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## Adaptation to social isolation Acute and long-term stress responses of growing gilts with different coping characteristics

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### Abstract

The present experiment studied the acute and long-term stress responses of reactive and proactive prepubertal gilts to social isolation. Gilts with either reactive or proactive features were identified according to behavioral resistance in a backtest at a young age (2–4 days), respectively being low (LR) and high resistant (HR) in this test. At 7 weeks of age, 12 gilts of each type were socially isolated. Initially, isolation was stressful for both types of gilts, as shown by increased cortisol concentrations and decreased body temperatures. Moreover, both types reacted with increases in exploration and vocalizations. Stress responses to isolation, however, differed in magnitude and/or duration between LR and HR gilts, which was in line with expected reaction patterns on the basis of preferred ways of coping. The cortisol response to isolation was higher in LR gilts, and they generally showed more explorative behavior. HR gilts seemed to be more engaged in walking/running behavior in the first hour after isolation, they generally vocalized more and their noradrenaline excretion in urine was higher at 3 weeks after the start of isolation. Several responses to isolation in the longer term pointed to a prolonged higher general state of stress of HR gilts. Body temperature in HR gilts, for instance, did not recover during 3 weeks of isolation, but values returned to “normal” within 1 day in LR gilts. At 1 week of isolation, relatively high parasympathetic responsivity to novelty was observed in HR gilts, probably due to stress-related high sympathetic reactivity. A shift in percentages of leucocyte subsets, typically occurring under conditions of stress, only developed in HR gilts during isolation. Finally, gastric ulceration was found in one HR gilt, but did not occur in LR gilts. To conclude, LR and HR gilts differed in their strategies to adapt to social isolation, and especially for HR gilts, this procedure seemed to become a chronic stressor. © 2001 Elsevier Science Inc. All rights reserved.

*Keywords:* Pigs; Coping; Stress; Social isolation; Behavior; Physiology

### 1. Introduction

Both animal and human studies have shown the existence of individual differences in cognitive appraisal of environmental stimuli. The individual's perception of the situation determines the level of aversiveness of a stimulus and whether a state of stress is induced. When a situation is

perceived as a threat, individuals differ in the way they cope with the challenge. Studies in feral populations of wild house mice and the great tit indicate the existence of basically two personality types of animals: reactive and proactive ones (discussed by Koolhaas et al. [1]). Both types differ fundamentally in their strategy to adapt to environmental conditions. Although each type may adapt successfully to the environment, reactive animals may have an advantage under environmental changes. From studies with rodents, it is concluded that the success of specific coping responses depends upon the stability or variability of the environment [1,2]. Reactive animals seem to adapt more

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easily to variable conditions and are more flexible. Proactive animals, on the other hand, develop routines and seem to anticipate situations, which is only of advantage in predictable (stable) conditions. In domesticated pigs, similar types can be distinguished [3,4], but they represent extremes within the pig population rather than being distinct categories of animals [4].

The aim of the present experiment was to study differences between reactive and proactive prepubertal gilts in acute and long-term stress responses to deprivation of social contact, i.e., social isolation. This experiment is part of a larger study, which investigates welfare problems of growing pigs that are related to (psycho) social factors in intensive pig production. The importance of having social contact with conspecifics as such is one important aspect of investigation, being related to our studies into processes of social support [5]. As for other social species [6–9], being socially isolated is known to be highly stressful for pigs [5,10–13]. Importantly, social isolation may have consequences for the animal in the longer term. We recently showed that, compared to socially housed pigs, isolated gilts generally develop a higher state of fearfulness, and become more responsive (more vulnerable) to environmental changes [5]. Social isolation may, thus, be considered as a long-term stressor, being of relevance for some pigs in intensive husbandry conditions, i.e., for individually kept sows and boars, but also for (growing) pigs which are singly kept for experimental purposes. The above reasoning led us to use social isolation as an environmental challenge or change. Individual differences in appraisal and adaptation (coping) were studied, and compared with expected stress responses on the basis of individual coping characteristics (see below). Gilts with specific coping characteristics were identified at a very young age (2–4 days) by means of a backtest. It was previously shown that for pigs with

extreme low (LR) or high resistance (HR) in the backtest, relationships exist between responses in this test and behavioral and physiological ways to cope with environmental changes at a much later age [3,4]. Extremely low and high resisting piglets in the backtest are considered to represent reactive and proactive animals, respectively [3,4]. It was shown, for instance, that HR pigs were the more aggressive animals in group-feeding competition tests at 10 and 25 weeks of age [4]. LR pigs, on the other hand, had a higher hypothalamic activation to a novel experiment (NE) test (at 10 weeks of age), to routine weighing (at 25 weeks of age) and to administration of a high dose of ACTH (at 24 weeks of age) [4]. LR pigs in the backtest were later also found to more inhibited to approach a novel object (NO) (at 3 and 8 weeks of age [3]) and to enter a novel surrounding (at 10 weeks of age [4]), leading to longer latencies (to contact).

In the present study, gilts with specific coping characteristics were socially (physically and visually) isolated by removal from their littermates at 7 weeks of age. Endocrine, behavioral and immunological effects were subsequently studied during 3 weeks. Moreover, production in terms of body growth and feed-efficiency (gain/feed) was examined. To assess their emotional state after 1 week of isolation, gilts were placed into a NE and exposed to a NO. Stress responses to these novel stimuli are often associated with emotions like fear or excitability [14–17]. After 5 weeks of isolation, postmortem observations were done to determine stomach wall ulceration and weights of adrenals and thymus.

