (Table 2). On average, latency to CO_2 exposure occured 17.4 ± 0.6 s after entry into the gondola and loss of posture (**LoP**) occured 22.5 ± 0.2 s later. There was no difference in the time of entry in the gondola to CO_2 exposure. In most abattoirs, 91.3 to 100% of the focal pigs lost posture before disappearing from the field of view (i.e. between the entry into the gondola and just after the first stop). However, in abattoir 2, the proportion of focal pigs that lost posture before disappearing from the camera's field of view occurred was low (21.2%), due to a change in the type of pigs stunned between the time of camera setup (when sows were slaughtered, not part of this study) and the time observations took place (finisher focal pigs), which appears

Table 2
Timeline of events in the gondola in the CO_2 chamber (means ± standard error). Pigs that did not lose posture on the footage before disappearing from the field of view were considered censored data in loss of posture analysis.

Abattoirs	A1	A2	A3	A4	A5	Overall	*P*-value
Time of entry in gondola to CO_2 exposure (s)[1]	19.7 ± 2.0[c]	15.4 ± 0.8[bc]	13.4 ± 1.4[a]	17.0 ± 0.4[b]	18.6 ± 0.3[d]	17.4 ± 0.6	*P* < 0.001[3]
Time CO_2 exposure to loss of posture (s)	16.5 ± 0.3[a]	22.2 ± 0.8[b]	27.1 ± 0.5[c]	24.4 ± 0.4[b]	23.3 ± 0.4[b]	22.5 ± 0.2	*P* < 0.001[3]
Time loss of posture to out of camera view (after first stop) (s)[1,2]	18.9 ± 0.9[c]	12.3 ± 4.9[a]	13.4 ± 0.9[b]	18.5 ± 1.1[c]	20.2 ± 0.6[d]	17.9 ± 0.5	*P* < 0.001[3]
Proportion of pigs that lost posture before disappearing out of camera view (%)*	94.3	21.2	91.3	100	99.7	81.3	*P* < 0.001[4]

[abcd]Means within a row with different superscript differ (*P* < 0.05).
*Subject to variation according to abattoir layout, hence not comparable to each other.
[1] These variables were log-transformed for statistical analysis and are presented as non-transformed means.
[2] These variables were dependent on the camera placement and field of view.
[3] ANOVA statistical analysis.
[4] Chi-square statistical analysis.

to affect time to LoP. Across all abattoirs, the pigs that disappeared from the field of view prior to LoP were in gondolas that moved significantly faster than for pigs that lost posture on the footage (time of CO_2 exposure to disappearance from the field of view: 30.4 ± 4.2 s vs 40.3 ± 0.2 s, *t*-test *P* = 0.02), most likely due to a shorter first stop.

The regression analysis revealed that latency to LoP differed according to the farm of origin (*P* < 0.001) and time of day (*P* < 0.001). In addition, latency to LoP was longer for pigs that displayed crawl and escape attempts in the gondola (*P* < 0.001), but shorter for pigs that displayed convulsions in the gondola (*P* = 0.004). Latency to LoP was also longer for pigs that received more electric prodding interactions in the forcing pen (P5, *P* = 0.004), but shorter for pigs that displayed crawl and escape attempts in the race (*P* = 0.01).

Crawl and escape attempt in the gondola

The occurrence of crawl and escape attempt in the gondola differed between abattoirs (*P* < 0.001), with the highest occurrence in A3 (46.2%) and the lowest occurrence in A4 (0.6%). A1, A2 (both 20%) and A5 (25%) were similar and intermediate between A3 and A4, with a difference between A3 and A5 (*P* = 0.03). The binary regression model correctly classified the occurrence of crawl or escape attempt in 32.4% of the focal pigs based on the variables retained in the model. Crawl and escape attempt in the gondola differed according to the sex of the focal pig (*P* = 0.001; sex distribution in Table 3), females being half as likely to crawl or attempt to escape than entire males (*P* < 0.0001, 'odds-ratio' **OR** = 0.519, odds ratio meaning X times more likely, if value >1, or X times less likely, if value <1), and barrows being intermediate and no different from other sexes. Crawl and escape attempts in the gondola were lower following higher amounts of highly aversive (P4, *P* = 0.024 OR = 0.681) and electric prod (P5, *P* = 0.024, OR = 0.805) interactions in the race. Finally, crawl and escape attempts were marginally higher in pigs that disappeared from the camera's field of view before losing posture compared to pigs that lost posture on the footage (*P* = 0.04, OR = 1.009).

