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# Effects of environmental enrichment on behavioral responses to novelty, learning, and memory, and the circadian rhythm in cortisol in growing pigs

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## Abstract

Previously we showed that pigs reared in an enriched environment had higher baseline salivary cortisol concentrations during the light period than pigs reared under barren conditions. In the present experiment, it was investigated whether these higher baseline salivary cortisol concentrations were a real difference in cortisol concentration or merely represented a phase difference in circadian rhythm. The effects of different cortisol concentrations on the behavioral responses to novelty and learning and long-term memory in a maze test were also studied in enriched and barren housed pigs. At 9 weeks of age enriched and barren housed pigs did not differ in baseline salivary cortisol concentrations nor in circadian rhythm, but at 22 weeks of age barren housed pigs had a blunted circadian rhythm in salivary cortisol as compared to enriched housed pigs. The differences in baseline salivary cortisol concentrations between enriched- and barren-housed pigs are age-dependent, and become visible after 15 weeks of age. Enriched- and barren-housed piglets did not differ in time spent on exploration in the novel environment test. Barren-housed pigs had an impaired long-term memory in the maze test compared to enriched-housed pigs; however, no differences in learning abilities between enriched- and barren-housed pigs were found. Because blunted circadian cortisol rhythms are often recorded during states of chronic stress in pigs and rats or during depression in humans, it is suggested that the blunted circadian rhythm in cortisol in barren-housed pigs similarily may reflect decreased welfare. 

*Keywords:* Circadian rhythm; Cortisol; Environmental enrichment; Memory; Novelty; Pigs

## 1. Introduction

In modern husbandry, growing pigs are often housed under "poor" conditions in barren pens, with little space allowance. Comparing the behavior of these pigs to the behavior of pigs housed under more enriched conditions, in larger pens with straw bedding, it was shown that barren housing conditions hamper the expression of normal behavior [1–4]. Pigs reared under barren conditions perform more manipulative social behavior like biting, nosing, and massaging of littermates [1,2,4,5], behave more aggressively [3], and develop more abnormal agonistic behavior [5] than pigs reared in an enriched environment. Pigs housed in a barren environment showed an increased amount of exploration of novel objects [6] or a novel environment [5,7] than enriched-housed pigs, and it has been suggested that pigs housed in a barren environment have a stronger motivation for exploration than enriched-housed pigs [5–7]. From these behavioral studies it was concluded that barren housing conditions have negative effects on pig welfare [1–3,6,7].

We showed that pigs housed in a barren environment differed not only behaviorally, but also physiologically from pigs housed under enriched conditions. Surprisingly, pigs housed in a barren environment had lower baseline salivary cortisol concentrations measured during the light period than enriched-housed pigs, especially at a later age [5]. Increased baseline plasma cortisol concentrations are often associated with conditions of chronic stress [8,9]. But, in view of the behavioral data, enriched-housed pigs were not supposed to suffer from chronic stress compared to pigs housed under barren conditions. It was, however, unclear if the higher baseline salivary cortisol concentrations in enriched-housed pigs were a real difference or merely represented a phase difference in circadian rhythm in cortisol. Therefore,

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in the present experiment we measured the circadian rhythm in salivary cortisol at different ages in pigs reared in an enriched and in a barren environment.

It has been shown in rats that disturbed corticosterone levels, i.e., very low or very high circulating corticosterone concentrations, impaired spatial learning [10,11]. Moreover, studies in rodents showed that environmental enrichment improves spatial abilities in a maze [12–14]. Therefore, it was also studied here if enriched-housed pigs have better spatial learning abilities than pigs housed in a barren environment in a maze test at different ages. Disturbed corticosterone levels in rats also increased the behavioral reactivity to novelty [15]. Earlier, it has been found that pigs housed in a barren environment spent more time on exploration of a novel environment or a novel object at a later age than enriched-housed pigs [5–7]. We investigated if pigs housed in a barren environment already differ from enriched-housed pigs in their behavioral response to novelty at a young age, by confronting enriched and barren housed piglets with a novel environment.

## 2. Materials and methods

All procedures in this study were approved by the ID-Lelystad Animal Care and Use Committee (Lelystad, The Netherlands).

