Fasting, feasting and the glutamatergic synapse

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Summary and comment on a recent Neuron paper entitled ‘Fasting activation of AgRP neurons requires NMDA receptors and involves spinogenesis and increased excitatory tone’ (Liu et al., 2012).

Obesity has reached pandemic proportions, with the United States being one of the nations most affected. Among many lines of investigation aiming to counter obesity is the examination of how energy homeostasis and feeding behavior are regulated by the brain.

Feeding behavior and weight regulation are largely controlled by the hypothalamic circuitry. Key components of this circuitry are two types of neurons in the arcuate nucleus (ARC) of the hypothalamus: the pro-feeding (anabolic) agouti-related protein (AgRP)- and neuropeptide Y (NPY)-expressing neurons (AgRP neurons), and the feeding-inhibitory (catabolic) proopio-melanocortin (POMC)-expressing neurons (POMC neurons). Circulating hormones are known to regulate the function of these neurons, including the neuropeomodulator ghrelin, which promotes AgRP neuron activation. Fasting has been shown to elicit activation of the anabolic AgRP neurons in part through ghrelin, resulting in an increase in feeding and subsequent weight gain. Conversely, the hormone leptin, which is secreted from adipocytes, activates the POMC neurons (Millington, 2007; Belgardt et al., 2009) to induce the release of melanocyte-stimulating hormone, which inhibits feeding. POMC neurons also release β-endorphin, which inhibits AgRP activation through a negative-feedback loop.

Despite detailed characterization of the feeding circuitry and its regulation by various peripheral hormones and metabolites, comparatively little attention has been paid to the synaptic regulation and plasticity of the neurons within the circuit, in which the firing rate of the circuit can be modulated by prior experience, including fasting. A recent pharmacological study by Yang et al. put forth a presynaptic mechanism to explain changes in the firing rate of this circuit (Yang et al., 2011), whereby an as-yet-unidentified hypothalamic neuron releases glutamate to activate AgRP neurons. According to this study, binding of the hormone ghrelin to the ghrelin receptor on this presynaptic neuron activates Ca2+/calmodulin-dependent protein kinase (CAMKK), leading to the direct activation of AMP-activated kinase (AMPK). Activation of AMPK causes the release of ryanodine-sensitive Ca2+ stores at the nerve terminal. The resulting increase in presynaptic Ca2+ at these synapses thereby increases the probability of synaptic vesicle release from these terminals, culminating in the increased firing rate of AgRP neurons and an accompanying increase in feeding behavior. This synaptic plasticity is reversible, because POMC neurons can release β-endorphin in response to leptin, activating opioid receptors on the presynaptic neuron. This, in turn, negatively regulates AMPK activity, reducing intracellular Ca2+ levels, depressing the firing rate and, in effect, diminishing feeding.

A more recent paper, and the focus of this article, offers a possible alternative mechanism of this circuit’s regulation. This landmark study by Liu et al. (Liu et al., 2012) builds on the findings of Yang et al. by ascertaining in more detail the events that occur following fasting-induced glutamate release onto AgRP and POMC neurons. They focus on addressing the role of N-methyl-D-aspartate receptors (NMDARs), which are known to regulate the strength and plasticity of glutamatergic signaling, by generating mice that lack NMDARs in either AgRP or POMC neurons. Strikingly, their results show that NMDAR signaling in AgRP neurons, but not in POMC neurons, is important for maintaining energy balance, and suggest a post-synaptic mechanism for plasticity within the feeding circuit.

A key aspect of the study is the use of different Cre mouse strains to selectively disrupt target gene expression – in this case the functionally essential NR1 subunit of NMDAR (encoded by Grin1) – in specific types of neurons. The selective deletion of NR1 specifically in AgRP or POMC neurons allowed for a refined study of the feeding circuit isolated from signaling found at other glutamatergic synapses throughout the brain. NR1 knockout mice showed no change in mini excitatory postsynaptic currents (mEPSCs) of AMPA-type receptors (AMPARs) compared with well-fed wild-type animals, regardless of whether NR1 was lacking in AgRP neurons or in POMC neurons. However, the mice lacking NR1 in AgRP neurons showed a specific loss of mEPSCs for NMDARs in AgRP neurons only. Phenotypically, these mice also showed diminished feeding and increased fat loss, demonstrating a role for NMDARs in the function of AgRP neurons.