Table 3
Distribution of the data in lairage, forcing pen and race for focal pigs per abattoirs and overall (means ± standard error). P3 corresponds to either slap, push, shout, piercing whistle, artificial noise, stomp; 'moderate aversive' interactions, P4 corresponds to either rhit, caught, hard push; 'highly aversive' interactions with stockpeople or gates, and P5 corresponds to the use of an electric prodder.

Abattoirs	A1	A2	A3	A4	A5	Overall	*P*-value
Lairage variables							
Stocking density scores (1/2/3; %)	24/60/16	35/51/14	29/57/14	39/53/8	15/82/3	29/60/11	*P* < 0.001[2]
Activity scores (1/2/3; %)	30/62/8	43/48/9	33/65/2	49/51/0	32/59/9	38/57/5	*P* < 0.001[2]
Pen sex composition (Both/Female/Male/Immunocastrated barrows; %)	53/25/22/0	41/20/39/0	40/23/0/37	49/38/13/0	67/24/9/0	51/27/16/6	*P* < 0.001[2]
Focal pig sex (Female/Male/Castrated male)	49/51/0	38/62/0	40/23/37	66/34/0	58/42/0	52/42/6	*P* < 0.001[3]
Skin injuries (1/2/3; %)	67/28/5	Not recorded	95/5/0	93/6/1	76/22/2	83/15/2	*P* < 0.001[2]
Forcing pen variables*,[1]							
Total time from start to end (s)	231 ± 11[c]	70 ± 9[b]	92 ± 11[b]	Not recorded	27 ± 2[a]	82 ± 7	*P* < 0.001[4]
P3 interaction (% of focal pigs)	100	86.0	7.3	59.9	36.2	43.3	*P* < 0.001[2]
P4 interaction (% of focal pigs)	0.0	3.5	57.3	7.6	2.0	18.8	*P* < 0.001[2]
P5 interaction (% of focal pigs)	45.6	69.8	0	0	0	8.7	*P* < 0.001[2]
Race pen variables*							
Total time from start to end (s)	93.0 ± 7.1[c]	74.0 ± 6.6[bc]	33.43 ± 2.4[a]	68.8 ± 3.0[b]	127.1 ± 6.7[d]	79.5 ± 2.5	*P* < 0.001[4]
P3 interaction (% of focal pigs)	27.3	4.2	76.9	59.4	38.9	45.0	*P* < 0.001[2]
P4 interaction (% of focal pigs)	1.6	0.0	4.1	0.0	49.3	10.4	*P* < 0.001[2]
P5 interaction (% of focal pigs)	98.6	92.1	52.4	0	0	42.2	*P* < 0.001[2]
Mount (% of focal pigs)	27.0	1.1	11.0	0.0	3.3	8.8	*P* < 0.001[2]
Crawl or escape attempts (% of focal pigs)	25.4	0.0	5.5	0	0	6.7	*P* < 0.001[2]
Slip or fall (% of focal pigs)	2.5	0	0.7	0.2	1.6	1.0	*P* = 0.004[2]
Post-stun variable							
Plasma cortisol concentration (ng/ml)	37.3 ± 1.1[c]	50.1 ± 2.3[b]	58.4 ± 1.5[a]	26.7 ± 0.9[d]	29.4 ± 1.1[d]	39.5 ± 0.7	*P* < 0.001[4]

[abcd]Means within a row with different superscript differ (*P* < 0.05).
*Subject to variation according to abattoir layout, hence not comparable to each other.
[1] Mount, slip or crawl or escape attempts in the forcing pen were only seen in 10, 7 and 7 pigs respectively across abattoirs.
[2] Chi-square statistical analysis.
[3] Fisher's Exact test statistical analysis.
[4] Kruskal–Wallis test statistical analysis.

