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The relationship between housing and social rank on cortisol, β-endorphin and dynorphin (1–13) secretion in sows

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Abstract

Endogenous opioids and glucocorticoids may be useful welfare indicators in pigs. Measures of plasma cortisol were carried out in plasma samples collected hourly, from 8:00 to 17:00 h from High Ranking (HR) ($n = 5$) Middle Ranking (MR) ($n = 7$) and Low Ranking (LR) ($n = 4$) group housed sows and from eight Stall Housed Sows (SHS). Cerebrospinal fluid (CSF) was collected after pentobarbital injection ($n = 17$; HR $n = 2$; MR $n = 3$; LR $n = 4$ and SHS $n = 8$) and the levels of β-endorphin and dynorphin monitored. Brains were removed, weighed and frozen. Cortisol, β-endorphin and dynorphin were measured in extracts of frontal cortex. LR sows had significantly lighter brains than HR sows (LR = 126.05 ± 2.18 and HR = 144.86 ± 3.97) and showed no circadian pattern in cortisol secretion. HR and LR sows had lower cortisol levels than MR sows. There was an indication that HR, MR and stall housed sows tended to have lower β-endorphin levels in the CSF than LR sows (0.83 pg/ml ± 0.39; 1.66 pg/ml ± 0.472; 1.07 pg/ml ± 0.267 and 6.81 pg/ml ± 3.27 respectively). Mean dynorphin (1–13) levels in the CSF

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were 12.56 pg/ml (±5.49). Sows kept in stalls had higher levels of dynorphin in the frontal cortex than group housed sows.

Keywords: Opioids; Endorphin; Dynorphin; Cortisol; Housing; Stress; Pig; Brain; Hierarchy; Welfare

1. Introduction

There have been many studies investigating behavioural and physiological responses of farm animals to short term problems during life and a smaller number on the effects of a sub optimal environment (Broom and Johnson, 1993). However, information about the brain mechanisms which were involved when animals cope with long lasting problems is surprisingly sparse. Breeding sows in modern farms are kept either singly housed or in groups for several years. There are indications that the welfare of singly housed sows is poor (Broom et al., 1995). The welfare of some sows kept in large groups with access to a computer operated feeding station may also be poor (Mendl et al., 1992). Behavioural and neurophysiological changes associated with life long experience may provide information about their welfare. Mendl et al. (1992, 1995) showed that group housed pigs built a stable social structure, which was evident in different lactations. Social rank was correlated with measures of productivity and hypothalamic pituitary adrenal axis activity (HPA axis activity). Middle ranking sows, the ones which were not very successful in competitive interactions had higher baseline cortisol levels, a marked suppression after dexamethasone injection and significantly higher cortisol levels after adrenocorticotrophic hormone challenge than High Ranking and Low Ranking sows (Mendl et al., 1992). Studying the same animals up to the 4th parity, Broom et al. (1995) were unable to find differences in cortisol responses between sows housed in groups and sows kept in stalls.

Opioid peptides inhibit the hypothalamic–pituitary–adrenocortical responsiveness in pigs and this inhibition is more evident in animals subjected to chronic stress (Janssens et al., 1995). Abnormal behaviour, such as stereotypies, are common in stall housed sows (Broom et al., 1995) and naloxone which is an opioid antagonist, reduces the performance of stereotypies in pigs (Cronin et al., 1985; Rushen et al., 1990). Additionally, sows which show high levels of stereotypies have a lower density of opioid receptors in the central nervous system (Zanella et al., 1991, 1996; Loyens et al., 1993). Sows kept in stalls have a higher density of μ opioid receptors in their brains (Zanella et al., 1991, 1996) and pigs kept in social isolation showed a decrease in μ opioid binding sites (Zanella et al., 1995). Based on these findings we monitored the relationship between endogenous opioids and cortisol and investigated whether or not some neurophysiological parameters were related to strategies adopted in group housing or were influenced by the sow's confinement in stalls, which limits its behavioural repertoire.

