Physiology & Behavior 198 (2019) 76–83

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Sick and grumpy: Changes in social behaviour after a controlled immune stimulation in group-housed gilts☆

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ARTICLE INFO

Keywords:
Sickness behaviour
Social behaviour
Sus scrofa
Pig
LPS
Tail biting

ABSTRACT

Poor health is associated with an increased risk of tail biting outbreaks in pigs. We propose that this is because illness changes social dynamics either by changing the behaviour of the sick pig towards its penmates, the behaviour of the healthy penmates towards the sick pig, or both. We tested the effect of immune stimulation (lipopolysaccharide (LPS) injection: O111:B4; 1.5 μg kg^{-1} IV) on social behaviour in gilts housed in triplets in a cross-over experiment. Each pen was subjected to the control treatment (all three pigs injected with saline) and then LPS treatment (one pig injected with LPS, two injected with saline), or vice versa. LPS injected pigs had a shift in social motivation and performed more tail- and ear- directed behaviour than saline pigs two days after injection. They seemed to fit the description of 'sick and grumpy'. This change was seen about 40 h after the signs of acute illness dissipated and was not accompanied by a similar increase in activity. We discuss possible mechanisms for this behavioural change in light of changes in neurotransmitter levels at three days after LPS injection described in a previous experiment.

1. Introduction

Sickness is considered one of the risk factors for tail biting in pigs [1,19,47–49]. Tail (biting) damage has been shown to correlate with rectal prolapse and respiratory disease on a farm level [30], and with leg disorders and respiratory disease on an individual level [27,34,36]. The mechanisms by which sickness works to increase the risk of a tail-biting outbreak is not known, but we hypothesise that one pathway is through the effect of cytokines on hormone and neurotransmitter levels, which are described by e.g. Dantzer et al. [16]. Changes in hormones and/or neurotransmitters have the potential to change behaviour. Sickness behaviour, or behavioural changes during illness, as well as mental side effects of immune therapy, are well described in humans [8–10,23].

Most papers reporting associations between sickness and tail biting in pigs are based on cross-sectional data. Although correlations have been reported [27,30,34,36], to date, no evidence for a causal relationship has been provided. To our knowledge, the only longitudinal study on the subject is Niemi et al. [35]. The authors document individual-level temporal associations between treatment for lameness and increased risk for having tail (biting) damage later. The odds ratio (OR) for risk of tail biting damage in pigs treated for lameness in this study was 1.6, whereas the OR of becoming lame after tail biting was higher (OR = 3.4).

If sickness has a role in the aetiology of tail biting it must be by increasing the likelihood of a pig becoming either a victim or a biter. A pig ill to such an extent that it shows social withdrawal and lethargy, both of which are parts of typical sickness behaviour, could be singled out as a victim as it would differ from the rest of the group. A study in hens reported increases in both gentle and severe feather pecks towards less mobile compared to active flock members, suggesting that the manipulation may be not only explorative but also include deliberate injurious pecking [44]. Sick animals may also actually be preferred opponents as victory is more certain when competing with a sick competitor than when competing with a healthy conspecific [7]. On the other hand, poor health may increase the propensity of a pig to become

☆ Summary statement: This paper is the first to provide preliminary evidence for a shift in motivation, evident as abnormal social behaviour, shortly after a bout of acute inflammation.

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https://doi.org/10.1016/j.physbeh.2018.09.018
Received 31 May 2018; Received in revised form 26 September 2018; Accepted 27 September 2018
Available online 02 October 2018
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a tail biter by increasing irritability, emotional lability and short temper.

Irritability, emotional lability and short temper have been observed as side effects of treatment with pro-inflammatory cytokines such as IL-2 and interferon alpha in humans [10,14,18,43]. There are also indications that inflammatory proteins may play a role in aggression. Elevated levels of IL-6 and C-reactive protein (CRP) have been found in psychiatric patients with a diagnosis of intermittent explosive disorder [13] and aggressive behavioural tendencies are correlated with inflammatory markers in healthy adults [28]. Furthermore, increased feather damage has been detected in hens after immunisation against the antigen human serum albumin [38]. Our previous finding that boars with a clinical respiratory infection, as compared to healthy controls, had an increased tendency for tail- and ear-biting 0–2 weeks before the clinical signs were visible, may indicate a change in behaviour already in the pre-pathologic state [33].

Most aspects of sickness behaviour are transient and resolve soon after the cessation of immune stimulation. Cognitive and mental dysfunction may, however, persist beyond the physiological inflammatory reaction [22]. Studies in mice have shown a peak in sickness behaviour two to six hours after treatment with the potent immunostimulator lipopolysaccharide (LPS), while depression-like behaviour did not peak until 24 h after injection [21]. Examples in humans include chronic hyperexcitability and altered pain perception after trauma-induced cytokine elevations [29], and post-vaccination depression [2]. In mice, spatial memory was impaired seven weeks after systemic inflammation [51], whereas systemic *E. coli* infection early in life appears to have enduring consequences for brain development [6]. Long-term post-inflammatory mental dysfunction is thought to be caused by neuroinflammation [42], which may lead to neurodegeneration [24,41].

