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Behavioural and physiological consequences of acute social defeat in growing gilts: effects of the social environment

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Abstract

Endocrine, behavioural and immunologic processes, together with body growth, were evaluated in gilts that were defeated at 10 weeks of age in resident–intruder tests. Immediately after defeat, gilts were either separated from or reunited with a familiar conspecific (litter-mate; always a barrow). Gilts were assigned to one of four treatments: (a) DI: defeat, followed by isolation (separation from original litter-mate; n=8); (b) I: no defeat, isolation (control group; n=9); (c) DP; defeat, followed by pair-housing (reunion with original litter-mate; n=8); and (d) P: no defeat, pair-housing (control group; n=8). The following general conclusions were derived: (1) social defeat caused pronounced short-term elevations in hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal medullary activities, and of prolactin levels. Moreover, as soon as 1 h after defeat, percentages of blood lymphocytes and neutrophilic granulocytes were, respectively, decreased and increased; (2) social defeat had some long-lasting influence on behaviour and physiology, but isolation predominantly determined responses in the longer term. Defeat, as well as isolation, resulted in increased cardiovascular activities compared to P controls, as observed in a novel object

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test (NOT: +7 days) and an aversion test (AVT: +14 days). Moreover, defeated as well as isolated gilts did not habituate to a repeated novel environment test (NET: −7, +2 and +7 days) in terms of frequencies of vocalising, whereas P controls did. Isolation, through the separation from any other pig, was responsible for the other observed long-term characteristics, which developed progressively. Isolated gilts showed high mobilities and high cortisol responses in the repeated NET (+7 days), not being habituated. This contrasted the reactions of pair-housed gilts, which were much reduced. In addition to their high cardiovascular activities in the NOT and the AVT, isolated gilts also displayed higher heart rates in the repeated NET and during human presence following the NOT, compared to pair-housed gilts. Finally, isolated gilts were more inhibited to approach a novel object (in the NOT) than pair-housed pigs; and (3) stress responses of defeated gilts were modulated by the subsequent social environment. Stimulation of the HPA-axis (plasma- and salivary cortisol) was prolonged in those defeated gilts which were isolated (observed in the first hour). Changes in leucocyte subsets were still observed after 3 days in DI, but were 'normalised' within 1 day in DP gilts. Two days after defeat, habituation to the repeated NET in terms of mobility and salivary cortisol responses occurred in control and DP gilts, but not in DI gilts. We argue that these effects of the social environment shortly after defeat were related to a stress-reducing effect of a stable social relationship, i.e. social support.

Keywords: Pig — social behaviour; Social defeat stress; Habituation; Sensitisation; Social support; Social isolation

1. Introduction

In modern pig husbandry, major welfare and production problems occur when pigs are regrouped and mixed with unfamiliar conspecifics. This practice, common with farmed pigs, leads to much aggression and vigorous fighting between strangers to establish a new social hierarchy of dominant and submissive animals (Meese and Ewbank, 1973). Most aggression occurs during the first few hours after mixing (Meese and Ewbank, 1973; Rushen, 1987; Stookey and Gonyou, 1994; Arey and Franklin, 1995; Olesen et al., 1996; Otten et al., 1997, 1999), with the establishment of a stable dominance hierarchy within 2 days (Meese and Ewbank, 1973; Friend et al., 1983; Olesen et al., 1996). In addition, chronically increased levels of less intense aggression for one (Stookey and Gonyou, 1994) or more weeks (Rushen, 1987; Arey and Jamieson, 1997; Ekkel et al., 1997) are observed.

The negative effects of mixing are often reflected in a reduction in body growth, which is not only seen within the first weeks after mixing (McGlone and Curtis, 1985; Stookey and Gonyou, 1994), but also over an extended period (months) (Ekkel et al., 1995, 1996; Lund et al., 1998). It is argued that a lower performance of a group depends on the inability of some individuals to cope with the new situation. There are indications that within pens of regrouped pigs, those animals showing a depressed growth, are also the ones which exhibit (social) defeat reactions (submissive behaviour) (Albinsson and Andersson, 1980). Instabilities of social relationships may lead to an aversive situation for submissive animals (persisting psychosocial pressure), while for dominants interactions are more predictable and can be controlled (Tuchscherer et al., 1998). On the other hand, acute social defeat may also have a long lasting negative impact on the loser animal, such as growth retardation, as shown in rodents (Miczek et al., 1990; Koolhaas et al., 1997; Ruis et al.,

1999). Defeated rodents display long term behavioural and physiological changes which much resemble symptoms of human psychopathologies, such as depression and anxiety. In pigs, Stookey and Gonyou (1994) demonstrated that a relatively short period of mixing (24 h) may have a negative effect on body weight gain for up to 2 weeks thereafter.

Observations of defeated rodents indicate that the dynamics of stress responses of individual animals may be modulated by the social environment. Long lasting effects of acute social defeat were observed in singly housed rats, being greatly reduced in rats that returned to stable social groups of litter-mates (Ruis et al., 1999). The ability to have stable social relationships with conspecifics may protect individuals against the adverse effects of stress (i.e. social support; Sachser et al., 1998). Studies in humans (Elmore, 1984; Cohen, 1988; Coyne and Downey, 1991; Paykel, 1994; Miller and Surtees, 1995; Biondi and Picardi, 1996) and non-human primates (Boccia et al., 1997) also show that social support permits individuals to cope more easily with stress factors. In pigs, it is not known whether social bonds with familiar pigs may enable animals to overcome social stress more easily, but Arnone and Dantzer (1980) suggest that an established social hierarchy protects pigs against behavioural and physiological consequences of emotional stimulation.

The present experiment reflects a fundamental design to gain more insight into social processes that occur in groups of pigs. Effects of social defeat and the importance of a (stable) social environment were investigated in pairs of pigs, for reasons of standardisation. Endocrine, behavioural and immunologic changes were observed in growing gilts that were defeated in resident–intruder tests. Effects of the social environment following defeats were studied by either separation from (social isolation) or permitting attachment to (pair-housing) an original familiar companion (litter-mate). Control animals were not defeated and either were isolated or remained pair-housed. In the course of 3 weeks, body growth was measured and animals were exposed to four challenge tests classically reported to induce emotional arousal.

2. Materials and methods

All procedures involving animal handling and testing were approved by the Animal Care and Use Committee of the Institute for Animal Science and Health in Lelystad, The Netherlands. Fig. 1 shows the timing of experimental procedures.

2.1. Experimental housing and animals

The experiment was carried out in three successive and identical trials (batches). Two weeks before the expected dates of farrowing, in each trial, three multiparous sows (Great Yorkshire×Dutch Landrace) were transported from the experimental farm 'Bantham' in Maartensdijk to the experimental farm in Lelystad. Both farms are part of the Institute for Animal Science and Health and are located in The Netherlands. The experiment took place in three adjacent experimental rooms (each trial in another room). Piglets (Great Yorkshire×(Great Yorkshire×Dutch Landrace)) were born in farrowing pens (2.35 m×1.70 m) with partly slatted concrete floors. They were weighed and ear tattooed for identification within 1 day after birth. Castration of male piglets, eye-teeth and tail clipping, and iron

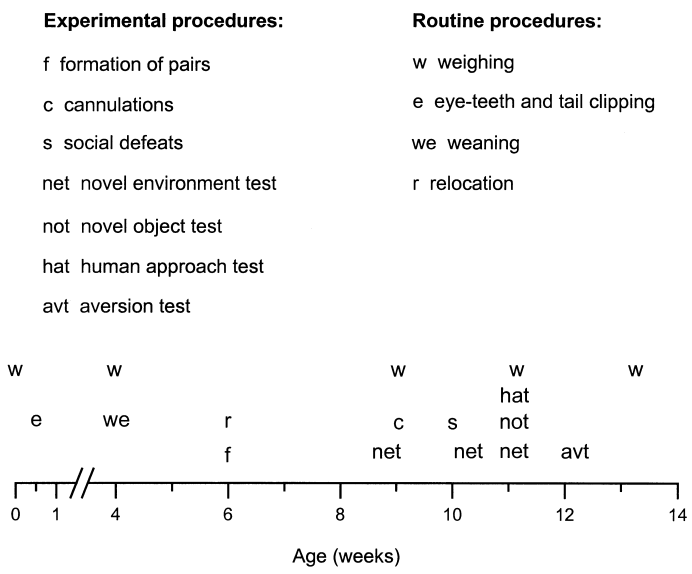

Fig. 1. Timing of experimental and routine procedures.

