Social isolation elicits deficits in the ability of newly weaned female piglets to recognise conspecifics

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Abstract

In pigs, weaning and social isolation can be highly stressful and may impair social recognition (SR). The goal of this study was to assess the ability of newly weaned female piglets to recognise familiar non-related conspecifics and to examine how this was affected by weaning age and/or acute social isolation. For that purpose, 48 female piglets were either weaned at day 11 (D11, n = 24) or at day 22 (D22, n = 24) of age and subjected to the familiarisation procedure during the first 2 days post-weaning. To examine whether social isolation affected SR, 24 piglets were isolated for 15 min after the last familiarisation procedure, while the control group (n = 24) remained with their littermates in the nursery pens. In the SR test, taking place immediately after social isolation, each subject was exposed to a familiar (n = 24) or an unfamiliar (n = 24) conspecific for a 3-min period. The animals were tested only once, 2 days after weaning, i.e. at 13 days of age for D11 piglets and 24 days of age for D22 piglets. The assessment of SR was based on the natural tendency of pigs to investigate unfamiliar conspecifics more intensely than familiar ones. Overall, during the SR test familiar piglets spent less time on social investigation (16.70 ± 2.86%) than unfamiliar ones (29.09 ± 3.71%). The results indicated a significant interaction between weaning age and social isolation among familiar pigs (F_{1,17} = 23.12, P < 0.001). Neither weaning age nor social isolation affected the duration of social investigation in piglets exposed to unfamiliar conspecifics (F_{1,17} = 0.24, P = 0.63). However, when isolated D11 piglets were exposed to familiar conspecifics they investigated more than non-isolated D11, isolated D22 and non-isolated D22 piglets. The amount of social investigation displayed by familiar isolated D11 piglets was similar to that displayed by unfamiliar animals. In conclusion, it may indicate that SR can be disrupted by 15 min of social isolation in piglets weaned at D11, but not in piglets weaned at D22.

Keywords: Pig; Weaning age; Recognition; Isolation; Stress

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1. Introduction

In feral and free-ranging systems pigs are weaned gradually. Weaning begins when the sow leaves the farrowing nest for foraging, but the complete cessation of suckling is normally seen around 8–17 weeks of age (Newberry and Wood-Gush, 1985; Jensen, 1986; Jensen and Recén, 1989), when young pigs continue to live in social contact with the sow and her social group. In commercial husbandry systems, piglets are abruptly weaned at much younger ages. The latest report of the NAHMS (2001) revealed that, in the USA, 63.9% of the piglets are weaned between 16 and 20 days of age and 15% are weaned before 16 days of age. In pigs, the consequences of abrupt weaning at such an early age, however, are not fully understood. In a previous study, Yuan et al. (2004) showed that piglets weaned between 9 and 12 days of age fought longer and initiated more fights that they did not subsequently win than piglets weaned between 21 and 23 days of age when they were regrouped at 9 weeks of age. They suggested that impairments in social recognition (SR) in animals weaned at around 12 days of age may have caused the increase in post-regrouping aggression.

In support of this, Laughlin and Zanella (2003) demonstrated that the ability to reach a hidden platform in a Morris Water Maze (spatial learning task) was hampered in female piglets that were weaned at D12, but only if they were socially isolated for 15 min immediately before testing. In contrast, the performance of female piglets weaned at D21 was not significantly affected by social isolation. These findings indicate that weaning at around 12 days of age do not impair learning per se, but may hamper cognitive functioning after an acute stressor, such as acute social isolation or regrouping.

Social recognition, the process by which animals identify and discriminate familiar conspecifics is a component of learning and memory that, in rodents, is sensitive to disruption by acute stressors (Burman and Mendl, 2000; Kogan et al., 2000). Based on the findings by Yuan et al. (2004) and Laughlin and Zanella (2003), however, we hypothesised that 15 min of social isolation does not disrupt SR in piglets weaned at 21 days of age, but only affects piglets weaned at 12 days of age. Similarly, we hypothesised that SR in piglets weaned at 12 days of age will not be impaired if the animal is not socially isolated prior to testing. In this study, we therefore aimed to assess the ability of newly weaned female piglets to recognise conspecifics and to examine how this is affected by weaning age and/or 15 min of social isolation, using the same stress paradigm previously used by Laughlin and Zanella (2003). To that end, we used a SR protocol developed by Souza et al. (2006).

