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Hunger-driven motivational state competition

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Summary

Behavioral choice is ubiquitous in the animal kingdom and is central to goal-oriented behavior. Hypothalamic Agouti-related peptide (AgRP) neurons are critical regulators of appetite. Hungry animals, bombarded by multiple sensory stimuli, are known to modify their behavior during times of caloric need, rapidly adapting to a consistently changing environment. Utilizing ARC^{AgRP} neurons as an entry point, we analyzed the hierarchical position of hunger related to rival drive states. Employing a battery of behavioral assays we found that hunger significantly increases its capacity to suppress competing motivational systems such as thirst, anxiety-related behavior, innate fear and social interactions often only when food is accessible. Furthermore, real-time monitoring of ARC^{AgRP} activity revealed time-locked responses to conspecific investigation in addition to food presentation, further establishing that even at the level of ARC^{AgRP} neurons

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Author Contributions

C.J.B., C.L., E.W. and M.J.K. conceived the project and designed the experiments. C.J.B., C.L. E.W., M.J.K. and S.X. performed experiments and analyzed the data. J.C.B. and E.T. assisted with pilot photometry experiments. M.J.K. wrote the paper.

choices are remarkably flexible computations integrating internal state, external factors and anticipated yield.

Introduction

Sherrington's "singleness of action" principle in which an organism must decide to pursue one behavior to the exclusion of others is central to the science of ethology (Sherington, 1906). This means that at some level in the nervous system, incentive stimuli must compete for expression. Hunger provides one of the strongest homeostatic motivations for behavior in the animal kingdom. Despite the wide diversity of stimuli and competing demands that naturally impinge upon animals, they must select and pursue food in times of caloric insufficiency. To address this homeostatic imbalance, animals must often navigate their environment in ways that require switching between exploratory, defensive, and competing behaviors, indicating tremendous plasticity in feeding behavior.

Agouti-related peptide (AgRP) neurons localized in the arcuate nucleus (ARC) display appetite-state-dependent, dynamic activity changes (Betley et al., 2015; Chen et al., 2015; Mandelblat-Cerf et al., 2015; Takahashi and Cone, 2005; Wu et al., 2014). Post-natal ablation of ARC^{AgRP} neurons results in cessation of feeding and ultimately starvation, establishing their necessity for survival (Gropp et al., 2005; Luquet et al., 2005). This function was further clarified using recent advances in neural manipulation and monitoring technologies. Selective activation of ARC^{AgRP} neurons rapidly and robustly promotes motivated feeding in calorically replete mice, while silencing these cells significantly attenuates food intake in mice with energy deficits (Aponte et al., 2011; Krashes et al., 2011). Importantly, in concert with human starvation studies demonstrating the physical, psychological and physiological damage due to caloric restriction (Keys, 1946), ARC^{AgRP} neural activity has been shown to induce a negative affective state, which is immediately suppressed in response to sensory cues anticipating food consumption (Betley et al., 2015). Taken together, these findings reinforce the role of ARC^{AgRP} neurons in both the need detection and response enactment of physiological hunger. In contrast, a subpopulation of GABAergic neurons marked by *Vgat* (vesicular GABA transporter) in the lateral hypothalamus (LH) have been shown to engage an undirected motor program encoding distinct sub-modalities of feeding and other motivated behaviors (Jennings et al., 2015; Navarro et al., 2015; Nieh et al., 2015; Nieh et al., 2016).

While feeding behavior remains complicated, it, like other motivations, has mostly been studied in experimental vacuums targeting a specified outcome (Calhoon and Tye, 2015; Falkner and Lin, 2014; Liu et al., 2014; Oka et al., 2015; Sternson, 2013; Yang and Shah, 2014). In nature, however, ethologically relevant motivations likely intersect in a highly complex manner and animals must select explicit actions at the expense of others. Thus, we sought to test the hypothesis that both physiological and ARC^{AgRP}-mediated hunger can subdue orthogonal motivated behaviors in favor of foraging. We employed optogenetic photoactivation to manipulate ARC^{AgRP} neuron activity in sated mice and compared responses to physiologically sated and hungry animals in a variety of behavioral assays. We found that even when motivated to pursue other behavioral programs, including water-

consumption, self-preservation in anxiogenic-like or fear-inducing contexts, or social interaction with conspecifics, hunger overrode competing incentives to promote feeding behavior. Furthermore, *in vivo* photometry analyses indicate that while these competing stimuli do not appreciably influence ARC^{AgRP} neural activity levels to the same extent as food, real-time network dynamics do respond to conspecifics, highlighting a novel way in which these neurons can integrate rival motivational systems.