injection, were carried out between 2 and 4 days of age. Piglets were weaned at 4 weeks of age. Within each trial, 10–12 pairs (litter-mates) of 6-week old animals were composed. Selected pigs were relocated but remained in the same room, while the other animals were removed. Pigs in each pair were weight matched and of both sexes. Whereas the barrow in each pair acted as a companion, the gilt was subjected to experimental treatments and observations, starting at 9 weeks and lasting until 13 weeks of age. Data were obtained from a total of 33 gilts. Pairs were housed in pens which measured 1.80 m×0.85 m, with partly slatted concrete floors and high (0.90 m) and solid wooden partitions between the pens preventing pigs from having visual and physical contact with other pigs. Experimental rooms were ventilated and temperature controlled, with temperatures kept between 22°C (at birth) and 19°C (at 14 weeks of age). Artificial lights were set on a 12 h:12 h light regime with lights on at 06.00 h, and with no daylight visible in the rooms (total lux in the rooms during the light period ranging from 50 to 100). Food (commercial pelleted dry diets) and water (from nipple drinkers) were available ad libitum. From 6 weeks of age onwards, pigs were accustomed to handling by experimenters. To observe body growth during the experimental period, pigs were weighed at 9, 11 and 13 weeks of age.

2.2. *Cannulations for repeated blood sampling*

A total of 1 week before the social defeats, all experimental gilts received an indwelling catheter under complete anaesthesia. Pigs were food deprived for 12 h, sedated with azaperone (2 ml i.m. per pig, Stresnil®, Janssen Pharmaceutica, Tilburg, The Netherlands) and anaesthetised with ketamine-HCL (10 ml i.m. per pig, Nimatek®, AUV, Cuijk, The Netherlands). A catheter was inserted through an ear vein into the jugular vein and the

external part was attached to the ear, according to a technique described by Freriksen et al. (1996). Surgery of one pig took approximately 20 min, and before return to its home pen an additional 1 ml azaperone was injected. An operated pig was separated from the companion through partitions in the home pen for a few hours, until fully recovered. Pigs were treated with antibiotics (2 ml i.m. per pig, Ampicillan® 20%, AUV, Cuijk, The Netherlands) for 4 days to prevent infections. Between blood samplings, catheters were filled with sterile saline with heparin (500 IU/ml).

2.3. Social defeats and social environments

At 10 weeks of age, experimental gilts were removed from their companions and gently transferred to an adjacent testroom (between 8.00 and 12.00 h in the morning). In this testroom, previously selected 2–3 week older gilts were housed individually (already for a period of 2–3 weeks) in pens measuring 2.35 m×1.70 m. Previous selection of these gilts was based on behavioural resistance in a backtest. Only high resisting gilts were selected, thereby increasing the chance of choosing potentially aggressive animals (Ruis et al., 2000). Moreover, we selected only those gilts for the fights which were the winner in all of three encounters with non-experimental pigs. In a resident–intruder paradigm, an experimental gilt (intruder) was individually introduced in the home pen of an aggressive gilt (resident), for a standard period of 15 min (see also De Jong et al., 2000). The start of fighting was subject to variation, but this period was sufficient to evoke vigorous and 'decisive' fighting. The fights involved behaviours such as parallel/inverse parallel pressings, head-to-head-knocks, head-to-body-knocks, head-tilts, bitings and displacements (Jensen, 1982; McGlone, 1985; Rushen and Pajor, 1987). An aggressive gilt was considered to be a winner when its opponent stopped fighting and started with defensive moves. At that time the winner was biting its opponent in the head region, particularly the ears (McGlone, 1985; Rushen and Pajor, 1987). By this submissive behaviour of the defeated pig, further aggression was inhibited. All experimental gilts were defeated in the social encounters. None of the catheters was lost during the social confrontations. Following defeats, experimental gilts were returned to their home pens and either separated from (isolation) or reunited with (pair-housed) the original companions (litter-mates). Separation took place by removal of the companion, shortly before the return of the gilt to its home pen. Control animals, which were not subjected to defeat, remained in their home pens and were either isolated (between 8.00 and 12.00 h in the morning; also by removal of the companion) or stayed together with their familiar conspecifics. Individuals and/or pairs were visually and physically separated. To summarise, the four treatments and numbers of animals assigned to the treatments were as follows: DI: defeat, followed by isolation (n=8); I: no defeat, isolation (n=9); DP: defeat, followed by pair-housing (n=8); P: no defeat, pair-housing (n=8).

2.4. Blood and saliva sampling

Blood samples of approximately 5 ml were withdrawn 5 min prior to and 5, 15, 30, 45 and 60 min after the social defeats. Due to loss of catheters, blood was only obtained from seven, six, seven, and six gilts in the DI, I, DP and P groups, respectively. Samples were

immediately transferred to polypropylene 10 ml centrifuge tubes containing EDTA (Vacuette®, Greiner B.V., The Netherlands) and placed on ice. Subsequently, the blood was centrifuged at 4°C for 10 min at 2000 g. Awaiting measurements of cortisol and catecholamines, half of each supernatant (approximately 1.5 ml) was stored at −20°C. The other half was stored at −80°C, awaiting ACTH and prolactin analyses. Hormone analyses were performed within 2 months after the collection of the plasma samples. Smaller amounts of blood (approximately 2 ml) were sampled at 3 days prior to, and also at 5 and 60 min, and 1 and 3 days after defeats. These samples were transferred to 5 ml evacuated centrifuge tubes containing heparin (Vacuette®, Greiner B.V., The Netherlands) and kept at room temperature. They were used within a few hours for leucocyte counts and differentiation of leucocyte subsets. By Day 3, due to loss of catheters, blood could only be drawn from six, six, five, and five gilts in the DI, I, DP, and P groups, respectively.

Saliva samples were taken simultaneously with blood samples, for determinations of (free) cortisol concentrations. Moreover, samples were taken during the (repeated) novel environment test (NET; see Section 2.5). This was done according to the same procedure as described before by Ruis et al. (1997). Briefly, saliva samples were collected by allowing animals to chew on two cotton buds simultaneously until the buds were thoroughly moistened. The buds were placed in special centrifuge tubes and kept on ice until centrifuged for 5 min at 400 g to remove the saliva. Saliva was then stored at −20°C until analysis.

2.5. Novel environment test (NET)

This test was performed at 7 days before, and 2 and 7 days after the social defeats. The novel environment consisted of an arena (3.8 m×3.0 m), with solid walls (1 m high) and a solid concrete floor (Fig. 2). The arena was connected with a startbox (0.8 m×0.8 m) and both were separated by a sliding door. The arena was situated in a closed room without visible daylight, with total lux of 400 at 0.5 m above the floor. A camera was mounted on

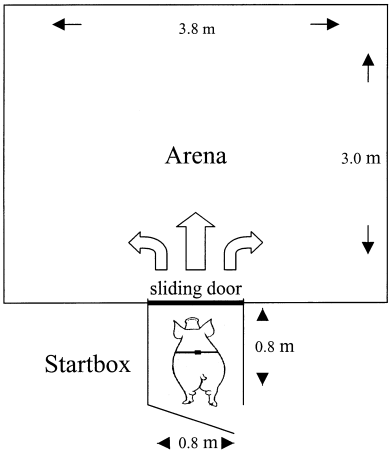

Fig. 2. Lay out of the novel environment test (NET; see Section 2).

the ceiling above the arena and was connected to a video recorder and a monitor in a separate room.

