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Hunger-Activated AgRP Neurons Inhibit MPOA Neurons Controlling Parenting

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Review of Li et al.

Parental care comprises several behavioral components directed toward fostering the survival of offspring. It is particularly essential for mammalian species, because infants' growth depends almost entirely on adult care. Mammalian parents nurse or forage to supply food. They also clean and carry their infants and provide warmth and protection against intruders or predators (Royle et al., 2014). In rodents, parents and virgin females display active pup-oriented behaviors, such as grooming, licking, and retrieving pups to the nest; passive pup-oriented behaviors, such as nursing and crouching over pups to provide warmth; and non-pup-oriented behaviors, such as collecting the material for and building nests (Table 1; Fang et al., 2018). These parental behaviors are governed by the medial preoptic area (MPOA). In rodents, MPOA lesions disrupt maternal care, and Fos expression (indicating activation) is induced in MPOA neurons during maternal behaviors (Numan, 1974; Tsuneoka et al., 2013).

Parental care consumes much time and resources. Parents therefore need to ensure food availability to sustain their

own survival (Kohl et al., 2017). Food scarcity induces hunger, which is driven by well characterized brain circuits (Lowell, 2019). Specifically, hunger stimulates the activity of AgRP (agouti-related peptide) inhibitory neurons in the arcuate nucleus of the hypothalamus, and this activity is suppressed by ingested calories (Morton et al., 2006; Su et al., 2017). Furthermore, optogenetic stimulation of AgRP neurons rapidly evokes compulsive food consumption in mice (Aponte et al., 2011). Notably, AgRP neurons also influence non-feeding-related behaviors, such as territorial aggression, via projections to multiple brain regions, thus suppressing these behaviors when animals are hungry (Betley et al., 2013; Padilla et al., 2016). A subset of MPOA neurons involved in parenting behaviors express galanin (Gal+) and receive a large input from the arcuate nucleus (Kohl et al., 2018). AgRP neurons might therefore regulate MPOA neurons controlling parental behaviors.

In a recent study, Li et al. (2019) tested the hypothesis that AgRP neurons regulate parental care based on the feeding state of the parent by projecting to the MPOA. The authors first established that 25 h of food deprivation in multiparous females blunted some aspects of maternal care, namely active pup-oriented and non-pup-oriented behaviors, while leaving passive pup-oriented behaviors intact. Specifically, the latency to retrieve pups scattered outside the nest was longer and

the percentage of successful trials was lower in food-deprived mothers than in mothers receiving food *ad libitum*. Hungry mothers also neglected nest building. But crouching over pups was unaffected (Li et al., 2019, their Table 1).

The authors next demonstrated that AgRP neurons are synaptically coupled with MPOA neurons and regulate some parental behaviors. First, a mouse line expressing the synaptic protein synaptophysin fused to tdTomato in AgRP neurons revealed the presence of synaptic terminals of AgRP neurons in the MPOA (Li et al., 2019, their Fig. 1). In addition, optogenetic stimulation of AgRP terminals in the MPOA in brain slices evoked IPSCs in ~30% of MPOA neurons in females and males (Li et al., 2019, their Fig. 2), indicating substantial functional connections between AgRP neurons and MPOA. Optogenetic stimulation of AgRP neurons in the arcuate decreased the time devoted to nest building in both virgin and lactating females (Li et al., 2019, their Fig. 3I,L). The percentage of trials in which females attempted to retrieve pups and how closely to the nest pups were retrieved, were not significantly altered, however (Li et al., 2019, their Fig. 3H,K). The authors then tested whether the projections of AgRP neurons to MPOA were specifically responsible for the effects on nest building. Optogenetic stimulation of AgRP terminals in the MPOA indeed decreased nest building (Li et al., 2019, their Fig.

