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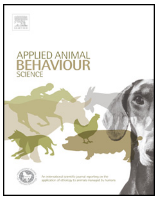

Effects of mother versus artificial rearing during the first 12 weeks of life on challenge responses of dairy cows☆

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ABSTRACT

We investigated the effects of mother versus artificial rearing on the responses to a social and a non-social challenge in adulthood. Rearing of treatment groups only differed during the first 12 weeks of life. Artificially reared animals were separated from their mothers within 24 h after birth and fed via an automatic milk feeder six times (A6, $n = 6$) or twice (A2, $n = 5$) a day. They were housed together with calves suckled by their mothers twice a day for 15 min (M2, $n = 9$) or with permanent access to the cow barn and thus to their mothers and the cow herd via selection gates (MP, $n = 6$). After weaning animals of all rearing treatments were kept together until integration into the dairy cow herd. About 4.5 months after calving (age 31 ± 1.4 months), cows were subjected to an isolation test and two novel objects tests (first: traffic cone, second: ball). ANOVA (behaviour; heart rate of novel object tests) and GLMM (heart rate and cortisol responses to isolation) were used for statistical analyses. During isolation, MP cows were more active: they walked significantly longer ($P = 0.036$), tended to enter more squares in the middle area of the test arena ($P = 0.059$), and to explore the arena or the outer environment for longer ($P = 0.056$) than cows of the other three treatments. In addition, MP and A6 cows had the lowest mean heart rate during isolation, whereas after return into the herd the MP cows showed the lowest heart rate. Cortisol levels differed between groups dependent on sampling time ($P = 0.001$), with MP cows having the lowest basal values but the highest after the isolation test. In the novel object tests, A6 cows tended to explore the traffic cone later ($P = 0.051$), focused the ball earlier ($P = 0.040$) and tended to use the area farther away from the ball more often than cows of the other three treatments ($P = 0.100$). These results in 2.5-year-old cows suggest that rearing with permanent access to the mother and the herd increases sociality leading to higher behavioural activity during isolation and affects physiological stress reactions so that they resemble a reactive coping style, while reaction to novel objects in the home environment is not affected by mother rearing.

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1. Introduction

Under natural conditions, young mammals usually have adequate physical and social contact with their mothers and herd. However, in farm animals social contact is often restricted or even prevented, which can have effects on their later behaviour and stress-responsiveness with maternal deprivation being linked, e.g. to higher reactivity of the HPA-axis, more anxiety-related and more stereotypic behaviour (for review see Latham and Mason, 2008; Newberry and Swanson, 2008; Sachser et al., 2011).

In dairy production, calves are usually separated from their mother within 24 h after birth (EFSA, 2009) and often kept individually for some weeks before being group-housed, whereas under natural conditions a strong bond between dam and calf is developed from day one that persists up to one year or beyond and calves have contact to conspecifics of varying age (Veissier and Le Neindre, 1990; Reinhardt and Reinhardt, 1981). Early separation and social deprivation was shown to have short- (in calves) and long-term (in heifers or cows) effects on behaviour, stress reactivity and the ability to cope with different challenges: Regarding short-term effects, calves kept in crates showed more open-field activity and greater adrenocortical reactivity than calves kept in groups (De Passillé and Rushen, 1997). Calves kept in isolation for 3 months had a longer latency to enter an open field compared to group-reared calves, and, in a social test, group-reared calves sniffed, mounted and had more mock fights than individually-reared calves (Jensen et al., 1999). Calves with contact with the mother for 2–12 weeks showed higher social activity and less abnormal oral behaviour, e.g. cross-sucking, than those separated from the mother within the first 24 h (Flower and Weary, 2001; Fröberg and Lidfors, 2009; Roth, 2008; Roth et al., 2009a; Wagner et al., 2013). Furthermore, calves that were reared in a pen together with their dams for 4 weeks and fed via bucket struggled less during a restraint test than single- or pair-reared calves (Duve et al., 2012). In addition, during an isolation test calves reared with their mothers for 12 weeks showed increased activity and higher motivation to rejoin their mothers and/or herd (more escape attempts, vigilance and vocalisation) at the age of 6 weeks (Wagner et al., 2013), and at the age of 11 weeks they showed higher cortisol response in an ACTH challenge test, interpreted as fewer signs of chronic stress than artificial-reared calves (Roth, 2008).

Concerning long-term effects in cattle, only a few studies investigated the effects of the early social environment on later behaviour during adolescence and adulthood. Le Neindre (1989a,b) found differences in maternal behaviour and in the responses to isolation in a novel environment between primiparous cows that had been group-reared with twice-daily (first two months of life) and permanent (from third month until weaning) contact with and suckling a foster cow for the first 8 months of life, or reared artificially being kept individually for the first 3 months of life and group housed with calves thereafter: Animals reared with a foster cow showed more pronounced maternal behaviour and were more active (more locomotion, exploration) during 5-min isolation tests. They also had a lower respiratory rate in an isolation test, although heart rate did not differ between rearing groups (Le Neindre, 1989b). These animals also showed more agonistic behaviour at the age of 2.5 years than individually reared ones (Le Neindre and Sourd, 1984). Recently, the behaviour and cortisol responses of dairy heifers reared either with some contact with the mother and, partly, other adult animals or reared artificially in groups during the first 12 weeks of life were compared when integrated into the cow herd shortly before their first parturition (Wagner et al., 2012). Mother-reared animals tended to self-groom more often and showed more submissive behaviour than group-reared heifers.

However, no information was available whether rearing with contact with the mother compared to group rearing also affected responses to social and non-social challenges after the first calving. Therefore the aim of the present study was to investigate possible long-term effects of the early social environment on the subsequent ability to cope with new situations by comparing behaviour and physiological reactions of mother- and artificial-reared animals after their first calving when isolated or confronted with novel objects. Based on results in previous studies in calves and cows we expected a higher activity in mother-reared cows in the isolation test and fewer stress reactions in both tests compared to artificial-reared cows.

