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Research report

Blocking the postpartum mouse dam's CB1 receptors impairs maternal behavior as well as offspring development and their adult social–emotional behavior

Michal Schechter[a,c,*], Albert Pinhasov[a], Aron Weller[d], Ester Fride[a,b]

[a] Department of Molecular Biology, Ariel University Center of Samaria, Ariel, Israel
[b] Department of Behavioral Sciences, Ariel University Center of Samaria, Ariel, Israel
[c] Faculty of Life Sciences, Bar Ilan University, Ramat-Gan, Israel
[d] Department of Psychology, Bar Ilan University, Ramat-Gan, Israel

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ABSTRACT

Maternal care is the newborns' first experience of social interaction, which affects their development and social competence throughout life. For the first time, we investigated the involvement of the endocannabinoid system (ECS) in mother–infant interaction in mice. We found that blocking the dam's CB1 receptors (CB1R) by the antagonist/inverse agonist rimonabant (SR141716) during postpartum days 1–8 affected maternal behavior as well as the social and emotional characteristics of the offspring as adults. Pups of rimonabant treated dams (RTD) had lower body weight during the first week of life and emitted fewer ultrasonic vocalizations (USVs) than vehicle treated dams (VTD). RTD crouched less over their pups and exhibited delayed pup retrieval. In Y-maze preference tests conducted at weaning age, females and males of both groups preferred their dam over milk. Males and females of RTD preferred dam over pup and pup over milk as opposed to the control group. At the age of 2.5 months, males of RTD displayed less motor activity. In the social behavior test, RTD male and female offspring were both more active, showing higher levels of active social interaction and rearing. These results indicate that the ECS is crucial for establishment of maternal behavior during the first postpartum week, with a long-term impact on the offspring's socio-emotional development.

1. Introduction

Maternal care is the newborns' first experience of social interaction, which affects their development and social competence throughout life [1]. Care and protection of infants determine the quality of the attachment process. Bowlby described a mother's love in infancy and childhood as important for mental health as are vitamins and proteins for physical health [2]. Furthermore, early postnatal development has long-lasting influences on the behavior of individuals in adulthood [3]. In animal models, alteration in maternal behaviors such as grooming and licking during the first 10 days postpartum affects the social and emotional behavior and relationships of the offspring as adults [4,5].

Maternal behavior in rodents is a complex set of activities, including nest repair, sniffing and exploration of pups, licking, pup retrieval, grooming, and nursing (arched-back nursing, prone nursing, blanket nursing) [6]. Mouse pups emit high frequency ultrasonic vocalizations (USVs) (20–70 kHz) to communicate with their mothers. It has been suggested that the quantity of USVs reflects the quality of maternal behavior in rodents [7].

Several hormones, neuropeptides and neurotransmitters are involved in the regulation of maternal care. These include mainly estrogen, progesterone, prolactin, oxytocin, vasopressin and dopamine [8–10]. Studies in rats and sheep suggest that oxytocin (OXT), a hypothalamic neuropeptide hormone, is involved in stimulating the rapid peripartum onset of maternal behavior [11–14]. Although intracerebroventricular (i.c.v.) injection of OXT antagonists is effective in preventing the induction of maternal behavior [15], once maternal behavior has been induced, i.c.v. injection of an OXT antagonist did not reduce maternal behavior [15,16]. Thus, other mechanisms are involved in the expression of maternal behavior.

The 'endocannabinoid system' (ECS) is present in many brain areas and is involved in basic processes including feeding, reproduction, addiction, stress and energy homeostasis [17–24]. Endocannabinoids are endogenous fatty acid-derived molecules that activate cannabinoid receptors (CB1, CB2) in the brain and organ systems [25]. The two major endocannabinoids are anandamide (AEA) and 2-arachidonyl glycerol (2-AG). High 2-AG levels in the brain have been observed immediately after birth, followed by a drastic decrease during the later stages of postnatal

* Corresponding author at: Ariel University Center, Department of Molecular Biology, Building 6, Ariel, 43000, Israel. Tel.: +972 54 2313808.
E-mail address: michalsc@ariel.ac.il (M. Schechter).

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development [26]. Levels of 2-AG in the brain of pups reach their highest levels together with OXT levels [27]. In dams, OXT enables the onset of maternal behavior [28]. Moreover, the newborn infant's ECS is associated with milk sucking [29–32]. Genetic deletion and pharmacological blockade of CB1 receptors (CB1Rs) on the very first days of life, lead to diminished milk sucking, which can be lethal [30,31,33]. Endocannabinoids have emerged as important regulators of extinction [34] and they play a role in social behavior [35] and in the ability to cope with stress [36].

Recent studies suggested that the ECS and the oxytocinergic systems interact [37] in the regulation of food intake [38] and in social behavior [35,39]. Research by Verty at al. found that the CB1R antagonist/inverse agonist rimonabant (SR141716) reduced body weight in male rats despite only a transient decrease in food intake. This was followed by an elevation of temperature assessed in the brown adipose tissue – a measure of thermogenesis. The authors suggest that the mechanism of action of rimonabant is by increasing energy expenditure and thermogenesis in particular [39].

In the present study, we examined the effect of blocking the brain's main endocannabinoid receptor (CB1R) on maternal behavior and attachment processes in mice. We hypothesized that blocking CB1 receptors will interfere with maternal–infant interactions, leading to abnormal offspring behavior.