Results

ARC^{AgRP} neural activation directs biased caloric consumption

To selectively mark and modulate ARC^{AgRP} neurons we injected Cre-dependent viruses encoding green fluorescent protein (GFP) or channelrhodopsin-2 (ChR2) into the ARC of *Agrp-IRES-Cre* mice (Figure S1A). For all experiments, *Agrp-IRES-Cre* animals were divided into three behavioral groups; physiologically sated mice with *ad libitum* access to food transduced with GFP (fed^{GFP}), physiologically hungry mice fasted for 24 hours transduced with GFP ($fasted^{GFP}$) and physiologically sated mice with *ad libitum* access to food transduced with ChR2 (fed^{ChR2}). All groups were tethered to patchcords and received the same light delivery protocol. Concordant with previous reports, home cage screening of light cycle food intake, a time when mice typically refrain from eating as seen in the fed^{GFP} group, revealed that photoactivation of ARC^{AgRP} neurons (fed^{ChR2}) promoted feeding comparable to fasted animals ($fasted^{GFP}$) (Figure S1B–C) (Aponte et al., 2011).

To evaluate whether this consumption was geared toward caloric sustenance, we acclimated mice to recognize and ingest 20 mg grain, sucrose and 1% saccharin pellets over the course of 3 consecutive dark cycle periods (Figure S1D). On study days, mice were exposed to each individual food source for one hour near the start of the light cycle. Both fed^{ChR2} and $fasted^{GFP}$ mice displayed significantly increased consumption of grain and sucrose, but not of 1% saccharin pellets, versus fed^{GFP} animals (Figure 1A), demonstrating that ARC^{AgRP} neurons are tuned to attaining caloric sufficiency. To further investigate this specificity, we used ChR2 to photostimulate LH^{VGAT} neurons in our pellet assay (Figure S1E). Unlike ARC^{AgRP} neurons, acute activation of LH^{VGAT} neurons significantly elevated consumption of all three types of pellets irrespective of caloric value (Figure S1F). Thus, unlike the broad consummatory spectrum evoked by LH^{VGAT} stimulation, ARC^{AgRP} neural activation is directed toward caloric intake.

Physiological hunger naturally promotes food-seeking responses across species, a calculated action whereby the organism must compute environmental costs and benefits, to appropriately ensure survival (Yapici et al., 2014). Head-dipping activity on a hole-board apparatus is frequently utilized as an indicator of exploratory tendencies in rodent studies (Boissier and Simon, 1962; Montgomery, 1955). To assess the explicit contribution of ARC^{AgRP} neurons toward exploration and foraging behavior, respectively, we tested our mice employing this assay when the 16 holes were either empty or baited with 20 mg grain pellets (Figure S1G–H). Fed^{ChR2} and $fasted^{GFP}$ mice drastically escalated the amount of exploratory head-dips into the empty wells compared to fed^{GFP} animals (Figure 1B). Furthermore, in a separate cohort of mice to avoid repeated exposure to the apparatus, fed^{ChR2} and $fasted^{GFP}$ animals displayed a fivefold increase in the retrieval and consumption

of the grain pellets, with several mice reaching the maximum limit of 16 pellets, compared to fed^{GFP} controls (Figure 1C). Therefore, both physiological and ARC^{AgRP}-mediated hunger enhanced exploratory tendencies and foraging behavior.

An animal's commerce with its environment depends on its ability to predict where and when incentive stimuli are likely to appear. To ensure viability organisms must form representations of their surroundings and learn relationships between a response and the consequences of the response (Kheifets and Gallistel, 2012). As acute ARC^{AgRP} stimulation motivates the seeking and consumption of food, we sought to determine if ARC^{AgRP} activity was sufficient to facilitate choice behavior and recall a learned preference for food location using a Y-maze apparatus (Figure 1D). Importantly, prior to any conditioning, each group of animals displayed an unbiased choice between the two arms of the maze, with a distribution comparable to chance (Figure 1E) (Binomial distribution, two-tailed P-test; fed^{GFP} > 0.9999, fasted^{GFP} = 0.4545, fed^{ChR2} = 0.8036). Throughout training, animals were restricted to 85% of their initial body weights to boost motivation in procuring food reward. Following conditioning trials, in which mice were trained to associate one arm of the maze with a 20 mg grain pellet utilizing visual and spatial cues, all groups of mice exhibited high proficiency in learning the task, with a weighted distribution toward the baited arm statistically significant from chance (Figure 1F) (Binomial distribution, two-tailed P-test; fed^{GFP} = 0.0213, fasted^{GFP} = 0.0042, fed^{ChR2} = 0.0042). After the last conditioning trial, animals were sorted into the respective groups and tested the following day under photostimulation conditions. Strikingly, while fed^{GFP} mice displayed random distribution between the two unbaited arms of the maze comparable to chance, both fasted^{GFP} and fed^{ChR2} mice continued to demonstrate a learned preference toward the arm associated with food reward (Figure 1G) (Binomial distribution, two-tailed P-test; fed^{GFP} = 0.8036, fasted^{GFP} = 0.0042, fed^{ChR2} < 0.0001). Accordingly, the foraging behavior elicited via physiological and ARC^{AgRP}-mediated hunger extends to learning and decision-making surrounding the exploration of an environment previously associated with food.