### 2.1. Animals and housing

The experiment was performed with 48 crossbred pigs [Great Yorkshire × (Great Yorkshire × Dutch Landrace)]. Pigs were either reared in an enriched (E) environment or in a barren (B) environment, as described earlier [5]. Two successive replicates were used in the experiment. Within each replicate, three groups of four pigs were assigned to the E environment, and three groups of four pigs were assigned to the B environment.

Six sows per replicate bred the piglets used in this experiment. One week before the expected date of farrowing the sows were housed in the farrowing pen. E piglets were born in farrowing pens (7.2 $m^2$) with a concrete lying area covered with straw (1.75 × 2.4 m) and a concrete slatted area (1.25 × 2.4 m). B piglets were born in standard farrowing pens where the sows were crated (3.1 $m^2$, half concrete area, half metal slats). Castration of male piglets, teeth clipping, ear tattooing, and tail docking were carried out at 3 days of age, following standard animal husbandry procedures at the experimental farm.

Piglets were weaned at 28 days of age and six piglets per sow (three barrows, three gilts) were randomly selected within a litter for use in this experiment. Piglets stayed in the same pen at weaning, and the sow and nonselected piglets were removed. At 10 weeks of age a final random selection within a litter of four experimental pigs per sow (two barrows, two gilts) was done. E pigs were relocated to enriched-fattening pens (4.64 $m^2$) with half concrete area covered with straw and half concrete slats. B pigs were relocated to barren fattening pens (3.36 $m^2$) with half concrete lying area and half concrete slatted floor. E and B fattening pens were in the same room. All pens were cleaned daily and fresh straw was provided in the E pens at 0830 h. Throughout the whole experiment, water and food were available ad lib. Environmental temperature was kept between 19-21°C in each room. Artificial lights were on from 0600–1800 h, with no daylight visible in the rooms.

Individual pigs could be recognized by a plastic ear tag and a number painted on their back. All pigs were accustomed to the experimenter by weekly handling from 5 weeks of age to avoid unwanted stress reactions to saliva sampling.

### 2.2. Saliva collection and cortisol analysis

Saliva was collected from all pigs every hour during 24 h at 9 and 22 weeks of age. In addition, saliva was collected from all pigs at 11, 13, 15, 17, and 19 weeks of age at the peak of the circadian cycle, i.e., at 1000 h. Saliva was collected by allowing the pigs to chew on two large cotton buds until they were thouroughly moistened (about 30–60 s per sample). The buds were placed in tubes and centrifuged 10 min at 400 × *g*. Saliva samples were stored at −20°C until analysis. Cortisol concentration in saliva samples was determined using a solid-phase radioimmunoassay kit (Coat-a-Count Cortisol TKCO, Diagnostic Products Corporation, Apeldoorn, The Netherlands) modified for pig salivary cortisol [16]. Cortisol in saliva is essentially in the free biologically active form, and is a good indication of levels of cortisol in blood plasma [17,18].

### 2.3. Behavioral tests

#### 2.3.1. Novel environment test

At 5 weeks of age, piglets were subjected to a novel environment test. The door of the pen was opened, and the piglets were allowed to move freely through the passageway (1.10 × 9.9 m) for 10 min. The passageway was divided in 10 imaginary sections. Behavior was recorded on videotape, and for each piglet the following elements were scored using the Observer software (Noldus, Wageningen, The Netherlands): (1) latency to leave the pen; (2) time spent exploring, i.e., rooting or nosing the passageway (expressed as % of time spent in the passageway); (3) time spent in a section of the passageway without other piglets (expressed as % of time spent in the passageway); (4) time spent in the home pen after entering the passageway (expressed as % of time spent in the passageway). At t = 10 min, an unfamiliar person entered the passageway and sat in the middle for another 5 min. In addition to time spent exploring, time spent in a section without other piglets and time spent in the home pen as described above, we scored (1) latency to enter the section of the person; (2) latency to touch the person, (3) frequency of touching the person; (4) time spent in the same section as the person (expressed as % of time spent in the passageway).

*2.3.2. Maze test*

At 11 and 20 weeks of age, the pigs were trained to run three different maze configurations. The maze configurations were developed using the concept of the Hebb–Williams maze [19], which is used to study learning and memory in rodents. In a pilot study, different types of Hebb–Williams maze configurations were adapted for pigs. Figure 1 presents the three different maze configurations used in this experiment. Each maze was divided in imaginary sections.