Mounting behaviour in the gondola

The occurrence of mounting other pigs in the gondola ranged from 1.0% (A4) to 24.3% (A2) of the focal pigs ($P > 0.05$), with the other abattoirs intermediate to these values (A1: 10%; A3: 23%; A5: 5%). The binary regression model correctly classified the occurrence of mounting behaviour in 66.4% of the focal pigs based on the variables retained in the model. The occurrence of mounting behaviour in the gondola differed according to the sex composition of lairage pens ($P = 0.004$), with more mounting in the gondola in pigs from lairage pens of mixed sexes compared to pigs from pens of males ($P = 0.006$, OR = 2427.428), and the least in pigs from lairage pens of females ($P = 0.013$ compared to males, OR = 0.002). Mounting behaviour in the gondola was higher following higher amounts of highly aversive interactions in the race (P4, $P < 0.001$, OR = 10.925). Mounting behaviour in the gondola was also higher with higher number of pigs in the gondola ($P < 0.001$, OR = 19.844). Finally, mounting behaviour was higher in pigs that disappeared from the camera's field of view before losing posture compared to pigs that lost posture on the footage ($P = 0.007$, OR = 6.984).

Gasping behaviour in the gondola

The occurrence of gasp behaviour in the gondola ranged from 63.1% (A1), 75% (A5), 80% (A4) to 81.8% (A3) of the focal pigs ($P > 0.05$), excluding A2 where only 22.2% of the pigs were observed gasping due to a camera placement problem (see earlier), which likely biased the population of pigs observed. The binary regression model correctly classified the occurrence of gasping in 92.0% of the focal pigs based on the variables retained in the model. The occurrence of gasping behaviour in the gondola differed according to activity in the lairage pen ($P = 0.01$), with pigs from moderately active lairage pens more likely to gasp than pigs from the least active lairage pens ($P = 0.05$, OR = 5.609), but pigs from the most active lairage pens being no different from either pigs from moderately active or least active lairage pens (and representing on average 5% of the cases). Gasping behaviour in the gondola differed according to skin injuries ($P = 0.01$), with pigs with moderate skin injuries in lairage more likely to gasp than pigs with no skin injuries ($P = 0.001$, OR: 3.306).

Convulsion behaviour in the gondola

The occurrence of convulsion behaviour in the gondola ranged from 60.1% (A3) to 69.6% (A5) of the focal pigs ($P > 0.05$), excluding abattoir 2 where only 28.0% of the pigs were observed convulsing due to a camera placement problem (see earlier), which likely biased the population of pigs observed. The binary regression model correctly classified the occurrence of convulsions in 85.8% of the focal pigs based on the variables retained in the model. The occurrence of convulsing behaviour in the gondola was lower for pigs that slipped or fell in the race ($P = 0.001$, OR = 0.265), lower with higher number of pigs in the gondola ($P = 0.05$, OR = 0.732) and lower for pigs that disappeared from the camera's field of view before losing posture compared to pigs that lost posture on the footage ($P < 0.001$, OR = 0.021). Convulsing behaviour in the gondola was marginally higher with longer time from entry in the gondola to the time pigs disappeared from the camera's field of view ($P = 0.03$, OR = 1.009).

Fighting behaviour in the gondola

Fighting behaviour in the gondola was rare, having been observed in three abattoirs (A5:1.3%, A2:2.1% and A1:5.4%) and absent in the other two abattoirs (A3, A4). Its rare occurrence made it unamenable for statistical analysis.

Relationship between behaviours in the gondola

The typical time course of behaviours observed in the gondola was first crawl, escape attempt or mount (when either of these behaviours occurred), with no specific order between these behaviours. Gasping occurred at the same time as these behaviours or afterwards, and convulsions were usually observed after pigs lost posture. Most behaviours observed in the gondola were weakly correlated with each other (Table 4). The only moderate positive correlation observed was between crawl/escape attempt and mounting. Correlations differed marginally across abattoirs.