After being removed from their home pens, individual gilts were gently driven into the startbox (through a corridor for 10–20 m). Immediately thereafter, a Polar® Sport Tester (PST; Polar Electro Oy, Kempele, Finland) was attached for recordings of heart rate (HR), according to a procedure described by Geverink et al. (1999). HR was recorded at 5 s intervals in beats per minute (bpm). After 2 min, the door of the startbox was opened and the gilt was allowed to enter the arena. The gilt was then allowed to explore the arena for 10 min. The order of testing was randomised and the test location was cleaned between tests. All tests were carried out between 09.00 and 14.00 h. Behaviour was recorded on videotape and analysed afterwards with the software programme EthoVision® (Noldus Information Technology, Wageningen, The Netherlands). Behavioural parameters that were determined were the latency time to leave the startbox (all four legs outside the box) and the distance travelled in the arena (locomotion). Number of vocalisations in the arena were scored during the test. At the end of testing, the PST was removed and the gilt was gently driven back to the home pen. Time series of HR measurements for each animal were summarised by the following parameters: average HR in the startbox and the arena (time point of) peak HR upon opening of the startbox, HR increase (increase to the peak value), acceleration rate upon opening of the startbox (relative increase to the peak value: regression coefficient (RC)), and deceleration rate (relative decrease to pre-peak levels: RC). The cortisol response to the overall test (in test 3, including the novel object and human approach test: see Section 2.6) was determined by sampling saliva 5 min prior to and, 5 and 15 min after testing. Samples at $t=-5$ min were also used to follow baseline salivary cortisol concentrations.

2.6. Novel object (NOT) and human approach test (HAT)

At the end of the third novel environment test, 7 days after the defeats, a novel object was introduced during 2 min. The novel object consisted of a yellow and a grey bucket (tied together) that was lowered from the ceiling onto the floor of the arena and then lifted to a height of approximately 0.5 m above the floor. After 2 min, one experimenter suddenly entered the arena (sudden human approach) and remained in a stationary posture at one end of the arena for 1 min. The latency time of each pig to initiate contact with the novel object and with the experimenter was determined afterwards from videotape. During both procedures, vocalisations were scored directly. HR in both tests was summarised in similar parameters as for the novel environment test.

2.7. Aversion test (AVT)

With this test, the degree of aversion of gilts was tested to a person wearing white overalls and having a syringe in one hand. Previously, antibiotic treatments were carried out following the cannulations (injections on four successive days). This was done by a person in white overalls. Since in all other situations, experimenters and farm personnel were wearing green overalls, gilts may predict, from the presence of the person in white, that they will be unpleasantly handled (aversion-learning; see Rushen, 1996). Two weeks

after the defeat procedure, a gilt was provided with a PST in its home pen for HR monitoring, done by a person in regular green overalls. Two minutes later, the person in white overalls entered the home pen, and remained in a stationary posture during 2 min. The syringe used for the antibiotic treatments was well visible, but was not used. Besides HR patterns (summarised in similar parameters as for the novel environment test), avoidance behaviour was directly recorded by scoring the latency time of the gilt to initiate contact with the handler, and total time contacting the latter.

2.8. Hormone and immunological determinations

2.8.1. Cortisol

Plasma cortisol was measured using a time-resolved fluoroimmunoassay (TR-FIA). The assay was performed as described for bovine plasma by Erkens et al. (1998), with the following modifications. Samples and steroid free plasma for the preparation of standards were diluted 1:10. Cortisol binding plasma proteins were inactivated at a temperature of 95°C. A low and a high control sample and a 50/50% mixture were analysed in all assays (n=40) to check for linearity and reproducibility. Mean concentrations and inter-assay coefficients of variation (CVs) were 19.7, 62.8 and 43.9 ng/ml and 11.3, 6.2 and 6.9%, for low, high and 50/50% control samples, respectively. Intra-assay CVs (n=14) of 4 samples with cortisol concentrations ranging between 20 and 70 ng/ml were 6.5±2.9% (mean±S.D.). Recovery of 20, 40, 80 and 160 ng of standard cortisol added per ml of three samples was 88±17, 96±12, 95±5 and 93±10%, for low, high and 50/50% control samples, respectively. The detection limit was 1.6 ng/ml. Salivary cortisol was measured in one assay on 1 day, by using a solid-phase radioimmunoassay (RIA) kit (Coat-A-Count Cortisol® TKCO, Diagnostic Products Corporation, Apeldoorn, The Netherlands), modified for pig salivary cortisol (Ruis et al., 1997). The detection limit of the assay was 0.13 ng/ml, and the intra-assay CV was 6.6%.

2.8.2. Plasma ACTH

A commercial ACTH assay for human plasma (Nichols Institute Diagnostics, San Juan Capistrano, USA) was used according to the instructions of the manufacturer and was validated for porcine plasma. To avoid a decline of the samples ACTH concentration that was observed for extended time intervals between thawing of the samples and start of the assay (but not for extended assay incubation), samples were thawed in series of 30, placed on ice and pipetted within 45 min, followed by the immediate start of the assay. Generally, assay incubation time at room temperature varied between 20 and 24 h; for series within one assay the maximal difference in incubation time was 1 h. Standards and samples (200 μl) were analysed in duplicate. Radioactivity of the tubes was measured for 1 min in a model 1470 Wizard® gamma counter and data were evaluated using Multicalc® software (Wallac Oy, Turku, Finland). A low and a high control sample and a 50/50% mixture were analysed in all assays (n=9). Mean concentrations and inter-assay CVs were 12.7, 152 and 78.2 pg/ml and 6.5, 3.2 and 2.9%, for low, high and 50/50% control samples, respectively. Intra-assay CV (n=14) of the mixed control sample was 3.9%. Recovery of 2.6, 7.75, 26.25 and 77.5 pg of ACTH (50 μl of kit standards E to H) added to 150 μl of the low control plasma was 114, 98, 105 and 96%, respectively. The detection limit was 1.0 pg/ml.

2.8.3. Plasma prolactin

For measurements of prolactin, a RIA was performed as reported by Van Landeghem and van de Wiel (1978), with minor modifications, using an automated procedure as described by Erkens et al. (1992). Porcine prolactin (code PRL-IVO-19-1-79), with a potency of 1.07 towards AEW-SP-162-C, was used as standard and for iodination. Labelling buffer was 0.5 M sodium phosphate, pH 7.4. Column elution and assay buffer was 0.05M sodium phosphate, 0.15 M sodium chloride, 0.1% (w/v) sodium azide, pH 7.2, containing 1% (w/v) bovine serum albumin (BSA, fraction V; Fluka AG, Buchs, Switzerland). One millilitre of a 1:8 dilution of donkey anti-rabbit solid phase (IDS, Boldon, England) with assay buffer, containing 0.1% (w/v) BSA, was used. Detection limit was 0.4 ng/ml. Intra-and interassay coefficients of variation were 10.7 (n=12) and 9.0% (n=5).