**2. Materials and methods**

All procedures in this study conformed with the requirements of the Animal Care and Use Committee of the

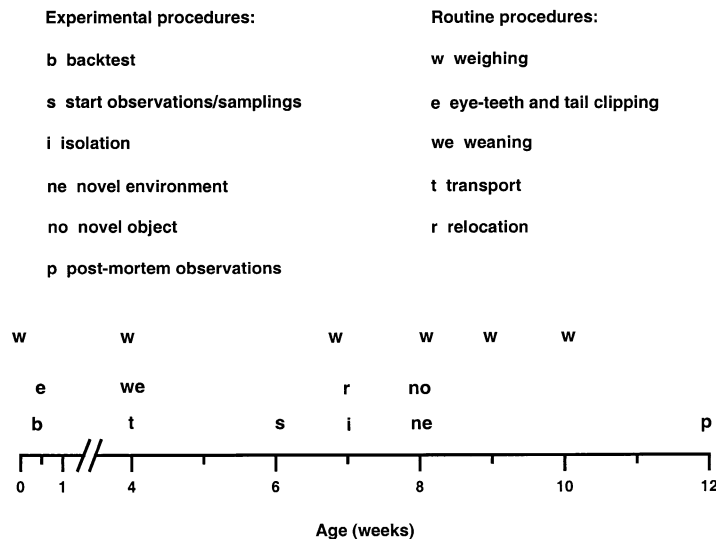


Fig. 1. Timing of experimental and routine procedures.

Institute for Animal Science and Health in Lelystad (ID-Lelystad), the Netherlands. Fig. 1 shows the timing of management and experimental procedures.

### 2.1. Selection of reactive and proactive gilts

The study was done in three identical and consecutive trials (batches) from January to July. Crossbred gilts (Great Yorkshire  $\times$  (Great Yorkshire  $\times$  Dutch Landrace)) from the Experimental Farm for Pig Husbandry at Raalte in the Netherlands were used. They were born in farrowing pens (3.60  $\times$  2.20 m) with partly (50%) slatted concrete floors. Within 1 day after birth, piglets were weighed and received an ear tattoo for identification. Prior to further routine procedures, piglets were subjected to a backtest (manual restraint) between 2 and 4 days of age, by the procedure described by Ruis et al. [4]. Briefly, in this test, a piglet is put on its back during 1 min and the number of escape attempts (behavioral resistance) is used to characterize the animal. Extreme responders, i.e., the LR (two or less

escape attempts) and HR (five or more escape attempts) were selected, representing the reactive and proactive gilts, respectively [4]. A total of 281 female piglets were tested, of which 74 animals (roughly the bottom 25% of the distribution) were classed as LR and 70 animals (roughly the top 25% of the distribution) as HR. The population distribution and the selection criteria were similar to that reported before by Ruis et al. [4] (see also Fig. 2). Selected piglets remained in their litters until weaning (at 4 weeks of age). Shortly after weaning, selected gilts were transported to an experimental farm in Lelystad, the Netherlands, which is part of the Institute for Animal Science and Health (ID-Lelystad), where the actual experiment took place. Littermates (3–5 animals in 38 litters) were kept together and were not mixed with animals of other litters. These litters, which lack “medium” responders, were standardized as much as possible according to penmates. In litters of three and four gilts, at least one LR and one HR gilt was present, and litters of five gilts consisted of at least two LR and two HR gilts.

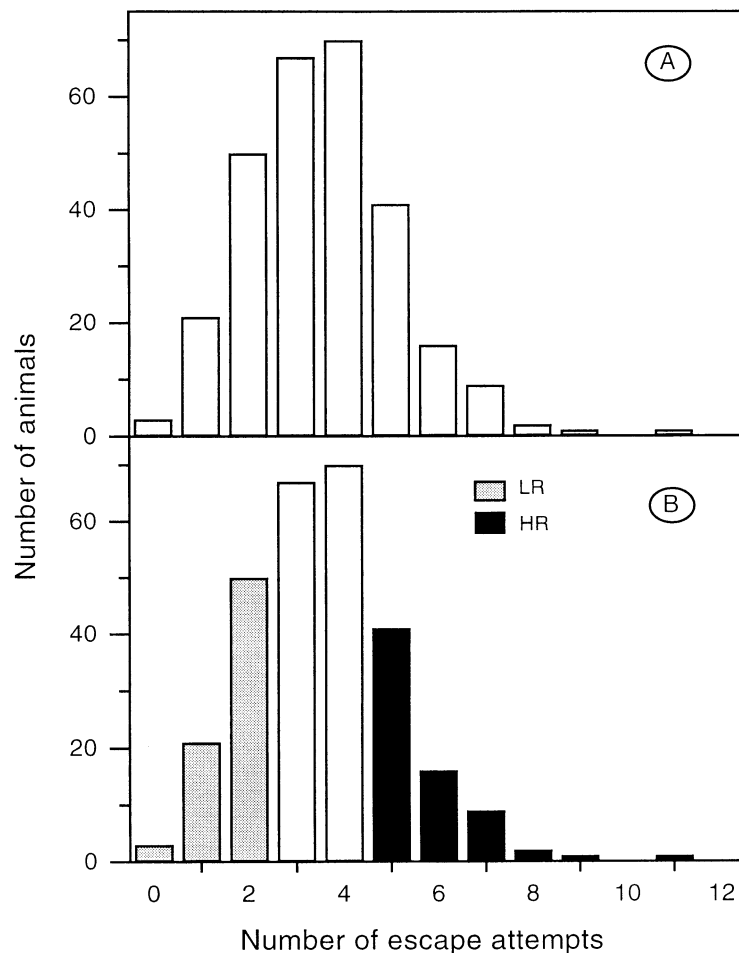


Fig. 2. (A) The histogram of escape behavior (number of escape attempts) of gilts in a 60-s backtest performed at 2–4 days of age ( $n=281$ ). (B) The same distribution as above, but after classification of extreme responding gilts as either LR (two or less escape attempts;  $n=70$ ) or HR (five or more escape attempts;  $n=74$ ) (see also Ruis et al. [4]).