2. Experimental procedures

2.1. Animals

Animal care and protocols met the guidelines of the U.S. National Institutes of Health, detailed in the Guide for the Care and Use of Laboratory Animals, and were applied in conformity with the Institutional Ethics Committee. All efforts were made to minimize animal suffering.

Two to three months old female Sabra outbred mice (Harlan, Israel) were used [40]. The colony room was maintained on a 12 h L:12 h D cycle (lights on 7:00–19:00 h). Five females were housed in each cage. For mating, three females and one male were placed together. Females were housed individually once pregnancy was observed (~day 14 of mating). The number of newborns in each cage was culled to eight. Pups were weaned from their dams at the age of 28 days.

2.2. Drugs and injections

The CB1R inverse agonist/antagonist SR141716 also known as rimonabant [41] was generously supplied by Sanofi-Aventis (France). Rimonabant was injected in a mixture of (1:1:18) Ethanol:Cremophor (Sigma, Israel):Saline (for dilution). Vehicle solutions (Ethanol:Cremophor:Saline) were used as appropriate. Daily intra peritoneal (i.p.) injections were performed for 8 consecutive days. Dams received either vehicle ($n = 7$) or 10 mg/kg of rimonabant ($n = 9$) daily during postnatal days (PND) 1–8. This is the first study to administer SR141716 to lactating dams.

Rimonabant displays a high selectivity for CB1 vs. CB2 receptors, and appears to antagonize/reverse-agonize most of the behavioral, electrophysiological and biochemical effects of cannabinomimetics [42,43]. Rimonabant's mechanisms of action as an inverse agonist and/or an antagonist include the ability to inhibit CB1R activity and also to competitively induce responses opposite to those of agonists. Each of these mechanisms will lead to effects in the opposite direction of the agonist [43]. In addition, most tissues and cells have endogenous cannabinoids which are antagonized by rimonabant. Thus it is difficult to dissociate the "antagonistic" activity from the "inverse agonistic" activity.

The 10 mg/kg dose of rimonabant was chosen because it had no effect on pup sucking as was already published [30]. Rimonabant was reported to decrease food consumption in the rat after administration of doses in the 1–10 mg/kg range (i.p. or i.g.) for 14–35 consecutive days [43,44].

3. Behavioral testing procedure

3.1. Pup's growth measurements

During the first two PNDs, pups were separated from their mothers only when the dam was injected with drug or vehicle.

On PNDs 3, 5, 7, pups were separated during injections and in addition, after 90 min pups were separated again from their mothers for recording of their weight, body temperature, and scoring of their "milk bands" in their stomachs. As the stomach area in mouse pups is transparent due to lack of hair and the thinness of the skin, the amount of milk consumed can be observed as a "milk band". Milk bands were scored according to their size (1 – full size, 0.75, 0.5, 0.25, 0 – absence of a milk band). After that the pups were kept in the incubator at 33 °C, in a room separate from the dams' holding cage for thirty min, until the onset of the pup retrieval test (Fig. 1A and B).

3.2. Pups retrieval test

On PNDs 3, 5, 7, pups were separated during dam's injections and after 90 min pups were separated again from their mothers for 30 min, until the onset of the pup retrieval test. Thirty minutes after separation from the dam, three pups were returned to the dam's cage and placed at the opposite corner of the nest. Dam's behavior during the test was video-recorded for off-line data analysis. Retrieval was scored when the dam picked up a pup in her mouth and transported it to the nest. Maximum retrieval time was 5 min. Other observed maternal behaviors: sniffing, licking, duration of crouching over pups and of maintaining a 12 cm distance from the pups and nest.

3.3. Measurement of maternal behavior

On PNDs 3, 5 and 7, each dam was observed twice for maternal behavior for 10 min with no disruption. The first observation was performed before drug/vehicle administration, and the second immediately after the pup retrieval test. Sniffing, licking, crouching over pups and distance of dam from pups in the nest were recorded. Social investigation (sniffing) and licking were counted by a manual counter, and duration of crouching time and of maintaining a distance of more than 12 cm from the nest were measured with stopwatches [45].

3.4. Pup's ultrasonic vocalizations (USVs)

Infant mice, like the young of all mammalian species including humans, exhibit distress-like reactions when they are separated from their dam and they emit maternal separation-induced USVs [46]. Their distress is signaled by the emission of 30–80 kHz USVs that can be heard by the dam and trigger retrieval [47–50]. USVs are an early communicative behavior of the pups and their mother, increased USVs have been viewed as a sign of an aversive affective state [51]. Prenatal exposure to the cannabinoid CB1R agonist WIN 55212-2 decreased the rate of separation-induced USVs in 10-day-old rats [52]. Behavioral analysis of CB1R knockout (KO) mice that included USVs, reported a total lack of the characteristic developmental peak in separation induced USVs [36].

In the present study, on PNDs 4, 6, and 8, individual pups' USVs were recorded 90 min after rimonabant or vehicle administration to the dam. Pups were separated from the dam and placed together in the incubator in a room separate from the dam. Pups were randomly taken individually from the incubator, placed in a small box (10 cm × 15 cm × 10 cm) padded with cotton and recorded for 2 min in a heated room (28 °C) [53]. USVs were recorded and analyzed by Ultravox hardware and software (Noldus, Wageningen – the Netherlands). Duration of 1 ms and above was considered as an event and the total number of events was calculated. The microphones were set to 20, 40, 60 and 70 kHz and placed about 10 cm over the pup. After testing, each pup was placed in a separate cage in the incubator. After completing a litter's testing, the pups were returned together to their dam (Fig. 1A and B).