2. Animals, material and methods

2.1. Animals

Twenty-four cross bred, Landrace × Large–White adult sows (Masterbreeders, Tring, UK), kept in the Department of Clinical Veterinary Medicine of the University of

Cambridge, were studied during this experiment. The animals were part of an investigation looking at the welfare of pigs in different housing conditions (Mendl et al., 1992; Broom et al., 1995). Sows were sampled after their 7th or 8th parturition. In order to avoid confounding effects due to hormonal changes related to reproductive cycle, samples were taken 8 to 12 days after estrus detection. During sampling time sows were kept in familiar pens where they had spent at least two weeks during each of the previous reproductive cycles.

2.2. Housing, feeding and management

Sixteen sows were kept in a group of 38 animals with access to a computerized feeding system (crate fabricated by Quality Equipment, Bury St. Edmunds, UK and electronics by Nedap Poiesz, Hengelo, Netherlands) divided into a strawed lying area (11.4 m × 5.5 m) and a dunging area (5.1 m × 5.5 m). Eight sows were kept singly in part slatted stalls with insulated floor (2 m × 0.6 m). Group housed and stall housed sows were fed 2.2 kg of pelleted food (Dalgety, UK) daily (160 g protein, 65 g oil, 60 g fibre, 65 g ash per kg). The feeding cycle for group housed sows started at 15.00 h and the same diet given to the sows housed in stalls was provided to them. Water was available ad libitum through nipple drinkers. Thermostatically controlled fans were used to ventilate the pig buildings and supplementary heating was provided when necessary. In addition to natural light, artificial lighting was on from 6.00 h to 22.00 h. Pens were cleaned daily. Ear tattoo and tags allowed individual identification of the animals. The animals were introduced into the systems when they were nine months old and in their seventh week of pregnancy. The pigs remained in the housing system except during farrowing, lactation, mating and the first two weeks after mating. The animals were looked after by the same stockperson and their management and feeding regime are described in Broom et al. (1995).

2.3. Behavioural observation

Behavioural observations took place during three weeks in the first and fourth parturition. A combination of focal and instantaneous sampling was used (Martin and Bateson, 1986). All agonistic interactions which took place in the group housed animals were recorded. Using the information collected during the behavioural observation the index of success was calculated as follows:

$$\frac{\text{Number of pigs than an individual is able to displace}}{\text{Number of pigs than an individual is able to displace} + \text{number of pigs that are able to displace the individual}} \times 100$$

The index of success in interactions calculated for the first pregnancy correlated well with the data obtained during the fourth pregnancy (Mendl et al., 1995). High rank (HR) sows won more competitive encounters than they lost (index > 50; $n = 5$). The middle rank (MR) sows (index < 50; $n = 7$) fought actively in competition, but were nor very successful, whereas the low rank (LR) sows (index $= 0$; $n = 4$) adopted a different

strategy and were passive avoiders. The behavioural data published by Mendl et al. (1992, 1995) was used in the present experiment.

2.4. Sample collection

Animals were fitted with an indwelling jugular catheter (Zanella and Mendl, 1992). Twenty-four hours after catheter placement blood samples were collected hourly, from 8:00 h to 17:00 h. On the day following plasma sample collection, pentobarbital sodium (24 mg/kg) was injected through the jugular catheter. When deep anesthesia was reached, cerebrospinal fluid (CSF) was collected by lumbar puncture from nine group housed sows (HR $n = 2$; MR $n = 3$; LR $n = 4$) and from eight stall housed sows.

After CSF collection the major vessels of the neck were sectioned leading to exsanguination and death. Brain and pituitary were removed, their weight recorded and they were frozen at $-80°C$, from HR ($n = 5$), MR ($n = 7$), LR ($n = 4$) and eight stall housed sows.