Most studies of immune stimulation by LPS-treatment in pigs follow the pigs for < 24 h [12,25,32,55]. We, however, recently found that there is a reduction in noradrenaline levels in the hippocampus, hypothalamus and frontal cortex, and an increase in serotonin levels in the right hippocampus in pigs 72 h after LPS injection [37]. The current experiment was designed to follow up these findings and test the hypothesis that an LPS injection influences social behaviour in small groups of pigs during the second day after LPS-treatment, which is shortly before the time at which we had found neurotransmitter levels to be affected. Pigs were housed in triplets, and the effect of LPS was tested with a cross-over design.

2. Materials and methods

2.1. Ethical statement

The experimental animals were housed and managed according to local animal welfare legislation. This study was approved by the Animal Care and Use Committee at the Norwegian Food Safety Authority under ID number 7138. Each pig was subjected to the following experimental procedures: videotaping as described below, daily spray-marking without restraint of the animals when videotaping, one intravenous injection of either LPS or saline twice (days INJ1 and INJ2 in Fig. 1), and euthanasia upon completion of both experimental periods.

2.2. Animals and study design

The experiment was run in two replicates, each consisting of eight groups of three gilts (24 animals in 8 pens per replicate, altogether 48 animals). The pigs were brought into the experimental facility in Oslo at between seven and 10 weeks of age and given at least two weeks (2–4.5) to habituate. They were purchased from a commercial farm less than a one-hour drive from the experimental facility. They and had no previous disease or treatment history. Pigs were selected from 24 different litters and were unfamiliar with their new pen-mates. They were housed in groups of three in pens (2.2 m^2) containing two drinking nipples and one ad-libitum feeder. The pens had a concrete floor and no slatted area. They were cleaned once per day. Fresh wood-shavings were added daily to a depth of 5 cm. The pigs were fed ad libitum with Format 110 (Felleskjøpet, Lillestrøm, Norway), a commercial growth diet for finishing pigs. The lights were turned on at 08:00 and off at 16:00, but natural light shone through the window, so the pigs were in dim daylight until the evening as this experiment was carried out in the springtime in Norway.

One pen had to be removed from the second replicate due to a severe umbilical hernia in one pig, leaving 15 pens and 45 animals for analysis. Within each replicate a two-period cross-over design was used. Both the pen status (control pen including no LPS-treated animal, or LPS-pen including one LPS-treated animal) and individual status (LPS-injected pig or saline-injected control pig) was switched between periods. An LPS-pen included one pig injected with LPS and two pigs injected with saline, whereas in the control pens all three pigs were injected with saline. In other words, one randomly chosen pig per pen was injected with LPS one time during the two periods of the replicate. If a pen contained an LPS-injected pig in one period, it contained only controls in the other. The experimental design is described in Fig. 1, and the number of pens and pigs in each treatment sequence (order of treatments in period 1 and period 2 for the given pen or individual) is given in Table 1.

The type and dose of LPS was chosen to produce a reliable but moderate response, as discussed in de Groot et al. [17] and Nordgreen et al. [37]. Lyophilized LPS (from *Escherichia coli* 0111:B4 [SigmaAldrich, Darmstadt, Germany]), dissolved in sterile 0.9% saline to a concentration of 2 mg ml^{-1} and frozen in glass vials, was thawed on the injection day and further diluted in saline to a concentration of 20 µg ml^{-1}. The injection volume was calculated individually to provide a dose of 1.5 µg kg^{-1} and injected into the ear vein in nose-snared animals using a Hamilton glass syringe. Control animals were handled equally and injected with a corresponding volume of saline. Injections were carried out in the morning after the completion of morning routines. The pens were treated in the same order in period one and period two, with the times of the first injection described in Fig. 1. Upon

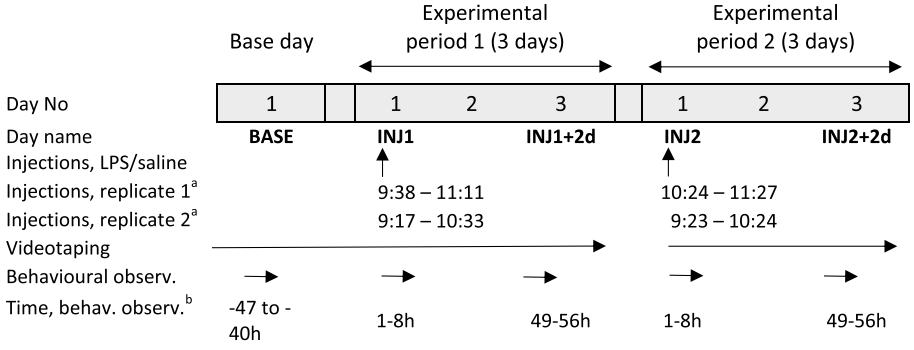

Fig. 1. An overview of the experimental design showing the procedures for one replicate of the study and times of injection of LPS and saline in both replicates. Each replicate was designed as a cross-over trial, where the same animals were observed for one BASE day, and then subjected to two experimental periods.
[a]Time of completion the last injection in the first and last pen [b]Time of behavioural observation in hours relative to the injection in the corresponding experimental period (INJ1 and INJ2).