2. Materials and methods

2.1. Animals, housing and management

The study was conducted at the Institute of Organic Farming of the Thünen-Institut (TI; Federal Research Institute for Rural Areas, Forestry and Fisheries) from April to October 2009. Two dairy cow herds were kept, separated by breed – Black-and-White German Holsteins (GH) and German Red Pieds (GRP) – in two identical parts (50 cows each) of an open-sided barn with cubicle loose housing. In 2009, GH had an average milk yield of 7621 kg/lactation and GRP 6157 kg/lactation. In this study, 26 cows of both cow herds (GH $n = 12$, GRP $n = 14$) were tested 5 ± 1 months (137 ± 21 days in lactation) after their first calving, i.e. at an age of 31 ± 1 months. The reactions of these animals when integrated into the cow herd shortly before calving had been studied before (Wagner et al., 2012).

Each part of the cubicle loose housing provided a total space of 785 m^2 (for more details and the scheme of the barn, see Wagner et al., 2012). A mixed ration (silage and concentrate) was provided once per day, additional concentrate was fed via concentrate feeder. In summer, the cows were pastured during the day. Cows were milked twice daily, starting at 5:15 and 15:45 h, respectively, and lasting about 2 h per milking. There were three calving pens per cow herd to which the animals were moved shortly before the expected calving until the first milking after calving. The experimental cows also had calved in these pens.

The calf area was located adjacently to the cow barn on each of the two sides (GH- or GRP-side), i.e. one calf group per cow herd existed. Shortly (at least within 4 h) after birth in a calving pen, all calves received one bottle of colostrum. Calves were moved to the calf groups directly from the calving pen (duration in calving pen, see Section 2.2.1). The calf area was subdivided into a deep litter resting area of 16 m^2 and a running area of 46 m^2 per calf group (for more details about the calf pen, see Roth et al., 2009a). Each calf pen was equipped with a milk feeder (FA Förster-Technik GmbH, Engen, Germany) and a selection gate connecting calf pen and cow barn; both were transponder-controlled. According to treatment (Section 2.2.1), the calves had access either to the milk feeder or to their mothers and the cow herd via the selection gate. Silage, hay, concentrate and water were available ad libitum. All calves were weaned at the age of 91 ± 2 days by being moved to another barn, where calves of both cow herds (and thus of both breeds) were mixed to form a single joint calf group until the age of seven months (group size: 25–30 animals). Thereafter, the experimental animals passed through three young cattle groups according to their age (age 7–16 months; month 16 until successful insemination; from identified pregnancy until integration into the cow herd). Group size varied between 25 and 30 animals in the younger group (7–16 months) and 30–40 animals in the two older groups. All groups of young cattle were housed in a barn with deep-bedded loose housing during winter and on pasture during summer.

2.2. *Experimental design and procedure*

2.2.1. *Treatment*

The 26 experimental cows (GH $n = 12$, GRP $n = 14$) had been part of the studies by Roth (2008) and Roth et al. (2009a) on short-term effects of contact with the mother comprising 57 calves (GH $n = 25$ and GRP $n = 32$) including males. These calves were divided into four different rearing treatments for the first 12 weeks of life. Only females were kept after weaning. Therefore the sample size of our experiment is reduced.

Calves of two rearing treatments were separated within the first 24 h from their mothers, spending the first 5 days alone in the calving pen and being fed colostrum four times a day. After moving to the calf area on day six, they were fed via an automatic milk feeder with a maximum milk amount of 8 l per day, divided into six (A6, $n = 6$) or two meals (A2, $n = 5$). The amount of milk of 8 l per day was gradually reduced to 3 l per day, from week eleven until weaning at day 91 ± 2 days.

Calves of the two other rearing treatments were kept with the mother in the calving pen for the first 5 days. On day six, they were moved to the calf area and had either permanent access to their mothers and the cow herd through selection gates (MP, $n = 6$) or restricted contact for suckling (M2, $n = 9$), i.e. the mothers were moved into the calf area twice a day for 15 min immediately before milking.

Calves of both cow herds (i.e. of both breeds) were allocated to the different rearing treatments (GH: A6 $n = 2$, A2 $n = 3$, M2 $n = 5$, MP $n = 2$; GRP: A6 $n = 4$ A2 $n = 2$, M2 $n = 4$, MP $n = 4$); thus in both calf groups calves of all four treatments were kept together with a dynamic group composition (newborn calves entering and weaned calves leaving the group). After the first twelve weeks of life, animals of the different treatments and breeds were kept together according to age as described in Section 2.1 until their integration into the cow herd. Thus, each test animal was exposed to the same conditions after weaning up to the time of the present study; only during the first 12 weeks of life rearing conditions differed. The integration in the dairy cow herd took place at the age of 25 ± 0.2 months (timeline, see Fig. 1).

2.2.2. *Procedure of testing*

We wanted to test responses to both a social and a non-social challenge. Thus, one social isolation and two novel object tests were performed. The social isolation test was performed in a calving box, thus largely avoiding the novelty, while social isolation was avoided in the novel object tests by testing in the home environment. Each experimental animal was tested on three days within one week. On the first day, the isolation test was conducted, followed by the two novel object tests.

2.2.2.1. *Isolation test.* One of the calving pens (3 m × 4 m) of each herd was used as test arena for the isolation test (Fig. 2a). Although acoustic contact with the herd was possible, visual contact was prevented by the windscreens of the cow barn. On one side of the test arena, a gate was constructed (metal fence; facing the barn), flanked by 2-m-high concrete walls on the remaining three sides. A part of the wall opposite the gate provided two feeding places with headlocks, which were closed during the test. The calving pen was cleaned with a high-pressure cleaner prior to the isolation test and between the individual tested cows. Before the test started, all cows were fixed in the feeding rack of the cow barn after morning milking, and all experimental cows for the test day (a maximum of 4 cows were tested consecutively on one day) were provided with the heart-rate equipment. Shortly before the first experimental cow was moved to the test arena, one experimenter took the first saliva sample (C0) for cortisol measurement from that cow, then the experimental cow was gently moved by three experimenters familiar to the animals (always the same people) through the home environment to the calving pen used as test arena. When the gate was closed and the three experimenters had left the visual field of the experimental cow, the isolation started for 15 min. The remaining cow herd was fixed in the feeding rack of the cow barn during the test. Immediately after the 15-min period of isolation, one experimenter used concentrate to lure the experimental cow into the headlock of the feeding place in the calving pen, where it was restrained and a second saliva sample (C1) was taken. Afterwards, the experimental cow was released and gently moved back by two experimenters and lured with concentrate to the feeding rack of the cow barn by one experimenter, where the other cows of the herd were still restrained. After 5 min of restraint, one experimenter took the third saliva sample (C2).