2.8.4. Plasma catecholamines

Plasma samples were analysed for adrenaline and noradrenaline by high pressure liquid chromatography (HPLC) with electrochemical detection following a liquid extraction (twice) according to the procedure described by Smedes et al. (1982). From each sample, a volume of 100 μl was used. DHBA (3,4-dihydroxybenzylamine) was used as an internal standard. The HPLC system consisted of an autosampler (Perkin-Elmer ISS-101®, Perkin-Elmer, Norwalk, CT, USA), a Perkin-Elmer 410® HPLC pump (Perkin-Elmer, Norwalk, CT, USA), a vacuum degasser (X-Act®, Jour Research, Onsala, Sweden), an EC-controller (INTRO®, Antec Leyden, Leiden, The Netherlands) with inbuilt columnoven, pulsdamper (SSI®, Antec Leyden, Leiden, The Netherlands) and detector cell (VT-03®, Antec Leyden, Leiden, The Netherlands). The potential was set at 0.611 V (versus Ag/AgCl in 2 mM [Cl^-]) using the in situ Ag/AgCl reference electrode (ISAAC®, Antec Leyden, Leiden, The Netherlands). The catecholamines were separated using a Nucleosil® C18 column (length 25 cm, 4 mm i.d., particle size 5 μm; Macherey Nagel, Düren, Germany). The mobile phase consisted of 72 mM citric acid monohydrate, 42 mM $Na_2HPO_4.2H_2O$, 1.1 mM 1-octanesulfonic acid (sodium salt), 0.4 mM EDTA, 2.0 mM NaCl, 3% acetonitryl. The pump was operated at a rate of 1 ml/min. The column, pulsdampener and detectorcell were maintained at a constant temperature of 30°C. The detector signal was recorded by a chartrecorder (BD112®, Kipp & Zn, Delft, The Netherlands). The detection limit (defined as the amount of compound producing a peak twice the basal noise) was typically 0.6 pg for noradrenaline and 1.0 pg for adrenaline.

2.8.5. White blood cell counts and differentiation

Total leucocyte counts were determined by means of an automatic cell counter (Sysmex®, F-800, TOA Medical Electronics, Kobe, Japan). White blood cells were differentiated in lymphocytes and monocytes/granulocytes by adding quicklyser II® (TOA Medical Electronics, Kobe, Japan). To control for accuracy of this method, blood smears were stained with a Hema-Tek slide-stainer (modified Giemsa). A total of 100 cells was counted microscopically, in which the lymphocytes and monocytes, and neutrophilic, eosinophilic and basophilic granulocytes were differentiated. Only 1–5% of leucocytes were eosinophilic and basophilic granulocytes, and monocytes. Therefore, the percent lymphocytes and monocytes/granulocytes from the cell counter quite accurately represent

the percent lymphocytes and neutrophilic granulocytes, and these were used for statistical analysis.

2.9. Statistical analysis

A mixed analysis of variance model was used to analyse data with respect to variables in the NOT, HAT, and AVT, and the development of weight. Effects for litters and animals within litters were entered as random effects in the model. These random effects account for possible correlation between litter-mates and correlation between observations on the same animal within a litter. Effects for social defeat (yes or no), housing (isolation or pairs), and trial (1–3) were entered as fixed effects in the model. The model included main effects and the interaction between social defeat and housing. In addition, for variables in the repeated NET, time relative to the (start of) treatments (−7, 2 and 7 days) was included as a fixed effect in the model. When no significant interaction was found, the model was reduced to main effects only. Data on latency times in the NET showed heterogeneity and were, therefore, logarithmically transformed prior to analysis. For this parameter, treatment effects were expressed by multiplicative factors between treatment means. Components of variance were estimated by restricted maximum likelihood (REML; Engel, 1990). Treatment effects (main effects and interactions) were tested with the Wald test (Buist and Engel, 1994). Acute hormone responses and changes in leucocyte subsets were analysed by summary statistics to circumvent the complicated dependence structures of closely spaced timepoints. For these variables, a paired t-test was used to test changes from baseline values. Moreover, analysis of variance was performed for treatment effects within separate timepoints. In case of significant results, comparisons between groups were made using t-tests with a pooled variance estimator. All calculations were performed with the statistical programming package Genstat 5 (1993)®. Effects were considered significant if $p<0.05$. Unless stated otherwise, data are presented as mean (±S.E.M.).

3. Results

3.1. HPA-axis activity

Compared to levels prior to stress procedures, social defeat caused significant increases ($p<0.01$) in ACTH and (plasma and salivary) cortisol concentrations, with peak values generally at $t=5$ min (Fig. 3). Undefeated controls either showed no (P gilts) or moderate (I gilts; elevated salivary cortisol concentration at $t=5$ min: $p<0.05$) hormone responses. Although not found for ACTH levels, the decline of cortisol concentrations to baseline values (recovery) following social defeat differed according to the way of housing. While (plasma and salivary) cortisol concentrations were recovered after $t=30$ min in DP gilts, cortisol concentrations remained significantly ($p<0.05$) elevated during the 60 min sampling period in DI animals. This effect of housing conditions following defeat was substantiated by comparisons between groups. At the end of the 60 min sampling period there was a significant interaction between the factors housing and defeat for plasma- ($p<0.05$) and salivary cortisol ($p<0.05$) concentrations. At this time, only those gilts which

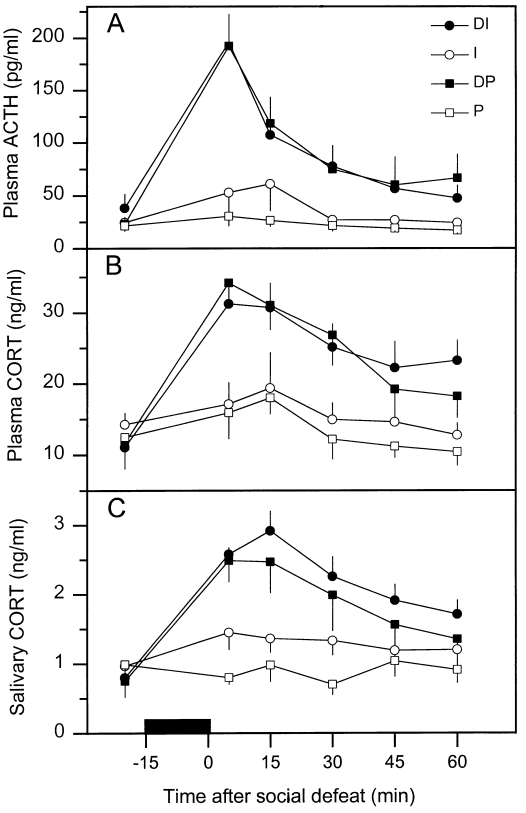

Fig. 3. Mean (±S.E.M.) concentrations of plasma ACTH (A) and cortisol (CORT) in plasma (B) and saliva (C) in the course of 1 h following the (start of) treatments. DI: defeat, isolation (sample size: n=7); I: isolation (controls; sample size: n=6); DP: defeat, pair-housing (sample size: n=7); P: pair-housing (controls; sample size: n=6). Horizontal black bar indicates the confrontation period. For significant changes within and differences between treatments, see Section 3.

were isolated following defeat showed higher plasma- and salivary cortisol concentrations, compared to both control groups (p<0.05) and P controls (p<0.05), respectively.

Baseline salivary cortisol concentrations, obtained from t=−5 min samplings of saliva in the repeated novel environment test, did not change in the course of 1 week following the (start of) treatments. Concentrations were 0.96 (±0.12), 0.81 (±0.08), 0.87 (±0.09) and 0.89 (±0.10) ng/ml at t=−7 days, 0.84 (±0.11), 0.89 (±0.13), 0.79 (±0.14) and 0.76 (±0.09) ng/ml at t=+2 days, and 0.90 (±0.10), 0.82 (±0.13), 0.74 (±0.14) and 0.71 (±0.13) ng/ml at t=+7 days, respectively, for DI, I, DP and P gilts.

