

Maternal behavior in a genetic animal model of absence epilepsy

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In this study we quantified maternal behavior in genetic epileptic rats with deficiencies in the DA system known to be involved in maternal behavior in order to assess whether these rats have disturbances in maternal care. Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats, a genetic model for generalized absence epilepsy and Wistar rats were compared. Maternal behavior parameters and activity scores were quantified from post-natal day (PND) 4–6 in an open field in the presence of pups. WAG/Rij rats had less approaches to the pups and higher latencies of approaching them compared to Wistar, while locomotion showed a different pattern over days. This confirms the hypothesis that animals with absence epilepsy show more poor maternal behavior and that these effects cannot be solely attributed to differences in locomotor activity. It is proposed that the reduction in maternal care is attributed to low activity in the mesolimbic DA system.

Key words: dopaminergic system, maternal behavior, WAG/Rij rats, genetic models, absence seizures

INTRODUCTION

It's well-known that parental motivation in mammals is one of the most important components of animal behavior. The initiation and regulation of maternal behavior is controlled by different hormonal and neuromodulatory brain systems, including the dopaminergic (DA) system (Leckman and Herman 2002, Numan 2007).

Evidence in favor of a role of the DA system in maternal behavior comes e.g. from pharmacological studies. Systemic injections of D1 (SCH 23390) and D2 (clebopride)-like antagonists show deficits in maternal behavior immediately postpartum but only clebopride reduces the full expression of maternal behavior (retrieving, grouping and crouching over pups) on the 7th day after delivery (Byrnes et al. 2002). Central bilateral intra-accumbens microinjections of the D2 receptor antagonist pemozide demonstrated longer

latencies for parameters of maternal care (Silva et al. 2003) and autoradiographic studies show elevated levels of D1 and D3 receptors in the shell region of the nucleus accumbens in high-licking/grooming dams. A previous study in our laboratory showed that intermittent administration of a low dose of the mixed DA antagonist haloperidol (0.1–0.2 mg/kg, which does not disturb motor activity in Wistar rats) to female rats before testing decreased the number of approaches to the pups and increased latencies of maternal behavior parameters (Dobryakova et al. 2006). The outcomes of these studies show that the DA system including the mesolimbic part is involved in the expression of maternal behavior.

The purpose of the present experiment was to assess differences in quantitative behavioral parameters of maternal behavior between Wistar Albino Glaxo/Rijswijk (WAG/Rij) and control Wistar rats. The WAG/Rij strain is one of the well validated genetic models for absence epilepsy in humans (van Luijtelaar and Coenen 1986, Depaulis and van Luijtelaar 2006, van Luijtelaar and Sitnikova 2006). Interestingly, some peculiarities of the DA system have been described in

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WAG/Rij rats. WAG/Rij show more and longer periods of catalepsy after audiogenic stimulation for which the striatum might be involved (Kuznetsova et al. 1996), behavioral studies in WAG/Rij pointed towards a high DA activity in the nigra-striatal system and low DA activity of the mesolimbic system (de Bruin et al. 2001). The latter conclusions were based on the sensitivity for apomorphine induced gnawing and the novelty/amphetamine ambulation score in the open field. Analysis of the different brain structures showed modified density of DA receptors: WAG/Rij rats have a lower number of D1-like DA receptors in the nucleus accumbens and increased density of D2-like receptors in the frontal and parietal cortical regions compared to non-epileptic ACI rats (Birioukova et al. 2005). The low reactivity of the mesolimbic DA system in WAG/Rij rats and the important role of the mesolimbic system as a reward system for rats, including reward experienced in maternal care (Lavi-Avnon et al. 2007), predict that WAG/Rij rats will show less maternal behavior than control rats.

METHODS

Subjects

Ninety days-old Nulliparous Wistar and WAG/Rij female rats, born and raised at the vivarium of Biological Psychology, Radboud University Nijmegen, were used. Animals were housed before mating in pairs in a room with 12-hours light-dark cycle (light on at 08.00 AM) with food and water always *ad libitum* available, the cages were enriched with Enviro Dry. After two weeks with Wistar and WAG/Rij male rats, pregnant females were housed individually in the same macrolon cages. The protocol was approved by the Medical-Ethical Committee of Radboud University Nijmegen (RU-DEC), all efforts were done to minimize the amount of discomfort for the animals.