2. Materials and methods

This is study was carried out at the Swine Teaching and Research Center (STRC) and the Intensive Metabolism Unit (IMU) at Michigan State University. All procedures used in the study were reviewed and approved by the All-University Committee for Animal Use and Care of MSU.

2.1. Weaning and isolation treatments

Three factors were studied, weaning age with two levels: 11 days of age (D11) and 22 days of age (D22), isolation with two levels: isolated and non-isolated and familiarity with two levels: familiar and unfamiliar. A total of eight treatments were used with six replicates per treatment group (n = 48).

2.2. Animals, housing, and weaning

Eight litters (Yorkshire × Duroc) were selected for this experiment and eight other litters were used to ensure that all experimental litters consisted of 10–11 female piglets. The cross-fostering of piglets between
litters occurred within 36 h post-farrowing. The cross-fostering protocol followed the standard operating procedures of the STRC. Only females were used because male piglets are usually subjected to castration within 48 h after birth, an additional stressor that may interfere with later stress responses. During the suckling period, sows and their piglets were housed in standard farrowing pens (1.8 m x 2.3 m).

Half the litter was weaned either at D11, while the remaining half was weaned at D22. Weaning was carried out by taking the selected piglets from their farrowing crates and driving them to the IMU (distance: 1.6 km, time: 5 min), where the experiment was conducted. There the piglets remained with their littermates in nursery pens, i.e. pen-mates were littermates, in nursery pens (1.5 m², randomised to four or five animals per pen). A heating pad, a nipple drinker and a five-space feeder were provided in each pen. All animals had ad libitum access to water and were fed according to the requirements of the National Research Council (NRC, 1998). Artificial lights were provided from 06:00 to 18:00 h. Room temperature was controlled (27 ± 2 °C).

Circular arenas (diameter, 1.5 m) were located between the nursery pens enabling piglets from neighbouring pens to access this common area when the doors were opened. Flexible netting dividing each arena into two halves allowed social interaction between non-penmates but mixing and agonistic interactions were prevented (for details see Souza et al., 2006).

2.3. Familiarisation

After weaning, the piglets were subjected to the familiarisation procedure, which was conducted in two phases: exposure between non-penmates (fam1) and exposure between pairs of penmates and pairs of non-penmates (fam2). Fam1 occurred on the day after weaning, when all piglets from each pen were allowed to interact with all piglets from an adjacent pen by allowing them to freely access the common arena divided by flexible netting from 08:00 to 12:00 and 14:00 to 18:00 h. Fam2 was carried out on the subsequent day (testing day), when pairs of piglets from adjacent pens (e.g. two siblings of pen-A with two siblings of pen-B) were placed in the arena twice for 10 min. After fam1 and fam2 piglets from adjacent pens that had been exposed to each other were considered familiar.

2.4. Social recognition test

Forty-eight female piglets were selected for the SR test. As part of the testing protocol, immediately after fam2 all litters were relocated (within 10 min) to novel nursery pens. This relocation to a novel pen ensured that piglets assigned to be tested together were housed in adjacent pens and shared the same arena. The novel pens were similar to their nursery pens and were located in the same room. The SR test began 4 h after fam2.