Table 1
The number of pens and animals in the different treatment sequences in period one and period two of the cross-over trial. CTR refers to saline treatment of an individual, or of all pigs in the same pen. LPS refers to the corresponding treatment of an individual pig in a pen with lipopolysaccharide.

Pen level treatment sequence	Number of pens[1] in replicate 1 + replicate 2	Individual level treatment sequence[2]	Number of individuals in replicate 1 + replicate 2
CTR → LPS	4 + 4	CTR → CTR	8 + 8
		CTR → LPS	4 + 4
LPS → CTR	4 + 3	CTR → CTR	8 + 6
		LPS → CTR	4 + 3

[1] Each pen has three pigs.
[2] Within pens with the treatment sequence given in the first column.

completion of both periods euthanasia was carried out by anaesthetising each pig to a surgical level using a combination of xylazine (2 mg kg^{-1}), ketamine (15 mg kg^{-1}) and butorphanol (0.2 mg kg^{-1}) injected intramuscularly. When the animals no longer responded to strong pressure applied between the hooves, they were euthanised with an intracardial injection of pentobarbital. Cardiac arrest was confirmed using a stethoscope.

2.3. Observation of behaviour

In each replicate, the animals were videotaped for 24 h per day over five days (Fig. 1). A baseline of behaviour was determined before any treatments except spray marking during the BASE day. BASE was followed by two experimental periods, during which videotaping took place on the day of LPS-injection (days INJ1 and INJ2 for experimental period 1 and 2, respectively), and on follow-up days two days later (INJ1 + 2d and INJ2 + 2d). An infrared camera was positioned above the centre of two pens, and recordings were performed using the Media Recorder system from Noldus (Wageningen, the Netherlands). Lights were on during the observations except for a small number of late 15-min slots in a few pens. The lack of artificial lighting caused no problems for the recording of behaviour as sufficient natural light entered the room through the windows.

Two classes of behaviours, including social and time budget behaviours, were recorded according to ethograms in Tables 2 and 3 by two observers blind to the treatments of the animals. For each animal, behavioural observations were conducted for 7 × 15 (=105) minutes per day, with each observation slot starting one hour after the previous one. To remove effects of the time of day, the observations were synchronised according to the time of injection in the pen. The starting point of the first registration period was determined pen-wise according to the exact time of the last injection in the pen plus one hour. The time of INJ1 determined the start of observations in BASE, INJ1 and INJ1 + 2d, whereas the time of INJ2 determined the start of observations that day and INJ2 + 2d. For times of injection and exact time slots of observation see Fig. 1.

All behavioural observations were carried out using video tapes. Social behaviours were event recorded, with a new occurrence of a repeated behaviour considered to begin after a pause of two seconds. The identity (colour codes) of the performer and receiver was recorded for each event to identify both social behaviours that were performed and received. Time budget behaviours were scan sampled at the beginning and end of each 15-min observation slot, summarising to 14 scans per day. If a person entered the pen, the observation was paused for a sufficient number of full minutes, and the observation time then extended accordingly. The beginning of the next observation slot was unaffected by the extension.

2.4. Statistical analyses

Variables describing social behaviours were summarised for each day and analysed at the individual level. Selected behaviours were summed as described in Table 2 to form the combined variables "total social behaviour", describing all kinds of active social behaviour initiated by the focal pig; as well as "tail -and ear-directed behaviour", including attention directed at these body parts. For all social behaviours, we differentiated between "performed" and "received", defined as the total number of occurrences the focal individual performed or received during the 105 min of observations taking place on one day.

Scans were summed by day. To describe the level of synchronization of activity in a pen, the percentage of scans with *all* three pigs either active or passive was considered, with activity defined as any behaviour in Table 3 other than lying inactive, which was considered passive. All other scan sampled behaviours were analysed on an individual level. The "activity percentage" of an individual was calculated as the percentage of active scans out of all scans.

Statistical analyses were conducted using SPSS 24.0 (SPSS Inc., Chicago, IL, USA). Linear mixed models (LMM, Linear Mixed -feature in SPSS) were fitted to the variables activity percentage and environmental exploration, and to performed and received total social behaviour after a square root transformation. Performed submissive behaviour, and performed and received tail- and ear-directed behaviour, exhibited a Poisson distribution and were analysed using the Generalized Linear -feature in SPSS (GLM). The majority of variables were zero-inflated, and as none of the distributions available in GLM enabled significant models to be fitted, non-parametric tests were

Table 2
Ethogram for event recording of social behaviour. No social behaviours were recorded when an animal was lying inactive, assumed to be sleeping. Combined variables were used for analysis.