Primiparous rats, thirteen WAG/Rij and ten Wistar rats with their own litters were used. The average litter size of WAG/Rij rats was 7.5 ± 0.64 pups (range: 4–11), the numbers of female and male were 4.2 ± 0.5 and 3.4 ± 0.4 , respectively (mean \pm SEM). The average litter size of Wistar rats was 9 ± 0.8 pups (range: 5–13); numbers of female and male were 5.8 ± 0.6 and 3.2 ± 0.6 , respectively (mean \pm SEM). All litters were included in the study independently of pup's number.

Procedures

Maternal behavior testing took place from post-natal day (PND) 4 till PND 6 in the "open field" test. The day of birth was chosen as PND 0. Maternal behavior is classically tested in the home cage, we have developed an alternative approach by challenging the mothers by placing 3 randomly chosen pups from her own litter in the centre of a round arena. In this way we provoke maternal behavior. We evaluated behavioral parameters such as latency of the first approach of the mother to the dish with pups, number of approaches, number of transfers of the pups and latency of transfer of the first pup (Dobryakova et al. 2006).

The Open Field is a round arena, 80 cm in diameter, divided into peripheral (Zone 1), middle (Zone 2) and central (Zone 3) sectors. The arena was encircled with a wall (height 40 cm). Maternal behavior was tested under bright and under red light. Two electric bulbs provided 180 lx in the centre and 140–160 lx along the edges of the arena and 15 W red illuminations providing 4.1 lx in the centre and 2.5–3.5 lx along the edges.

The first observation occurred under red light. Each rat was placed into the arena (near the wall) in the presence of an empty Petry dish in the centre of the arena and its activity (distance moved, velocity, duration and frequency of movements, heading and rearing) was observed and recorded for 2 min on video using the Ethovision software (Noldus, Wageningen, NL). Analysed were horizontal (total number of sectors entered) and vertical activity (rearing), time of grooming and the latency of leaving Zone 1 of the arena, total distance moved, movement frequency and movement duration.

Next, the mother was removed from the arena and stayed in her home cage for 1 min. The second observation period in the arena started and maternal reactions of the pups were monitored. First, behavior was recorded for 2 min under red light. The test began when 3 pups were placed in the Petri dish. In this part of the experiment the following parameters were recorded: latency of the first approach to the dish, number of approaches, number of transfers of the pups and latencies to transfer of the first pup from the Petri dish to the wall. During each test session 3 randomly selected pups (independently of sex) from the mother's litter were placed in the Petry dish in order to induce rat's maternal behavior.

The third observation period in the arena started again after 1 min rest during which the mothers stayed in their home cage, maternal behavior was now observed under bright light. The same pups were used in the same dish, than the mothers were placed back into the arena. Here we recorded for 2 min the same parameters of maternal behavior but under bright light conditions.

Statistical analysis

An analysis of variance (ANOVA) with 'strain' as between subject factor and days (3 levels) and light (red-white) as within subject factor was used for the evaluation of maternal behavior, activity in the open field was evaluated with a two way ANOVA with days and strain as within and between subjects factors respectively. *Post-hoc* test were LSD tests.

RESULTS

Maternal behavior

The 3-way ANOVA showed that Wistar rats showed more approaches to the pups than WAG/Rij rats ($F_{1,21}=34.42$, $P<0.001$), see Fig. 1.

There was also a day effect ($F_{2,42}=7.72$, $P<0.001$): there were less approaches on PND 4 than on PND 5, an illumination effect ($F_{1,21}=12.94$, $P<0.01$), more approaches in the light and a day \times illumination interaction ($F_{2,42}=3.32$, $P<0.05$), see Fig. 2. *Post hoc* tests showed that approaches increased from PND 4 to PND 5 for bright light condition, and that the increase continued for the light condition on PND 6, but not for the dark condition. The difference between light and dark was significant on PND 5 ($P<0.05$).

A significant strain effect was found for latencies of the first approach to the Petri dish ($F_{1,21}=20.2$, $P<0.001$), the Wistar rats were quicker than WAG/Rij dams, see Fig. 3. The ANOVA showed also a significant day ($F_{2,42}=6.23$, $P<0.01$) effect, latencies were longer on PND 4 than on PND 5, and an illumination effect ($F_{1,21}=11.81$, $P<0.01$), the latencies were longer in the dark condition. There was also an interaction between light and strain ($F_{1,21}=8.93$, $P<0.01$), as depicted in Fig. 4: WAG/Rij's were slow when tested under red light and significantly faster under bright light, Wistar's were faster than WAG/Rij rats under both light conditions.