Prior to testing, the 24 female piglets selected for the isolation treatment were individually removed from their nursery pen and placed in a weighing cart just outside the nursery pen and stayed there, alone, for 15 min. After isolation each pig was immediately subjected to the SR test. The non-isolated piglets were left undisturbed with their littermates in their home pens during this time and only placed in the arena for the testing. The SR test consisted of a single 3-min exposure of piglets in the arena without the netting, which allowed the animals for the first time to have full body contact. The testing exposure was conducted in pairs of non-penmates. For the familiar treatment, each pair was composed of two female piglets that were familiarised together, whereas for the unfamiliar treatment, each pair was composed of female piglets that were completely unfamiliar to each other. The piglets were tested 2 days after weaning; i.e. animals weaned at D11 were tested at 13 days of age and animals weaned at day D22 piglets at 24 days of age. Every pig was tested only once.

2.5. Behavioural observations

The social behaviour of individual piglets in the arena during the SR test was video recorded and subsequently analysed using “The Observer 3.1” behavioural recording software (Noldus Information Technology, Wageningen, The Netherlands).
The duration of social investigation was used for SR assessment. Social investigation was defined as
direct contact with the other piglet and consisted of engagement on behaviours such as nosing, sniffing, and
close following.

The total amount of general behavioural activity was also recorded to detect possible non-specific effects
of social isolation, such as changes in activity patterns that could alter the amount of social investigative
behaviour. It was calculated by subtracting the duration of general inactivity from the total duration of the
exposure and in the results is presented as a percentage. Inactivity was defined as lying on side or belly
without visibly nosing or sniffing the arena or the other pig.

2.6. Statistical analysis

The distribution of the duration of social investigation met the criteria for the normality and homogeneity
of variance. The social investigation behavioural data were analysed using a mixed model analysis of
variance procedure (PROC MIXED) in SAS 9.1 (Statistical Analysis System Institute and Inc., 2003) to test
for effects of familiarity, weaning age, and social isolation and all possible interactions between these three
factors. All effects were tested against the random effect litter. Results are presented as mean ± S.E.M.
Differences between the experimental groups were considered to be significant if \( P < 0.05 \).

Due to the lack of normality and homogeneity of variance of general behavioural activity, the difference
between groups (isolated D11, non-isolated D11, isolated D22, and non-isolated D22) was analysed using a
two-tailed Wilcoxon two sample test.

3. Results

Neither isolation nor weaning age affected general behavioural activity (\( P > 0.31 \)).

The results regarding the percentage of social investigative behaviour in familiar and
unfamiliar exposures during the SR test is presented in Fig. 1. During the SR test the percentage of
time female piglets spent on social investigative behaviour was significantly affected by
familiarity (\( F_{1,40} = 6.99, P = 0.01 \)). Familiar piglets spent less time on social investigation
(16.70 ± 2.86%) than unfamiliar piglets (29.09 ± 3.71%). Because of the significant effect of
familiarity (see below) further analysis were performed for each level of familiarity (familiar and
unfamiliar) separately. Within familiar exposures, the percentage of social investigative
behaviour was significantly affected by an interaction between weaning age and social isolation
(\( F_{1,17} = 23.12, P < 0.001 \)), such that female piglets weaned at D11 that were socially isolated
prior to testing spent a larger percentage of time on social investigative behaviour than non-
isolated D11 (\( P < 0.0001 \)) and isolated (\( P < 0.0001 \)) and non-isolated D22 animals
(\( P < 0.0001 \)). Within unfamiliar dyads, neither weaning age (\( F_{1,17} = 0.24, P = 0.63 \), social
isolation (\( F_{1,17} = 1.11, P = 0.30 \)), nor their interaction (\( F_{1,17} = 1.20, P = 0.29 \)) significantly
altered the percentage of time spent on social investigation.

4. Discussion

In this study we assessed the ability of newly weaned female piglets to recognise conspecifics and
investigated whether this was affected by weaning age and/or 15 min of social isolation.

Female piglets were subjected to a familiarisation procedure for 2 days post-weaning. During
these 2 days, piglets manifest abrupt changes in behaviour, such as decreased play (Herskin and
Jensen, 2000). Prior to our experiment, we could not rule out the possibility that weaning would
hamper social learning ability or motivation to explore during the familiarisation process, which
in turn could eliminate possible differences in social investigation between familiar and
unfamiliar pairs. Although we did not design this experiment to investigate this hypothesis, the outcome of the test showed that the SR ability was not impaired by weaning. Newly weaned female piglets showed clear differences in time spent on social investigative behaviour during the SR test, overall unfamiliar pairs investigated the conspecifics more than familiar ones.