2.5. Physiological measures

For the measurement of opioid peptides, plasma and CSF samples (1000 μl) were thawed and an equal amount of 0.1% trifluroacetic acid (Sigma, St. Louis, MO, USA) was added. The samples were vortexed and centrifuged at 17.000 g for 15 min. C18 Sep columns (Peninsula, Belmont, CA) were activated by two successive passages of 4% acetic acid and methanol. Plasma and CSF samples were passed into the columns. Distilled water (20 ml) was passed into the columns. The peptides were eluted in polypropylene tubes with methanol and dried under vacuum (Savant Vacuum Concentrator, Holbrook, NY, USA). The dried peptides were eluted in 250 μl of assay buffer (Peninsula), and assayed for β-endorphin or dynorphin using radioimmunoassay kits (Peninsula). Plasma cortisol was measured using radio immunoassay (Zanella, 1992). Brain frontal cortex was homogenized with 0.1 N acetic acid (35 mg tissue/ml) and part of the homogenized tissue was mixed with an equal amount 0.1% trifluroacetic acid. β-endorphin and dynorphin (1–13) were measured by radio immunoassay after concentrating the samples by reverse phase chromatography using C_{18} Sep-Pak cartridges (Waters, Milford, MA, USA). Cortisol was extracted from the homogenized tissue using dichloromethane (BDH, Poole, Dorset, UK) (Cham et al., 1980), dried under nitrogen and assayed using radioimmunoassay (Zanella, 1992). The protein concentration in the tissues used for steroid and peptide determination was quantified using a modified Bradford assay (Duhamel et al., 1981). For every physiological measure taken samples were assayed in duplicate.

2.6. Data analysis

After analyzing cortisol and β-endorphin levels in plasma samples taken from 8.00 to 17.00 h the results were presented either as mean AM (8.00 to 12.00) or mean PM (13.00 to 17.00) values. The results reported were obtained using the statistical package

Table 1
Plasma cortisol (ng/ml ± SEM) levels in sows kept in two housing conditions

Housing	Cortisol AM	Cortisol PM
Stalls	44.66 ± 6.24	31.28 ± 4.28
Group housing	45.62 ± 6.06	29.37 ± 3.16

AM values are mean from samples taken hourly from 8.00 h to 12.00 h.
PM values are mean from samples taken hourly from 13.00 h to 17.00 h.

Stat View (Abacus Concepts, Berkeley, CA, USA). *t*-Test was used to compare cortisol and β-endorphin plasma AM and PM values. Comparison was made between the measures taken for sows housed in stalls and sows housed in groups using analysis of variance. The same statistical analysis was used to compare sows with different success in competitive interactions (e.g. High Ranking, Middle Ranking and Low Ranking).

3. Results

3.1. Plasma cortisol

There was a significant difference in cortisol levels from plasma samples taken in the morning and samples taken in the afternoon (df:24; $t = 5.65$, $p = 0.001$) (Table 1). Both, stall housed sows and group housed sows showed higher cortisol levels in samples taken from 8.00 h to 12.00 than in samples taken from 13.00 h to 17.00 h. There was no significant difference between group housed sows and sows kept in stalls. Although most of the sows showed higher cortisol levels in the morning than in the afternoon samples, low ranking group housed sows did not. Low ranking sows had the lowest AM and PM cortisol values but no difference between samples taken in the morning and samples taken in the afternoon (df:3; $t = 0.609$; $t = 0.58$ NS) (Table 2). The highest AM and PM levels were found in the middle ranking sows and the lowest in the low ranking ones (Table 2). The difference between middle ranking animals and low ranking ones reached significance (df: 2; f: 5.091; $p = 0.023$).

Table 2
Plasma cortisol levels (ng/ml ± SEM) in relation to social rank

Social rank	Cortisol AM	Cortisol PM
High rank	45.79 ± 8.29	27.78 ± 2.74
Middle rank	59.72 ± 8.92	36.46 ± 5.24
Low rank	20.74 ± 4.25	18.95 ± 4.85

Data available only for sows housed in groups.
AM values are means from samples taken hourly from 8.00 h to 12.00 h.
PM values are means from samples taken hourly from 13.00 h to 17.00 h.