2.2.2.2. *Novel object tests.* The novel object test took place in the home environment of the experimental animals, in a part of the alley where cows walked back from the milking parlour to the cow barn. The alley was 2 m wide and 20 m long, for testing a 10 m long part was used (test alley; Fig. 2b). To reach the test alley, the experimental cows had

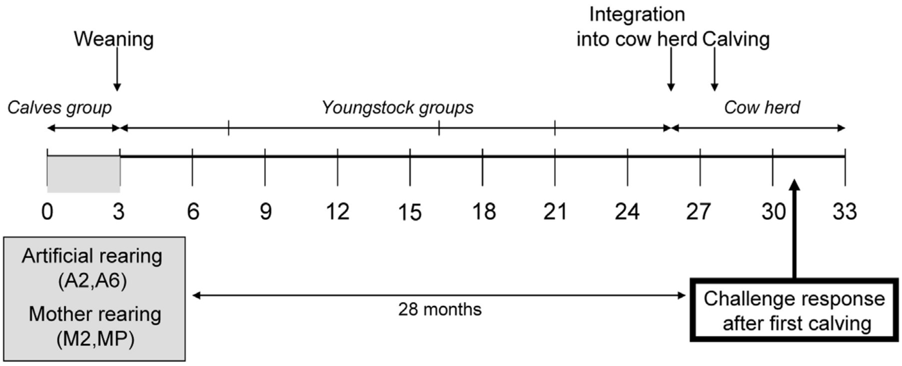

Fig. 1. Timeline from birth to challenge response in months. Grey part illustrates time of rearing treatment, two-way arrows on the timeline illustrate keeping of animals, and vertical arrows illustrate major events.

to turn the corner at a distance of 10 m from the exit of the milking parlour, so that the novel object was first visible for tested cows after the corner (starting line). In the test alley, the novel objects were placed at a distance of 8 m from the starting line (Fig. 2b number 5). They were positioned on the side of the test alley to allow the cows to pass them at smaller or greater distances (Fig. 2b number 6). Each cow was tested twice at an interval of 1 or 2 days; the novel object in the first test was always a traffic cone (height 46 cm, base 28 cm × 28 cm, orange with white stripes) standing on the ground, in the second test it was a pink ball (diameter 60 cm) hanging at the height of the cow's head. After afternoon milking the experimental cow was released individually into the alley leading to the test alley. The next cow was released from the parlour after the test was finished, so that each experimental cow was alone in the test alley. The test ended when the experimental cow had left the test alley on its own. If the time exceeded 3 min, the cow was gently moved by staff out of the alley (this situation occurred only once).

2.3. Data recording

2.3.1. Behavioural observations

Behaviour was recorded using the software XT9 Observer® (Noldus Information Technology) with continuous focal-animal behaviour sampling (Martin and Bateson, 1993) from video recordings for both tests. Observations from videotapes were performed by one person blind for the rearing treatments of the cows. Additionally, the vocalisation was recorded directly for the isolation test. For definitions of behavioural parameters, see Table 1. The behaviours 'vigilant' and 'exploration environment' were merged for analysis in the isolation test (exploration total), because the general function of vigilant behaviour is to obtain information about the environment (Boissy and Dumont, 2002). The latency was set to 200 s when the parameters did not occur. In addition, the localisation of the experimental cow was recorded by using a grid laid over the test areas. The floor space of the calving pen was divided into 12 equal squares (1 m × 1 m) which were categorised

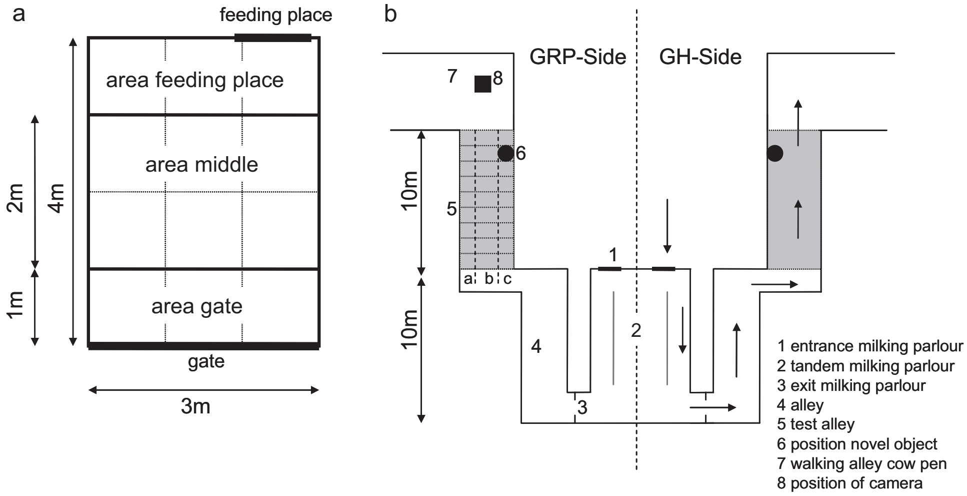

Fig. 2. Sketch of the test arena for the isolation test (a) with dimensions and allocation of grid squares and areas. Furthermore, a scheme of the alley with the milking parlour for the novel object test (b), the left side (GRP-side) showing the dimension, description in numbers and the allocations of grid cells and areas (three rows: a, b, c). The test alley is marked in grey. The arrows on the right side (GH-Side) indicate the way of the tested cows from the waiting area through the milking parlour and alley with test alley back to the walking alley of the cow barn.

Table 1
Definitions of behaviours observed during the isolation and novel object tests. F, frequency; D, duration, L, latency.

Behaviour	Definition	Isolation	Novel object
Walk	Lifting one claw from the ground with forward or backward movement of the extremity, followed by putting down the claw to the ground	F, D	F, D
Head normal[a]	Head position horizontal to the withers or above, up to a maximum of 10–15°	F, D	F, D
Head down[a]	Head position below horizontal to the withers, but not exploring	F, D	
Vigilant[a,b]	Head position above horizontal to the withers (over 10–15°) and external ears upright, not pendulous, both while standing or walking	F, D	F, D
Exploration environment[a,b]	Sniffing or licking the environment except the novel object	F, D	F, D
Exploration object[a]	Sniffing or licking the novel object		F, D, L
Self-grooming[a]	Licking, rubbing or scratching its own body	F, D	F, D
Focus	Virtual extension of the median plane of the head strikes the novel object		F, D, L
Elimination	Defecation and urination	F	F
Vocalisation	Any vocalisation of the cow	F	F
Entering squares	Square was counted if one front extremity entered a square/cell; if a second extremity entered the same area, this was not counted again	F	F

[a] Behaviours were mutually exclusive.