3.2. Plasma catecholamine concentrations

Fig. 4 shows that social defeat significantly increased plasma catecholamine concentrations, both compared to baseline values (p<0.05 at least), and to control treatments (main

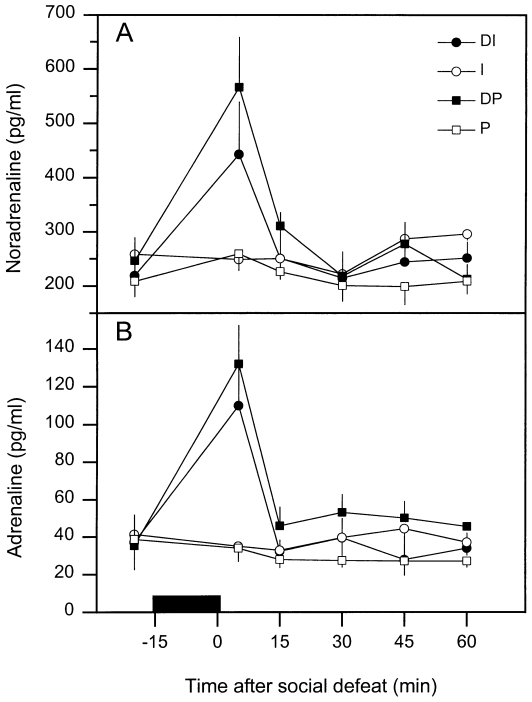

Fig. 4. Mean (±S.E.M.) plasma noradrenaline (A) and adrenaline (B) concentrations in the course of 1 h following the (start of) treatments. DI: defeat, isolation (sample size: n=7); I: isolation (controls; sample size: n=6); DP: defeat, pair-housing (sample size: n=7); P: pair-housing (controls; sample size: n=6). Horizontal black bar indicates the confrontation period. For significant changes within and differences between treatments, see Section 3.

effect for defeat: $p<0.05$). Peak values were observed at 5 min following the end of the fights, and concentrations were recovered thereafter. In undefeated controls, no significant increases in catecholamine concentrations were observed. Interestingly, at t=60 min, noradrenaline levels in isolated gilts were higher than in pair-housed animals (significant main effect for housing: $p<0.05$).

3.3. Plasma prolactin concentrations

Following social defeat, prolactin concentrations were significantly elevated at t=5 min, but not thereafter, both compared to baseline values ($p<0.05$) and to control treatments (main effect for defeat: $p<0.05$) (Fig. 5).

3.4. Changes in peripheral blood leucocytes

Fig. 6 shows the changes in percentages of leucocyte subsets in the four treatment groups. The changes are presented relative to baseline values (presented as the 0-line in the

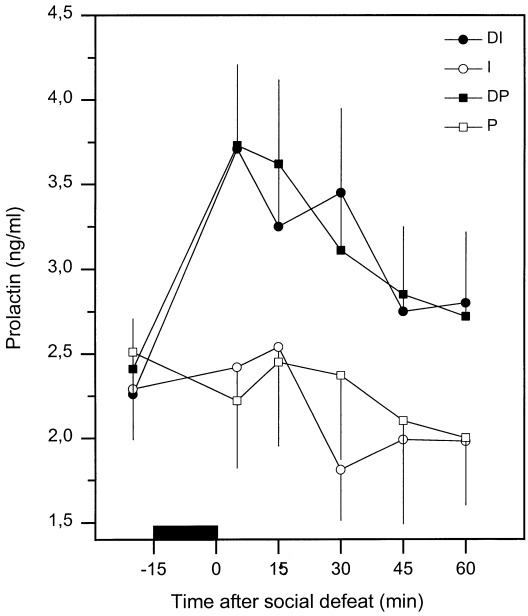

Fig. 5. Mean (±S.E.M.) plasma prolactin concentrations in the course of 1 h following the (start of) treatments. DI: defeat, isolation (sample size: n=7); I: isolation (controls; sample size: n=6); DP: defeat, pair-housing (sample size: n=7); P: pair-housing (controls; sample size: n=6). Horizontal black bar indicates the confrontation period. For significant changes within and differences between treatments, see Section 3.

figure). In both control groups, no significant changes from baseline values in percentages of leucocyte subsets were observed. However, as soon as 1 h after defeat, defeat stress resulted in significant lowerance in percentage of lymphocytes, compared to baseline values (p<0.05) and to those of control groups (main effect for defeat: p<0.05). Simultaneously, percentages of neutrophilic granulocytes were significantly enhanced, compared to baseline (p<0.05) and control (main effect for defeat: p<0.05) values. On Days 1 and 3, values were returned to baseline and to those of controls in DP, but not in DI gilts. On Days 1 and 3, in DI gilts there were still tendencies for a lowered lymphocyte percentage (p=0.06 and 0.08, respectively) and for an enhanced neutrophilic granulocyte percentage (both p=0.07). Moreover, at these timepoints, between-group comparisons showed significant (p<0.05 at least) interaction effects between the factors defeat and housing. In DI gilts, lymphocyte percentages were significantly decreased (p<0.05 at least) compared to those of both control groups. The increase in granulocyte percentages in DI gilts was only significant (p<0.05 at least) compared to those of P controls.

3.5. Responses to the repeated novel environment test (NET)

Table 1 shows the behavioural characteristics and cortisol responses of gilts to the repeated NET. HR patterns during the tests are shown in Fig. 7.

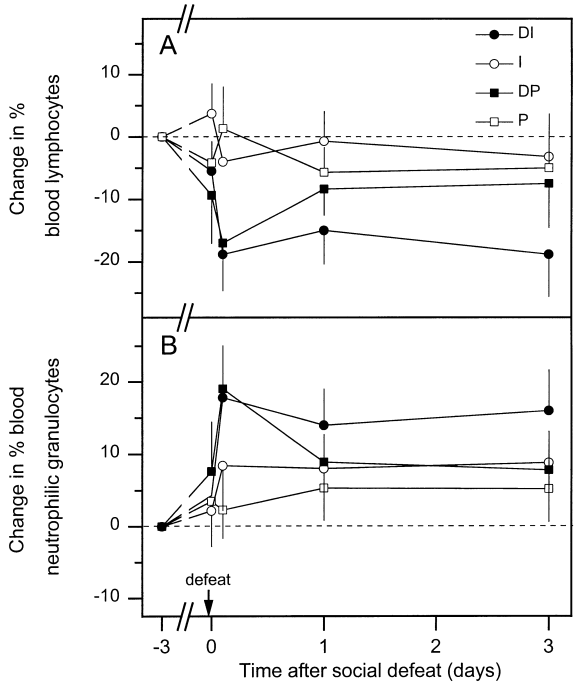

Fig. 6. Mean (±S.E.M.) changes in percentages of blood lymphocytes (A) and blood neutrophilic granulocytes (B) in the course of 3 days following the (start of) treatments. The changes are presented relative to baseline values (0-line). DI: defeat, isolation (sample size: n=6); I: isolation (controls; sample size: n=6); DP: defeat, pair-housing (sample size: n=6); P: pair-housing (controls; sample size: n=5). For significant changes within and differences between treatments, see Section 3.

Table 1
Behavioural and cortisol responses (mean±S.E.M.) to the repeated novel environment test (NET)

	Test at (days)	DI	I	DP	P
Latency time (s)	−7	31.6 (18.3)	31.3 (11.7)	18.5 (11.5)	24.5 (13.8)
	2	5.9 (1.7)**	7.1 (1.5)***	5.0 (1.3)**	5.6 (0.8)**
	7	5.0 (1.3)**	4.8 (1.1)***	4.8 (0.6)**	4.9 (0.8)**
Locomotion (m)	−7	100 (7)	102 (12)	109 (6)	107 (3)
	2	102 (6)^{a}	88 (11) ^{a,b}	84 (9)^{**,b}	96 (8)^{a,b}
	7	100 (11)	98 (6)	90 (6)**	80 (13)**
Vocalisations (number)	−7	109 (20)	96 (20)	106 (15)	114 (18)
	2	141 (13)	133 (21)	146 (12)**	122 (20)
	7	121 (18)^{a,b}	123 (20)^{a,b}	141 (25)^{a}	83 (14)^{**,b}
CORT response (ng/ml)	−7	1.83 (0.33)	2.03 (0.26)	2.13 (0.37)	1.87 (0.25)
	2	1.63 (0.13)	1.58 (0.25)**	1.49 (0.24)**	1.30 (0.17)**
	7	1.83 (0.18)^{a}	1.77 (0.21)^{a}	1.24 (0.16)^{***,b}	1.42 (0.11)^{**,a}

^{a,b} Means with different superscripts within the same row differ (p<0.05). Asterisks indicate changes within groups (vertically), compared to values in the first test (at −7 days): $^{**}p$<0.05, $^{***}p$<0.01. Interaction and main effects are mentioned in Section 3.

