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Maternal high-fat diet leads to persistent synaptic instability in mouse offspring via oxidative stress during lactation

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ABSTRACT

Maternal obesity has negative effects on the neurodevelopment of the offspring. Pups from high-fat diet (HFD)-fed mice exhibit peroxidized lipid accumulations in the brain and behavioral impairments. However, the synaptic basis of maternal HFD-induced brain dysfunction in offspring remains unclear. In the present study, we focused on the dynamics and morphology of postsynaptic dendritic spines and filopodia in the offspring of HFD-fed mouse dams, using *in vivo* two-photon imaging, chosen because of the involvement of peripheral organs and non-neuronal cells in the abnormal metabolic state. We observed instability of dendritic spines and filopodia in the cerebral cortex of offspring from HFD-fed dams. Interestingly, the synaptic instability persisted into adulthood with a lower spine density even when the offspring were fed with a normal diet after weaning. HFD-fed offspring from HFD-fed dams showed a severe disruption of dendritic spines. Synaptic instability and loss of spines were caused even by HFD exposure exclusively during lactation. The treatment of ascorbic acid, an antioxidant, during lactation ameliorated the synaptic impairments. These results suggest that maternal obesity leads to persistent synaptic impairments in the offspring, which may be associated with behavioral deficits in adulthood, and that these synaptic deficits may be due to oxidative stress from peroxidized lipid accumulations during the lactation period.

1. Introduction

Obesity is a worldwide health problem and a major contributor to the increased incidence of coronary artery disease, hypertension and type 2 diabetes (Kopelman, 2000). Epidemiological studies have shown that maternal obesity has negative effects on the neurodevelopment of children (Van Lieshout et al., 2011). Animal studies have also demonstrated the effects of maternal obesity on the brain, as well as in the peripheral organs of offspring (Williams et al., 2013). However, little is known about the effects of maternal obesity on brain development in offspring. We previously reported that mouse pups from dams fed with a high-fat diet (HFD) show peroxidized lipid accumulations in many brain regions, impaired adult neurogenesis in the hippocampus and deficits in spatial learning performance (Tozuka et al., 2009, 2010). Other animal model studies have also demonstrated cognitive impairment of offspring from HFD-fed dams (Rodriguez et al., 2012; Wu et al., 2013). However, the synaptic basis for maternal HFD-induced brain dysfunction remains unclear. It is possible that postsynaptic dendritic protrusions may be impaired in offspring born to HFD-fed dams, because many behavioral deficits are associated with protrusion impairment (Penzes et al., 2011). In our preceding study, peroxidized lipid accumulations in the brain of the offspring from HFD-fed dams disappear when the offspring are raised on a normal diet (ND) after weaning, whereas impaired progenitor cell proliferation in the hippocampus and elevated locomotor activity persist into adulthood (Tozuka et al., 2009, 2010). Thus, there may be reversible and irreversible components of maternal HFD-induced brain impairment in offspring. Accordingly, these lines of evidence raise the following questions about the impact of maternal HFD on the synapses of the offspring: Are the synaptic impairments

Abbreviations: HFD, high-fat diet; ND, normal diet.

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induced by maternal HFD reversible or persistent? When is the critical period for the synaptic impairment in the offspring from HFD-fed dams? Is oxidative stress from peroxidized lipids during the critical period a key factor in developing synaptic impairment?

In the present study, we analyzed the dynamics and morphology of dendritic protrusions, classified into mature spines or immature filopodia, using *in vivo* two-photon laser-scanning microscopy. We evaluated the synaptic deficits induced by HFD *in vivo* because animals exposed to HFD show various pathologies in many peripheral organs and non-neuronal cells in the brain (Bilbo and Tsang, 2010; Williams et al., 2013). We used not only ND-fed offspring from HFD-fed dams but also HFD-fed offspring from HFD-fed dams, to model western eating habits. Furthermore, in order to elucidate the critical period when maternal HFD disrupts the synapses of the offspring, we used ND-fed offspring from HFD-fed dams exclusively during lactation. We examined the involvement of oxidative stress from peroxidized lipids during the critical period by antioxidant-treatment. Our findings demonstrate that maternal HFD lead to synaptic instability in the offspring mediated by oxidative stress during lactation, and that the synaptic instability persisted into adulthood with developing loss of dendritic spines. The present study is the first to examine the synaptic basis of brain dysfunction in offspring born to HFD-fed dams.