[b] Behaviours merged for analysis in the isolation test to “exploration_total”.

into three sections after observation (Fig. 2a). In the novel object test, the grid laid over the test alley consisted of 27 grid cells (size: 1.1 m × 0.7 m, i.e. the width and length of the test alley comprised three and nine cells, respectively). For further analysis, the grid cells were categorised into three rows after observation (Fig. 2b); each row was 0.7 m wide and 10 m long and consisted of 9 cells.

In the isolation test, video analysis started when the experimenters were out of the visual field of the cow to be tested and was finished after 15 min. In the novel object tests, the observation started when the cow appeared at the starting line, i.e. when the novel object was visible for the test cow, and ended when the tested cow had left the test alley.

2.3.2. Physiological measurements

2.3.2.1. Heart rate. The Polar®-equipment (Polar Electro Oy, Helsinki, Finland, S810 monitors and horse trainer transmitters with electrodes) were used for non-invasive heart rate measurement in both tests. The horse trainer transmitter and electrodes were attached to a horse girth and fitted to the cows as described by Hopster and Blokhuis (1994). The electrodes were applied with ample electrode gel (to ensure contact) and covered by a wide elastic band with an integrated pocket for the monitor. To habituate the animals to the procedure of fitting and wearing the heart rate equipment, it was fitted for at least 8 h one week before the test was conducted. The interbeat intervals recorded were transferred to a computer, and the data were corrected for measurement errors (artefacts) using the Polar Precision Performance software (Version 4.03.050) by Polar Electro® AG.

2.3.2.1.1. Isolation test. After morning milking, the heart rate equipment was fixed to all experimental cows of the test day. The average heart rate was calculated for 6 time periods. The last 10 min of the experimental cow's restraint in the feeding rack before being moved to the test arena was used as baseline (base). As the duration of moving the animals to the test arena differed between animals (126 s, min–max 60–300), the last minute of moving was used (move). The period of 15 min of isolation was divided into three 5-min periods (0–5, 5–10, 10–15). As recovery phase (recov) we used the first 4 min of restraint in the feeding rack after the experimental animal had been moved back to the herd.

2.3.2.1.2. Novel object test. After morning milking, the heart rate equipment was fixed to the cows while they were restrained in the feeding rack. During afternoon milking, the monitors for heart rate recording were started. The mean heart rate during the last 5 min before the start of the experiment (i.e. the cow was standing in the milking parlour, exited it and walked along the alley until it reached the test alley) was used as baseline. To determine the possible increase in heart rate during the novel object test, the difference between the mean heart rate during the testing period in the test alley and the baseline was calculated for further analysis (heart rate increase).

2.3.2.2. Adrenocortical activity. Saliva for cortisol analysis was sampled only during the isolation test. The first sample was collected immediately before moving the experimental cow to the test arena (C0, baseline), the second sample was taken directly after the 15 min of isolation (C1) and the last one 5 min after the animal was moved back to the herd (C2). Saliva was collected using an absorbent cotton (Salivette®, Sarstedt AG, Nürnbrecht) held in a forceps that was inserted into the cow's mouth for 5 s. The samples were stored in a tube in the freezer (−20 °C) to prevent drying until analysis. For the analysis, the Salivette® tubes were defrosted at room temperature, and saliva was removed from the absorbent cotton by centrifugation (10 min at 2500 g). Afterwards, aliquots of the saliva samples were analysed using a cortisol enzyme immunoassay as described by Palme and Möstl (1997).

2.4. Statistical analysis

All statistical analyses were carried out with the software package PASW Statistics, Version 17. For analysing behavioural parameters and heart rate difference from baseline in the novel object test, we computed an ANOVA (analysis of variance) with the fixed factors *rearing treatment* (A6, A2, M2, MP) and *breed* (GH, GRP). Breed was included as a potentially confounding factor, therefore breed effects are not presented in the results. To verify the assumption of the models, residuals were checked for normal distribution using a Shapiro–Wilk test and for homogeneity of variance using a Levene test.

We used log 10 (cone: area farther NO_D; ball: head normalD, heart rate) or rank transformation (isolation: walkD, head downF, area gateF, area feeding placeL; cone: vigilantD, focus NO D; ball: head normalF, vigilantD, area farther NO D) to get normally distributed residuals and homogenous variances in the isolation and novel object tests. However, even after transformation assumption of the model regarding normal distribution of residuals was not fulfilled for several variables (isolation: walk_F, head down_D, self-grooming_F and D; novel object cone: self-grooming_F and D, vigilant_F, exploration NO_F, heart rate; novel object ball: vigilant_F, exploration NO_F, focus NO_F and D). For these variables we used a Kruskal–Wallis test for analysing rearing effects.

To compare physiological reactions in the two novel object tests, we calculated non-parametric statistics (Wilcoxon test) for dependent data of heart rate-differences due to non-normality of data. Heart rate and cortisol levels in saliva in the isolation test were analysed using a generalised linear mixed model (GLMM) with *rearing treatment* (A6, A2, M2, MP), breed (GH, GRP) and *time period* (base, move, 0–5, 0–10, 0–15, recov for heart rate; C0, C1, C2 for adrenocortical activity) and interactions as fixed effects. The individual nested within its rearing was included as a random effect. In the GLMM, fixed factors and interactions were stepwise excluded from the models when they had no significant effect ($P > 0.05$). Accordingly, *rearing treatment, breed* and *time period* and the *interaction between time period and breed* were included as fixed factors in the final model for heart rate. For cortisol levels in saliva, *rearing treatment, time period* and the *interaction between time period and rearing treatment* were finally included. To verify the assumption of the models, residuals were checked visually for normal distribution and for homogeneity of variance. We used for heart rate and cortisol levels log 10 to get homogenous variances in the isolation test.