## 2.2. Isolation procedure and management

In each trial, experimental testings took place in three adjacent rooms. Groups of littermates were randomly allocated to these rooms, in which temperature (kept between 19°C and 21°C) and lighting (lights on from 06:00 to 18:00 h; total lux varying from 50 to 100) were controlled. Pen size was  $2.35 \times 1.70$  m and the concrete floors were partly slatted. Food (commercial pelleted dry diets) and water (from nipple drinkers) were available ad libitum. During 2 weeks, pigs were kept in this environment without experimental intervention, but with habituation to housing and human presence. For the isolation procedures, starting at the age of 7 weeks, 12 LR and 12 HR gilts were removed from their litters and housed individually in  $1.80 \times 0.85$ -m pens on partly slatted floors. To minimize litter effects, gilts were chosen from as many litters as possible (maximally two gilts from one litter: 12 LR gilts from 11 litters and 12 HR gilts from 10 litters), with initial weight being balanced across the two experimental groups. A change of room (relocation) was always part of the isolation procedure, and numbers of LR and HR were equal in each room. Within each trial, isolations occurred on 4 different days, with one LR and one HR gilt being housed individually on 1 day. During the individual housing, lasting for 3 weeks, gilts were able to hear other pigs, but they were not able to have visual and physical contact (social isolation). Regular contact (frequency and length) between caretakers and animals was maintained, and should not have confounded with the outcome of the experiment. Isolation always started in the morning between 08:00 and 10:30 h. Gilts which were not isolated were allocated to mixing procedures described elsewhere [18].

## 2.3. Sampling procedures for hormonal and immunological measurements

Blood, saliva and urine samplings and processings took place according to procedures described by Ruis et al. [18]. Blood samples were collected 2 days prior to, and after 1 and 3 weeks of isolation (between 09:00 and 11:00 h). Blood was obtained by puncturing the jugular vein. The duration of handling and sampling took approximately 1 min/pig, and should not have confounded with measurements of baseline cortisol. Before isolation, however, in some cases, two gilts of the same group were sampled (see Section 2.2). In these few cases, order of samplings were randomized, and blood sampling of one gilt may have affected the hormone levels measured in the other pig. The greater portions (8 ml) of the blood samples were transferred to ice-cold EDTA coated tubes and centrifuged (at 4°C, 10 min,  $2000 \times g$ ) within 30 min. Then, 1.5-ml aliquots of plasma were either frozen at  $-20^\circ\text{C}$  (for cortisol measurements) or at  $-80^\circ\text{C}$  (for ACTH and prolactin determinations). Blood samples (2 ml) originating from samplings at the above timepoints were transferred to

heparin-coated tubes and kept at room temperature. They were assayed for leucocyte counts within a few hours.

Saliva samples were taken by allowing animals to chew on cotton buds, according to a procedure described by Ruis et al. [12]. Samples were taken 15 min before and 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after the start of isolation. Some of these samplings (at 15, 30 and 45 min) coincided with behavioral samplings. At these timepoints, behavioral observations were interrupted, leading to one or two missing samples. Further saliva sampling was done on days  $-2$ , 1, 2, 7, 14 and 21, when a single sample was taken between 08:00 and 10:00 h. These samplings did not interfere with behavioral observations. Finally, saliva was gathered in the novelty test (see Section 2.7). Saliva was stored at  $-20^\circ\text{C}$  until analysis for cortisol.

Urine samples were collected in early morning periods (between 06:00 and 08:00 h). Collections, by awaiting spontaneous voidings [18], took place 2 days before and 1, 3, 7, 14 and 21 days after the start of isolation. On average, 10 gilts of each type were successfully sampled at the different timepoints. Before storage at  $-20^\circ\text{C}$  (for measurements of catecholamines and creatinine), samples were adjusted to pH 3 using formic acid.

## 2.4. Hormonal and immunological measurements

To assess hypothalamic–pituitary–adrenal (HPA) activity, concentrations of plasma ACTH, plasma cortisol and salivary cortisol were determined as previously described [5]. Plasma ACTH and salivary cortisol concentrations were measured by radioimmunoassay procedures, and plasma cortisol concentrations by means of a fluoroimmunoassay. Plasma concentrations of prolactin were quantified in one assay by means of a radioimmunoassay [5,19]. Urinary catecholamine (noradrenaline and adrenaline) and creatinine concentrations were determined as described elsewhere [18]. Briefly, catecholamines were assayed using a high-performance liquid chromatography (HPLC) procedure with electrochemical detection, following a two-step extraction. Creatinine levels were determined using a colorimetric quantitative reaction (Boehringer PAP-method). Catecholamines levels were expressed as ratios to creatinine concentrations: noradrenaline/creatinine (NC) and adrenaline/creatinine (AC) ratios. Blood cellular immunological characteristics were determined by measures of percentages of lymphocytes and neutrophils [5,18]. For this purpose, a total of 100 cells was counted microscopically, in which these leucocytes were differentiated.

## 2.5. Body temperature

To estimate body temperature, a thermometer was used which was inserted in the ear (ThermoScan, IRT 3020, Braun, Germany). As a validation of this type of thermometry, comparisons were made with rectal temperatures [18]. Within 10 s, temperature was measured twice, and the

average value was used for analysis. Temperatures were measured twice before isolation (at  $-7$  days and  $-15$  min) and 1, 3 and 5 h, and 1, 2, 7, 14 and 21 days after the start of isolation. Except for those on the day of isolation, measurements were always done between 09:00 and 11:00 h, and did not overlap with the collection of behavioral data. Temperature measurements at 3 and 5 h after the start of isolation were done just before behavioral observations in the home pen (see Section 2.6).

## 2.6. Home pen behavior

Gilts were observed in their home pens during specific 30-min periods, in which the behavior of each animal was scan sampled at 1-min intervals (a total of 31 observations for each 30-min period). The ethogram of recorded behaviors is listed in Table 1. On the day of isolation, observation periods were started from time 0 of isolation and then at 30 min and 3 and 5 h after the start of isolation. Additionally, behavior was observed 2 days prior to isolation and 1, 2, 7, 14 and 21 days after the start of isolation. On each of these observation days, behavior was scan sampled at 1-min intervals during a single 30-min period (always between 08:00 and 10:00 h). Behavioral data were expressed in percentages of all (total) behavioral observations (except for vocalizing, which could coincide with other behaviors).