The maze was located in a separate room without olfactory, auditory, and visual contact with other pigs. Pigs were food deprived for 12 h, and were trained to find a food reward (30 g of standard pelleted pig food) at the end of the maze. Pigs were randomly taken out of their pen and led through a passageway to the start of the maze. The start box was opened and the pig was allowed to find and eat the food reward. During a trial for each pig was scored: (1) number of wrong line crossings (i.e., crossing the line of an imaginairy section in the wrong direction); (2) time to reach the food; (3) frequency of defaecating and urinating. If the pig did not find the food reward in a trial within 10 min, it was gently led to the food, allowed to eat, and given the maximum time score. The maze was cleaned after each pig.

At 11 weeks of age, pigs were trained to run maze I and maze II (Fig. 1). In the first session, they were allowed to explore maze I and to find the reward. If a pig did not find the food within 15 min, it was led to the reward. Thereafter, each pig received six trials (two trials/day, 3-h interval between trials on the same day). After six trials, pigs were subjected to maze type II (Fig. 1) during six trials.

At 20 weeks of age, pigs were subjected to maze II, which they already were subjected to at 11 weeks of age. After three trials, they were subjected to maze III during five trials (Fig. 1).They were subjected to two trials per day, with an intertrial interval of 3 h.

*2.4. Statistical analysis*

Differences in cortisol concentration in the saliva and behavior in the maze between E and B pigs were analyzed with a mixed analysis of variance model with treatment, replicate, and sex as fixed effects in the model and group entered as random effect. Components were estimated with Restricted Maximum Likelihood Model (REML) procedure [20]. No replicate or sex effects were found. The residuals were checked for homogeneity of variance. Data on latencies and number of errors in the maze test showed heterogeneity due to an increased variance with increasing mean, and were logarithmically transformed and reanalyzed. Group means of behavioral variables in the novel environment test were analyzed with a mixed analysis of variance model with treatment and replicate as fixed effects in the model. No replicate effects were found after analysis. Components were estimated with the REML procedure [20]. Differences were considered significant if $p < 0.05$.

## 3. Results

*3.1. Cortisol*

At 9 weeks of age, E and B pigs did not differ significantly in baseline salivary cortisol concentrations measured over 24 h (Fig. 2, upper panel). The integrated 24-h salivary cortisol concentration is 2.24 ± 0.16 ng/mL for E pigs and 2.15 ± 0.13 ng/mL for B pigs at 9 weeks of age. However, at 22 weeks of age E pigs had a clear circadian rhythm in salivary cortisol, whereas B pigs had a blunted circadian rhythm in salivary cortisol compared to E pigs (Fig. 2, lower panel). E pigs had a significantly higher baseline salivary cortisol concentration during the light period than B pigs: at 0900 h: $p < 0.05$; at 1000 h: $p < 0.05$; at 1100 h:

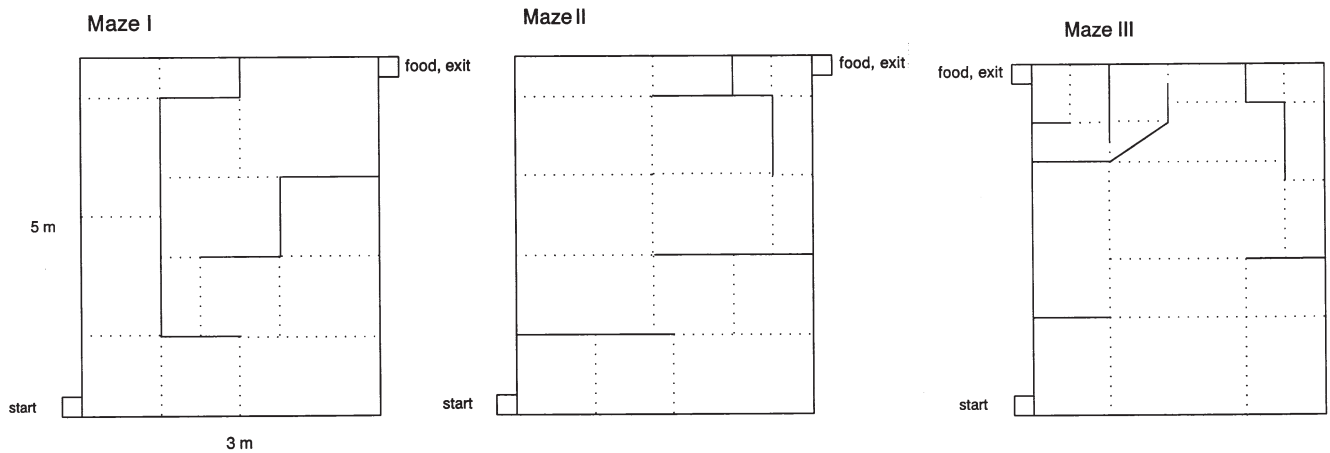


Fig. 1. Configurations of maze I, II, and III used in the maze test. Dotted lines show the imaginary sections of a maze.

$p < 0.05$; at 1200 h: $p < 0.10$; at 1300 h: $p < 0.05$; at 1600 h: $p < 0.01$; at 1700 h: $p < 0.05$; at 1800 h: $p < 0.10$. Moreover, E pigs had a significantly higher baseline salivary cortisol concentration than B pigs during the dark period at 0500 h ($p < 0.01$) and at 1900 h ($p < 0.01$). The integrated 24-h cortisol concentration is 2.82 ± 0.25 ng/mL for E pigs and 1.51 ± 0.08 ng/mL for B pigs at 22 weeks of age.

In addition, baseline salivary cortisol concentrations were measured at 11, 13, 15, 17, and 19 weeks of age at the peak of the circadian cycle, i.e., at 1000 h. Figure 3 shows an overview of the baseline salivary cortisol concentrations at 1000 h from 9 to 22 weeks of age for E and B pigs. Between 9 and 11 weeks of age salivary cortisol concentrations slightly increase for all pigs ($p < 0.01$ at least for both E and B pigs), at 15 weeks of age salivary cortisol concentrations suddenly increase for all pigs, and between 15 and 22 weeks of age salivary cortisol concentrations decrease for all pigs ($p < 0.001$ for both E and B pigs). E and B pigs significantly differed in their baseline salivary cortisol concentration from 17 weeks of age: E pigs had a significantly higher baseline salivary cortisol concentration than B pigs at 17 weeks of age ($p < 0.01$) and at 22 weeks of age (see above; $p < 0.05$), and tended to have a higher baseline salivary cortisol concentration at 19 weeks of age ($p < 0.10$).

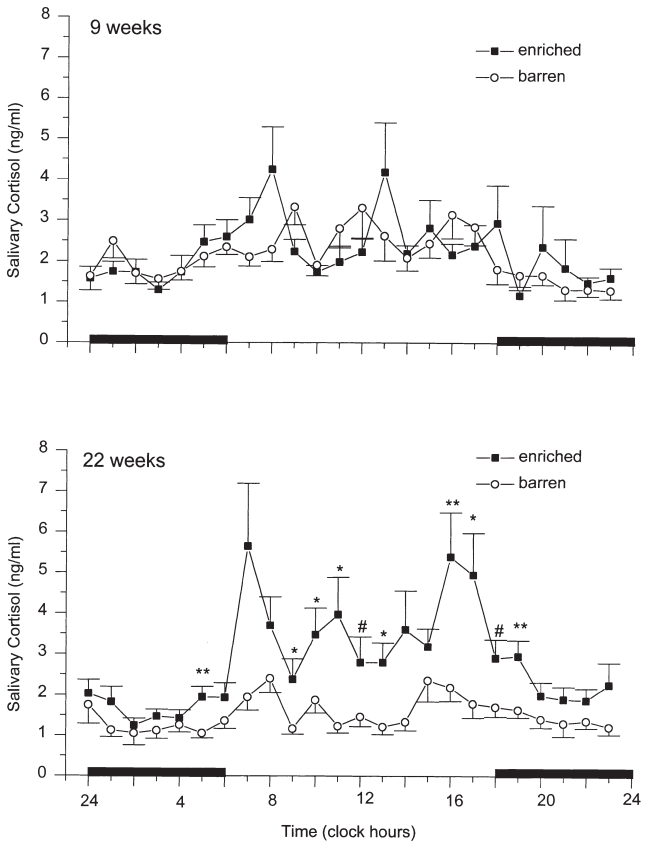


Fig. 2. Baseline salivary cortisol concentrations (mean ± SEM) measured over 24 h at 9 weeks of age (upper panel) and at 22 weeks of age (lower panel) for enriched and barren-housed pigs. Black bars indicate the dark period. $^{\#}p < 0.10$ (tendency), $^{*}p < 0.05$, $^{**}p < 0.01$.

