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Research report

Neonatal whisker trimming in WAG/Rij rat pups causes developmental delay, encourages maternal care and affects exploratory activity in adulthood

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ABSTRACT

WAG/Rij rats are genetically predisposed to absence epilepsy. Maternal behavior in WAG/Rij female rats is known to differ from that in non-epileptic females. We hypothesize that (1) mother's behavior may be changed as response to changes in pup's conditions; (2) sensory deprivation at the neonatal age affect learning and behavior in adulthood. All whiskers in WAG/Rij rat pups were trimmed daily during PN1-PN8. Maternal behavior was examined during the same period. It was found that in the control group, WAG/Rij females often demonstrated abnormally long (> 1 min) repetitive purposeless stereotypical actions that were roughly classified as compulsive-like behavior. Mothers of the trimmed pups showed less compulsive-like behavior and more intensively interacted with pups and built better nests. Rat pups in the trimmed group had lower body weight on PN7-PN19 as compared to the control. In the trimmed group, maturation of motor skills and early behavioral patterns (i.e. walking, grooming, vertical activity, motor functions of forelimbs) showed 1–2 days delay in comparison to the control. At the age of 2–2.5 months, the locomotor activity in the trimmed rats differed from the control, but the level of anxiety was the same (the open field and the elevated plus maze). At the age of 6 months, the trimmed and control rats showed no differences in conditioned avoidance learning test, therefore, neonatal whisker trimming did not influence fear-based learning abilities in adulthood. It is hypothesized that an enhanced maternal care is capable to modulate development of brain functions in sensory deprived progeny.

1. Introduction

Mother-infant bonding is one of the most powerful evolutionary tools for children's survival. Maternal care effectively modulates development of neuronal functions in progeny (reviewed in Nephew and Murgatroyd, 2013). In mammals, maternal care-giving behavior continuous all over the day, i.e., it takes up to 18 h per day in lactating rat (Perez-Torrero and Rubio-Navarro, 2015). Deficiency of maternal care is deleterious for child development. It is well established that mother's neglect or maltreatment cause emotional abnormalities such as anxiety and depressiveness, cognitive impairments, higher stress susceptibility and total ill-being in upgrown children (Taylor et al., 2011; Spratt et al., 2012) as well in animals (Borrow and Cameron, 2017; Callaghan and Richardson, 2013; Vetulani, 2013). Ill-being of mother is the major reason of maternal childhood maltreatment. Mothers who suffer from chronic illness have limited abilities to provide appropriate care for their children (Bågedahl-Strindlund, 1988; Sieh et al., 2010) in a manner similar to animals. For example, WAG/Rij rats with genetic predisposition to absence epilepsy showed less maternal care in comparison to control Wistar dams (Dobriakova et al., 2010, 2014). Previously in the cross-fostering study we showed that improvement of maternal care caused a reduction of genetically predetermined absence epilepsy in WAG/Rij rats fostered by Wistar dams (Sitnikova et al., 2015, 2016). Similar results were obtained in APO-SUS rat model for schizophrenia, in which cross-fostering to phenotypic counterpart APO-UNSUS mothers led to normalization of its extreme behavior (Van Vugt et al., 2014).

In the current study we examine developmental aspects of behavior in well recognized genetic model of absence epilepsy, WAG/Rij rats (Coenen and Van Luijtelaar, 2003). Epileptic activity in WAG/Rij rats is known to be fully developed at the age of 5–6 months (Coenen and van Luijtelaar, 1987). Behavioral manifestations of absence seizures in WAG/Rij rats are reminiscent of human absence epilepsy such as immobility, loss of awareness and responsiveness (i.e., behavioral "absences"). Therefore, it is likely that absences in lactating dams may interrupt activity and worsen the quality of maternal care. In WAG/Rij females, epileptic activity is known to be present throughout the reproductive cycle (Tolmacheva et al., 2004; Kovács et al., 2017). During

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weaning period, epileptic activity in WAG/Rij females is known to be influenced by the presence/absence of pups. For example, on 12–13 postpartum days, the number of epileptic discharges in WAG/Rij females did not change during 4-h separation from pups, but an increased number of epileptic discharges was found in females after reunion with pups (Kovács et al., 2017). In addition to that, an increased epileptic activity was found in females suckling in kyphosis posture (Kovács et al., 2017). Therefore, the pup-directed activity in mothers with genetic predisposition to absence of epilepsy influences intensity of seizures. However, it is not known whether the quality of maternal care correlates with the severity of epileptic activity.

All infants are attached to their primary caregivers. Newborn rats primarily use tactile and olfactory sensory systems to find their mothers (Hofer and Sullivan, 2001). Facial (or mystacial) vibrissae is the main tactile sensory input in rats. Even though whisker system starts to actively participate in perception only at postnatal days 11–13 (Welker, 1964; Landers and Philip Zeigler, 2006), rats are born with whiskers which are involved in feeding behavior and mother-infant communication (Sullivan et al., 2003). Tactile cues in mammalian newborns are crucial for nipple attachment and consequently for successive feeding. For example, nipple attachment in weanling rat pups is known to be impaired after sensory deprivation of whisker pad with lidocaine injections or by whisker trimming (Kenyon et al., 1982; Sullivan et al., 2003). In the current study we modeled neonatal tactile sensory deprivation by means of bilateral daily trimming of all whiskers in WAG/Rij rat pups during PN1-PN8 and controlled their body weight.