Liu et al. further probed the mechanism underlying these pronounced phenotypic changes in mice lacking functional NMDARs on AgRP neurons (Liu et al., 2012). They explored neuroanatomical changes, revealing a decrease in the number of dendritic spines (which are enriched in glutamate receptors) and diminished spine size. An increase in dendritic spine formation and synaptogenesis are indicative of postsynaptic adaptive responses, and reflect enhanced synaptic strength and activity. Wild-type mice showed increases in spine number and size following fasting. Consistent with these spine dynamics, the authors detected a change in AMPAR mEPSC frequency in response to fasting. These changes were blocked by the disruption of NMDARs in AgRP neurons, indicating that postsynaptic NMDARs are required for the alterations in spine dynamics of AgRP neurons following fasting. Interestingly, this NMDAR-dependent...
fasting-induced plasticity was reversible: re-feeding of fasted animals allowed for the reversion of AMPAR mEPSC frequency to normal levels, which could have important therapeutic implications for individuals suffering from obesity. NMDAR activation was also shown to increase the production of mRNAs encoding AgRP and NPY, an additional parameter that indicates enhanced AgRP neuron function.

The net effect presented here is that fasting regulates the spine growth of AgRP neurons so as to set the excitatory tone of the connection that governs AgRP neuron firing. Collectively, these data demonstrate the role of the fast-acting glutamatergic synapse in the regulation of AgRP neuronal activity and functional output over long periods of time.

Liu et al. raise the possibility that their results contradict the presynaptic mechanism proposed by Yang et al. (Yang et al., 2011). Instead, they argue for a postsynaptic mechanism of AgRP neuron regulation, supported by a failure to find a difference in paired pulse facilitation after fasting. It is conceivable, however, that both mechanisms take place. For example, it is possible that fasting causes ghrelin release to induce presynaptic facilitation acutely (within a few hours), leading to an elevated AgRP neuron firing rate. This short-term presynaptic activation of the excitatory inputs into the AgRP neurons might activate NMDARs to increase spine growth, leading to an increase in the post-synaptic response of AgRP neurons and thus an elevated AgRP neuron firing rate over the long term.

Overall, the study by Liu et al. suggests that a baseline excitatory tone, modulated by plastic glutamatergic synapses, functions in AgRP neurons to set the fasting behavioral response (Liu et al., 2012). The authors applied an elegant approach in generating mice defective for the NMDAR-dependent feeding circuit to show that AgRP neurons are specifically involved in NMDAR-dependent regulation. This approach allowed the investigators to minimize the impact of the negative feedback loop from POMC back to AgRP neurons in their analysis. It also identified increased spinogenesis as a direct cell biological readout of increased activation and synaptic plasticity, resulting in the increased firing of AgRP neurons.

What still remains elusive is the identity of the presynaptic neuron or neurons that release glutamate for AgRP neuron excitation. Also of interest is a more detailed examination of the presynaptic and postsynaptic events that govern feeding. Finally, it is also unclear precisely how fasting induces the activation of NMDARs.

In summary, Liu et al. (Liu et al., 2012) present a significant study that brings to light the importance of the post-synaptic events of the glutamatergic synapse of AgRP neurons in the regulation of metabolic memory (Spanswick et al., 2012). The findings offer additional support for the idea of an overall central regulation of energy homeostasis (Belgardt et al., 2009) and reveal another regulatory sector of an already complicated feeding circuit. As an extension of this, feeding and fasting can be viewed as experiences that control hunger and weight that are subject to glutamatergic regulation and reversible plasticity. Identifying the role of NMDARs and the details of postsynaptic regulation in this context could open new therapeutic avenues to control feeding behavior and weight.

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**REFERENCES**