Next, we probed the integrative role of ARC^{AgRP} neurons on thirst regulation using a lickometer to analyze drinking behavior. These experiments were executed both in the presence or absence of food, adding a within-subject scheme in addition to the between-subject design (Figure S2A). All groups of mice that had *ad libitum* access to water before the 1 hour test exhibited modest interactions (licking) with a water spout when a non-food object was present, demonstrating that acute ARC^{AgRP} neural activation was not sufficient to orchestrate water drinking behavior (Figure 2A). However, when food was available, fed^{ChR2} and fasted^{GFP} mice significantly increased the number of spout interactions (prandial thirst) contrasted to fed^{GFP} controls, an effect correlated to food intake (Figures 2A–B, E). Thus, ARC^{AgRP} activation, akin to physiological hunger, does not stimulate water intake independently of food consumption.

In a separate set of experiments, mice were deprived of water for 24 hours before testing (water restricted) in the presence or absence of food. As expected, water-restricted fed^{GFP} mice revealed a higher number of spout interactions in the object condition compared to the same group of animals with *ad lib* access to water before the assay (Figures 2C, S2D). Interestingly, in the non-food object condition, water-deprived fasted^{GFP} animals displayed a

sharp reduction in spout interactions compared to fed^{GFP} mice (Figure 2C), a phenomenon previously reported in rodents attributed to differences in cellular dehydration and plasma osmolality or alternatively, neurological inhibition of the drinking system due to hunger (Siegel and Talantis, 1950; Verplanck and Hayes, 1953). In support of a competing drive-state hypothesis, whereby two motivational states contend to promote a defined goal-oriented behavior, we found that water-restricted fed^{ChR2} animals, presumably with similar physiology (blood volume and tonicity) as fed^{GFP} mice, mimicked the decline in spout interactions observed in the fasted^{GFP} mice (Figure 2C). All water-restricted groups of mice demonstrated comparable spout interactions when food was made available, a result correlated with the amount of food consumed (Figures 2C–E). Moreover, in agreement with prior findings (Kutscher, 1972), water-restricted animals likely voluntarily curbed their food intake leading up to the experiment, as water-restricted fed^{GFP} mice ate significantly more than the same group of animals with *ad lib* access to water before the assay (Figure 2F).

Within subject comparisons showed that while the fed^{GFP} group did not alter drinking behavior in the presence or absence of food in either the *ad lib* water or water restricted conditions, both fasted^{GFP} and fed^{ChR2} groups consumed more water when food was present in either condition (Figure S3B–C, F–G, J–K). All groups exhibited increased water intake in the absence of food when water restricted (Figure S2D, H, L). Finally, while the fed^{GFP} group enhanced drinking behavior when food was accessible in the water restricted condition, both fasted^{GFP} and fed^{ChR2} groups displayed comparable water intake in the presence of food regardless of thirst state (Figure S2E, I, M). These studies reveal the complex contestation between water- and caloric-deprivation, indicating ARC^{AgRP} neural activation mimics physiological hunger in responding to thirst imbalances.

ARC^{AgRP} neural stimulation suppresses anxiety-like behavior depending on food location

It has been shown that ARC^{AgRP} activation decreased anxiety-related behavior (Dietrich et al., 2015; Padilla et al., 2016), yet whether this increased risk behavior is linked with food acquisition remains to be elucidated. Here we employed a range of apparati known to induce anxiety-related behavior in mice to explore whether physiological and/or artificial hunger could subdue anxiety-like behavior in the absence or presence of food positioned in anxiogenic open zones versus anxiolytic “safe” zones. We found that fed^{ChR2} and fasted^{GFP} mice spent more time in the center of a standard mouse open field apparatus, when a non-food object was located in the middle, than fed^{GFP} controls (Figures 3A–B, S3A). However, time spent in the center by fasted^{GFP} and fed^{ChR2} mice was heightened when food was located in the middle, an effect reflected by food consumption levels (Figures 3A–C, S3B–D).

To better illuminate total time spent in an open zone and further strengthen anxiety-like behavior, we tested our groups in a larger open field apparatus (2.5 x bigger) where we found comparable levels of time spent in the center zone in the presence of a non-food object between all groups (Figures 3D–E, S3E). Conversely, the addition of food in the middle of the apparatus significantly elevated the time spent in the center zone in fasted^{GFP} and fed^{ChR2} mice compared to fed^{GFP} controls, again indicated by total food consumption (Figures 3D–F, S3F–H). Interestingly, although fed^{GFP} mice significantly escalated the time

spent in the center zone in the presence of food versus object in the small open field apparatus, this enhancement was abolished using the big open field (Figure S3B, F). To gauge the context specificity of ARC^{AgRP} activation to reduce anxiety-related behavior, we examined another subset of cells (LH^{VGAT}) demonstrated to impact acute feeding (Jennings et al., 2015; Navarro et al., 2015; Nieh et al., 2015). Importantly we failed to detect a significant difference between the total time spent in the center zone interacting with the object or food irrespective of context during LH^{VGAT} photostimulation (Figure S3I).