Prevalence of modalities for the variables in lairage, forcing pen and race

Prevalences of the various variables recorded in lairage, forcing pens and race leading to the gondola are presented in Table 3. Handling bouts are presented as percentage of focal pigs receiving the handling, rather than the number of bouts. Forcing pen variables did not return significant relationships with behaviours displayed in the gondola.

CO_2 external sensor data

Results of the CO_2 measurements by a portable CO_2 meter attached to the gondola varied between 0.1 and 4.1% for the minimum concentration at the entrance into the chamber to 84.5 to 99.6% at the maximum concentration (Table 5).

Cortisol concentration

A linear regression model for plasma cortisol concentration explained 35% of the variance ($P < 0.001$), with significant positive effects of abattoir ($P < 0.001$), system ($P < 0.001$), farm of origin ($P < 0.001$), P5 (i.e. electric prod) interactions in the forcing pen ($P = 0.05$), and mounting in the race ($P = 0.02$), and trends for total time in the forcing pen ($P = 0.07$), P4 interactions in the forcing pen ($P = 0.09$) and latency to lose posture ($P = 0.07$).

Discussion

Current criticism against CO_2 stunning based on animal welfare concerns focuses on its aversiveness, and therefore, the behavioural reaction of pigs to the process, along with the time to loss of

Table 4
Spearman Rho correlation coefficient (r) and *P*-values (*P*) between the behaviours of pigs observed in the gondola in the CO_2 chamber.

Behaviour in the gondola	Crawl and escape attempt	Mount	Convulsion
Mount	r = 0.20 $P < 0.001$		
Convulsion	r = 0.01 $P = 0.65$	r = −0.10 $P < 0.001$	
Gasp	r = 0.001 $P = 0.98$	r = −0.08 $P = 0.001$	r = 0.12 $P < 0.001$

Table 5
Carbon dioxide concentrations measured from a single external CO_2 gas sensor per abattoir in gondola cycling through the CO_2 gas chamber without pigs.

Abattoirs	A1	A2	A3	A4	A5	Overall
Maximum CO_2 concentration (%)	84.5	88.6	87.9	99.6	99.6	94.5
Minimum CO_2 concentration (%)	4.1	0.3	0.6	0.1	0.2	1.1

consciousness. Nevertheless, CO_2 stunning is part of an overall slaughter system, and the present findings showed that the variation in behavioural response to CO_2 exposure relates not only to conditions in the CO_2 chamber but also to handling, animal characteristics (sex, farm and/or transport), lairage conditions, facility design and management, which all play a role in determining the pigs' reaction to CO_2 stunning and ultimately animal welfare. However, this is an observational study and while relationships suggest the possibility of causality, evidence of causality can only be demonstrated by changes, for example in handling, resulting in changes in animal behaviour and/or stress. Furthermore, these variables may be a mixture of independent and mediating variables.

Four main behaviours were recorded during the stunning process. The typical time course of behaviours observed in the gondola started with crawl and escape attempts or mount (when either of these behaviours occurred), with no specific order between these behaviours, simultaneously or followed by gasping, while convulsions usually occurred after pigs lost posture. Crawling and escape attempt are indicative of an aversive reaction, which along with mounting other pigs raise animal welfare concerns as presumably conscious reactions (Rault et al., 2020). These behaviours ranged from rare (≤1% of cases) up to almost a quarter of the cases for mounting and almost half of the cases for crawl and escape attempt, but with large variation between abattoirs. Both crawling and escape attempts and mounting related to either the sex composition of the pen or sex of the focal pig, with females being less likely to display these behaviours than males, and mounting in the gondola being much more likely in pigs from lairage pens of mixed sexes. The likelihood of mounting in the gondola also increased with greater amounts of highly aversive handling in the race by the stockperson or after being trapped by the automatic gates. However, the likelihood of crawl and escape attempts in the gondola decreased with greater amounts of highly aversive handling and electric prodding in the race. Hence, aversive handling in the race was correlated with greater mounting but fewer crawl or escape attempts in the gondola, against the simple prediction that increased aversive handling would encourage both behaviours.