Behaviour	Combined variable[1]	Description
Sniffing/ manipulating	T	Snout in contact with or very close to another pig, excluding the tail. Includes gentle manipulation such as rooting or licking. Not including the behaviour bellynosing.
Sniffing/ manipulating tail	T,S[2]	Snout in contact with or very close to/ rooting or licking the tail of another pig. Taking the tail in the mouth *without any reaction* by the receiver.
Biting tail	T,S	Taking the tail of another pig in the mouth *followed by an immediate avoidance reaction* by the receiver.
Biting ear	T,S	Taking the ear of another pig in the mouth *followed by an immediate avoidance reaction* by the receiver.
Fighting/ attacking	T	Forceful biting, or hitting/knocking of another pig with the head. Includes chasing performed immediately after biting/ hitting/ knocking. Not including the behaviours tail and ear biting.
Submissive		Escaping or moving away, or attempting to do so, *immediately* upon being subjected to social behaviour by another pig.
Bellynosing	T	Using the snout repetitively to lift, root or massage the belly or groin area of another pig.
Other social behaviour	T	Behaviour involving contact with another pig not listed above, e. g. mounting (chest contact with the back of another pig), standing with forelegs on another pig, touching another pig with another body part than the head, or responding to social behaviour by turning the head towards the active pig without touching it.

[1] T indicates that the behaviour is included in the combined variable "Total social behaviour".
[2] S that it is included in "Tail- and ear -directed behaviours".

Table 3
Ethogram for scan sampling of behaviour for time budgets.

Behaviour	Behaviour description
Environmental exploration	Snout in contact with the floor, enrichment material, pen fittings or other objects in the pen in a pig sitting or standing. If the pig is lying down snout contact and movement of the head, mouth or snout is required. Sitting, standing or moving with the head down, apparently touching the ground even if the snout cannot be seen.
Sitting inactive	Dog-sitting doing nothing except from turning of the head as when watching something
Lying inactive	Lying inactive in any posture
Other behaviour	Any behaviour not listed above, such as feeding, drinking, defecating or walking with the head up

applied as described below.

LMM and GLM on individual-level variables were set up with pig within pen as the subject ($n = 45$), pen as a random effect ($n = 15$) and day within pig (BASE, INJ1, INJ1 + 2d, INJ2, INJ2 + 2d) as a repeated effect. Fixed effects included replicate ($n = 2$, pen level), day ($n = 5$), pen level treatment ($n = 3$ including BASE at BASE day and for the other days LPS if the pig was in a pen with an LPS-treated pig, or CTR if no pig in the pen was LPS-treated), individual-level treatment (BASE at base day, thereafter either LPS or CTR), as well as individual-level treatment sequence (BASE on base day, INJ1 and INJ1 + 2d, thereafter either LPS → CTR, CTR → LPS or CTR → CTR). Interactions that were included were replicate x day, replicate x individual-level treatment, and individual-level treatment x day. The only pen-level variable included in the study, synchronization of activity, was analysed using LMM with pen as the subject, day as repeated effect, and the fixed effects replicate, day, and pen-level treatment. LMM and GLM were built by backward step-wise elimination and variables kept in the model if significant at the 10% level, or if they significantly improved the fit of the model (see below) although failing to reach this level of significance. Covariance structures including diagonal and autoregressive with and without moving average were tested for each model, and the one providing the best fit was chosen. LMM fit and absence of outliers or cases with excessive leverage were ensured by investigating residuals, homoscedasticity plots and leverage values, as well as Akaike (AIC) and Bayesian (BIC) information criteria. GLM were built using Poisson distribution in this study. For these models (over)dispersion was assessed using the Pearson Chi-squared statistic, and different models compared using the corrected AIC and BIC. The meanings of significant interactions were clarified by plotting predicted values with 95% confidence intervals (CI). For significant interactions of particular interest, differences in predicted continuous variables (behaviour) between different levels of categorical variables (days, treatments and treatment sequences) were tested using post-hoc *t*-tests.

Univariate non-parametric tests were applied to test for differences between treatment and treatment sequence on performed and received tail biting, fighting and belly-nosing; as well as received submissive behaviour. The analyses were carried out separately per day (INJ1, INJ2, INJ1 + 2d and INJ2 + 2d for treatment, and the two last days for treatment sequence), and results interpreted following a Holm-Bonferroni correction for multiple comparisons. The Wilcoxon Rank Sum test was applied to test for differences between treatments within pen. This analysis was possible only in LPS -pens. The test negotiates only one observation per treatment and pen, thus, if two individuals in a pen represented the same treatment (sequence), their variables were averaged. The pen effect was controlled for by considering the two treatments or treatment sequences within pen as repeated measures. The Mann-Whitney *U* test was used to test for differences between pen level treatments (i.e. comparing LPS and CTR pens). Only performance of the analysed behaviours was included in the analysis on pen level, as the number of similar received actions in the pen by definition was equal. In these analyses, each variable was summed per day for all animals in the pen.