## 2.7. Behavioral, cortisol and cardiac responses to novelty

After 1 week of isolation, each gilt was subjected to a novelty test consisting of two novel stimuli, according to procedures described by Ruis et al. [5,18]. The order of testing of individual gilts was randomized. Handling and transport before the test was standardized as much as possible. After removal from their home pens, individual gilts were gently driven into a startbox (through a corridor for 10–20 m). Pigs were introduced into a novel arena ( $3.8 \times 3.0$  m) following opening of the startbox (NE). A pig was left in the novel arena for a total of 15 min during which its behavior was recorded via a video camera. Latency to

leave the startbox and locomotion were analysed afterwards (Ethovision, Noldus Information Technology, Wageningen, the Netherlands). Number of vocalizations was recorded directly throughout testing. Ten minutes after opening the startbox, a NO (a yellow and a gray bucket tied together) was lowered from the ceiling onto the floor and then lifted to approximately 0.5 m above the floor. Behavioral parameters used in this NO test were contact latency, number of contacts, total time of contact and number of vocalizations. To determine the cortisol response to the novelty test (NE and NO), saliva was sampled 5 min before and 5 and 15 min after testing. Two minutes before allowing gilts to enter the novel arena, i.e., immediately after being driven into the startbox, they were equipped with a commercial heart rate (HR) monitor (Vantage NV, Polar Electro Oy, Kempele, Finland). This monitor allowed to measure HR and heart rate variability (HRV) in the time domain. The following indices of cardiac activity were determined [20]: (1) mean HR (beats per minute: bpm), as measured from the time between two successive R peaks of the ECG (R–R intervals: RR, ms); (2) overall HRV (sympathetic–parasympathetic autonomic balance), as estimated by (a) the standard deviation of the mean RR (S.D., ms) and (b) the ratio between the standard deviation of the mean RR and the mean RR (SD/RR, coefficient of variance); and (3) root mean square of successive RR differences (r-MSSD, ms), which estimates the parasympathetic influence on HRV. To gain knowledge on cardiac reactivity prior to isolation, HR and HRV were determined in the home pen during 9-min periods. This was done between 3 and 5 days before isolation. Because at this time gilts were still housed in groups, HR monitors were protected from damage by fastening them under a belt made of elastic band. This procedure caused some disturbance and, accordingly, may have had the potential to lead to (mild) stress [5].

## 2.8. Production

Shortly before the start of isolation, and once a week during 3 weeks thereafter, all pigs were weighed. Feed intake was determined by keeping a daily record of all feed added to, and the weight of, the feed hoppers. Feed intake, live-weight gain and gain/feed ratio were calculated per week.

## 2.9. Postmortem examinations

Five weeks after the start of isolation, pigs of trials 1 and 3 were sacrificed for examinations of pathological changes in the pars oesophagea of the stomach, weights of adrenal glands and thymus and permeability of gut epithelium. The appearance of the pars oesophagea of the stomach was scored for any development of hyperkeratosis and ulceration. A scoring protocol ranging from 0 to 5 was used [21]. Adrenal glands and thymus were weighed and these weights were expressed relative to body weights.

Table 1  
Ethogram of the behavioral measures

Behavior	Definition
Exploring	Rooting, sniffing, touching the pen
Defecation/urination	Self-explanatory
Inactive	
Sleeping	Lying with eyes closed
Lying	Lying with eyes open
Sitting	Standing on forelegs, hind quarter on the floor
Standing	Standing inactive, may be between activities
Ingestive	
Feeding	Time spent with head in the feeder and chewing feed
Drinking	Use of water nipple to obtain water
Vocalizing	Total vocalizations: grunts and squeals
Walking	Walking or running through the pen

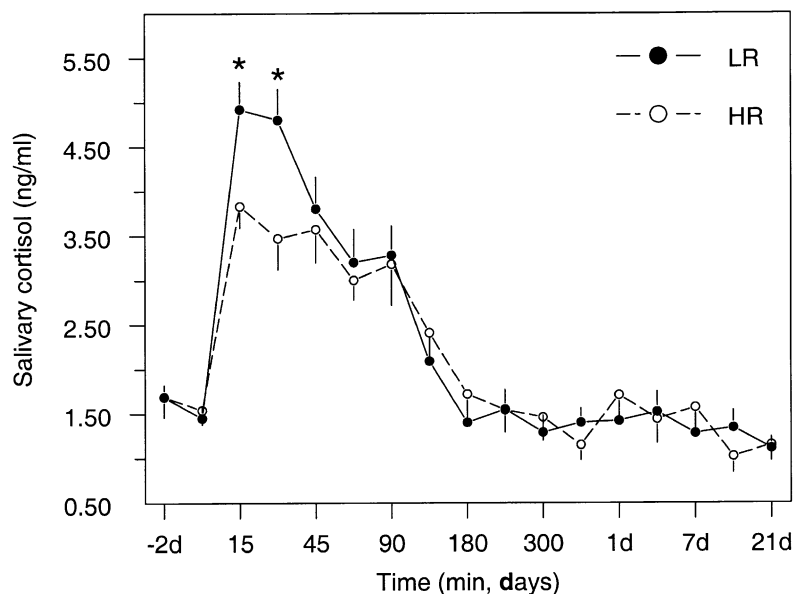


Fig. 3. Mean ( $\pm$ S.E.M.) salivary cortisol concentrations of LR ( $n=12$ ) and HR ( $n=12$ ) gilts during 3 weeks of social isolation. \* Significant difference ( $P<.05$ ) between LR and HR gilts. For significant changes within LR and HR gilts and significant differences in changes between LR and HR gilts, see Section 3.

Methodology and results of gut permeability will be described elsewhere (in preparation).

### 2.10. Statistical analysis

Data was analyzed with an analysis of variance model with main effects for type (LR or HR) and trial (1–3). For analysis of percentages a logistic regression model was employed with a multiplicative overdispersion factor. Counts were analyzed as overdispersed Poisson data on a logarithmic scale. Latency times were also analyzed on a logarithmic scale. Due to the low incidence of stomach ulceration, only descriptive statistics are given for this variable (percentages, numbers). Hormonal and temperature changes within animals were analyzed with a paired  $t$  test. All calculations were performed with the statistical programming package Genstat 5 [22]. Differences were considered significant if  $P<.05$ . Data are presented as means  $\pm$  S.E.M.