Gasping was relatively common, occurring in 63–82% of the pigs observed, respectively, and with little variation between abattoirs. Gasping is considered to occur at the onset of breathlessness (Velarde et al., 2007), and CO_2 is likely to lead to severe air hunger, which is reported to be the most unpleasant sensation of breathlessness (Beausoleil and Mellor, 2015). However, gasping has also been reported to persist beyond isoelectric EEG, and therefore may not necessarily be indicative of consciousness (Rault et al., 2020). Regular gasping is nevertheless one of the most frequent symptoms of inadequate stun along with the corneal reflex (Atkinson et al., 2012). The present findings show that gasping during the CO_2 stunning process was correlated with higher pen activity in lairage and higher fresh skin injuries, indicating a possible effect of lairage conditions.

Convulsions were also prevalent, occurring in 60–70% of cases, but showed only marginal links to variables related to the timing of the process in the gondola. They generally occurred after loss of posture and are considered the earliest sign of onset of unconsciousness and insensibility during exposure of pigs to CO_2 (EFSA, 2013). This muscular excitation could start as an aversive response through vigorous escape attempts and continued as convulsions during unconsciousness (Llonch et al., 2013). Accordingly, in the present study, convulsions were most often seen after loss of posture; however, they were not correlated with crawl and escape attempts.

Stockpeople handling of animals pre-slaughter can affect the animals' behaviour and cortisol concentration (Coleman et al., 1998; Hemsworth et al., 2011). Aversive interactions occurred quite frequently throughout the forcing pen and race. While it was assumed that the electric prod was active when it was used, this could not be established for observation and prods may have been used while inactive at times. Nevertheless, pigs may find the use of prods highly aversive, and their behavioural response often indicated this (Jongman et al., 2000). However, aversive interactions in the race did not appear to strongly affect pig behaviour in the gondola. Still, aversive interactions in the race were associated with increased mounting of other pigs in the gondola, while it was also associated with decreased crawl and escape attempts.

Although the type of CO_2 system (group-wise vs single-file loading) had no significant effect on behaviour in the gondola, the two abattoirs with a group-wise loading system and automatic gates, which minimized stockperson handling, had significantly lower cortisol concentrations post-stunning. This may reflect the advantage of group-wise systems that allows to move pigs as a group all the way to the gondola, taking full advantage of one of the benefits of CO_2 stunning. However, intervals between the commencement of handling in the forcing pen and time of death varied between abattoirs, and the time spend in the gondola was longer in group-wise system as these were larger CO_2 chambers. Therefore, differences in cortisol may not necessarily reflect differences in stress immediately pre-slaughter as cortisol takes up to 3 min to rise, so care should be taken when interpreting this result. Nevertheless, variation in stockmanship handling in single-file systems and research in stockpeople handling indicates that better handling practices can be achieved, for instance with appropriate cognitive-behaviour training interventions (Hemsworth and Coleman, 2011) resulting in lower stress levels of slaughter pigs and better animal welfare outcomes.

Loss of posture is considered the first indicator of loss of consciousness (Raj and Gregory, 1995, 1996; Velarde et al., 2007; Llonch et al., 2012) and is characterized by the inability of the animal to remain in a standing position. In the present study, pigs lost posture on average within 22.5 s of the time the gondola started lowering down, which is in accordance with the literature ((Raj, 1999): 17 s at 90% CO_2; Velarde et al., 2007: 22.4 s at 80% CO_2; Verhoeven et al., 2016: 37 s at 80% and 23 s at 95%). However, latency to loss of posture differed between and within abattoirs. Latency to loss of posture was associated with farm of origin, which highlights the role of background characteristics linked to the farm. Further research should investigate the specific factors relating to the farm, such as genetics, previous experience, etc., or transport conditions on the pigs' susceptibility to succumb to CO_2. Latency to loss of posture also varied according to the time of the day, which could be linked to various factors such as CO_2 chamber concentration, speed of the line, length of time in lairage and other factors that vary over the course of the day. Finally, pigs that crawled or attempted to escape while in the gondola were associated with longer time to lose posture. Noteworthy, the number of pigs in the gondola was not associated with latency to loss of posture.