There are missing values due to technical problems (e.g. failed video-recordings due to disruptions or failed heart rate measurements due to shifted electrodes). In the isolation test, heart rate measurement was not possible for one cow of the A6 and one of the A2 treatment and cortisol measurement failed for one cow of the MP treatment. In the novel object test with the traffic cone, values for behaviour and heart rate are lacking for three animals of the A6, one of the A2 and three of the M2 treatment; with the ball, behaviour and heart rate could not be measured in one animal of the A2 treatment. For all data presented in boxplots, line charts and in the text, we used the original non-transformed values.

3. Results

3.1. Isolation test

3.1.1. Behaviour

Rearing had an effect on the duration of walking ($F_{3,26} = 3.42$, $P = 0.036$), with cows of the permanent rearing treatment (MP) showing longer duration of walking than those of the three other treatments (Fig. 3a). Accordingly, the frequency of entering the squares of the middle area of the test arena tended to differ between rearing treatments ($F_{3,26} = 2.90$, $P = 0.059$), with MP cows entering them more often than the cows of the other three rearing treatments (Fig. 3e). Furthermore, rearing tended to affect the head position ‘head normal’ (duration: $F_{3,26} = 2.64$, $P = 0.076$, Fig. 3c; frequency: $F_{3,26} = 2.95$, $P = 0.056$, Fig. 3d) and ‘exploration total’ (duration: $F_{3,26} = 2.95$, $P = 0.056$, Fig. 3b). Compared to the three other rearing treatments MP cows kept the head in its normal position for the shortest time and with the lowest frequency, but showed exploration total for the longest duration. In all other recorded behavioural parameters, no differences between the rearing treatments were observed (for all $P > 0.1$; overall mean ± st.dev. in s/15 min (duration D) or events/15 min (frequency F): walking F 30.81 ± 7.54; head down F 0.62 ± 0.98, D 6.16 ± 9.91; exploration total F 52.27 ± 10.52; self-grooming F 0.69 ± 1.26, D 2.14 ± 3.39; elimination F 0.69 ± 0.74; vocalisation 23.69 ± 17.05; area gate F 21.15 ± 12.16; area feeding place 11.85 ± 4.21).

3.1.2. Physiological parameters

The mean heart rate was highest during the moving phase and decreased in the course of isolation. However, in the recovery phase it was still higher than the baseline for most treatments (effect of time period $F_{5,24} = 61.21$; $P < 0.001$; Fig. 4a). We found a significant rearing effect ($F_{3,24} = 16.46$; $P < 0.001$ Fig. 4a): Cows of the A6 and MP treatments had a lower mean heart rate than cows of the A2 and M2 treatments overall periods. No interaction of time period with treatment was detected, although MP was the only rearing treatment where heart rate did not increase from isolation 10–15 to recovery.

Concerning adrenocortical activity, all cows exhibited an increase in cortisol levels directly after isolation (C1 compared to baseline C0). We found an effect of time period ($F_{2,25} = 67.18$; $P < 0.001$), a tendency for treatment ($F_{2,25} = 2.45$; $P = 0.090$) and an interaction between time period and rearing treatment ($F_{5,25} = 2.24$; $P = 0.001$). MP cows had the lowest baseline values but the highest values at C2 (Fig. 4b). Over the course of time, the baseline C0 was lowest for all four treatments, C1 was highest for all treatments and there was a clear decrease from C1 to C2 for all treatments except for MP, for which C2 was nearly as high as C1 (Fig. 4b).

3.2. Novel object test

3.2.1. Behaviour

Most of the animals explored the novel objects in the test, except two cows (one of the M2 and one of the A6) with the traffic cone and six cows (three of the M2 and three of the A6) with the ball. We found a rearing effect on latency and a tendency on frequency of the exploration of the traffic cone (latency: $F_{3,19} = 3.33$; $P = 0.051$; Fig. 5a; frequency: $P = 0.080$; Fig. 5b): Cows of the A6 treatment explored the traffic cone later than cows of the three other treatments, whereas cows of the A2 treatment explored it more often than cows of the other treatments.