## 3. Results

### 3.1. Hormones and immunology

Salivary cortisol concentrations increased significantly ( $P<.01$ ) after isolation in both types of gilts. However, during the first 30 min, the increase was higher in LR gilts than in HR gilts (Fig. 3). LR and HR gilts did not differ in salivary cortisol values following the initial 30-min period, and concentrations returned to preisolation values within 3 h. At 1 and 3 weeks of isolation, (changes in) plasma ACTH, cortisol, and prolactin concentrations did not differ between

LR and HR gilts (see also Table 2). However, when compared to values before isolation, isolation caused significant ( $P<.05$ ) changes in percentages of lymphocytes and neutrophils in HR gilts at 3 weeks after the start of isolation (changes in %:  $-5.58 \pm 3.18$  and  $5.33 \pm 3.37$ , respectively) and not in LR gilts (changes in %:  $1.75 \pm 3.17$  and  $-1.33 \pm 3.37$ ).

Following isolation, changes in urinary NC ratios differed significantly ( $P<.05$ ) between LR and HR gilts, with the NC ratio being more elevated in LR pigs at 1 week of isolation (Fig. 4). After 3 weeks of isolation, the NC ratio tended ( $P=.06$ ) to be higher in HR than in LR gilts. No significant differences in (changes in) AC ratios between LR and HR gilts were found.

Table 2

Plasma hormone concentrations and percentages of circulating leucocyte subsets (means  $\pm$  S.E.M.) for LR ( $n=12$ ) and HR ( $n=12$ ) gilts during 3 weeks of social isolation

Variable	Type	Time relative to the start of social isolation		
		-2 days	1 week	3 weeks
ACTH (pg/ml)	LR	59.0 $\pm$ 29.5	62.7 $\pm$ 10.4	48.4 $\pm$ 18.8
	HR	102 $\pm$ 30.9	49.7 $\pm$ 10.3	61.0 $\pm$ 18.8
Cortisol (ng/ml)	LR	30.1 $\pm$ 3.7	31.4 $\pm$ 3.3	30.0 $\pm$ 3.7
	HR	29.5 $\pm$ 3.9	30.3 $\pm$ 3.4	28.0 $\pm$ 3.7
Prolactin (ng/ml)	LR	1.20 $\pm$ 0.16	1.30 $\pm$ 0.24	1.42 $\pm$ 0.23
	HR	1.52 $\pm$ 0.18	1.58 $\pm$ 0.25	1.68 $\pm$ 0.22
Lymphocytes* (%)	LR	58.41 $\pm$ 3.16	57.08 $\pm$ 2.91	60.16 $\pm$ 3.50
	HR	63.62 $\pm$ 3.22	62.25 $\pm$ 2.98	58.04 $\pm$ 3.61
Neutrophils* (%)	LR	39.42 $\pm$ 3.04	41.60 $\pm$ 2.91	38.09 $\pm$ 2.79
	HR	35.42 $\pm$ 3.07	36.92 $\pm$ 3.02	40.75 $\pm$ 2.78

\* Significant ( $P<.05$ ) difference between LR and HR gilts in changes in percentage leucocyte subsets: values at 3 weeks compared with those at -2 days (see also Section 3).

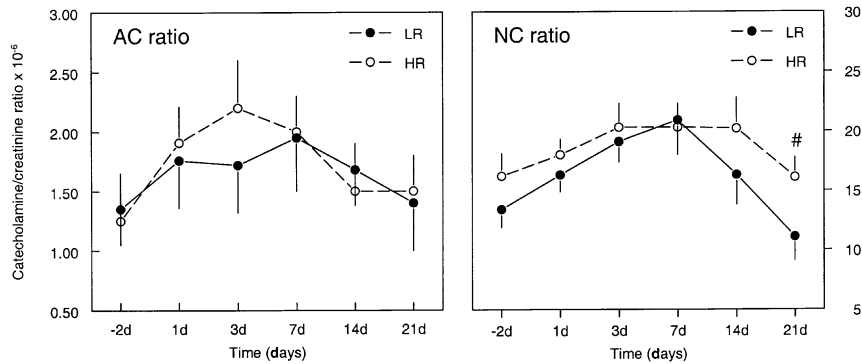


Fig. 4. Mean ( $\pm$ S.E.M.) urinary catecholamine concentrations of LR (average sample size:  $n=10$ ) and HR (average sample size:  $n=10$ ) gilts during 3 weeks of social isolation. #Tendency for a difference ( $P=.06$ ) between LR and HR gilts. For significant changes within LR and HR gilts and significant differences in changes between LR and HR gilts, see Section 3.

### 3.2. Body temperature

Body temperature decreased significantly ( $P<.05$ ) in response to isolation in both LR and HR gilts (Fig. 5). After 3 weeks of isolation, body temperatures in HR gilts were still lowered, while in LR gilts body temperatures did not differ from preisolation values beyond the first day of isolation. At day 7 of isolation, the difference between the two types of gilts was significant ( $P<.05$ ).