Table 3
Plasma β-endorphin (pg/ml ± SEM) levels in sows kept in two housing conditions

Housing	β-Endorphin AM	β-Endorphin PM
Stalls	4.65 ± 1.03	7.95 ± 3.35
Group housing	7.72 ± 2.03	8.74 ± 2.22

AM values are mean from samples taken hourly from 8.00 h to 12.00 h.
PM values are mean from samples taken hourly from 13.00 h to 17.00 h.

3.2. Plasma β-endorphin

β-endorphin levels in plasma were not significantly different between sows housed in stalks or kept in group (Table 3). No significant differences were found associated with social rank, but there was a trend in AM and PM samples suggesting that low ranking animals had the highest β-endorphin levels and middle ranking animals the lowest (Table 4).

3.3. Endogenous opioids in the cerebrospinal fluid (CSF)

Given the small number of sows in which successful collection of CSF took place, the only possible comparison was between low ranking sows and sows kept in stalls. Group housed low ranking sows had a significantly higher β-endorphin concentration in the CSF than stall housed sows (Tables 5 and 6). Mean dynorphin (1–13) levels in the CSF were 12.56 pg/ml (±5.49) and there was no significant difference between sows from different social rank and housing system (Tables 5 and 6).

3.4. Endogenous opioids, cortisol concentration and brain weight

Low ranking sows (LR) had significantly lighter brains when compared with high ranking animals (HR) (126.05 ± 2.18 and 144.86 ± 3.97, respectively) (df: 2; f: 5.096; $p = 0.023$). There was no significant difference between mean brain weight taken from animals kept in groups and sows kept in stalls (137.54 ± 3.079 and 136.16 ± 3.36; df: 1; f: 0.77; $p = 0.78$ NS).

Table 4
Plasma β-endorphin levels (pg/ml ± SEM) in relation to social rank

Social rank	β-Endorphin AM	β-Endorphin PM
High rank	7.55 ± 3.80	13.29 ± 6.74
Middle rank	3.87 ± 0.359	5.40 ± 1.55
Low rank	13.65 ± 5.38	10.02 ± 4.35

Data available only for sows housed in groups.
AM values are means from samples taken hourly from 8.00 h to 12.00 h.
PM values are means from samples taken hourly from 13.00 h to 17.00 h.

Table 5
Concentrations of β-endorphin and dynorphin in the cerebrospinal fluid of sows kept in stalls ($n = 8$) and in group ($n = 9$)

Housing	β-Endorphin	Dynorphin
Stalls	1.07 ± 0.26	10.90 ± 1.80
Group housing	3.72 ± 1.60	12.38 ± 1.50

Data are presented in pg/ml ± SEM.

Table 6
Concentration of β-endorphin and dynorphin in the cerebrospinal fluid of high ($n = 2$), middle ($n = 3$) and low ranking ($n = 4$) sows

Social rank	β-Endorphin	Dynorphin
High	0.83 ± 0.39	14.47 ± 2.43
Middle	1.66 ± 0.47	13.26 ± 3.77
Low	6.72 ± 3.14	10.68 ± 1.89

Data are presented in pg/ml ± SEM.

There was no significant difference between sows housed in stalls and sows kept in groups in the cortisol and β-endorphin concentration in the frontal cortex (Table 7). Stall housed sows had significant higher levels of dynorphin (1–13) in the frontal cortex than sows kept in groups (102.43 ± 20.03 and 54.76 ± 7.28) (df: 1; f: 7.57; $p = 0.0116$) (Table 7).

Table 7
Levels of β-endorphin, dynorphin and cortisol in the frontal cortex of stall and group housed sows.

Housing	β-Endorphin	Dynorphin	Cortisol
Stalls	23.29 ± 6.038	102.43 ± 20.03	139.94 ± 27.95
Group	19.41 ± 4.72	54.76 ± 7.28	190.27 ± 64.72

β-Endorphin and dynorphin are presented as pg/mg of tissue and cortisol as ng/mg of tissue.