DI: defeat, isolation (n=8); I: isolation (controls; n=9); DP: defeat, pair-housing (n=8); P: pair-housing (controls; n=8).

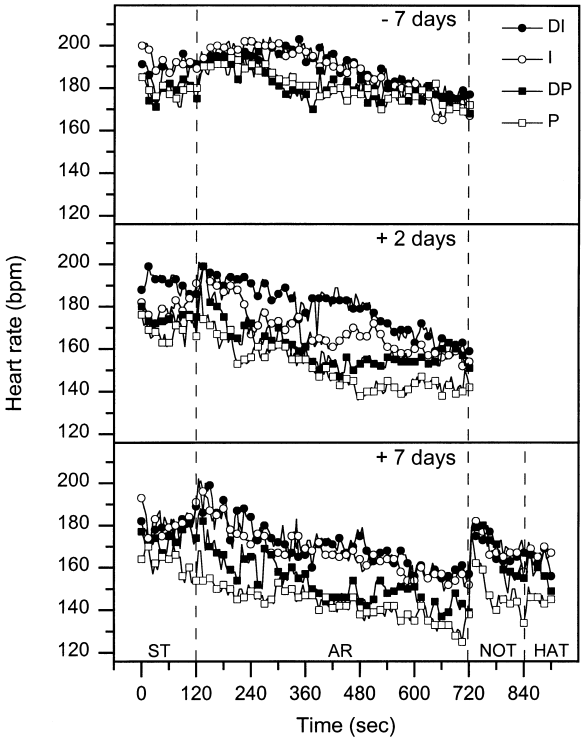

Fig. 7. Mean heart rates (HRs) during the repeated novel environment test (NET) at 7 days before (upper panel), 2 days after (middle panel) and 7 days after the (start of) treatments (lower panel). Every third data point is shown. The latter test was inclusive the novel object test (NOT) and human approach test (HAT). For a detailed description, see Section 2. ST: startbox; AR: arena; DI: defeat, isolation (n=8); I: isolation (controls; n=9); DP: defeat, pair-housing (n=8); P: pair-housing (controls; n=8). For significant changes within and differences between treatments, see Section 3.

3.5.1. Latency times to enter the arena

No differences were observed between test groups, but following the first exposure to the NET, latency times to leave the startbox were much reduced (about a factor 3.4 ($\pm$0.5)) in the subsequent tests (significant main effect for the factor time: p<0.01).

3.5.2. Locomotions

Changes in locomotions depended on defeat and housing procedures, and were characteristic for each test (three-factor interaction: p<0.05). While at 2 days after the (start of) treatments mobility was reduced in most animals when compared to the first test (being significant in DP gilts; p<0.05), DI gilts were still highly mobile. A comparison of groups showed a significantly (p<0.05) higher mobility for DI gilts than for DP animals. At 1 week after the (start of) treatments, both DI and I gilts showed high mobilities, whereas locomotion had declined significantly (p<0.05) in both groups of pair-housed gilts when compared to the first test. Within this timepoint, no significant differences were observed between the treatment groups.

3.5.3. Vocalisations

There was a tendency for an interaction ($p=0.06$) between the factors defeat and housing for the change in numbers of vocalisations. Two days after the (start of) treatments, most animals except P controls vocalised more often than they did in the first test, being significant ($p<0.05$) for DP gilts. One week after the (start of) treatments, P controls vocalised significantly ($p<0.05$) less, whereas in the other groups numbers of vocalisations did not differ from those in the first test. Within this timepoint, DP gilts vocalised significantly ($p<0.05$) more than P controls.

3.5.4. Cortisol responses

For this parameter, the three-factor interaction was significant ($p<0.05$) and patterns of changes followed those for locomotions. DI gilts at 2 days, and DI and I gilts at 7 days after the (start of) treatments, showed cortisol responses as high as in the first test. Responses in the other groups, however, were significantly ($p<0.05$) reduced at these timepoints. One week after the (start of) treatments, cortisol responses of isolated (DI and I) gilts were significantly ($p<0.05$) higher than those of pair-housed (DP and P) animals.

3.5.5. Heart rate (HR)

HR decreased in all animals with repeated exposure to the test (Fig. 7). This was shown by significant ($p<0.01$ at least) main effects for the factor time (relative to the (start of) treatments) for average HR in the startbox (185.0 ($\pm$3.7), 179.4 ($\pm$3.9) and 175.3 ($\pm$3.6)) and in the arena (185.7 ($\pm$3.2), 164.6 ($\pm$3.4), 158.3 ($\pm$3.2), at -7, 2 and 7 days, respectively). However, HR patterns diverged according to experimental procedures, with housing conditions as the predominant factor. Overall, the average HR in the startbox tended ($p=0.09$) to be higher in isolated than in pair-housed gilts: 182.0 ($\pm$4.2) and 176.7 ($\pm$3.2), respectively. Isolated animals also displayed higher HR in the arena. Here, a significant ($p<0.001$) interaction between the factors housing and time was observed. Whereas the average HR was 185.7 ($\pm$3.2) bpm before the (start of) treatments, average HR for isolated and pair-housed gilts were, respectively, 172.3 ($\pm$4.4) and 157.0 ($\pm$4.2) bpm at 2 days (within-test comparison: significant difference, $p<0.05$), and 167.8 ($\pm$4.0) and 148.8 ($\pm$4.1) at 7 days after the (start of) treatments (within-test comparison: significant difference, $p<0.001$). For responses to the opening of the startbox, there was a significant ($p<0.01$) interaction between the factors time and housing for peak HR. By Day 7, the difference between isolated and pair-housed gilts was significant ($p<0.01$): 208.0 ($\pm$5.6) and 186.6 ($\pm$5.8) bpm, respectively. No significant effects were observed for timepoints of peak HR, HR increases, and HR acceleration and deceleration rates.

3.6. Responses to the novel object (NOT) and human approach test (HAT)

3.6.1. Latencies to initiate contact

At 7 days after the (start of) treatments, isolated gilts, irrespective of previous social defeat, took significantly longer to contact the novel object than those that were pair-housed (significant main effect for the factor housing, $p<0.01$). Latency times were 39.6 ($\pm$8.6) and 19.7 ($\pm$3.2) seconds for individually and pair-housed gilts, respectively. Upon

sudden entrance of an experimenter in the arena, latency times to contact the latter did not differ between the experimental groups.

3.6.2. Vocalisations

There were no differences in numbers of vocalisations between the experimental groups, neither in response to the novel object, nor in response to the sudden approach of an experimenter. Frequencies of vocalising were 27 (±11), 32 (±14), 35 (±12) and 23 (±7) in the NOT, and 20 (±6), 25 (±10), 27 (±9) and 16 (±7) in the HAT, respectively, for DI, I, DP and P gilts.

3.6.3. Heart rate (HR)

For responses to the novel object, HR acceleration rates and peak HR did not differ between groups, but increases in HR were significantly (main effect for housing: $p<0.05$) higher in pair-housed (48.2 (±5.3) bpm) than in isolated animals (35.6 (±5.2) bpm) (see the lower panel of Fig. 7). Animals being defeated showed significantly (main effect for the factor defeat: $p<0.01$) lowered HR deceleration rates (but their acceleration rates did not differ). RCs of HR deceleration rates for defeated and non-defeated gilts were, respectively, −0.12 (±0.1) and −0.50 (±0.1). However, during the overall test HR was significantly ($p<0.05$ at least) lower in the P gilts than in gilts of the other groups; average HRs were 168.8 (±7.4), 169.9 (±6.8), 164.9 (±5.5) and 148.5 (±5.5) bpm, in DI, I and DP and P gilts, respectively (interaction not significant ($p=0.10$)).