### 3.3. Behavior in the home pen

Before isolation, patterns of different behavior did not differ between LR and HR gilts (Fig. 6). Isolation caused a significant ( $P<.01$ ) increase in exploratory behavior. The two types did not differ in this behavior on the first day of isolation, as observed for specific 30-min periods and for

pooled 30-min periods on the first day of isolation. However, thereafter, LR gilts were generally more often observed to explore than HR gilts (% of exploration for pooled 30-min periods beyond the first day of isolation:  $20.3\pm 2.6$  vs.  $12.7\pm 1.8$ ;  $P<.05$ ). With regard to specific 30-min periods, a significant difference in exploration was observed at 1 day of isolation. Initially, HR gilts walked more than LR gilts, but this difference disappeared after 1 h of isolation. At 1 day of isolation, HR gilts showed a higher level of behavioral inactivity, while no differences were observed at the other timepoints. Vocalizing was significantly increased in response to isolation, being elevated during the entire 3-week observation period ( $P<.05$  at least) for both types of gilts. Characteristically, HR gilts vocalized more than LR gilts, which was demonstrated for pooled 30-min periods on the first day of isolation (% vocalizing:  $39.3\pm 3.1$  vs.  $31.2\pm 3.3$ ;  $P<.05$ ), for pooled 30-min periods beyond the

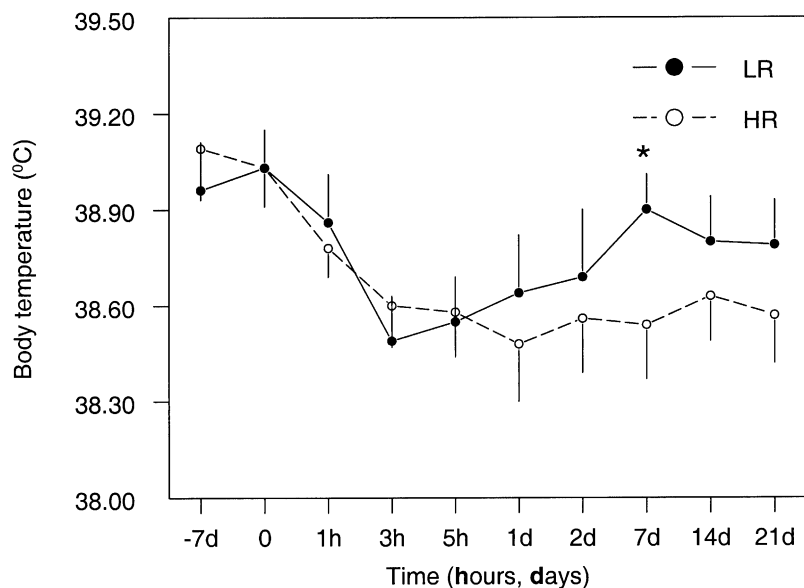


Fig. 5. Mean ( $\pm$ S.E.M.) body temperatures of LR ( $n=12$ ) and HR ( $n=12$ ) gilts during 3 weeks of social isolation. \* Significant difference ( $P<.05$ ) between LR and HR gilts. For significant changes within LR and HR gilts and significant differences in changes between LR and HR gilts, see Section 3.



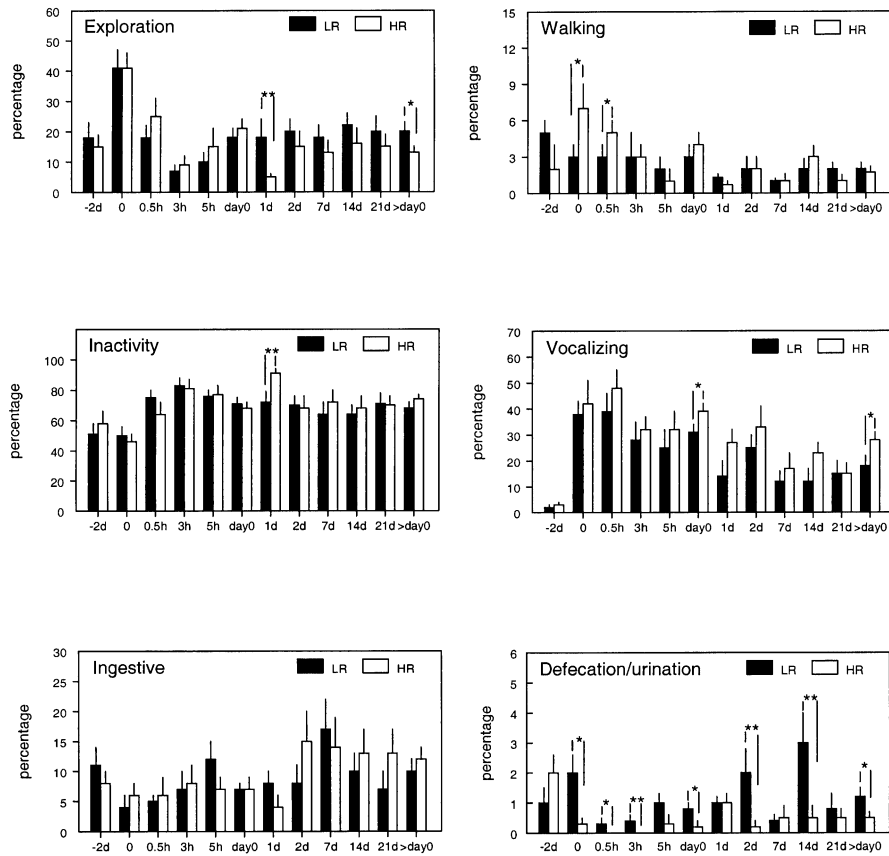


Fig. 6. Behavior of LR ( $n = 12$ ) and HR ( $n = 12$ ) gilts during 3 weeks of social isolation. Gilts were observed in their home pens during specific 30-min intervals (timepoints: hours, days). Pooled 30-min periods on the first day of isolation: day 0. Pooled 30-min periods beyond the first day of isolation: >day 0. Behavioral elements were expressed in percentage (means  $\pm$  S.E.M.) of total behaviors. Significant differences within timepoints between LR and HR gilts: \*\*  $P < .01$ , \*  $P < .05$ . For significant changes within LR and HR gilts and significant differences in changes between LR and HR gilts, including pooled observations, see Section 3.

first day of isolation (% vocalizing:  $28.4 \pm 3.1$  vs.  $18.1 \pm 3.7$ ;  $P < .05$ ), but not for specific 30-min periods. Feed and water intake (ingestive behavior) did not differ between both types of gilts. Finally, LR gilts were generally more often observed to defecate/urinate compared to HR gilts (on the first day of isolation:  $0.78 \pm 0.2$  vs.  $0.21 \pm 0.15\%$ , pooled 30-min periods:  $P < .05$ ; beyond the first day of isolation:  $1.24 \pm 0.3$  vs.  $0.48 \pm 0.18\%$ , pooled 30-min periods:  $P < .05$ ). However, this behavior represented only a very small percentage of total behavioral observations.

