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PRESENCE OF PUPS SUPPRESSES HUNGER-INDUCED FEEDING IN VIRGIN ADULT MICE OF BOTH SEXES

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Abstract—Despite recent progress on neural pathways underlying individual behaviors, how an animal balances and prioritizes behavioral outputs remains poorly understood. While studying the relationship between hunger-induced feeding and pup-induced maternal behaviors in virgin female mice, we made the unexpected discovery that presence of pups strongly delayed and decreased food consumption. Strikingly, presence of pups also suppressed feeding induced by optogenetic activation of Agrp neurons. Such a suppressive effect inversely correlated with the extents of maternal behaviors, but did not rely on the display of these behaviors, and was also present in virgin males. Furthermore, chemogenetic activation of Vglut2+ neurons in the medial preoptic area (mPOA), a region critical for maternal behaviors and motivation, was sufficient to suppress hunger-induced feeding. However, muscimol inhibition of the mPOA, while disrupting maternal behaviors, did not prevent pup suppression of feeding, indicating that neural pathways in other brain regions may also mediate such an effect. Together, these results provide novel insights into neural coordination of pup care and feeding in mice and organizations of animal behaviors in general. © **2017 Published by Elsevier Ltd on behalf of IBRO.**

Key words: anorexia, maternal behaviors, hunger, preoptic, feeding, Vglut2.

INTRODUCTION

Mammalian newborns are altricial and rely on parental or alloparental care provided by mature conspecifics to survive (Numan, 2003). In laboratory mice, pup care behaviors are readily observed in virgin or postpartum females, and include retrieval of pups to a secure nest site, crouching over pups, building and maintaining a nest site, and defending pups against intruders (Lonstein and Gammie, 2002; Kuroda et al., 2011; Dulac et al., 2014). By contrast, male mice ignore or even attack pups unless they become fathers, at which point they do transiently display pup care behaviors similar to females (Brown, 1993; Lonstein and De Vries, 2000; Wu et al., 2014). Although promoting the survival of the young, pup care behaviors pose considerable time and energy demands to the provider. How an animal coordinates pup care with self-directed needs and the neural mechanisms that govern such behavioral orchestration remains poorly understood.

Caloric insufficiency elicits a sense of hunger, which in turn drives food forage and feeding behaviors via activation of hypothalamic neurons that express Agouti-related peptide (Agrp) (Betley et al., 2013; Zha and Xu, 2015). Ablation of Agrp neurons in adult mice results in cessation of feeding and starvation while selective activation of Agrp neurons can drive feeding in satiated animals (Aponte et al., 2011; Atasoy et al., 2012; Chen et al., 2015; Krashes et al., 2011, 2013; Luquet et al., 2005). More importantly, hunger or activation of Agrp neurons competes with other motivational systems and suppresses non-feeding-related behaviors such as thirst, innate fear, social interactions and territorial aggression (Burnett et al., 2016; Padilla et al., 2016). Thus, it seems that female individuals may prioritize feeding and self-preservation over pup care during period of caloric insufficiency.

On the other hand, in species such as mouth-breeding cichlid fish or domestic chicken, females actually undergo lengthy voluntary anorexia during brood care (Mrosovsky and Sherry, 1980; Mrowka, 1986). Female cichlid fish when ready to brood take up eggs into her buccal cavity to spawn and carry them continuously for days, during which period food intake is greatly reduced (Mrowka, 1984, 1986). Similarly, over a three-week egg incubation period hens direct majority of their activities sitting in the nest and spend very little time on feeding, which leads to loss of body weight despite the ready availability of food (Sherry et al., 1980). These observations demonstrate that females of some species do prioritize care of offspring over feeding and raise the question whether a similar mechanism may also be in place in mammals.

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Abbreviations: Agrp, Agouti-related peptide; AMG, amygdala; CeA, central amygdala; CGRP, calcitonin-related peptide; ChR2, Channelrhodopsin-2; D1R, dopamine receptor 1; LH, lateral hypothalamus; mPOA, medial preoptic area; NAC, nucleus accumbens; NTS, nucleus of the solitary tract; PBel, parabrachial nucleus; PFA, paraformaldehyde; PFC, prefrontal cortex; PKCδ, protein kinase C delta; Vglut2, vesicular glutamate transporter 2; VTA, ventral tegmental area; WT, wild-type.

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In fact, previous studies have established that the mPOA, a region essential for maternal behaviors and motivation (Tsuneoka et al., 2013; Wu et al., 2014), is reciprocally connected with Agrp neurons in mice (Betley et al., 2013; Wang et al., 2015). Inspired by such anatomical connections, we examined relationship between hunger-induced feeding and pup-induced maternal care in virgin female mice by presenting an animal with both food pellets and pups after 10-h of food deprivation. Surprisingly, we found that presence of pups strongly delayed and suppressed food consumption. Strikingly, feeding induced by optogenetic activation of Agrp neurons was also dramatically reduced by the presence of pups. Furthermore, although activation of the mPOA Vglut2+ neurons was sufficient to suppress hunger-induced feeding, inhibition of the mPOA did not prevent pup suppression of feeding. To our knowledge, these results provide the first evidence for a possible anorexigenic effect of pups in mammals.