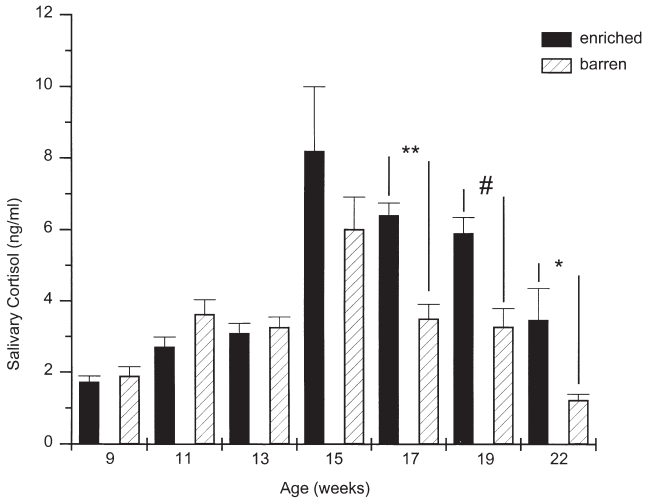


Fig. 3. Baseline salivary cortisol concentrations (mean ± SEM) at 1000 h from 9 to 22 weeks of age for enriched and barren-housed pigs. $^{\#}p < 0.10$ (tendency), $^{*}p < 0.05$, $^{**}p < 0.01$.

At 9, 11, 13, and 15 weeks of age baseline salivary cortisol concentrations at 1000 h did not differ significantly between E and B pigs.

### 3.2. Behavior

#### 3.2.1. Novel environment test

E pigs spent significantly more time in a section without other pigs of the group than B pigs ($p < 0.01$; Table 1). Although B pigs had a longer latency to leave the home pen and spend more time in the home pen after entering the passageway once, the differences were not significant (Table 1). In contrast, after the introduction of the person, B pigs tended to spend more time in a section without other pigs of the group than E pigs ($p < 0.10$; Table 1). Latency to touch the person tended to be shorter for B pigs ($p < 0.10$), but E and B pigs did not differ significantly in other behavioral parameters measured after the introduction of the person (Table 1).

#### 3.2.2. Maze test

Both E and B pigs quickly learned the configuration of maze I and II at 11 weeks of age (Table 2). E and B pigs did not differ significantly in latency to reach the food, frequency of defecating and urinating (data not shown), and the number of incorrect line crossings (Table 2) in maze I and maze II at 11 weeks of age. However, when maze II was repeated at 20 weeks of age, B pigs had significantly more incorrect line crossings during the first trial than E pigs ($p < 0.05$; Table 2). E and B pigs did not differ significantly in latency to reach the food and frequency of defecating or urinating during the first trial of maze II at 20 weeks (data not shown). During subsequent trials, and in maze III E and B pigs did not significantly differ in the latency to reach the food, frequency of defecating and urinating (data not shown), and the number of incorrect line crossings (Table 2).

Table 1
Mean values ± SEM for behavioural parameters measured during the novel environment test for E and B piglets before and after the introduction of an unfamiliar person

| | | Mean values ± SEM | |
|---|---|---|---|
| | Parameter | E piglets | B piglets |
| Without person | latency to leave pen(s) | 95.11 ± 10.84 | 152.48 ± 25.34 |
| | % time exploring | 20.11 ± 1.62 | 17.41 ± 2.90 |
| | % time in one section without other pigs | 8.66 ± 1.02 | 6.35 ± 1.05** |
| | % time in home pen | 17.46 ± 1.65 | 32.68 ± 5.44 |
| With person | latency to enter section with person(s) | 102.36 ± 16.28 | 94.85 ± 15.08 |
| | latency to touch(s) | 226.99 ± 16.58 | 148.42 ± 24.73* |
| | % time in section with person | 4.26 ± 0.67 | 3.67 ± 0.61 |
| | frequency of touching the person | 1.28 ± 0.28 | 2.50 ± 0.41 |
| | % time exploring | 27.85 ± 3.35 | 29.94 ± 4.99 |
| | % time in one section without other pigs | 5.58 ± 1.21 | 8.15 ± 1.35* |
| | % time in home pen | 2.24 ± 0.72 | 13.41 ± 2.23 |