3.4. Behavioral, cortisol and cardiac responses to novelty

Table 3 shows the behavioral and cortisol responses to NE and the NO. With respect to behavioral observations in these novelty tests, the only significant difference between LR and HR gilts was noticed in the NO, in which HR gilts vocalized more often. The salivary cortisol response to overall testing was higher in LR gilts compared to HR gilts. With regard to cardiac activities, overall HRV (SD/RR; during the NE), and parasympathetic activity (r-MSSD; during the NE and NO) were higher in HR gilts (Table 4). HR did not differ between the two types of gilts. Before

isolation, LR and HR gilts did not differ in HRV and average HR, as observed in their home pens. Parasympathetic

Table 3 Behavioral and cortisol responses (means  $\pm$  S.E.M.) of LR and HR gilts to the novelty test at 1 week of isolation

Variable	Type	
	LR ( $n = 12$ )	HR ( $n = 12$ )
<i>NE</i>		
Latency to enter (s)	$24.2 \pm 6.6$	$24.3 \pm 6.7$
Locomotion (m)	$100 \pm 7.3$	$101 \pm 7.7$
Vocalizations (number)	$124 \pm 22$	$137 \pm 21$
<i>NO</i>		
Contact latency (s)	$21.3 \pm 8.0$	$23.9 \pm 7.9$
Number of contacts	$10.8 \pm 1.6$	$12.6 \pm 1.6$
Contact time (s)	$37.4 \pm 7.2$	$47.3 \pm 7.2$
Vocalizations (number)**	$71 \pm 10$	$113 \pm 10$
<i>NE + NO</i>		
Cortisol response (ng/ml)*	$2.54 \pm 0.31$	$1.60 \pm 0.35$

NE = 10-min period, NO = 5-min period.

\* Significant difference between LR and HR gilts:  $P < .05$ .

\*\* Significant difference between LR and HR gilts:  $P < .01$ .

activity (r-MSSD), however, tended to be higher in LR gilts before isolation ( $P < .1$ ; Table 4).

### 3.5. Production

LR and HR gilts did not differ significantly in body weight gain and feed intake (Table 5). However, in the second week of isolation, the gain/feed ratio was significantly ( $P < .01$ ) lower in LR gilts compared to HR animals. This effect of coping characteristics on gain/feed was not observed in the other weeks, nor when averaged over the whole 3-week isolation period.

### 3.6. Postmortem observations

Most isolated gilts had intact (69%; score of 0) or slightly damaged (25%; hyperkeratosis and no ulceration; scores of 1 or 2) stomach walls. The prevalence of more severe (score of 3 or more) stomach wall damage was only determined for one HR gilt, which showed severe hyperkeratosis and ulceration (score of 4) of the pars oesophagea. Weights of adrenals (in mg/kg:  $66 \pm 2.7$  and  $68 \pm 4.1$ , respectively) and thymus (in g/kg:  $2.76 \pm 0.11$  and  $3.02 \pm 0.27$ , respectively) did not differ between LR and HR gilts.

## 4. Discussion

Our results show that social isolation was perceived as a stressful condition by both types of gilts. This was indicated by physiological changes which were considered indicative for a higher state of stress, such as a acute release of cortisol [23,24] and a (less) acute decrease in body temperature [10,25]. Consistent with earlier findings, social isolation also induced behavioral changes like an increase in exploration [26–28] and more vocalizing [9,15,17]. Whereas exploration may represent a search for social contact (social motivation), vocalizing may be guided by both social motivation and fear [5].

Table 4

HR and HRV (means  $\pm$  S.E.M.) of LR ( $n = 12$ ) and HR ( $n = 12$ ) gilts, before isolation and during the novelty test at 1 week of isolation

Variable	Type	Test		
		Before isolation	NE	NO
HR (bpm)	LR	$173 \pm 4.9$	$165 \pm 4.6$	$163 \pm 5.0$
	HR	$178 \pm 5.1$	$169 \pm 4.7$	$162 \pm 4.7$
S.D. (ms)	LR	$24.2 \pm 2.6$	$34.0 \pm 5.0$	$38.8 \pm 5.3$
	HR	$21.4 \pm 2.7$	$42.7 \pm 5.1$	$39.1 \pm 5.1$
SD/RR	LR	$0.07 \pm 0.01$	$0.07 \pm 0.01^*$	$0.10 \pm 0.01$
	HR	$0.06 \pm 0.01$	$0.11 \pm 0.01$	$0.10 \pm 0.01$
r-MSSD (ms)	LR	$0.35 \pm 0.04^\dagger$	$0.39 \pm 0.13^*$	$0.58 \pm 0.14^*$
	HR	$0.23 \pm 0.05$	$0.81 \pm 0.14$	$0.97 \pm 0.14$

HR, S.D., coefficient of variance (SD/RR) and r-MSSD before isolation (in the home pen: 9-min period) during the NE and NO.

\* Significant difference between LR and HR gilts:  $P < .05$ .

† Significant difference between LR and HR gilts:  $P < .01$  (tendency).

Table 5

Production characteristics (means  $\pm$  S.E.M.) of LR and HR gilts during 3 weeks of social isolation

Variable	Type	
	LR ( $n = 12$ )	HR ( $n = 12$ )
<i>Feed intake (kg)</i>		
Week 1	$8.99 \pm 0.52$	$8.32 \pm 0.50$
Week 2	$10.0 \pm 0.65$	$9.35 \pm 0.64$
Week 3	$10.75 \pm 0.69$	$11.08 \pm 0.68$
Total period	$29.74 \pm 2.04$	$28.75 \pm 2.01$
<i>Weight gain (kg)</i>		
Week 1	$7.47 \pm 0.47$	$6.93 \pm 0.47$
Week 2	$5.74 \pm 0.42$	$6.03 \pm 0.40$
Week 3	$6.18 \pm 0.38$	$6.86 \pm 0.38$
Total period	$19.39 \pm 1.19$	$19.83 \pm 1.19$
<i>Gain/feed (kg/kg)</i>		
Week 1	$0.83 \pm 0.01$	$0.83 \pm 0.02$
Week 2 **	$0.57 \pm 0.02$	$0.64 \pm 0.02$
Week 3	$0.59 \pm 0.04$	$0.63 \pm 0.04$
Total period	$0.66 \pm 0.02$	$0.70 \pm 0.02$

\*\* Significant ( $P < .01$ ) difference between LR and HR gilts.