2. Materials and methods

2.1. Experimental animals

Mice expressing yellow fluorescent protein (YFP) predominantly in layer (L) 5 pyramidal neurons (*Thy1-YFP* H-line) were purchased from the Jackson Laboratory and backcrossed with C57BL/6J mice. Mice were housed four per cage under controlled temperature (25 ± 1 °C) and lighting (12 h light/dark cycle), and were provided with food and water ad libitum. The procedure of maternal HFD feeding and the macronutrient composition and caloric distribution in the ND and HFD have been described previously (Tozuka et al., 2009). Briefly, heterozygous *Thy1-YFP* mice were fed ND (CE-2, CLEA Japan) or HFD (HFD-32, CLEA Japan) (Oike et al., 2005) for 6 weeks until mating. The same diet was maintained during pregnancy and for 15 days of lactation. From lactational day 16, when pups first start to eat a solid food, pups were assigned as follows: those born to HFD-fed dams but consuming ND (HFD/ND), those from ND-fed dams but consuming HFD (ND/HFD), those from HFD-fed dams and consuming HFD (HFD/HFD), and those from ND-fed dams and consuming ND (ND/ND). Pups born to HFD-fed dams exclusively during lactation and consuming ND were assigned to HFD_{lac}/ND group. Pups from HFD-fed dams treated with 1 g/L L-ascorbic acid (Vitamin C; Nacalai Tesque, Japan) in drinking water during lactation and consuming ND were assigned to HFD_{AA}/ND group. The fluid intake of mice received normal drinking water or drinking water supplemented with ascorbic acid is approximately 10 mL/day (Abdel-Wahab et al., 2002). On the day of parturition (day 0), litters were culled to 6–8 offspring per mother. Pups were kept with their dams until weaning on day 22. Only male offspring heterozygous for YFP were used for all analyses. To avoid autoxidation of the HFD, diets were stored at −80 °C until use, and food was renewed every 2 d. Animal procedures were in strict accordance with the guidelines of the National Institute of Neuroscience, National Center of Neurology and Psychiatry (Japan).

2.2. Surgical procedure for in vivo imaging

We used the thinned-skull cranial window technique (Yang et al., 2010) because it is less invasive than the open-skull method (Xu et al., 2007). Male pups expressing YFP were deeply anesthetized with urethane (2 mg/g body weight, i.p.). Body temperature was maintained at 37 °C with a heating pad during the surgery and following imaging. After the scalp incision, both eyes were lubricated with eye ointment, and the primary somatosensory area (1.1 mm posterior to bregma and 3.4 mm lateral from midline) was identified with stereotactic coordinates. A small metal plate with a round hole was glued onto the skull with cyanoacrylate glue and acrylic resin dental cement (UNIFAST; GC, Japan), and mice were fixed to a custom-made skull immobilization stage via the metal plate. The skull above the imaging area, located in the center of the hole in the metal plate, was thinned to a thickness of approximately 20 μm with a high-speed microdrill (UG23A/UC210C; Urawa, Japan) and microsurgical blade (USM-6400; Sable Industries, Vista, CA, USA). The hole in the metal plate was filled with artificial cerebrospinal fluid during surgery and imaging.

2.3. In vivo transcranial two-photon imaging

Thy1-YFP pups were imaged under anesthesia using two-photon laser scanning microscopy (FV1000-MPE; Olympus, Japan) with a water-immersion objective lens (25×, NA 1.05) at 8× digital zoom yielding high-magnification images suitable for quantification of dendritic spines. A Ti-sapphire laser (MaiTai HP DeepSee-OL; Spectra-Physics, Mountain View, CA, USA) was tuned to 950 nm. Laser intensity was kept in the range of 10–30 mW at the focus to minimize phototoxicity. Image-stacks (512 × 512 pixels; 0.124 μm/pixels; 0.75 μm z-step) were taken at approximately 70 μm below the pial surface, where L1 dendrites of L5 pyramidal neurons are located. Dendritic segments were imaged for each experiment at time 0, and then the same dendritic segments were imaged again after an interval of 1 h. All images were acquired carefully to approximate similar fluorescence levels across imaged regions within each experiment and across imaging sessions in different animals. The animals were sacrificed immediately after imaging sessions.

2.4. Image analysis

The turnover rate, density, head width, and neck length of dendritic protrusions were analyzed with Neurolucida (MicroBrightField, Williston, VT, USA) from three-dimensional two-photon z-stacks. Morphometric analysis of dendritic protrusions was in accordance with a previous report (Grutzendler et al., 2002). Dendritic protrusions were classified into two groups, spines and filopodia, and formation/elimination rate were analyzed in each group. Filopodia were identified as long, thin structures (ratio of head width to neck width <1.2:1; ratio of length to neck width >3:1). The remaining protrusions were classified as spines. These protrusions were considered the same between two views on the basis of their spatial relationship to adjacent landmarks and their relative position to immediately adjacent protrusions. Protrusions were considered different if they were >0.7 μm from their expected positions based on the first view. The formation and elimination rates for the protrusions were defined as the fraction of protrusions that appeared and disappeared, respectively, between two successive frames, relative to the total protrusion number. Protrusion turnover rate was defined as the sum of the protrusions formed and eliminated divided by twice the total number of protrusions. Data were collected from 10 to 21 dendrites and approximately 1000 protrusions in four to seven mice.

2.5. Statistical analysis

To determine statistical significance, we used Student's *t* test, a one-way ANOVA followed by Tukey's multiple-comparison test, a