*$p < 0.10$ (tendency) E versus B pigs.
**$p < 0.01$ E versus B pigs.

## 4. Discussion

The present experiment demonstrates that pigs housed in a barren environment have a blunted circadian rhythm in salivary cortisol compared to pigs housed under enriched conditions at 22 weeks of age. The differences in baseline salivary cortisol concentration between enriched- and barren-housed pigs are age dependent, and become visible from 15 weeks of age. Enriched- and barren-housed piglets did not differ in time spent on exploration in the novel environment test. In addition, the results indicated that pigs housed in a barren environment had an impaired long-term memory in the maze test compared to enriched-housed pigs.

Table 2
Number of incorrect line crossings for E and B pigs in maze I and maze II (11 weeks of age), and maze II and maze III (20 weeks of age)

| | | | No. of incorrect crossings (mean ± SEM) | |
|---|---|---|---|---|
| Maze type | Age | Trail | E pigs | B pigs |
| I | 11 weeks | 1 | 7.21 ± 1.18 | 8.33 ± 1.91 |
| | | 2 | 4.12 ± 0.95 | 3.62 ± 0.68 |
| | | 3 | 3.45 ± 0.64 | 2.70 ± 0.41 |
| | | 4 | 2.25 ± 0.29 | 2.83 ± 0.51 |
| | | 5 | 2.12 ± 0.36 | 2.29 ± 0.34 |
| | | 6 | 2.16 ± 0.26 | 2.00 ± 0.34 |
| II | 11 weeks | 1 | 15.45 ± 3.41 | 18.67 ± 3.39 |
| | | 2 | 7.54 ± 2.07 | 4.79 ± 1.14 |
| | | 3 | 1.87 ± 0.32 | 2.58 ± 0.80 |
| | | 4 | 0.91 ± 0.19 | 2.70 ± 0.91 |
| | | 5 | 1.79 ± 0.83 | 1.08 ± 0.28 |
| | | 6 | 0.70 ± 0.23 | 1.95 ± 0.69 |
| II | 20 weeks | 1 | 3.12 ± 1.05 | 11.95 ± 3.6* |
| | | 2 | 1.04 ± 0.29 | 1.00 ± 0.40 |
| | | 3 | 1.25 ± 0.48 | 0.54 ± 0.52 |
| III | 20 weeks | 1 | 13.75 ± 3.03 | 16.18 ± 4.22 |
| | | 2 | 4.83 ± 0.87 | 3.86 ± 0.85 |
| | | 3 | 2.87 ± 0.45 | 3.59 ± 1.02 |
| | | 4 | 1.41 ± 0.28 | 4.40 ± 2.08 |
| | | 5 | 1.41 ± 0.34 | 2.04 ± 0.43 |

* $p < 0.05$ E versus B pigs.

### 4.1. Cortisol

Cortisol measurements showed that the higher baseline salivary cortisol concentrations during the light period in E pigs compared to B pigs are age dependent, and become visible from 15 weeks of age. In addition, we showed that baseline cortisol concentrations slightly increased between 9 and 11 weeks of age, also increased between 13 and 15 weeks, and then gradually decreased until 22 weeks of age for both E and B pigs. These data confirm the results of other studies [16,21–23] that salivary and plasma cortisol concentrations in pigs initially increase followed by a decrease with age. In children, it was also shown that an initial increase in cortisol concentration until 1–6 years of age is followed by a decrease until 15 years of age [24,25]. In rats, baseline corticosterone concentrations are low between 4 and 14 days of age, and in this period (the so-called stress hyporesponsive period) corticosterone responses to stressors are blunted [26,27]. Such a stress hyporesponsive period may also exist in pigs during the period of low baseline cortisol concentrations; however, further research is necessary to study the relationship between baseline cortisol concentrations and HPA axis responses to stressors in young pigs. We showed that differences in baseline cortisol concentrations between enriched- and barren-housed pigs become visible after 15 weeks, indicating that it may be possible that the HPA axis in pigs is less sensitive to environmental stress before this age.