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Table 1. Cell-type-specific interventions in the MPOA affect distinct components of parenting

Molecular marker	Interventions	Parenting behaviors						References
		Active pup-oriented				Passive pup-oriented	Non pup-oriented	
		Retrieval	Sniffing	Grooming	Motivation	Crouching	Nest building	
Vgat	Ablation	↓	N/A	N/A	N/A	N/A	N/A	Li et al. (2019); Wei et al. (2018)
	Optogenetic inhibition	↓	N/A	N/A	N/A	N/A	↓	
	Chemogenetic inhibition	=	N/A	N/A	N/A	N/A	↓	
	Optogenetic activation	↑	N/A	N/A	N/A	N/A	↑	
Esr1	Ablation	↓	N/A	N/A	N/A	N/A	N/A	Fang et al. (2018); Li et al. (2019); Wei et al. (2018)
	Optogenetic inhibition	↓	N/A	N/A	N/A	=	N/A	
	Chemogenetic inhibition	↓	=	=	↓	=	N/A	
	Optogenetic activation	↑	↑	=	N/A	=	=	
Gal	Ablation	↓	N/A	↓	N/A	↓	↓	Wu et al. (2014)
	Optogenetic activation	N/A	N/A	↑	N/A	↓	N/A	

↓ Indicates that the corresponding behavioral component is down-regulated, ↑ is up-regulated, = indicates that no changes were observed. N/A indicates data not available.

4D). Moreover, stimulation of AgRP terminals in the MPOA did not affect the percentage of trials in which females attempted to retrieve pups. But pups were less closely (therefore less successfully) retrieved to the nest (Li et al., 2019, their Fig. 4 C).

The authors then sought to determine which subsets of neurons within the MPOA were targeted by AgRP neurons. Consistent with previous studies showing that MPOA neurons controlling parental behaviors are mostly GABAergic (Tsuneoka et al., 2013; Wu et al., 2014), Li et al. (2019) found that MPOA cells targeted by AgRP neurons express Vgat (Li et al., 2019, their Fig. 5B). Optogenetic stimulation of MPOA Vgat+ neurons augmented the percentage of pup retrieval attempts and promoted nest building (Li et al., 2019, their Fig. 8B,C). Conversely, optogenetic inhibition of MPOA Vgat+ neurons decreased the percentage of pup retrieval attempts and the number of pups retrieved, and inhibited nest building (Li et al., 2019, their Fig. 8H–J). To further test the effect of inhibiting MPOA Vgat+ neurons on parental care, the authors expressed the silencing designer receptor exclusively activated by designer drug (DREADD) hM4D in MPOA Vgat+ neurons. Injecting clozapine-*N*-oxide (the designer drug) in females decreased nest building, without affecting the percentage of pup retrieval attempts or how closely to the nest pups were retrieved (Li et al., 2019, their Fig. 5 G,F). The discrepancy of effect on pup retrieval between chemogenetic (unaffected) and optogenetic (affected) silencing of MPOA Vgat+ neurons might be caused by different temporal modulations of neuronal activity with these methods: optogenetic modulation is acute while chemogenetics modulates neuronal activity over several hours, po-

tentially allowing compensatory circuits to mask the silencing of these neurons.

Finally, the authors asked whether the decline in maternal care resulting from stimulation of AgRP neurons depended solely on projections to the MPOA. They compared the optogenetic stimulation of AgRP terminals in the MPOA (Li et al., 2019, their Fig. 4 C,D) to the stimulation of terminals in the paraventricular nucleus of the hypothalamus (PVH) and the lateral hypothalamic area (LHA), two nuclei receiving major AgRP neuron inputs inducing feeding (Betley et al., 2013). Stimulation of AgRP projections to LHA had no effect on maternal care (Li et al., 2019, their Fig. 4 K,L), whereas stimulation to PVH specifically reduced nest building without affecting pup retrieval (Li et al., 2019, their Fig. 4 G,H). Therefore, stimulation of AgRP terminals represses nest building in both PVH and MPOA, but represses pup retrieval in MPOA only.