Furthermore, the results suggested that SR of piglets weaned at D22 was not impaired 4 h after the familiarisation procedure and that 15 min of social isolation prior to testing did not affect SR. Although, non-isolated piglets weaned at D11 demonstrated clear SR ability, socially isolated animals showed possible deficits in recognising familiar conspecifics, indicated by a significant increase in the percentage of time spent on social investigation.

It could be argued that after social isolation, isolated D11 piglets were more motivated to socially investigate, irrespective of whether they recognised a familiar individual. If so, we would expect to find an increase in social investigation time in piglets weaned at both D11 and D22. However, only animals weaned at D11 were affected by social isolation. Furthermore, results by Laughlin and Zanella (2003) indicated that 15 min of social isolation may impair cognition, as isolated female piglets weaned at 12 days of age failed to learn a non-social spatial task.

The increase in social investigation may also be a result of alterations in general behavioural activity caused by social isolation rather than a deficit in recognising familiar conspecifics. Repeated social isolation of piglets during the first days of life caused a decrease in their behavioural activity (Kanitz et al., 2004). However, in contrast with their findings, in the present study a single 15-min period of social isolation had no significant effect on general behavioural...
activity. Therefore, it is unlikely that the increased social investigation found in piglets weaned at D11 was side-effect of changes in general behavioural activity.

Interestingly, the amount of social investigation of familiar isolated D11 piglets was similar to the amounts found when unfamiliar animals were tested. Therefore, it is possible that SR in D11 piglets, but not in D22 piglets, is susceptible to interference by social isolation prior to testing and that the difference in social investigation found in the present study may reflect impairments in short-term SR ability. The mechanism for this impairment is still not known but might be related to stress hormones (e.g. cortisol, catecholamines) released during isolation (e.g. Ruis et al., 2001).

A previous study conducted in our lab (Laughlin and Zanella, 2002) demonstrated that cognitive impairments in socially isolated female piglets weaned at 12 days of age were not caused by differences in the HPA-reactivity, as their salivary cortisol levels did not differ from those of isolated control animals that were either unweaned or weaned at 21 days of age. Therefore, it is possible that weaning around 11 days of age does not affect the HPA-axis per se, but may affect brain sensitivity to increases in central cortisol levels.

A follow-up study by Poletto et al. (2005) suggesting that weaning at 10 days of age suppressed mRNA expression of stress-related hippocampal genes, while no changes were observed in piglets weaned at 21 days of age is therefore very interesting and deserves further exploration.

Little information is available regarding social recognition in piglets, particularly regarding sex differences. In a previous study (Souza et al., 2006) we did not find significant differences in SR between sexes. However, due to the small sample size and the occurrence of castration in male piglets we did not use male pigs in this experiment. It is premature to draw conclusions regarding the responses of male piglets to weaning and social isolation as the result by Siegford et al. (2008) indicated that there was a significant interaction between sex and weaning age. Therefore, these results should not be extrapolated to male piglets.

5. Conclusions

Weaning at D11 and social isolation do not impair SR per se, but socially isolated D11 piglets show impairments in the ability to recognise familiar conspecifics as indicated by increases in time spent on social investigative behaviour of familiar animals to amounts comparable to those found in unfamiliar dyads. The SR deficits after social isolation may indicate possible changes in sensitivity to stress hormones in piglets weaned at D11 and this altered sensitivity to acute stress may be caused by the age at which the developing brain is exposed to increases in stress hormones. Further research is necessary to investigate possible mechanisms through which weaning at a very young age may affect both brain normal development and stress sensitivity. From a welfare point of view, it should be further investigated whether the increased stress sensitivity is long lasting effect, potentially, compromising pig welfare.

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