Stress responses to isolation, however, differed in magnitude and/or duration between LR and HR gilts. The acute increase in salivary cortisol, for instance, was higher in LR gilts as compared to HR gilts. The same comparison showed that LR animals were generally more explorative, as shown for pooled observations beyond the first day of isolation. HR gilts, on the other hand, were more “restless” than LR gilts, which was especially seen shortly after isolation. HR gilts performed more walking/running behavior during the first hour, and showed a higher level of vocalizing (pooled observations) during the first day of isolation. In the longer term, HR gilts vocalized on average more than LR gilts did (pooled observations beyond the first day of isolation; Fig. 6). Moreover, after 3 weeks of isolation, the urinary NC ratio was higher in HR gilts. This difference could not be explained by a difference in behavioral activity [29]. These characteristics of LR and HR gilts agree with expected reaction patterns on the basis of preferred ways of coping. Reactive copers, here represented by the LR gilts, were previously shown to have a relatively high HPA axis reactivity and a high explorative motivation under challenging conditions [1,4,30]. Responses of HR gilts, on the other hand, were more characterized by proactivity. Proactive rodents were observed to be more active in response to a stressor, by actively seeking a way to remove themselves from the source of stress [1,2,31,32]. This may resemble the higher level of “restlessness” of HR gilts at the start of isolation. Physiologically, the higher domination by the sympathetic nervous system in HR pigs agrees with observations of proactive rodents [1,2,31,32] and pigs [3], which predominantly react with a sympathetic stress response.

A dominance of the sympathetic nervous system in HR gilts was not observed in the novelty test, during which average HR did not differ between the two types of gilts.

Nevertheless, we argue for a higher sympathetic activity in HR gilts, but this was accompanied by an increase in parasympathetic activity. The latter was evidenced by a higher r-MSSD in HR gilts compared to LR gilts. The r-MSSD only takes the high frequency variations of RR intervals into account, which specifically quantify the influence on HR of the parasympathetic branch of the autonomic nervous system [20]. The higher HRV (SD/RR) in HR gilts, observed in NE, may substantiate this vagal counter regulation of sympathetic activation [20]. A predominant parasympathetic reactivity, however, has often been ascribed to the more reactive type of animal [1,31,33]. Indeed, prior to isolation, during cardiac monitoring in groups of pigs, parasympathetic activity tended to be higher in LR compared to HR gilts. We, therefore, suggest that the relatively high parasympathetic activity in HR gilts represented a way to compensate for an increase in sympathetic tone during stress caused by novelty. A maintenance in sympathovagal balance during stress-inducing situations was reported before [10,18]. In addition, it may be argued that the novelty test was less stressful for the LR gilts, leading to a relatively small parasympathetic response. This may be substantiated by behavioral observations. On the basis of preferred coping responses to environmental challenges [3,4], it may be expected that LR gilts more gradually explore the NE or NO, leading to longer latencies to contact [1,3,4]. However, differences in latencies to leave the startbox and to contact the NO were not observed in the present experiment. This possibly indicates that the novelty challenges were relatively more demanding for HR gilts. In the present study, several long-term observations in the home pen support a difference in the state of stress between LR and HR gilts, as shown by differences in the temporal patterns of stress responses. In general, these differences point to a prolonged (chronically) higher general state of stress of HR gilts. Body temperature, for instance, did not recover in HR gilts within the 3-week observation period. In contrast, these values were not found to differ from preisolation values beyond the first day of isolation in LR gilts. Moreover, when comparing values at 3 weeks with those prior to isolation, a decrease in percentage of lymphocytes and an increase in percentage of neutrophils indicated a higher state of stress in HR gilts [5,34,35]. In LR gilts, no changes in percentages of these leucocyte subsets were observed. The incidence of stomach ulceration was very low, and no statistically founded conclusions can be derived. However, the only animal showing ulceration was a HR gilt, which may support the thought of a higher vulnerability of proactive animals to the formation of ulcers, when stress is uncontrollable [1,36]. Our arguments for a situation of chronic stress in HR gilts, but not in LR animals, could not be supported by data on weights of adrenals and thymus. It was previously reported that chronic stress conditions are able to, respectively, enlarge and reduce the size of adrenals and thymus [37–39], but we were not able to demonstrate differences between the two types in the weights of these organs.

LR gilts showed a lower gain/feed ratio in the second week of isolation and a more elevated NC ratio at 1 week of isolation. At least to some extent, a higher behavioral activity of LR gilts may have accounted for the effect on these variables, rather than being solely attributed to stress [18,29,40]. Defecation/urination behavior was only rarely observed, and it may be questioned whether registration of this short-lasting behavior can be done properly with scan sampling. Nevertheless, defecation/urination was slightly more often observed in LR gilts. Again, a higher behavioral activity may have played a role: higher activity in itself may lead to more time spent in the dunging area, thereby triggering defecation/urination behavior.

To conclude, our results indicate that LR and HR gilts differed in their ways to adapt to the social isolation challenge, as seen by several differences in the temporal dynamics of stress responses. Some variables may point to a higher state of stress in LR or reactive gilts, but the general impression is that these animals recovered more quickly from the imposed social isolation than HR or proactive gilts did. Especially for the latter animals, this social challenge seemed to become a chronic stressor. Although we cannot simply generalize between stressors, it may be hypothesized that a better adaptation of LR gilts to social isolation may represent a general better ability to adapt to a variety of challenges, occurring in intensive husbandry conditions. Conditions that are difficult to control may especially impose a risk for welfare and health of HR or proactive pigs. However, further research is needed to confirm this hypothesis.

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