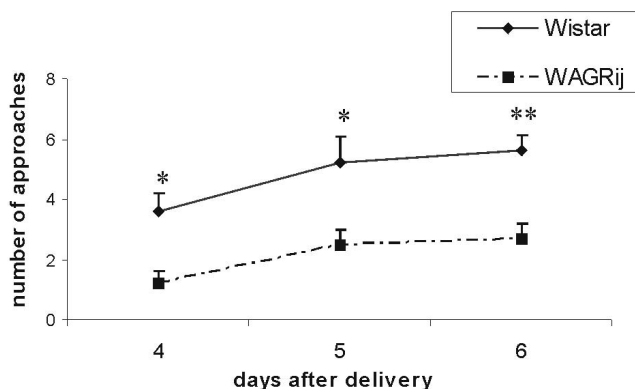


Fig. 1. Maternal behavior parameters in the open field test in WAG/Rij and Wistar rats at PND 4–6. Number of approaches (Mean and SEM) to the pups are represented on the Y-axis and days (PND 4–6) on the X-axis; ** $P<0.001$, * $P<0.01$, Wistar versus WAG/Rij, strain difference.

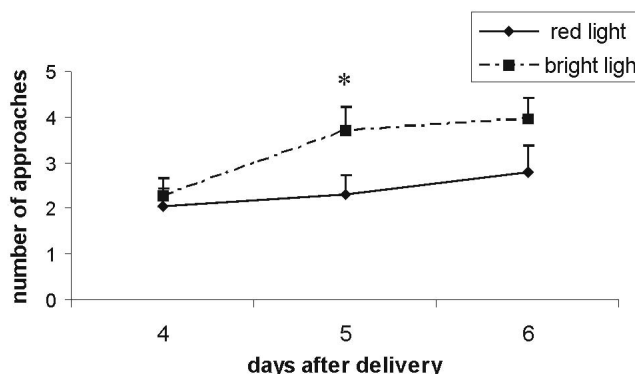


Fig. 2. Number of approaches (Mean and SEM) in the open field test in WAG/Rij and Wistar rats at PND 4 – PND 6 under red and bright light. * $P<0.05$.

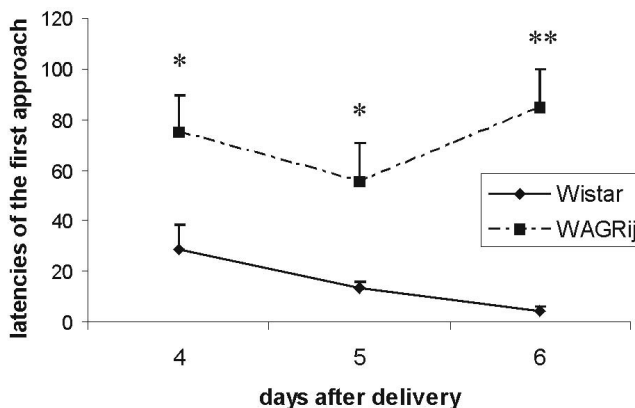


Fig. 3. Latencies of the first approach to the pups (in s) (Mean and SEM) on the open field test in WAG/Rij and Wistar rats under red light conditions, * $P<0.05$, ** $P<0.01$: WAG/Rij versus Wistar, strain difference.

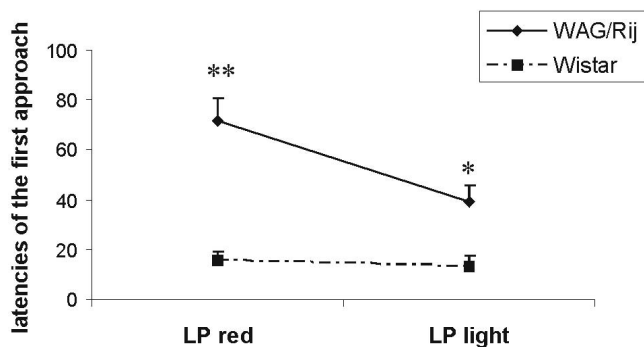


Fig. 4. Latencies of the first approach to the pups (in s) (Mean and SEM) on the open field test in WAG/Rij and Wistar rats under red and bright light. Number of approaches to the pups is represented on the Y-axis. Effect of bright and red light on the latencies are represented on the X-axis; * $P<0.01$, ** $P<0.001$, strain difference.

A significant strain effect was also found for the number of transfers of the pups ($F_{1,21}=17.63$, $P<0.001$): Wistar dams showed more transfers compared with WAG/Rij dams.