3. Results

3.1. Pen- and individual-level effects of day and treatment

Data on all behaviours are given per day and treatment in the Supplementary Table. Univariate analyses indicated no significant differences between treatments or treatment sequences in performed tail biting, fighting or belly-nosing; nor in receiving these behaviours, or in receiving submissive behaviour.

Day had a significant effect on total performed and received social behaviour, performed tail- and ear-directed behaviours, performed submissive behaviour; as well as scan-sampled activity percentage, environmental exploration and pen-level synchronization of activity (Tables 4 and 5). The effect of day on received tail- and ear-directed behaviours was non-significant, but still kept in the model due to a favourable effect on fit (Table 4). Details on the main effects of day are of minor importance in this study and thus not discussed further. To summarize, LPS-treated pigs showed marked passiveness in INJ1 and INJ2. Apart from this (expected) effect there appeared to be a general increase in activity over the whole study period. No clear pattern could be recognized in pen-level synchronization of activity (see Fig. 2 and 3, and the Supplementary Table).

The main effect of individual-level treatment was significant for most variables analysed by LMM or GLM. As compared to LPS, CTR was

Table 4
Results from linear mixed or Poisson regression analyses on event recorded day-time social behaviours of pigs ($n = 45$) in pens ($n = 15$) with either one (LPS-pen) or no (CTR-pen) individual(s) treated with lipopolysaccharide.

Predictor	Performed total social	Received total social	Performed tail- and ear-directed	Received tail- and ear-directed	Performed submissive
Pen level					
Replicate[a]	ns	ns	–	–	*
Day[b]	***	***	*	ns	*
Replicate x Day	***	***	–	–	–
Pen treatment[c]	–	†	–	–	–
Individual level					
Treatment[d]	*	*	ns	†	*
Treatm. sequence[e]	–	–	–	–	*
Treatment x Day	*	–	**	–	

[a]n = 2 [b]BASE, INJ1, INJ1 + 2d, INJ2, INJ2 + 2d [c]n = 3: NO on BASE day, then according to period LPS- pen or CTR-pen [d]NO on BASE day, then according to period LPS or CTR [e]NO on BASE day, then according to period CTR → CTR, CTR → LPS or LPS → CTR (treatment in INJ1 → treatment in INJ2). Symbols indicate significance: *** $p < .001$, ** $p < .01$, * $p < .05$, † $p < .1$, ns: $p > .1$ but included in the model, – not included in the model.

Table 5
Results from linear mixed or Poisson regression analyses of day-time time budgets of pigs (n = 45) in pens with either one (LPS-pen, n = 8) or no (CTR-pen, n = 7) individual(s) treated with lipopolysaccharide. Synchronization of activity is a group-level variable.

Predictor	Activity percentage[f]	Environmental exploration	Synchronization of activity
Pen level			
Replicate[a]	ns	–	ns
Day[b]	***	***	ns
Individual level			
Treatment[d]	–	*	
Treatment sequence[e]	*	–	
Treatment x Day	†	†	
Group level			
Treatment[d]	–	–	*
Treatment sequence[e]	–	–	–

[a]n = 2 [b]BASE, INJ1, INJ1 + 2d, INJ2, INJ2 + 2d [c]n = 3: NO on BASE day, then according to period LPS- pen or CTR-pen [d]NO on BASE day, then according to period LPS or CTR. [e]Order of treatment in INJ1 and INJ2. Symbols indicate significance: *** p < .001, *p < .05, †p < .1, ns: p > .1 but included in the model, – not included in the model. [f]The percentage of scans where the pig is doing something else than lying inactive.

more active both socially (variable performed total social behaviour, p = .004, Fig. 2a) and in environmental exploration (p = .04, Table 6), whereas LPS performed more submissive behaviour (p = .02) and received more attention by others (variable received total social behaviour, p = .02) than CTR.

Pen-level treatment affected only received total social behaviour. The effect was non-significant (p = .06, Table 4). Average predicted values were higher for CTR animals in pens without an LPS-animal than in pens with one throughout the experimental periods, however, 95% CI:s overlapped (data not shown).