Analyses of the circadian rhythm in salivary cortisol showed that B pigs have a blunted circadian rhythm in salivary cortisol at 22 weeks of age compared to E pigs. Blunted circadian rhythms in cortisol are found in situations of chronic stress in pigs or rodents (e.g., [28–31]), and during some disease states in humans like certain types of depression (e.g., [32–35]). However, a difference between the blunted circadian cortisol rhythm during chronic stress in pigs and rodents or depression in humans, and the blunted rhythm of B pigs in our study is that during chronic stress or depression there is an elevated circadian trough [30,33,35],

whereas in this experiment B pigs have a decreased circadian peak. But, like in chronically stressed animals or depressed patients, the blunted circadian rhythm in cortisol in B pigs may be an endocrine sign of decreased welfare. This is supported by behavioral studies showing that barren housing conditions have negative effects on pig welfare [1–3,6,7]. If the blunted circadian rhytm in cortisol in B pigs means that their psychological state can be compared to depression, treatment with antidepressants may normalize their HPA axis function (e.g., [32,36,37]). Further research is necessary to study if B pigs indeed have depressive symptoms.

Chronic disturbance of the circadian rhythm in corticosterone in rats has been shown to have effects on the HPA axis responses to stress. Chronic disturbance of the circadian rhythm in corticosterone and maintenance of the corticosterone concentration at a low level causes augmented ACTH responses to different stressors [38,39]. Thus, circadian increases in corticosterone seem to be required for normal termination of ACTH responses to stress [39], and thus for normal physiological functioning of the animal. Although we did not measure ACTH responses to stressors in E and B pigs yet, we observed increased cortisol responses to transport stress in B pigs (De Jong, unpublished results), possibly indicating that also in pigs chronic disturbance of the circadian rhythm in cortisol has effects on the HPA axis responses to stress.

The blunted circadian rhythm in cortisol in B pigs may reflect a difference in activity level between E and B pigs. E pigs may be more active and more aroused because of the daily supplement of straw, in contrast to the B pigs that are housed in a pen without environmental stimuli. However, the stronger motivation for exploration in B pigs suggests a stronger arousal in B pigs compared to E pigs [5–7]. In a similar previous experiment it was shown that the total time spent active during the light period did not differ between E and B pigs [5], although a higher activity level of pigs reared in straw compared to barren-reared pigs has been reported by others [40]. It may also be possible that E pigs differ in the circadian pattern of activity when compared to B pigs. E pigs may show an increased activity when the fresh straw is supplied. Moreover, it has been shown that the frequency of visits to the feed trough is increased during daytime in pigs housed on strawbedding compared to barren-housed pigs [40]. However, it remains to be investigated if the circadian pattern of activity in pigs is related to the circadian pattern in cortisol.

In the present expriment, E pigs were born from enriched-housed sows, and B pigs were born from barren-housed sows, to allow the pigs to experience the different environments from birth. We do not exclude that not only the rearing environment, but also prenatal effects, affect the physiology and behavior of the pigs studied in the present experiment. It has been shown that housing conditions of the sow affected pig behavior until 13 weeks of age [41]. In rodents, it has been shown that prenatal stress affects the HPA axis activity (e.g. [42]), but these effects have not been studied in pigs yet. The prenatal effects of housing conditions of the sow on physiology and behavior of the offspring need to be further investigated.

### *4.2. Behavior*

In rats, it has been shown that corticosterone has an effect on the behavioral response to novelty, which is mediated by central mineralocorticoid receptors (MRs). Disturbed corticosterone levels, i.e., very low or very high circulating corticosterone concentrations increase the behavioral reactivity to novelty [15]. Previous research showed that barren-housed pigs spent more time on exploration [5,6], and had a higher locomotor activity [43], in response to novelty compared to enriched-housed pigs at an age of 26–28 weeks. In the present experiment, enriched- and barren-housed piglets did not differ in time spent on exploration in response to novelty at 5 weeks of age. However, no differences in baseline salivary cortisol concentrations between enriched- and barren-housed pigs were found before 15 weeks of age. These data suggest that a relationship between circulating cortisol concentrations and the behavioral response to novelty may exist in pigs; however, further research is necessary to test this hypothesis.