Responses to the human approach were characterised by higher peak HR in isolated animals (178.5 (±4.9) bpm) compared to that in pair-housed ones (164.8 (±5.0) bpm) (significant main effect for housing: $p<0.05$) (see the lower panel of Fig. 7). HR increases, and HR acceleration and deceleration rates, did not differ between groups. Average HR during the presence of the experimenter was higher in isolated (165.2 (±4.5) bpm) than in pair-housed animals (151.7 (±4.6) bpm) (significant main effect for housing: $p<0.05$)).

3.7. Responses to the aversion test (AVT)

3.7.1. Latencies to contact experimenter

Latency times to contact an experimenter dressed in white overalls and total time contacting the latter did not differ between treatments. Latency times were 12.5 (±5.1), 16.0 (±8.2), 9.9 (±6.7) and 7.3 (±4.9) s, and total times of contact were 83.5 (±18.3), 92.2 (±12.4), 98.8 (±15.6) and 102.4 (±15.0) s, for DI, I, DP and P gilts, respectively.

3.7.2. Heart rate (HR)

For average HR during the 2 min prior to the entrance of the person in white overalls, a tendency ($p=0.07$) for an interaction effect was observed between the factors housing and defeat (Fig. 8). Compared to other treatment groups, average HR in P controls was lower, being significantly ($p<0.05$) lower than in I controls. The interaction was significant ($p<0.05$) for the average HR during the 2 min exposure to the experimenter in white overalls. Again, P controls showed lower HR values, being significantly ($p<0.05$ at least) lower compared to all other groups. Peak HRs were affected by housing conditions

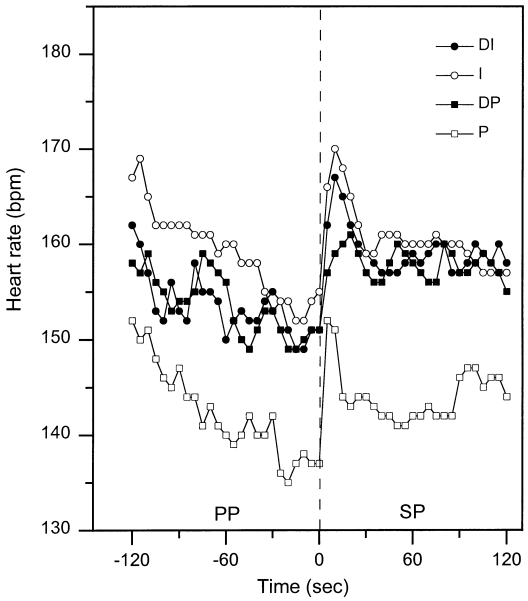

Fig. 8. Mean heart rates (HRs) in the aversion test (AVT) at 2 weeks after the (start of) treatments. At t=0 a person in white overalls entered the home pen. PP: pre-stimulation period; SP: stimulation period; DI: defeat, isolation (n=8); I: isolation (controls; n=9); DP: defeat, pair-housing (n=8); P: pair-housing (controls; n=8). For significant changes within and differences between treatments, see Section 3.

(significant main effect: $p<0.05$), and were 171.5 (±2.9) and 161.2 (±3.2) bpm, in isolated and pair-housed gilts, respectively.

3.8. Body growth

Body weights did not differ between treatment groups at 1 week before the (start of) treatments (21.7 (±1.7), 22.9 (±2.1), 21.3 (±1.8) and 23.2 (±2.2) kg), 1 week thereafter (36.0 (±2.0), 35.9 (±2.2), 34.9 (±2.1) and 37.3 (±2.8) kg), and 3 weeks thereafter (47.0 (±2.8), 46.6 (±2.8), 45.4 (±2.5) and 47.8 (±3.5) kg), respectively, for DI, I, DP, and P gilts.

4. Discussion

We will discuss our findings by raising three issues. Firstly, we describe the severe acute consequences of social defeat, causing strong endocrine and immunological changes in the loser animal. Secondly, we discuss the behavioural and physiological effects of acute social defeat, and of social isolation, in the longer term. Finally, some indications are given for the occurrence of social support following social defeat.

1. Socially defeated gilts show strong acute endocrine changes, as shown by elevations in hypothalamic-pituitary-adrenal (HPA) activity, sympathetic-adrenal medullary

activity, and prolactin levels. Elevations in HPA- and sympathetic-adrenal medullary activities during agonistic interactions have been reported before in the pig (Otten et al., 1997, 1999), and in other species (Von Holst, 1985; Sachser et al., 1998). Most pronounced endocrine changes have been found to occur in those animals being defeated (Sachser, 1987; Marchant et al., 1995). The hormonal changes measured in this study were generally short-lived following termination of the acute social stress, which was most pronounced for plasma catecholamine levels. In fact, catecholamine levels might already be much decreased at the first sampling point after the fighting period. This may also explain the relatively low peak levels, compared to, for instance, those reported by Otten et al. (1997, 1999). Little is known about the effects of social stress on plasma prolactin levels in pigs, but in rats it was shown that prolactin release is augmented under conditions of fear (Zou et al., 1998; Meerlo et al., 1999). When extrapolated to pigs, this may substantiate that the loser pigs are highly fearful during encounters. This fearfulness may be related to the unpredictability and uncontrollability of the situation, which are factors held responsible for increments in prolactin (Muir and Pfister, 1987). Consequently, measurements of prolactin may provide a valuable tool for stress assessments in pigs. Our data also demonstrate defeat-induced changes in blood cellular immunological characteristics, by shifts in percentages of leucocyte subsets. While percentage of lymphocytes was decreased (lymphopenia), that of neutrophilic granulocytes was increased (granulocytosis), visible as soon as one hour after the encounters. Similar changes in circulating leucocyte subsets have been found in socially stressed rats (Stefanski and Engler, 1998) and chickens (Gross and Siegel, 1983), and in pigs being shipped (McGlone et al., 1993). It is likely that endocrine factors released during stress, modulate leucocyte trafficking, which results in a redistribution of leucocytes between blood and other immune compartments (Dhabhar et al., 1995). Corticosteroids seem to be the major mediators of this effect (Dhabhar et al., 1995; Wallgren et al., 1994).

2. Some long-term behavioural and physiological changes in defeated gilts may represent long lasting effects of the acute defeat stress, but social isolation predominantly determined the responses of gilts in the course of time. Neither social defeat, nor social isolation, did affect body growth and baseline cortisol concentrations in the course of time. However, some characteristics of defeated gilts, also observed for isolated gilts, involved increases in cardiovascular activity and vocal responsivity. In reaction to certain stimuli (NOT, AVT), higher cardiovascular activities were maintained in defeated as well as in isolated gilts, compared to those of pair-housed control gilts. Although, we cannot entirely distinguish between physical and emotional causes of these cardiovascular activities, we suggest that at least some emotional arousal was involved. Gross indices of locomotory behaviour did not differ between, for example, defeated and undefeated gilts (no differences in inhibitory (latency) behaviours). Both the NOT (Hopster et al., 1999) and tests which resemble the AVT (Rushen, 1996) are considered to be fear-eliciting challenges, and higher levels of fear in defeated as well as isolated gilts, may therefore, also maintain higher HRs in these animals. Surprisingly, we also measured high HRs during the two-minute pre-exposure period in the AVT. Because our HR measuring technique may induce some disturbance, we suggest that this reflected a higher cardiac responsiveness of defeated

and isolated gilts, rather than higher resting (baseline) HR values of these gilts in their home environment. Consequently, it may be suggested that even minor challenges elicit fear-related HR responses in defeated as well as in isolated animals. Another characteristic of defeated as well as isolated gilts was that they did not show habituation to the repeated NET with respect to the number of vocalisations, while pair-housed controls did so. Vocalising has been shown to be positively correlated to locomotory behaviour and adrenocortical responses (Désautes et al., 1997). However, the present study does not provide evidence for such a relationship, since patterns of vocalisations did not necessarily parallel those of locomotions and cortisol responses. We believe that different emotions, i.e. social motivation (search for social contact) and general fearfulness, are contributing to vocal responsiveness.