The latency to loss of posture is known to vary according to CO_2 concentration (Raj and Gregory, 1996; Raj, 1999; Velarde et al., 2007; Llonch et al., 2013). While we attempted to measure actual CO_2 concentration in the gondola during the full cycle, it was logistically impractical to measure CO_2 concentrations continuously, while the pigs were being stunned. Furthermore, lack of reliability of the CO_2 meter prevented us from drawing accurate conclusions from those measures, as the device would at times get stuck at high concentrations. It appears that abattoirs 1 and 3 had lower maximum CO_2 concentrations; however, this did not impact on latency to loss of posture, possibly because all abattoirs used maximum CO_2 concentrations in excess of the recommended minimal levels of 85% CO_2. While loss of latency was found to be affected by CO_2 concentrations of either 80 or 95% in a study by Verhoeven et al. (2016) under experimental conditions, there is a lot of variation in variables (such as speed of decend and size of the pit) between abattoirs under commercial conditions that may affect this latency. Re-stuns due to signs of return to consciousness were rare occurrences, indicating that the required high concentrations of CO_2 were consistently achieved.

Atkinson et al. (2012) conducted a thorough assessment of signs of stun quality and return to consciousness in CO_2 systems, but it remains difficult to compare it with the results of the present study as they assessed pigs after stunning, whereas we assessed pigs during the stunning process. Camera recording remained challenging in commercial conditions, and the placement of the cameras allowed tracking pigs from their entry into the gondola to just after the first stop, by which time most, but not all, pigs lost posture. The most likely cause for pigs disappearing from the field of view before loss of posture was linked to the gondola stopping for a significantly shorter amount of time at the first stop, due to quicker or no loading of the pigs in the next gondola. This is because the speed of the system is dictated by the loading of live pigs in the gondola rather than the unloading of stunned pigs at the other end. As a result, the behavioural time course of these pigs that disappeared from the field of view before losing posture was truncated, but it affected to a greater extent the observation of convulsions that occurred later in the process. Nevertheless, relationships were observed between pigs that showed crawl or escape attempts and later loss of posture, and pigs that crawled, attempted to escape or mounted other pigs and disappearance from the field of view, suggesting that the pigs that react more aversively to CO_2 also take longer to succumb to it. However, these relationships may not necessarily be causal and may be explained by other related factors.

This study, through measuring the reaction of pigs to CO_2 stunning in five abattoirs with a uniform protocol for data collection, allowed assessing variation between and within abattoirs while minimizing idiosyncratic findings that could be due to a specific abattoir. Interestingly, abattoir 4 had minimal crawl, escape attempts, mounting behaviour and the lowest cortisol concentration, and this abattoir anecdotally ran at lower than normal gondola capacity during the experiment (3 pigs rather than the intended use of 8 pigs per gondola).

Conclusion

In conclusion, substantial variation in the reaction of pigs to CO_2 stunning was observed between and within abattoirs using a uniform protocol for data collection. This variation in outcomes between abattoirs and stunning systems, and the relationships between preslaughter handling and behavioural outcomes indicate that improvements can be made to reduce aversive responses to CO_2 stunning. It opens the way to more controlled studies to investigate in particular the causal factors explaining variation in the latency to loss of poture, crawl and escape attempts, such as variables linked to farm of origin, lairage conditions and handling.

Ethics approval

This experiment was approved by the University of Melbourne Animal Ethics Committees in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and the Human Ethics Committee for the observations of handling by stockpeople (Project ID 1513706.1). All procedures contributing to this work complied with the ethical standards of the relevant national and institutional committees on animal experimentation.

Data and model availability statement

The data were not deposited in an official repository but are available upon request.

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Author contributions

J-LR and EJ conceptualizes the study and obtained the funding. RW conducted a literature review. J-LR, EJ, RW and MR developed the methodology and collected the data. J-LR and EJ wrote the initial draft of the manuscript. J-LR, EJ and MR edited the manuscript. J-LR, EJ, RW and MR reviewed and approved the final manuscript.

Declaration of interest

The authors declare no conflict of interest.

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