3.2. Pen- and individual-level effects of the interaction between day and treatment

Significant interactions between treatment and day were clarified by plotting predicted values and their 95% CI:s (Fig. 2 and 3). The number of performed total social actions (p = .02 for the treatment x day -interaction, Table 4) was lower in LPS as compared to CTR on both injection days (p < .01 and p < .001 for the treatment effect on INJ1 and INJ2, respectively, t-test, Fig. 2a), to return to equal levels on days INJ1 + 2d and INJ2 + 2d (p > .5 on both days, t-test). The number of performed tail- and ear-directed behaviours (p = .007 for treatment x day, Table 4) appeared relatively constant in CTR, whereas LPS-treated pigs showed lower levels on the injection days, followed by higher levels in INJ1 + 2d and INJ2 + 2d (p < .001 for the treatment effect on both days, respectively, t-test, Fig. 2b).

Treatment x day tended to affect the percentage of active scans (p = .099) and the number of scans in environmental exploration (p = .05, Table 5). Predicted values for the latter were numerically smaller in LPS than CTR on injection days (Table 6).

3.3. Effects of treatment sequence

Treatment sequence effects were analysed in order to find possible delayed effects of LPS, which would be evident as differences in behaviour between LPS → CTR and CTR → CTR during the second treatment period. Treatment sequence affected the number of performed submissive actions (p = .04, Table 4) and the percentage of active scans (p = .02, Table 5), however, evidence for a delayed effect of LPS was present only for the latter. LPS → CTR performed more submissive behaviour than CTR → CTR in INJ2 and INJ2 + 2d (p < .001 on both INJ2 and INJ2 + 2, t-test, Fig. 3).

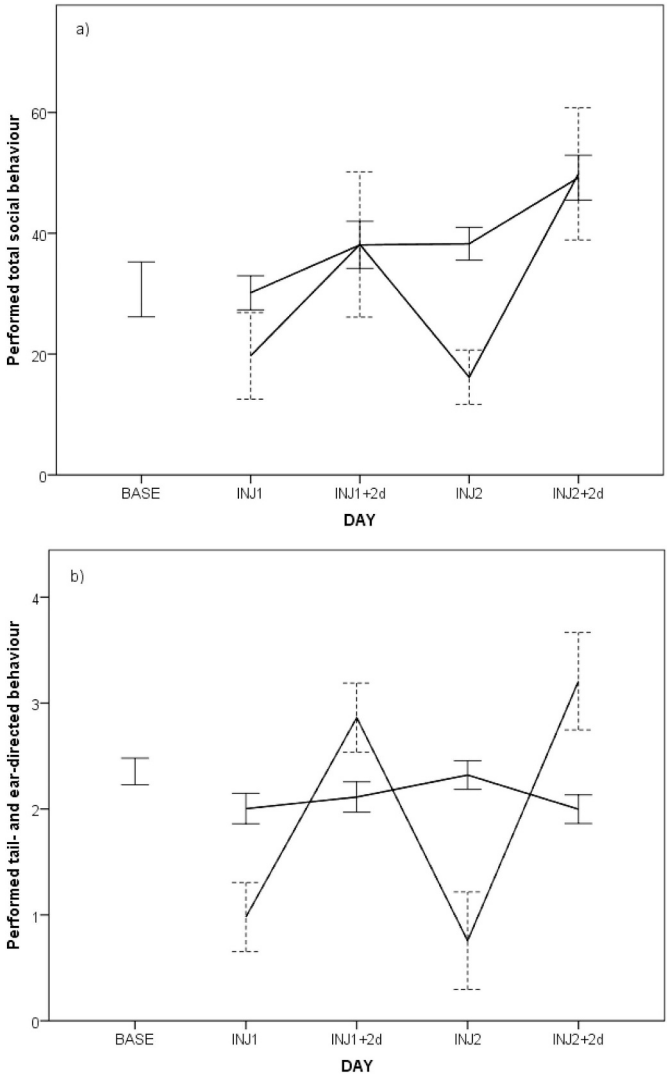

Fig. 2. Average predicted number of event-recorded a) total social behaviour and b) tail- and ear -directed behaviour during 105 min per day in LPS-treated animals (n = 8, dashed error bars) and their controls (n = 37, solid error bars), with all animals pooled on BASE day. INJ1 and INJ2 refer to two injection days, each with a follow-up two days later (INJ1 + 2d and INJ2 + 2d). Error bars represent the 95% confidence interval.

4. Discussion

4.1. General comments

Previous studies suggest statistical associations between poor health and tail biting damage in pigs. This study describes behavioural responses to the LPS-induced acute sickness of one pig in a group, with the aim of identifying possible causal links between sickness and changes in social behaviour that could increase the risk of tail biting. The animals were observed until the second day after the bout of illness, when overt behavioural and physiological symptoms had dissipated, in order to test the hypothesis that an LPS injection influences social behaviour during this day. In a previous experiment, we found changes in noradrenaline levels, a tendency to an increase in right hippocampal serotonin levels and an increase in the levels of one pro-inflammatory cytokine (IFN-γ) in the brain [37] 72 h after LPS injection. The present results do support our hypothesis, and additionally indicate that