EXPERIMENTAL PROCEDURES

Animal

$Agrp^{Cre}$ (#012899), Ai32 (#012569), Vgat-Ires-Cre (#016962) and Vglut2-Ires-Cre (#016963) mice were purchased from the Jackson Laboratory. C57BL/6 animals were purchased from the Slac Laboratory Animal (Shanghai). All experimental animals used in the study were adults (>8 weeks old) and were bred onto C57BL/6 background for at least one generation. Animals were housed in the Institute of Neuroscience animal facility on 12 h:12 h light/dark cycle with food and water *ad libitum* unless otherwise specified. A maximum of five animals of the same sex were allowed for each cage. Animals heterozygous for the $Agrp^{cre}$ and Ai32 allele were denoted as $Agrp^{ChR2}$ mice. All experimental protocols were approved by the Animal Care and Use Committee of the Institute of Neuroscience, Chinese Academy of Sciences, Shanghai, China (IACUC No. NA-016-2016).

Stereotactic surgery

Mice were anesthetized using isoflurane and placed onto a stereotactic frame (David Kopf Instrument, Model 1900). The skull was exposed with a small incision and holes were drilled in the skull to implant optic fibers or cannulas or to inject virus with glass pipettes (15–25 μm in diameter at the tip). Unilateral optical fibers (200 μm in diameter, N.A. 0.37, length 7 mm, AniLab Software and Instruments Co., Ltd.), bilateral cannulas (C235GS-5-0.8/sp 7 mm, Plastic One Inc.) were inserted through the drilled holes and secured onto the skull with dental cement. The coordinate of bregma: AP: −1.700 mm, ML: −0.250 mm, DV: −5.650 mm was used to target the arcuate nucleus unilaterally. The coordinate of bregma: AP: +0.120 mm, ML: ±0.400 mm, DV: −4.600 mm was used to target the mPOA bilaterally with cannulas. For viral injections, the coordinate of bregma: AP: +0.160 mm, DV: −5.100 mm, ML: ±0.400 mm was used to target the mPOA bilaterally. Injections of 200 nl virus per side were made with a hydraulic pump at a speed of 40 nl per minute. Optical fiber or cannula implanted animals were allowed at least one week to recover before behavioral tests. Virus-injected animals were allowed at least three weeks for viral expression before behavioral tests.

Virus

Viruses used in the study include AAV-hSyn–HA-HM3D(Gq)-Ires-mCitrine (titer 2.3×10^{12} genomic copies/ml, UNC Gene Therapy Center Vector Core), AAV–hSyn–mcs–mCherry-3flag (titer 3.38×10^{12} genomic copies/ml, Obio Technology Co., Shanghai), AAV-hSyn-DIO-HM3D-mCherry (titer 7.9×10^{12} genomic copies/ml, UNC Vector Core), AAV-EF1a-DIO-mCherry (titer 8.93×10^{12} genomic copies/ml, Obio Technology Co., Shanghai). All viruses used were of Serotype 2/8.

Behavioral test

Animals were singly housed for at least three days before the behavioral tests. Pup-directed behaviors were induced by scattering three pups aged between P0 and P4 at the edge away from the nest or under a barrier and the animal was videotaped for at least 30 min. Food consumption was measured by weighing the food pellets using a scale before and after the behavioral tests, or with an automated feeder system (Biolink Optics system). For food restriction experiment, animals were moved to a new cage two and a half hours after the onset of light cycle and left without access to food but with access to water for 10 h, till half an hour after the onset of dark cycle, after which behavioral tests were carried out. Animals were allowed at least five days to recover between each food restriction experiment. All experiments with different stimuli were carried out in a balanced manner to minimize order effects. Videotapes were scored by an experimenter blind to the treatment of the animal using a custom written Matlab program.

Specifically, to test the effects of pups on hunger-induced feeding, C57BL/6 virgin females or males were food deprived for 10 h as described above then tested with food alone or with one of the following stimuli: scattered pups, scattered glass balls, barrier, barrier plus grouped pups or barrier plus a female for a period of 30 min. After recovery, animals were starved again and shuffled to give a different stimulus in the next round. To test the effects of pup ultrasound on hunger-induced feeding, C57BL/6 virgin females were food deprived for 10 h and tested with or without ultrasound for a period of 30 min. The ultrasound was recorded and played by Avisoft Bioacoustics ultrasound microphones and players. To test long-term effects of pups on feeding, C57BL/6 virgin females were tested with food alone, scattered glass balls with food or scattered pups with food, and food intake (45 mg for each pellet) was automatically recorded in single-housed mice with the Biolink Optics system from 2 h before the onset of dark cycle for a continuous12-h period or weighted before and after a 24-h behavioral test period.