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Review article

Sexually-dimorphic alterations in cannabinoid receptor density depend upon prenatal/early postnatal history

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ABSTRACT

Recent research has demonstrated that the endogenous cannabinoid system is central to the brain's response to stress. As part of an ongoing collaboration, we sought to examine the effects of prenatal and early postnatal rearing and housing conditions on developing endocannabinoid systems. We compare brain cannabinoid receptors (CBR) in offspring of either prenatal vehicle intubated or non-treated dams (Experiment 1) or in rats derived from a vendor and shipped at weaning to a collaborating lab (Experiment 2). From postnatal day (PND) 23, all rats were either housed in isolated conditions or enriched conditions with 3 rats/cage and a variety of stimulus objects changed twice a week. All rats underwent 5 days of handling as controls for a behavior study and all rats were sacrificed at approximately PND48–50 within 2 hours of the last behavioral test. All brains were processed together for CB1 receptor binding using ^{3}H CP55,940 in prefrontal cortex, striatum, amygdala and hippocampus. Conditions in the two labs were as similar as possible since the two studies were intentionally designed to be comparable and contemporary. Results show that 1) comparing offspring of non-treated dams to offspring of dams receiving daily vehicle intubations, males show decreased CB1 binding in most brain regions while females only showed alterations in the hippocampus and these were increases in the offspring of the vehicle-intubated dams. 2) When comparing offspring of non-treated dams in NY with those derived from a vendor, shipped and maintained in the collaborating lab, this latter group showed reduced CB1 binding in prefrontal cortex in males and increased binding in all four brain regions in females. Therefore, overall, both prenatal handling (intubations) and being vendor-derived, shipped and maintained in a collaborating facility reduced CB1 receptors in males and increased them in females in key limbic brain regions. Effects of environmental enrichment or isolation were minor with only the prefrontal cortex showing an increase in binding in the isolated animals that were offspring of the vehicle-intubated dams. These results support the ideas that prenatal/early postnatal conditions produce different effects in males and females and override the effects of enrichment/isolation on cannabinoid receptors. Behavioral responses to cannabinoid challenges would therefore be expected to vary depending on sex, prenatal/early postnatal history and postweaning conditions of the rats. Since exogenous cannabinoids act through the CBR, the present data may provide a molecular basis for discrepant behavioral effects reported across various labs in the literature as well as sex differences seen following stress and/or manipulation of the cannabinoid system.

1. Introduction

The endogenous cannabinoid system, which develops during prenatal and postnatal life in humans and animals, is essential to mount appropriate behavioral and neuroendocrine responses to the environment. Cannabinoid receptors and their endogenous ligands, *N*-arachidonoylethanolamide (anandamide/AEA) and 2-arachidonoylglycerol (2-AG), are present in the rat brain as early as gestational days 11–14 (Rodriguez de Fonseca et al., 1993; Berrendero et al., 1999), while in humans, cannabinoid receptors have been found from week 14 of gestation (Glass et al., 1997; Wang et al., 2003). The endocannabinoid system influences many processes during development through the modulation of neurotransmitter release and action (Viveros et al., 2005; Gaffuri et al., 2012). Additionally, many studies have shown the importance of the endocannabinoid system in modulating hypothalamic-pituitary-adrenal (HPA) axis activity throughout development (for review, Lee and Gorzalka, 2012). CB1 receptor signaling is stress responsive, and repeated exposure to chronic unpredictable stress in adulthood has been shown to reduce CB1 receptor signaling, a change associated with anhedonia and anxiety (for review, Hillard,

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2014; McEwen et al., 2015). Prenatal stress is widely known to alter behavior and neuroendocrine responses throughout life. While the types of stressors utilized in studies of rodents during the latter third of pregnancy have varied (immobilization, isolation on a platform), alterations in behavior, neuroendocrine responses and gene expression have been described in the offspring and these alterations are frequently sexually dimorphic (Bowman et al., 2004; Mychasiuk et al., 2011, Muhammad and Kolb, 2011; Vallee et al., 1997). Postnatal stress, usually in the form of maternal separation or deprivation, typically induces hyper-reactive HPA axis activity, as well as increases in anxiety and depressive-like measures (Maccari et al., 2014). Maternal separation during the early postnatal period has been shown to have long lasting effects on stress responses and the responses of the cannabinoid system (Llorente-Berzal et al., 2011; Marco et al., 2014). Numerous types of stressors can alter the subsequent responses to drugs. Although typically not considered as stressors, transport and shipping of mice and rats can increase both behavioral and endocrine measures of stress (Tuli et al., 1995; van Ruiven et al., 1998). Shipping during the peripubertal period produces alterations in response to gonadal hormones that persist into adulthood (Laroche et al., 2009) and shipping at weaning produces sex-specific alterations in behavioral responses to THC (Wiley and Evans, 2009).

Housing conditions of the rats can be varied to examine the effects of enriched or isolated conditions on cognition, emotion and reward (see review by (Simpson and Kelly, 2011). While the conditions can vary from one study to the next, enrichment typically occurs from postnatal day (PND) 21/22 (weaning) until the behavioral test is conducted. Isolation is generally believed to be stressful for a naturally social animal like the rat (Fox et al., 2006) while housing with novel objects and with peers is considered less stressful (Belz et al., 2003). Maternal isolation has also been shown to alter offspring play behavior compared to social maternal housing during pregnancy (Honeycutt and Alberts, 2005). Therefore, housing conditions both during prenatal and postnatal life can impact behavior and stress responsivity.