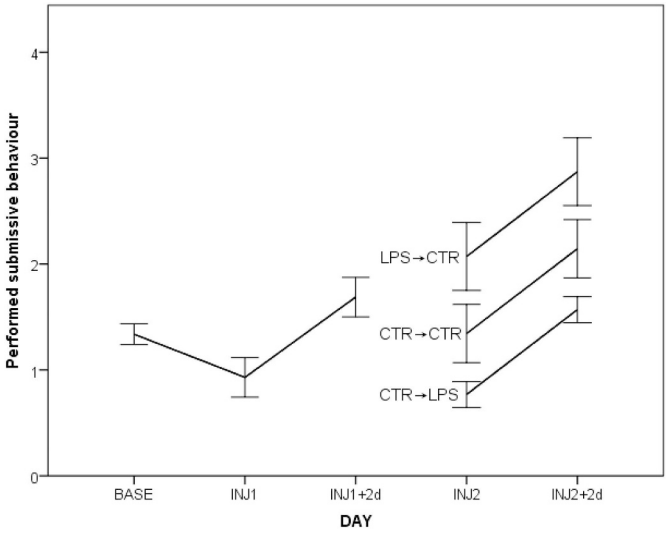

Fig. 3. Average predicted number of event-recorded submissive behaviour during 105 min per day with error bars representing the 95% confidence interval according to treatment sequence, defined as the order of LPS and saline (CTR) treatment in injection days INJ1 and INJ2. Sequences include CTR → CTR (n = 30) CTR → LPS (n = 8) and LPS → CTR (n = 7). INJ1 + 2d and INJ2 + 2d are follow-up days two days after the respective injections. All sequences are pooled in BASE, INJ1 and INJ1 + 2.

Table 6
Predicted values for the number of scans in environmental exploration out of 14 scans per day according to treatment during two injection days INJ1 and INJ2 and their respective follow-up days INJ1 + 2d and INJ2 + 2d.

Day	Treatment	
	CTR[a] n = 37	LPS[b] n = 8
BASE	2.4	2.4
INJ1	1.8	1.0
INJ1 + 2d	2.5	2.8
INJ2	2.8	0.9
INJ2 + 2d	3.2	3.1

[a] Control, saline injection.
[b] lipopolysaccharide injection.

sickness may alter behaviours that increase both the risk of biting and the risk of being tail bitten.

4.2. Effects of LPS on overall activity

Immediate changes in behaviour in response to a low dose of LPS in this study can be summarised as a short-term decrease in activity. This effect was expected based on previous experiments reporting a marked decrease in activity lasting for a few hours following LPS injection [25,37]. It also coincides with the peaks in a number of pro-inflammatory cytokines that are seen following LPS injection [12,32,37,55].

The present experiment failed to reproduce the behavioural synchronization previously seen in healthy pigs in response to LPS-induced sudden passiveness of a penmate [37]. The social environment did, however, differ to some extent between these two studies. The current CTR animals may have been stimulated to remain active as they were allowed to interact with another healthy penmate, in contrast to the experimental set-up in Nordgreen et al. [37] in which pigs were housed in pairs with visual but not tactile contact. Behavioural synchronization was also more unlikely in the present experiment with three (as compared to two) individuals per group when the whole group was required to be in the same category of behaviour for synchronization to be present.

4.3. LPS-effects on social behaviour

LPS had clear effects on tail- and ear-directed behaviours. This kind of activity decreased along with the general increase in social passiveness on the injection day but showed a marked increase two days later, which was not due to increased social activity and suggests that LPS-treated pigs experienced a shift in social motivation after recovery that was not an artefact of changes in activity. Although the physiological background for these late effects can only be hypothesised, it is worthwhile noticing that they are temporally closely associated with differences in noradrenaline concentrations in the hippocampus, hypothalamus and frontal cortex and an asymmetry in serotonin levels in the hippocampus as compared to saline-treated controls 72 h post-LPS injection [37]. Noradrenaline may influence mood and is also important for frontal cortex function with both high and low levels adversely influencing performance in frontal cortex-dependent tests [3–5,20,26,31]. As the frontal cortex is important for selecting context-appropriate behaviours [3], a decrease in cortical function may make it harder for the pigs to interact functionally with their penmates. The asymmetry in serotonin levels may also suggest a possible physiological mechanism behind the motivational shift in LPS injected pigs. Recent experiments have shown changes in the serotonergic system in tail biters and their victims both centrally and peripherally [50]. Biters had increased serotonin turn-over in the frontal cortex, and lower blood serotonin storage than controls whereas victims showed several changes in brain dopamine and serotonin metabolism [52]. The serotonergic system is involved in the regulation of mood, eating behaviour, aggressive behaviour and mental disorders in humans and other species (for a review, see [11]). In pigs, the serotonergic system has also been linked to aggressive behaviour [39,40].