Social isolation predominantly determined behavioural and physiological characteristics of gilts in the longer term. The effects of social isolation developed progressively which was shown by the absence of (pronounced) acute hormonal and immunological responses to this procedure. Staying in its home environment may be responsible for this initial non-responsiveness to isolation. This is supported by Puppe et al. (1997), who reported that piglets appear to have more problems in coping with a new housing environment than with social disturbances. In our study, after 1 h of isolation, increments in noradrenaline levels (relative to pair-housed animals) occurred. Houpt et al. (1988) and Parrott et al. (1994) also found that isolation induced noradrenaline release. An increase in physical activity may mainly be responsible for this (Goldstein, 1987), since isolation in a known environment was observed to increase explorative behaviour (Carbonaro et al., 1992; Poindron et al., 1994). This suggests that social motivation underlies this behaviour, as a search for interactions with conspecifics. As argued by Jensen et al. (1999), animals deprived of social stimuli in the home environment may also be more responsive to novel environmental stimuli. Testing of isolated control gilts in the repeated NET showed that they initially (at 2 days) habituated well in terms of locomotory and adrenocortical responses, but less habituation was observed at 7 days. Regarding these reactions, pair-housed gilts habituated well to the repeated tests at 2 and 7 days. In pigs, Von Borell and Ladewig (1992) interpreted increased locomotory activities and greater adrenocortical responsiveness in a novel environment (being positively related like in the present study) in terms of excitability. This excitability may represent components of emotional arousal such as fear and/or exploratory motivation, known to occur in tests involving novelty (Boissy and Bouissou, 1995), such as the NET. We have repeated the NET and consequently expected that the novelty of the test decreased. When the test becomes less frightening, then persistence of, for example, high locomotory behaviour rather reflects exploratory motivation than fear. Alternatively, a higher emotional arousal may slow down habituation-like processes, and therefore, fearfulness towards the challenge may be maintained. Thus, reactions to the NET of isolated gilts are not easily explained in terms of specific emotions, but their responses to the NOT suggest that the animals are more fearful. Regardless of previous social defeat, they were more inhibited to approach the novel object, which is a validated measure of fear in cows (Hopster et al., 1999). Besides the already mentioned cardiovascular characteristics of isolated gilts, isolated gilts also displayed

higher HRs than pair-housed gilts in the startbox and the arena of the repeated NET, with the most pronounced difference at 7 days. Whereas the higher HR of isolates in the arena may be related to their higher amount of activity, a primarily emotional response, i.e fear, may determine their HR response in the startbox, in which animals were more or less restricted to move. Additionally, differences in average HR between animals under the respective housing conditions existed in the HAT, but in this test it was more difficult to discriminate between physical and emotional causes. Peak HRs in isolated gilts were found to be higher in several tests, except the NOT, presumably related to higher HR levels prior to the latter stimulus.

To conclude, socially defeated, and to a greater extent socially isolated gilts, seemed to be more responsive to changes in their environment (sensitisation process: Post, 1992; Koolhaas et al., 1997). We have raised some points here for an increased emotional reactivity of defeated gilts, but body growth as an important indicator of performance, was not affected. This contrasts findings in rodents, in which acute social defeat causes suppression of body growth for several weeks (Miczek et al., 1990; Koolhaas et al., 1997; Ruis et al., 1999). In pigs, however, depressed body growth is observed following mixing (McGlone and Curtis, 1985; Stookey and Gonyou, 1994; Ekkel et al., 1995, 1996; Lund et al., 1998), which may substantiate that a lower performance is not (only) related to short-term vigorous fighting, but to a prolonged coexistence of dominant and submissive animals. For the loser pigs, the stress of being defeated together with the continuing stress of threats and submission may be primarily responsible for a reduction in performance. This is supported by Stookey and Gonyou (1994), although the same authors also suggest that a relatively short period of mixing, that is 1 day, partly attributes to a long lasting (up to 2 weeks thereafter) setback in body weight gain. Finally, increases in emotional arousal and decreases in habituative abilities of socially isolated gilts, may emphasise that pigs deprived from any social contact, which is a rather unnatural situation, are less able to cope with environmental stimuli than those animals which are socially housed.

3. Some characteristics of stress responses to social defeat were indicative for a modulating effect of the subsequent social environment. Although not reflected in catecholamine and prolactin response profiles, stimulation of the HPA-axis (plasma- and salivary cortisol) was prolonged in those defeated gilts which were isolated (observed in the first hour). Measures of circulating ACTH, however, did not differ between both groups of defeated gilts and levels had returned within 30 min. Apparently, hypercortisolism is maintained in defeated isolates, despite recovery in ACTH levels. This may either present an enhancement in adrenal responsiveness to ACTH, regulatory changes in hypothalamic-pituitary hormones other than ACTH, or extrapituitary mechanisms (Levine et al., 1997). Not only hypercortisolism was prolonged in defeated isolates: this was also observed for changes in blood cellular immunity. While percentages of circulating leucocytes subsets were changed for at least 3 days in defeated isolates, values were ‘normalised’ within 1 day in defeated pair-housed animals. It may be suggested that a prolonged hypercortisolism maintains a redistribution in circulating leucocytes, but we were not able to detect differences in baseline (salivary) cortisol between the groups at 2 days after the defeat stress. When challenged in the repeated NET at 2 days after defeats, adrenocortical responsiveness

of defeated isolates was similar to that in the test prior to defeats. However, in the test at Day 2, the response was decreased in their pair-housed counterparts, as was also found in the control groups. A similar pattern was observed for locomotory behaviour in the latter NET, remaining at high levels in defeated isolates, but being reduced in the other groups.

We argue that the effect of the social environment shortly after defeat is related to a stress-reducing effect of an established and stable social relationship rather than being a consequence of additional stress caused by social isolation. The latter procedure had no (or little) consequences for gilts in the short term (isolated controls). Restoring a stable social relationship, following a highly unstable social situation (during defeat), may offer defeated animals an improved ability to overcome the effects of the social stress. Such an effect of housing conditions has also been shown in a study with defeated rats (Ruis et al., 1999), in which adverse effects of acute social defeat were greatly reduced in animals which returned to stable social groups, compared to isolated animals. The amelioration of stress responses by the presence of members of the same species is called social support. In general, social support cannot be provided by any conspecific, but the ability to act as an 'arousal-reducing structure' is restricted to bonding partners (Sachser et al., 1998). In our study, social bonds existed between litter-mates, and it has been shown that this type of relationship is among the strongest in pigs (Petersen et al., 1989). Our results indicate that the presence of a familiar companion per se, irrespective of dominance relationships, may ameliorate responses to defeat stress. This may be due to high social stabilities, provided by established relationships between the members of a pair, resulting in predictable behaviour (Sachser et al., 1998). We finally hypothesise that, when benefiting from processes of social support, negative effects of mixing may be moderated by bringing subgroups of litter-mates together. This may reduce the number of defeats and may increase social buffering. As shown in an outdoor environment, familiar pigs tend to form subgroups, whereas unfamiliar pigs maintain separate rest areas for over 6 months (Stolba and Wood-Gush, 1984).

5. Conclusions

Our results emphasise the importance of established and stable social relationships between conspecifics. Instability, leading to social defeat, induces pronounced behavioural, endocrine and immunological changes. A stable social environment, on the other hand, not only reduces the occurrence of social stress, but group members may also buffer against the adverse effects of (social) stress (social support). Our data further emphasise that the pig is a socially living animal which requires (stable) social contact with conspecifics: social isolation leads to a higher vulnerability of gilts to subsequent environmental challenges.

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