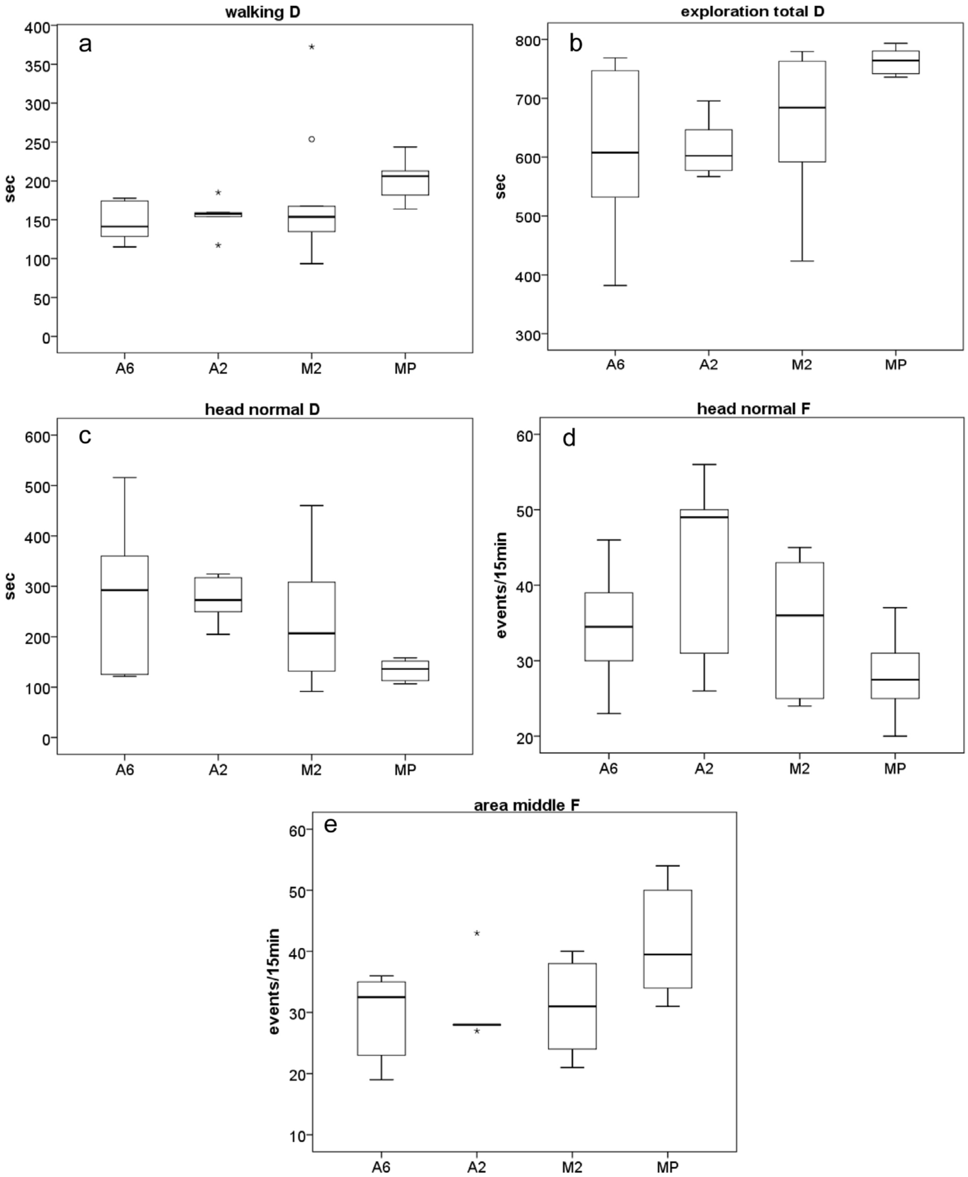

Fig. 3. Duration (D) of walking (a), exploration total (b), and of the head position head normal (c) in s, frequency (F) per 15 min of head position normal (d) and entering squares of the middle area (e) during 15 min of isolation of the cows of the different rearing treatments (A6 $n = 6$, A2 $n = 5$, M2 $n = 9$, MP $n = 6$). Data are presented as box-and-whisker plots, with boxes representing the first and third quartiles, the central bar being the median, and the whiskers extending to the minimum and maximum values except outliers (dots) and extremes (stars).

When using the ball as a novel object, a rearing effect was found on the latency of focusing the ball ($F_{3,25} = 3.35$; $P = 0.040$;) and on the frequency of entering the squares farther away from the ball ($F_{3,25} = 2.38$; $P = 0.100$): Cows of the A6 treatment focused the NO earlier than most cows of the other three treatments (Fig. 5c) and used the area farther away from the ball more often (Fig. 5d). None of the other recorded behaviours differed between treatments when tested with the traffic cone (for all $P > 0.1$; overall mean ± st.dev. in s/test (duration D) or events/test (frequency F) or s (latency L): walking F 2.68 ± 1.06, D 20.58 ± 5.79; head normal F 2.53 ± 0.90, D 2.53 ± 0.90; vigilant F 1.63 ± 0.68, D 15.88 ± 12.64; exploration environment F 0.32 ± 0.58, D 1.38 ± 2.91; exploration object D 5.80 ± 3.22; self-grooming F 0.05 ± 0.23, D 0.12 ± 0.54; focus F 1.89 ± 0.88, D 18.98 ± 12.35, L 2.07 ± 0.64; area farther NO F 2.58 ± 1.39, area middle F 6.53 ± 1.50; area close NO F 1.63 ± 1.07) or ball (walking F 2.38 ± 0.77, D 17.89 ± 3.67; head normal F 2.33 ± 1.37, D 10.66 ± 12.39; vigilant F 1.63 ± 1.28, D 16.40 ± 15.08; exploration environment F 0.29 ± 0.55, D 1.48 ± 3.12; exploration object F 0.75 ± 0.44, D 3.31 ± 3.21, L 63.13 ± 82.78; self-grooming F 0, D 0; focus F 2.17 ± 1.17, D 11.91 ± 7.79; area middle F 5.79 ± 1.93; area close NO F 1.33 ± 1.01).

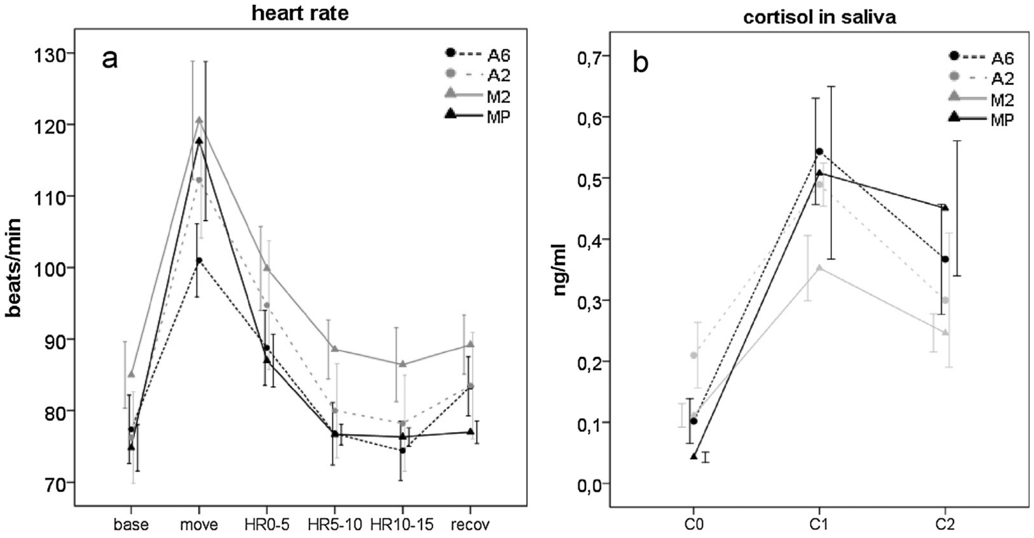

Fig. 4. Mean (±standard error) heart rate (beats/min, a) from baseline (base), moving (move), first (0–5), second (5–10) and third (10–15) 5-min period during isolation and the recovery phase (recov) of the cows of the four rearing treatments (A6 $n = 5$; A2 $n = 4$; M2 $n = 9$; MP $n = 6$). Cortisol concentrations in saliva (ng/ml, b, mean ± standard error) from baseline (C0), directly after isolation (C1) and 5 min after the tested cow was back in the feeding rack (C2) for cows of the different rearing treatments (A6 $n = 6$; A2 $n = 5$; M2 $n = 9$; MP $n = 5$).