It has been hypothesised that immunostimulation may lead to tail biting behaviour due to a shortage of the serotonin precursor tryptophan which may increase the motivation to explore [54]. The present results do not, however, show increases in environmental exploration, nor in overall social activity in response to LPS. It is, however, possible that the duration of our experiment was too short to detect changes in exploratory motivation.

Another change in the quality of social behaviour in LPS-treated pigs was seen for submissive actions, which were performed more frequently by LPS treated pigs than CTR pigs both on the days of injection and two days later. As these actions by definition required that the animal is subjected to social approach, the effect may be a response to the increase in received social activity that was evident both on the injection days and on the follow-up days. The increase in tail- and ear-directed behaviour in LPS-treated pigs upon recovery may also have been a reaction to the increased social attention by penmates. Although tail- and ear-biting are not considered to have been aggressively motivated or to be immediate responses to social approach, they are thought to be provoked by stress (e. g. [48]), which may very well be experienced in response to increased manipulation by others.

Healthy pigs diverted some of their attention from the other healthy penmate to the individual expressing sickness behaviour. Long-lasting bouts of gentle manipulation of passive animals were frequently observed on the videos, possibly due to inquisitive exploration, which may function to cause a change in the environment rather than being a response to a stimulus. These extensive bouts were not captured in our analyses due to event recording of behaviours. Irrespective of the nature of the relative increase of attention towards LPS-treated pigs, it indicates that signs of sickness failed to provoke avoidance, which is considered normal behaviour in some species [15,46]. The avoidance reaction may in the present animals have been overridden by curiosity, a characteristic thought to be strongly expressed in pigs [56].

Another reason for the increased attention towards the sick penmate may be related to perceived competitive ability. As a sick animal exhibits signs of weakness, others may prefer to compete with it for resources or as an attempt to raise its status in the social hierarchy, as

success is more certain when competing with a sick competitor than when engaging in a contest with a healthy conspecific. This mechanism has been described for male finches [7], and may be a type of fighting caused by uncertainty about relative fighting ability [45].

The increase in interest by CTR pigs towards LPS pigs remained two days after the injections, which is about 40 h after physiological signs of sickness had dissipated. The reason for this is unclear. The change in the quality of social activity by LPS-treated pigs, evident by the increase in tail- and ear-directed behaviours and submissive actions, may have attracted the CTR animals. The order of these behaviours may also have been the opposite with the increased attention (by CTR) provoking a change in social behaviour (in LPS).

This study provided evidence for a delayed effect of LPS in the form of more submissive behaviour in saline-injected pigs with experience of LPS-injection in the previous experimental period compared to pigs without experience of a LPS-injection. Although the number of animals in sequence CTR → LPS was only four, the result is worth looking into in further studies, given that inflammation has been associated with a number of long-term consequences including, for example, chronic hyperexcitability and altered pain perception in humans [29] and impaired spatial memory in mice [51]. Neuroinflammation is considered to be the cause of long-term post-inflammatory mental dysfunction [42].

4.4. Implications for links between sickness and tail biting

The present results suggest two possible links between sickness and tail biting. The most evident pathway is the increase in tail- and ear-directed behaviours in animals after a bout of illness, indicating that recovered animals may have an increased propensity to become tail biters. The other pathway is indirect and comes in the form of increased attention towards a sick animal, which may increase the risk for the sick individual to become a victim of tail biting. Although tail-directed activity remained unchanged after LPS-treatment in the present study, we know that other types of manipulation, e.g. social or inquisitive, may turn into tail biting. This two-stage type of tail biting is considered the most prevalent in a global perspective [48]. If the sick animal attracts aggressive behaviour by healthy conspecifics, sickness may also lead to tail biting of the sudden-forceful type [48].

The present results indicate that the pen-level risk for tail biting may be higher *after* a bout of illness in the group than during the acute stage, as both increased attention from penmates towards LPS treated pigs and increased tail- and ear- directed behaviour from LPS-treated pigs towards penmates were evident two days after LPS-treatment. Previous knowledge about longer-lasting effects of immune stimulation in the form of cognitive and mental dysfunction may provide indirect support to these findings, although most of the literature describes far longer time spans between cause and effect [22,42]. This paper provides preliminary evidence for causal pathways from sickness related behaviour to tail biting in pigs.

Acknowledgements

We thank Anastasija Popova for assisting with the experimental procedures and caretaking of the pigs. This study was part of the ANIHWA ERA-net project FareWellDock, funded by the Norwegian Research Council (NRC project number 236518) and the Finnish Ministry of Agriculture and Forestry (project numbers 1564/311/2013 and 1697/312/2014). Networking activities related to this work were partly funded by COST Action CA15134 - Synergy for preventing damaging behaviour in group housed pigs and chickens (GroupHouseNet), supported by COST (European Cooperation in Science and Technology: www.cost.eu). The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.physbeh.2018.09.018.

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