Since both exogenous and endogenous cannabinoids act through the CB1R, alterations in receptor expression suggest changes in the endogenous cannabinoid system, which may produce altered responses to stressors and to drugs. The brain regions that are involved in the response of the endocannabinoid system to the environment include the hippocampus, amygdala and prefrontal cortex (McLaughlin and Gobbi, 2012). In the present study, we measured CB1R densities in these brain regions in rats that either were bred in house and experienced prenatal stress (dams intubated) or were offspring of non-treated dams (Experiment 1) or were vendor-derived, shipped at weaning and raised in a collaborating facility operating with parallel procedures (Experiment 2). In addition we studied the effects of different post-weaning housing conditions in both facilities on CB1R. The findings show that prenatal stress of the mothers and being derived from a vendor and shipped at weaning do alter CB1R densities and that the effects are different in males and females.

2. Materials & methods

2.1. Experiment 1

Sprague-Dawley rats (VAF strain, Charles River Laboratories, Raleigh, NC) were assigned to treatment groups (see prenatal dosing below). Rats were kept under a 12 h light-dark cycle (lights on at 7:00 h) and temperature of 20–22 °C. Females in proestrus were placed with males of the same strain at 4:00 PM. The next morning, rats were checked for sperm by vaginal smear. If sperm was present, that day was designated as gestational day 1 (G1). Pregnant dams were individually housed in plastic cages with bedding and randomly assigned to receive daily intragastric (IG) intubations of vehicle or no-treatment. Daily intubations of water (sterile water, Baxter, the vehicle-intubated group) began 1 week after arrival of the rats in the vivarium using a 16 gauge straight feeding needle and continued through mating and up to the day before delivery (G22). The non-treated dams received no handling for the duration of the pregnancy. These dams were controls for a large prenatal dosing study described in Dow-Edwards et al., 2014. On the day of birth (usually G23), designated as postnatal day 1 (PND1), all pups were sexed, toe-clipped, and weighed. Litters were culled to 10 pups (5 males, 5 females) and pups from vehicle-intubated dams were surrogate fostered to non-treated dams on that same day. Cross-fostering was not used and pups from non-treated dams were not fostered due to absence of available dams. At PND 21 animals were weaned, ear punched and separated into same sex cages containing 5 littermates until PND 23. On PND 23, rats were housed in one of two conditions: either one rat/cage (isolated environment) or with 3 same-sex littermates/cage which also contained stimulus objects (enriched environment) (see details of housing conditions in Dow-Edwards et al. (2014). Rats in both housing conditions had access to food and water ad lib. Subjects were weighed at weekly intervals from PND 8 to PND 42 prior to behavior testing. All procedures were approved by the IACUC in accord with the recommendations of the American Academy of Lab Animal Science. All experiments were carried out in accordance to the Guide for the Care and Use of Laboratory Animals, Eighth Edition, National Research Council, Department of Health and Human Services, 2011.

As part of their postnatal experience on PND 44, pups in the current study served as saline-saline controls for conditioned place preference (CPP) testing. The results have been previously reported (Dow-Edwards et al., 2014; Zakharova et al., 2009a, 2009b) and the 5-day paradigm is presented here for informational purposes only. For this procedure, rats were individually placed in 42 × 42 cm Plexiglas boxes with solid white walls on one side and vertical black/white stripes on the other for a period of 30 min. On the following 3 days, the boxes were partitioned to prevent access to both sides; the rats were injected with saline (1 ml/kg body weight) and placed on one side of the box for 30 min in the morning and 3–4 h later, injected with saline and placed on the opposite side of the box for 30 min. On the 5th day, the partition in the box was removed and the rats were placed in the box for 30 min under observation. Within 2 h of this final test session, subjects were decapitated, brains removed, frozen and stored at -80 °C until shipment to U. Miami for sectioning and processing.

2.2. Experiment 2

Sprague-Dawley rats (vendor-derived) (VAF strain, Charles River Laboratories, Raleigh, NC) were from the same facility in Raleigh that supplied the breeders for Experiment 1. The standard operating procedure of this vendor is to house each pregnant female separately in a single cage. Litters are culled to 14 most often retaining only the males and remain housed in these cages until the day of shipping on PND 20. The pups are placed in shipping crates (5–8 same sex per crate) and trucked to Miami overnight in climate controlled vehicles. For this experiment, only non-littermate pups were ordered (that is, in each shipment, rats from different litters were requested). The pups arrived in the Miami lab on PND21 and were housed in same sex cages until PND23. On PND 23, housing conditions were identical as those described above in Experiment 1; isolated or enriched. The objects and rotation cycle of the objects were intentionally designed to be identical to those of the NY lab described above. As in Experiment I, on PND 44, pups underwent CPP training as described above (again these rats were the saline-saline controls) and were sacrificed within 2 h of the final CPP test. Brain tissue was harvested and handled as described below.

2.3. Receptor autoradiography

Tissue processing for Experiments 1 and 2: All brains were processed for receptor densities at the University of Miami. Brains were sectioned