In a novel environment test not only the motivation to explore, but also fear is measured [44]. We observed that E piglets seem to respond less fearfully to the novel environment, as they tended to have a shorter latency to leave the pen and tended to spent less time in their home pen than B pigs. E piglets spent significantly less time with their group mates in one section compared to B piglets, suggesting that they needed less social support of their group mates in the novel situation than B piglets [45]. However, when the piglets were used to the novel environment and the unfamiliar person entered the passageway, B piglets approached this person more rapidly and spent less time with support of their group mates than E piglets. These results suggest that at a young age, B piglets initially seem to be more fearful to a novel environment than E piglets, but rapidly habituate to the novelty.

Corticosteroids not only influence the behavioral responses to novelty, but corticosteroids also influence learning and memory. It has been shown that very low or very high concentrations of circulating corticosterone impair spatial learning in rats through actions via the MR and glucocorticoid receptor (GR) [10,11]. Central MRs and GRs play a role in specific aspects of spatial learning [10,11], and in performance of working and reference memory in a spatial learning paradigm [46]. Differences in baseline salivary cortisol concentrations between enriched- and barren-housed pigs suggest that differences in central MR and GR concentration between enriched- and barren-housed pigs may exist. Thus, it may be possible that also in pigs there is a relationship between circulating cortisol concentrations and spatial learning and memory in a maze test.

Training the pigs in a maze to find a food reward did not show differences in learning abilities between E and B pigs at 11 as well as at 20 weeks of age. However, data suggested that B pigs had an impaired long-term spatial memory compared to E pigs, because they made more mistakes when the maze test was repeated at 20 weeks of age. These results indicate that in pigs a relationship between baseline circulating cortisol levels and long-term spatial memory may exist. However, further research studying MR and GR concentration and function in enriched- and barren-housed pigs is necessary to support this hypothesis.

In rodents, it has been shown that animals reared in a more complex environment had more dendritic branches in certain areas of the temporal cortex and hippocampus [13,47,48] and a higher weight of regions of the cortex and subcortex [49] than animals reared in a barren environment. Thus, rearing conditions may affect brain morphology. In addition, it had been shown that rats reared in a more complex environment had a better performance in a radial maze test than rats reared under impoverished conditions; thus, there may be a functional relationship between brain morphology and performance in a maze test [12,14]. Studies of brain morphology in pigs are needed to conlude if rearing conditions affect brain morphology, and if this is related to long-term spatial memory.

It has been found that pigs housed in an enriched environment moved more rapidly to the food in a T-maze, whereas pigs housed in a barren environment explored more the environment. Moreover, pigs housed in a barren environment could more rapidly change their behavior when the T-maze was changed [7]. In our experiment, E and B pigs did not differ in latency to reach the food, nor did they differ in the number of mistakes before and after changing the maze. This does not support the suggestion that E pigs behave more fixed and routine-like than B pigs [7].

## 5. Conclusions

The present experiment shows that the decreased baseline salivary cortisol concentrations found in pigs housed in a barren environment in a previous experiment [5] can be ascribed to a blunted circadian rhythm in salivary cortisol in barren-housed pigs compared to enriched-housed pigs. As a blunted circadian rhythm in cortisol is often measured during situations of chronic stress in pigs and rodents (e.g., [30,31]) or depression in humans (e.g., [33,35]), it is suggested that similarily the blunted circadian rhythm in cortisol in barren-housed pigs may reflect decreased welfare. The present experiment indicated that a relationship between circulating cortisol levels and behavioral response to novelty and long-term spatial memory may exist in pigs; however, further research is necessary to support this hypothesis.

Cortisol levels are often used to assess chronic stress and subsequently judge animal welfare [9,50]. However, the present experiment demonstrates that the assessment of stress should not be based on increased baseline cortisol levels only, but should also consider the shape of the circadian rhythm in cortisol.

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