3.2.2. Physiological parameter

No significant differences were found in the increase in heart rate for both novel object tests (heart rate increase mean ± SE in beats/min for traffic cone: A6: 49.33 ± 42.39; A2: 18.25 ± 16.99; M2: 10.33 ± 3.31; MP: 21.17 ± 9.68; $Chi^2 = 1.65$; $P = 0.647$; ball: A6: 4.83 ± 2.76; A2: 11.75 ± 8.71; M2: 3.45 ± 1.58; MP: 19.80 ± 12.12; $F_{3,25} = 0.75$; $P = 0.535$). Over all treatments, heart rate

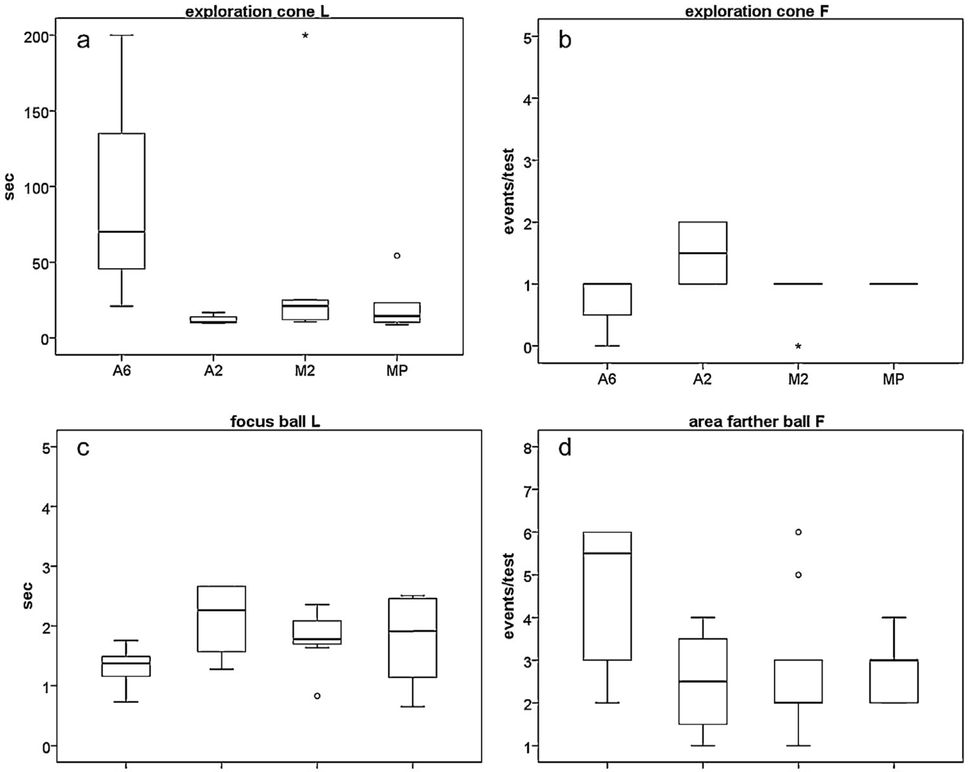

Fig. 5. Behaviour of the cows of the different rearing treatments for (a) latency (L) of exploring the traffic cone and (b) frequency (F) of exploring the traffic cone during the first novel object test (A6 $n = 3$, A2 $n = 4$, M2 $n = 6$, MP $n = 6$), (c) latency (L) of focusing the ball and (d) frequency (F) of entering the squares of the area farther from the ball during the second novel object test (A6 $n = 6$, A2 $n = 4$, M2 $n = 9$, MP $n = 6$).

increase was greater in the first NO test (with the traffic cone) than in the second test with the ball ($Z = -1.966$, $P = 0.049$, $N = 18$).

4. Discussion

4.1. Isolation test

The hypothesis of higher activity in mother reared animals was confirmed for cows with permanent access to their mothers and the cow herd (MP): They walked longer, tended to enter more squares in the middle area of the test arena and tended to show more exploration behaviour than cows of the other three treatments. This is in line with a previous study where cows reared with a foster cow walked longer, stood less in the central area and explored a wall in a 5-min isolation test more often than cows reared individually during the first 8 weeks of life (Le Neindre, 1989b). In addition, Le Neindre (1989b) found correlations between locomotion in the isolation test and social activity in the herd, which reflects high social motivation. Factors contributing to stress in an isolation test are, generally, social isolation and the novelty of the test arena (Boissy and Le Neindre, 1990; De Passillé et al., 1995; Hopster, 1998; Veissier and Le Neindre, 1992), and in group housed cattle social motivation is expected to be the most important factor influencing behaviour in a novel arena test (Forkman et al., 2007). In our study the environmental novelty was limited, as all cows had calved in one of the calving pens of identical design, although some differences existed between the situations during calving and during the isolation test (during the latter: lack of straw and food, closed headlocks and closed windscreens, hindering visual contact with the herd during isolation). Therefore differences between treatments might have been strongly influenced by differences in social motivation. Cows of the MP treatment not only had contact with their mothers but also with the cow herd, offering them a more diverse social environment with more diverse social experiences, which is likely to affect not only social behaviour (Wagner et al., 2012), but also social motivation later in life. Young replacement dairy goats reared with their mothers showed greater group cohesion when integrated into the lactating herd at the age of about 2 years than artificially reared animals, suggesting higher sociality in these animals (Szabò et al., 2013). Calves reared with access to their mothers and the cow herd (comparable to MP in our experiment) showed a higher motivation to rejoin their social partners in an isolation test and were socially more active in a confrontation test than group-reared, automat-fed calves (Wagner et al., 2013). These findings support the notion of stronger social motivation and more active coping with isolation in MP cows.

Nevertheless, isolation is a stressor for gregarious animals such as cattle, even in familiar environments (Duve and Jensen, 2011) and regardless of differences in social motivation. This is reflected in the increase in heart rate and cortisol in saliva in response to the isolation test in most cows. Cows of all treatments had the highest heart rate during moving, and heart rate decreased during isolation. Similarly, in other studies with cattle heart rate decreased during the period of isolation (Boissy and Le Neindre, 1997; Hopster and Blokhuis, 1994; Roth, 2008). Treatment had an effect: MP cows, as well as A6 cows, had the lowest mean heart rate in the isolation test. A lower heart rate during isolation is interpreted to reflect a lower stress level (Hopster and Blokhuis, 1994), but the difficulty with heart rate is that it reflects both emotional stress and locomotor activity. Although MP cows walked longer than cows of the other three treatments, they had a relatively lower heart rate. Further, heart rate of MP cows during recovery did not differ from the last 5 min during isolation (HR10-15), while for all other treatments including A6 heart rate increased. This might indicate that MP cows have developed a more efficient and flexible cardiovascular system due to more locomotion (longer distances to gain access to mother) and more locomotor play behaviour (Waiblinger et al., 2013) in their first three months of life.