Activity

Both strains showed a decrease of rearing over days ($F_{2,42}=48.18$, $P<0.001$), data not shown. WAG/Rij rats showed less rearing than Wistar rats ($F_{1,21}=4.92$, $P<0.05$), this effect persisted over days.

An increase over days was found for grooming ($F_{2,42}=3.48$, $P<0.05$), a decrease over days for total number of sectors entered and leaving Zone 1 ($F_{2,42}=71.35$, $P<0.001$; $F_{2,42}=6.28$, $P<0.01$, respectively). A significant interaction between strain and days was

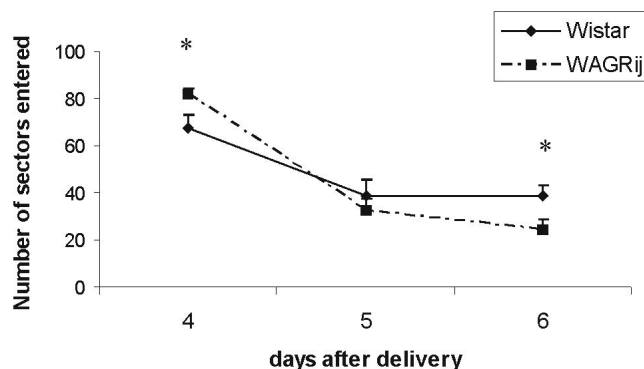


Fig. 5. Number of sectors entered (Mean and SEM), on the open field test in WAG/Rij and Wistar rats at PND 4–6. Number of sectors is represented on the Y-axis. * $P<0.05$, strain difference.

found for total distance moved ($F_{2,42}=6.73$, $P<0.01$). Post hoc tests showed that WAG/Rij were more active ($P<0.05$) than Wistar rats on PND 4 the first test day, Wistar were more active ($P<0.05$) than WAG/Rij rats on PND 6, as depicted in Fig. 5.

The ANOVA showed strain differences for movement frequency. Wistar rats showed more frequent movements ($F_{1,21}=6.28$, $P<0.05$) than WAG/Rij rats. Furthermore, the ANOVA showed significant day effect ($F_{2,42}=5.99$, $P<0.01$) for movement duration. Both strains showed a decrease of movement duration over days.

Correlations between locomotor activity (rearing, total distance moved, movement frequency) and maternal behavior parameters (latencies, approaches and transfers) were computed for both strains separately. The correlations were between 0 and 0.3 and not significant.

DISCUSSION

Most relevant is that WAG/Rij rats had less approaches to the pups and were slower in approaching their pups compared to Wistar rats. This demonstrates that WAG/Rij rats show less maternal behavior. Reduced maternal behavior was predicted based on abnormalities in the DA system of WAG/Rij rats (Kuznetsova et al. 1996, de Bruin et al. 2001). The reduction, in maternal behavior of WAG/Rij rats might be related with other behavioral differences between the two strains, a passive behavioral strategy in stressful situations has been found in WAG/Rij rats in comparison with Wistar (Sarkisova et al. 2003, Midzyanovskaya et al. 2006).

Wistar rats didn't show significant differences between the two illumination conditions, while WAG/Rij rats did. WAG/Rij rats were slower under red light compared to bright light conditions. The lack of differences in parameters of maternal care within the Wistar rats suggests that mild aversive stimulation (the presence of bright light) does not affect maternal behavior of Wistar rats, however, the bright light as a stressor might have motivated WAG/Rij rats to be quicker in reaching out for the pups. Another possibility is that WAG/Rij rats were slow in adapting: the first challenge to retrieve the pups was done under red light, the second under bright light (van Luijcklaar et al. 2007).

Another related explanation for the reduced maternal care in genetic epileptic rats could be that rats of the WAG/Rij strain have some symptoms of depres-

sion. They show enhanced immobility in Porsolt's forced swimming test ("behavioral despair") and reduced preference for sugar water (anhedonia), both symptoms disappear after chronic but not acute treatment with desipramine (Sarkisova et al. 2003, van Luijtelaar et al. 2007). Interestingly, also Flinders rats show similar depressive symptoms as WAG/Rij rats and also Flinders rats, which are considered as a genetic model of depression exhibit abnormalities in maternal care (Lavi-Avnon et al. 2005). Therefore, we propose that the low DA reactivity of the mesolimbic system (de Bruin et al. 2001) and specifically the nucleus accumbens in WAG/Rij rats might explain the strain difference in maternal care, we assume that WAG/Rij dams are less rewarded by their pups compared to Wistar dams and that changes in the reward system are accompanied by depressive symptoms.