The article published by Li et al. (2019) adds to a series of recent publications investigating the contribution of distinct MPOA cell types to specific elements of parenting (Table 1). Approximately 80% of MPOA cells are Vgat+ neurons (Wei et al., 2018). Ablation of Vgat+ neurons decreases pup retrieval (Wei et al., 2018), whereas optogenetic interventions affect components of active pup-oriented and non-pup-oriented behaviors (Li et al., 2019; Table 1). A partially overlapping population of MPOA neurons expressing the estrogen receptor α (Esr1+) represents 50% of MPOA neurons (Wei et al., 2018). Several studies converge to establish that MPOA Esr1+ neurons selectively control active pup-oriented behaviors (Fang et al., 2018; Wei et al., 2018; Li et al., 2019; Table 1). A third MPOA cell type identified in the control of parenting, also

mostly GABAergic, expresses the neuropeptide Galanin (Wu et al., 2014). Ablation of MPOA Gal+ neurons decreases pup retrieval and grooming, crouching over pups, and nest building (Table 1; Wu et al., 2014). Given that ~50% of MPOA Gal+ neurons coexpress Esr1 (Wei et al., 2018), future intersectional molecular studies will help to determine whether the dual-positive Gal+/Esr1+ fraction is selectively involved in the active pup-oriented component of parenting. Recent publications using *in vivo* recording techniques have begun to provide insight into the architecture of neuronal activity within the MPOA. MPOA neurons activated during pup retrieval are inhibited during nest building (Fang et al., 2018). Furthermore, MPOA Esr1+ neurons are activated when a mother approaches or retrieves a pup, and their activity decreases when building nests or crouching over pups (Fang et al., 2018; Wei et al., 2018). Importantly, MPOA itself provides the strongest neuronal input to MPOA Gal+ neurons (Kohl et al., 2018). These findings suggest a basic organization of the MPOA in which active neuronal clusters controlling pup-oriented behaviors send collaterals to inhibit clusters controlling non-pup-oriented behaviors, hence organizing distinct components of parenting sequentially.

The identification of a functional input from the hunger-stimulated AgRP neurons to the MPOA adds another layer of complexity to the organization of MPOA circuits governing parenting. The difference in pup retrieval success depending on stimulation of AgRP neurons in the arcuate (unaffected) or terminals in the MPOA (decreased) is surprising (Li et al., 2019, their Figs. 3, 4C). Using supplementary measurements, such as the latency to retrieve the first pup (Li et al., 2019, their

Table 1; Fang et al. 2018), might reveal that stimulation of AgRP neurons in the arcuate also affects pup retrieval, recapitulating the effect of food deprivation (Li et al., 2019, their Table 1). Nevertheless, Li et al. (2019) provide evidence that the AgRP input to the MPOA preferentially modulates nest building, a non-pup-oriented behavior, and affect less consistently pup retrieval, an active pup-oriented behavior governed by *Esr1*+ neurons. Therefore, AgRP neurons are likely to inhibit preferentially a subset of MPOA *Vgat*+ neurons that may express Galanin, but not *Esr1*. Refined molecular markers and tracing techniques will help to characterize MPOA neuronal cluster(s) (Moffitt et al., 2018) inhibited by hunger-stimulated AgRP neurons.

Beside the MPOA, Li et al. (2019) found that AgRP projections to the PVH specifically repress nest building without affecting pup retrieval. The PVH regulates many physiologic functions such as feeding and energy expenditure through heterogeneous neuronal clusters expressing neuropeptides, including oxytocin and vasopressin affecting parental care (Qin et al., 2018). In mammalian females, oxytocin is well known for regulating uterine contraction and breast milk release peripherally, and, in neural circuits, enhances the salience of pup calls to promote pup retrieval (Marlin et al., 2015). Moreover, vasopressin expression is negatively correlated with nest building, and chemogenetic stimulation of PVH vasopressin neurons represses nest building (Bendesky et al., 2017). AgRP neurons substantially connect and inhibit PVH oxytocin neurons, but it is yet unknown whether AgRP neurons inhibit vasopressin neurons too (Atasoy et al., 2012). Further studies could also explain how AgRP projections to the PVH specifically reduce one component of parenting, nest building, without blunting pup retrieval.

Because the authors found similar connections from AgRP neurons to the MPOA in both males and females (Li et

al., 2019, their Fig. 2), future studies should also ask whether parenting would be similarly attenuated by hunger in fathers or, alternatively, whether hunger unveils pup-directed aggression, which is a typical response for virgin males. For mammalian mothers exposed to food scarcity, the repressing effects of hunger-stimulated AgRP neurons on parental behaviors are likely to take place as early as during pregnancy, where hormonal fluctuations stimulate nest building in anticipation of the arrival of the pups (Broida and Svare, 1983; Rilling and Young, 2014).

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