Concerning the time course of cortisol levels, MP cows showed the strongest HPA reaction, as there was a sharp increase from baseline to directly after isolation and values stayed nearly as high after returning to the feeding rack (C2, i.e. 20–25 min after the start of the isolation test), while in cows of the other three treatments levels decreased from C1 to C2. Cortisol increase in response to different stressors and after an ACTH administration often peaks around 20 min after the start of the stressor (disbudding: Graf and Senn, 1999; milking or ACTH: Negrão et al., 2004; novel arena test: Veissier and Le Neindre, 1988). Thus, the higher level of cortisol in MP in C2, might indicate a more prolonged and stronger activation of the HPA axis. As calves, at the age of 11 weeks, the same animals had been subjected to an ACTH challenge. Then MP and M2 showed higher cortisol responses than A2 and A6 animals (Roth, 2008) – the results for MP, but not for M2, correspond to our findings of stronger HPA reactivity. The state of the HPA axis may not be the same today as it was more than 2 years ago. Another reason may be found in the differences in social motivation discussed above, which can result in different perceptions of isolation by MP and M2 cows. Despite a similar HPA-axis sensitivity, this may lead to a higher activation of the HPA axis (Mormède et al., 2007; Veissier and Boissy, 2007) in MP cows.

When summarising physiological and behavioural responses to isolation, these cannot be interpreted solely in terms of different levels of stress – higher activity and lower heart rate in MP cows can be interpreted as a lower level of stress, while a higher level of cortisol is generally seen as a sign of higher (physiological) stress levels. However, the individual perception of the situation, its predictability and controllability or, in other words, expectancies about the outcome and the available reactions may trigger different physiological stress responses (Veissier and Boissy, 2007). Interestingly, in MP cows the variability within the rearing treatment was especially low for heart rate, but high for cortisol. Furthermore, the physiological reactions of MP cows correspond to those of a reactive coping style – with low sympathetic but high HPA-axis reactivity (Koolhaas et al., 1999). Although our small sample size requires cautious interpretation, this aspect merits further study.

4.2. Novel object test

The majority of animals explored the novel objects, except two cows (one of the M2 and one of the A6) with the traffic cone and six cows (three of the M2 and three of the A6) with the ball. A6 cows had a higher latency and a lower frequency of exploring the traffic cone, focused the ball earlier and used the areas farther away from the ball more often, i.e. showed avoidance – these behaviours are in line with an interpretation as a higher level of fear (exploration: Boissy and Bouissou, 1995; Vandenheede et al., 1998; focussing: Winther Christensen et al., 2005; avoiding: Van Reenen et al., 2005). However, as sample size was very small with the traffic cone (only three cows representing the A6 treatment), these results should be interpreted with caution. Further, no differences between treatments were observed for other behavioural parameters and heart rate. Heart rate was higher during the novel object test than baseline in all but one animal (A2) when tested with the traffic cone and in all but seven animals (2 A6, 1 A2, 2 M2, 2 MP) when challenged by the ball. As baseline included not only walking in the alley but also a part of milking (i.e. lower locomotor activity and lower heart rate, e.g. Hagen et al., 2005; Von Borell et al., 2007), the increase in heart rate might be explained by a higher locomotor activity. It is nevertheless likely that a psychological component was involved: in all cows, the increase in heart rate was lower in the second novel object test, which can be explained by habituation due to repeated testing (Forkman et al., 2007; Welp et al., 2004). However, heart rate increase was moderate in most animals, suggesting a relatively low level of arousal, probably because of the familiar environment. Moreover, the cows were able to show some form of avoidance behaviour by moving farther away and thus had some control over the situation, which reduces stress (Veissier and Boissy, 2007; Wiepkema and van Adrichem, 1986). Therefore the level of fear or arousal might have been too low to allow for a differentiation between physiological reactions of different rearing treatments. Using similar tests, Gibbons et al. (2009) concluded that these tests do not allow for a reliable temperament assessment. In sum we could not confirm the hypothesis of lower stress reactions for mother reared cows in the novel object test.

4.3. General discussion

The isolation test was chosen as a social challenge, while the novel object tests were deliberately performed in the familiar physical and social environments but without involving other cows – thus avoiding social aspects during testing. While in the social challenge (isolation) MP cows differed most significantly from the other three treatments, in the non-social novel object test this was the case with the A6 treatment. As discussed above this points to the fact that social motivation or sociality can be seen as the underlying causes of differences between MP and other treatments thus being evident in social challenges only. By contrast, contact with the mother twice a day for only 15 min (M2 cows) and no contact with the herd seemed to have no effect on social motivation, although there was a stress-reducing effect of this type of rearing during integration into the cow herd (Wagner et al., 2012).

Arguing that the main difference between MP cows and cows of other treatments can be explained by social factors, one would expect that they would not differ from the other three groups in the responses to novel objects. While this was confirmed, it remains unclear why A6 cows showed more fear of novelty than the other three treatments. The only difference from the A2 treatment was the number of meals during rearing treatment. Yet a greater number of meals may even result in a higher level of frustration as compared to twice-daily meals, because the amount of milk per meal is lower and thus unsatisfied hunger and sucking motivation may increase (Keil and Langhans, 2001; Miller-Chuson et al., 2012; Roth et al., 2009b). Further studies with a higher number of animals are needed to examine the possible long-term effects of such differences in rearing management.

5. Conclusion

The results indicate that the rearing conditions during the first twelve weeks of life have long-term effects on the challenge responses of 2.5-year-old cows. Permanent access to the mother and the adult herd seems to increase sociality triggering higher activity during the social challenge of isolation. Further the physiological reactions to isolation with lower sympathetic and higher HPA-axis reactivity might indicate that these cows have developed a reactive coping style. However, rearing with the mother did not affect reactions to a novel object in the home environment.

Conflict of interest

The authors declare that there are no conflicts of interest.

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