Tactile discrimination functions of whisker system is known to be impaired due to whisker trimming during the 1 st week of life (e.g., Carvell and Simons, 1996; Chu et al., 2013). For instance, in gap-crossing test, it was shown that whisker trimming in Wistar rats from PN0 to PN3 reduced whisker-specific tactile functions on PN30-PN35 (Lee at al., 2009). Probably neonatal whisker trimming affects only whisker-oriented learning, and does not influence general abilities to learn. Here we tested this hypothesis using fear-based learning (active avoidance test) that did not require whisker-oriented tactile discrimination.

Manifestation of anxiety phenomenon in rats with absence epilepsy is rather questionable. Among WAG/Rij rats, the high level of anxiety was found only in subjects with audiogenic seizures (Sarkisova et al., 2005). Although the Genetic Absence Epilepsy Rat from Strasbourg (GAERS, a valid animal model of absence epilepsy, similar to WAG/Rij) demonstrated an increased level of anxiety in the elevated plus maze and the open field in comparison to non-epileptic control rats (Jones et al., 2008; Powell et al., 2014). For the other hand, the level of anxiety in rats may be modulated by sensory inflow from their whiskers during the first weeks of postnatal life. It is known that trimming of whiskers in mice for 10 days after birth lead to an increased activation of amygdala neuronal circuitry engaged in anxiety (Soumiya et al., 2016). Daily whisker trimming from PN9 to PN20 resulted in reduced emotional reactivity to potentially dangerous stimuli at the adolescent age (Shishelova, 2005). Consequently we suppose that the level of behavioral anxiety is increased in rats after neonatal whisker trimming. In order to examine the putative effect of neonatal whisker trimming on locomotor activity and anxiety in young adult WAG/Rij rats, we used standard tests, such as the elevated plus maze and open field.

A great number of studies are focused on harmful effects of weakening of maternal care on infant's development (e.g., Callaghan and Richardson, 2013; Rüedi-Bettschen et al., 2006; Vetulani, 2013; reviewed in Kaffman and Meaney, 2007), but the role of infant in mother-infant interactions is almost ignored. It remains unknown at what extent infants influence behavior of their mothers. We hypothesize that mother's behavior may be changed as response to changes in pup's conditions. Complete trimming of whiskers deprives rat pups from tactile information, but it may also stimulate their mothers for additional care. Inasmuch as active whisking begins on postnatal days 11–13 (Welker, 1964), sensory information from vibrissae during the 1 st postnatal week is known to be necessary for maturation of somatosensory system and behavior (Sullivan et al., 2003; Lee at al., 2009). Here we investigate natural forms of pup-oriented maternal behavior in natural conditions at their home cage. The current study aims to determine the effect of whisker trimming of rat pups (1) on maternal behavior of their mothers, (2) on development of their motor skills and early behavioral patterns in the childhood, (3) on behavioral features and learning in the adulthood.

2. Methods

2.1. Animals

The study was performed in WAG/Rij rats that were bred and maintained at Institute of Higher Nervous Activity and Neurophysiology RAS (Moscow, Russia) and approved by our institution's animal ethics committee.

The progeny was obtained from 14 primiparous female WAG/Rij rats at the age of 4–6 months. The day of delivery was defined as P0. On the next day after birth the number of pups per litter was reduced to 7–9 and the number of males and females was set to equal as close as possible. The body weight of pups was measured daily.

In the experimental group (4 litters, 35 pups) each subject underwent daily whisker trimming between PN1 and PN8. The mothers were removed from the home cage before whisker trimming in pups. The trimming lasted approximately 10–20 s per pup (1.5–2 min per litter). Pups were held in one hand to limit as much as possible any movement (especially of the head). All whiskers on the both sides of the snout were trimmed directly at the skin surface. In the control group (4 litters, 33 pups), trimming was imitated by means of gentle mechanic stimulation of the surface of whisker pads with scissors. Durations of sham and real trimming was the same.

On PN26 rat pups were separated from mothers and housed together with same-sex littermates (2–5 individuals per cage).

2.2. Maternal behavior

Before whisker trimming/sham procedure, the lactating female was removed from the home cage and put into a clean cage. After about 1.5–2 min the female was returned to the home cage and its behavior was video recorded by Canon IXUS 220HS in a period between 10 and 12 a.m. Recordings were made right after whisker trimming. This short lasting maternal separation is known to encourage natural pattern of maternal behavior (Shishelova and Raevskii, 2016). Mother's behavior was video recorded for 10 min daily from PN1 to PN8 and analyzed offline. We investigated the following parameters of pup-oriented maternal behavior and nest-building activity.

1 General parameters of maternal behavior:
- the latency of the first approach to the pups, seconds;
- the latency of the beginning of the pup currying, seconds;
- the duration of pup currying, seconds;
- percent of carried pups from the total number of pups in the litter;
- the total duration of active contacts with pups per minute (such as pup licking, sniffing, carrying), seconds;
- the latency of the first return to the nest, seconds;
- the latency of the return to the nest after which the dam stays in the nest for more than a minute (prolonged stay), seconds.

1 Strategy of pup-oriented behavior:
Strategy of pup-oriented behavior
Strategy 0 – a dam did not carry pups;
Strategy 1 – a dam carried some pups to another place and does not return them back to the nest;
Strategy 2 – a dam carries the pups over the cage and returns back them to the nest;