Table 8
Levels of β-endorphin, dynorphin and cortisol in the frontal cortex of high, middle and low rank group housed sows

Social rank	β-Endorphin	Dynorphin	Cortisol
High rank	26.66 ± 8.26	65.88 ± 15.13	94.65 ± 14.01
Middle rank	9.88 ± 2.56	48.88 ± 13.48	135.24 ± 26.88
Low rank	23.58 ± 12.02	50.68 ± 9.47	351.19 ± 198.15

β-Endorphin and dynorphin are presented as pg/mg (± SEM) of tissue and cortisol as ng/mg (± SEM)of tissue.

There were no significant differences among HR, MR and LR group housed sows and stall housed sows in the levels of β-endorphin, dynorphin and cortisol in the frontal cortex (Table 8).

4. Discussion and conclusions

The measurement of peripheral and central levels of β-endorphin, which is an endogenous μ opioid agonist, dynorphin (1–13), which acts on κ opioid receptors, and cortisol, which is the major glucocorticoid secreted by the adrenal cortex of pigs was made in order to understand their validity as welfare indicators. One criticism of previous opioid system data used to assess animal welfare, is that the work has been carried out either in vitro (Zanella, 1992) or by using an antagonist with marked side effects (Cronin et al., 1985; Janssens et al., 1995). Absolute changes in cortisol levels after dynamic challenge, either suppression with dexamethasone or stimulation with synthetic adrenocorticotrophic hormone, may be of limited use to assess the animal's response to real life events (Mendl et al., 1992; Broom et al., 1995). In addition these data must be complemented by the measurement of cortisol levels during a period where the animal is undisturbed. Schönreiter and Zanella, in prep. found that salivary cortisol levels were increased after social isolation, in pigs but additionally there was a disruption in the circadian pattern when the animals were kept isolated from their penmates. Hence monitoring the circadian pattern by plasma or salivary cortisol seems to be a useful measure. Point samples of plasma would have failed to identify the lack of circadian pattern for cortisol in LR sows. Even dynamic tests such as dexamethasone suppression and ACTH challenge did not find differences between HR and LR group housed sows (Mendl et al., 1992).

In agreement with the results presented by Mendl et al. (1992) low ranking and high ranking sows had lower cortisol levels than middle ranking animals during daytime. The lack of a circadian pattern in plasma cortisol levels found in LR sows may be under hypothalamic control. LR and HR sows responded similarly to dexamethasone suppression and ACTH challenge (Mendl et al., 1992) suggesting normal pituitary and adrenal cortex responsiveness. The findings that middle ranking sows tend to have lower AM and PM peripheral plasma β-endorphin levels than high and low ranking sows and the highest plasma cortisol concentration may be of significance. Opioids may inhibit the HPA-axis responsiveness. Chronically stressed pigs had a greater cortisol response to restraint when they were pre-treated with naloxone, which is a μ opioid antagonist, than control animals (Janssens et al., 1995).

Considering that peripheral β-endorphin is mainly produced by the pituitary (Smyth and Zakarian, 1980) where it is co-released with ACTH (Pickar et al., 1983) it is surprising that no clear circadian pattern was found.

Changes in β-endorphin levels in the cerebrospinal fluid associated with grooming behaviour in monkeys were reported by Keverne et al. (1989). Martensz et al. (1986) demonstrated that subordinate male talapoin monkeys had three times higher intracerebral extracellular levels of β-endorphin than did dominant animals. Our data support Martensz et al. (1986) findings, where sows which had no success in competitive interaction had significantly higher levels of β-endorphin in the cerebrospinal fluid.

κ Opioid agonists are related to aversion and the higher levels in frontal cortex sows kept in stalls may be an indicator of poor welfare. Zanella et al. (1991, 1996) showed that κ opioid receptor density was negatively correlated with stereotypies in sows. The present findings add further evidence to the relevance of κ opioid agonists in the adaptive mechanisms of domestic animals.

The social and physical environment can induce behavioural changes that are correlated with physiological measures. Peripheral and central levels of endogenous opioids and glucocorticoids are useful welfare indicators in pigs.

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