WAG/Rij rats showed consistently poorer maternal behavior over the testing days compared to Wistar rats, for latency to approach the pups and for number of approaches, while there were no consistent strain differences for total distance moved, the most reliable indicator of total amount of locomotor activity. In fact an interaction between strain and days was found for this variable: sometimes Wistar were more active, sometimes WAG/Rij rats were more active. These findings demonstrate that there is no simple relationship between total distance moved and maternal behavior and that maternal behavior and horizontal locomotor activity are differently sensitive for experimental manipulations such as strain and repeated testing over days in the same arena. Moreover, there were no significant correlations between parameters of maternal behavior and locomotor activity. However, consistent strain differences were found for rearing and movement duration. Therefore, it can be assumed that maternal behavior and some aspects of activity such as vertical movements might be partly correlated with maternal behavior. However, considering that total distance moved and maternal parameters are controlled by independent experimental parameters, it can be concluded that the differences in maternal behavior cannot be attributed solely to differences in activity. The latter conclusion and the effects on which this is based are in agreement with the outcomes of our previous study with haloperidol in Wistar rats (Dobryakova et al. 2006): haloperidol did not affect locomotor activity score but disrupted maternal care.

It is acknowledged that also the reaction of the pups towards the removal of the mother from her litter and or the reaction of the pups when being removed from the home cage and placed in the Petri dish could have played a role in the altered maternal care of the WAG/Rij mothers. Body weight, temperature, ultrasonic vocalization and the physical state of pups could influence maternal behavior of the rat dams (Hashimoto et al. 2001, Lavi-Avnon et al. 2005). Therefore, reduced maternal care can be due to both the maternal or pups strain characteristics as response to the experimental manipulations.

Our study showed that rats of the Wistar strain were more active in the first locomotor activity test (first 2 min in the Open Field) and had more rearings compared to WAG/Rij rats; this difference in explorative behavior is in agreement with Midzyanovskaya and colleagues (2005) data. The higher level of total distance moved on PND 4 (1st day of testing) in Wistar rats compared to WAG/Rij rats is in agreement with the higher amount of exploratory behavior in the elevated plus-maze and in the hole board when Wistar and WAG/Rij rats were compared (Kliueva et al. 1999).

This lower activity level of WAG/Rij rats might be due to high basic anxiety level of WAG/Rij rats when tested in new environment (van Luijtelaar et al. 2007). However, the anxiety level of the WAG/Rij rats decreased rapidly in the course of the experiment and on PND 6 locomotor activity of WAG/Rij rats was higher than in Wistar's. Alternatively de Bruin and colleagues (2001) showed that apomorphine-susceptible rats (APO-SUS) and WAG/Rij rats had higher novelty-induced level of locomotion (in a large open field) compared to apomorphine-unsusceptible (APO-UNSUS) and rats of the ACI strain. Therefore it seems that the control strain is one of the crucial variables in strain comparative studies concerning the interpretation of the locomotor activity data in terms of anxiety, an increase or decrease.

WAG/Rij rats show hundreds SWDs per 24 h, and the number of SWDs might be even higher than normal considering the altered endocrinological state during and after pregnancy (Tolmacheva et al. 2004). It is also known that DA activity in the ventral striatum is critical for the control of absence seizures since local administration of DA agonists in the core of the nucleus Accumbens reduce absence seizures (Deransart et al. 2000). These two factors facilitate the occurrence

of absence seizures. However, it is not very likely that WAG/Rij rats will have absence seizures during the behavioral testing. Absences preferably occur during periods of low vigilance, such as passive wakefulness and drowsiness and are actively suppressed during an operant learning task (Coenen et al. 1991, van Luijtelaar et al. 1991). Therefore it is unlikely that the quality of maternal behavior is decreased due to the presence of seizures during the challenge. However, this can be experimentally verified.

An equal interesting topic is whether epilepsy *per se* could be the cause of the quality of the maternal care. There are no human studies on this issue, but the comparison with the Flinders rats might give a first clue. To the best of our knowledge, Flinders rats have no spontaneous absence or other seizures, not they have epilepsy, while the quality of the maternal care is also reduced. This strongly suggests that it is merely the depressive-like symptoms and not the seizures or this type of epilepsy that are responsible for the reduced quality of maternal behavior in WAG/Rij rats.

CONCLUSION

Maternal behavior of WAG/Rij rats is poorer than in Wistar. It is proposed that this strain differences in maternal behavior are due to the low DA activity in the mesolimbic system in genetic epileptic rats.

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