Next, to deconstruct general anxiety-related behavior from food accessibility, we repeated the above experiment in the big open field placing the object or food in a random corner ("safe" zone) of the apparatus (Figure S4A). We then measured the total time each animal spent in either the open center zone or the corner object/food zone. Interestingly, we found no differences between the three groups in the total time spent in the center zone either in the presence of an object or food in the corner of the apparatus (Figure S4B). Although all groups displayed similar time spent in the corner object zone, $fasted^{GFP}$ and fed^{ChR2} mice spent significantly more time in the corner food zone than fed^{GFP} controls (Figure S4F), presumably due to elevated food intake (Figure S4J). Notably, within-subject comparisons revealed a statistical decrease in the time spent in the center zone in $fasted^{GFP}$ and fed^{ChR2} mice when food was made available (Figure S4D–E), a probable reflection of the statistical increase spent in the food zone (Figure S4H–I). No such alterations were detected in fed^{GFP} mice (Figure S4C, G).

Lastly, we used a zero-maze apparatus to interrogate anxiety-related behavior, which is inversely correlated with the time that animals spend in the open arms (Figure S3J). Again, when objects were placed in the middle zones of the open arms, all groups displayed a similar amount of time in these designated areas (Figure 3G–H). In contrast, fed^{ChR2} and $fasted^{GFP}$ animals spent considerably more time in these middle zones, when they were baited with food, than fed^{GFP} mice, reflected by increased food intake (Figures 3G–I, S3K–M). Thus, the capability of hunger to curtail anxiety-like behavior in the absence of food seems to be dependent on the assay adopted, but regardless, the presence of food in open/exposed zones is sufficient to override anxiogenic responses.

ARC^{AgRP} neural activation competes with innate motivational drives

Innate fear is a basic and natural mechanism by which organisms evade danger. A volatile chemical produced by foxes, trimethylthiazoline (TMT), has been shown to incite fear-like and aversive behavior in mice, enabling remote or contact-based detection of predator cues (Fendt et al., 2005; Yang et al., 2016). We assessed whether physiological or artificial hunger could supersede the inherent avoidance of this odorant in the absence or presence of food. Importantly, we validated that mice exposed to TMT exhibit diminished locomotor activity and time spent in both the TMT paired chamber and designated TMT zone while increasing immobility (freezing), compared to the same mice exposed to a neutral odorant (water) (Figure 4A–E).

When a non-food object was placed in the TMT zone, fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} animals showed a strong preference for the neutral side of the chamber compared to the TMT-paired side (Figures 4F–G, S5A). Moreover, all groups intensely avoided the TMT

zone (Figure 4F, H). However, presentation of food in the TMT zone significantly increased fasted^{GFP} and fed^{ChR2} animals' preference for the TMT-paired chamber, time spent in the TMT zone and total food intake compared to fed^{GFP} mice (Figures 4F–H, S5B). Notably, within-subject analysis unveiled fed^{GFP} subjects avoided both the TMT-paired chamber and TMT zone irrespective of object or food, while fasted^{GFP} and fed^{ChR2} animals shifted preference toward the TMT-paired chamber and TMT zone in the presence of food versus object (Figure S5C–E, G–I). In contrast food-dependent shifts in chamber preference and time spent in the TMT zone observed with ARC^{AgRP}-mediated hunger, no statistical differences were observed with LH^{VGAT} activation in the presence of a non-food object versus food (Figure S5F, J). These experiments establish the capacity of both physiological and ARC^{AgRP}-, but not LH^{VGAT}-, mediated hunger to suppress innate fear when the acquisition of food is a likely outcome.

Mice are naturally social animals demonstrating high interaction rates with conspecifics. Not only do mice prefer social to isolated housing, but also the aversive state of isolation generates the motivation to seek and engage in social contact (Niesink and van Ree, 1982; Van Loo et al., 2004). To analyze the effects of hunger on social interactions (a proxy of mating or aggression), we exposed experimental animals (socially isolated naïve males) to either a receptive female or juvenile male, respectively, confined to a social cage in the absence or presence of food (Figures S6A). Importantly, social approach was controlled by the experimental animal, as containment of the receptive female or juvenile male eliminated the potential of copulation or territorial attack, respectively, enabling an unbiased readout of social interest. Fed^{GFP}, fasted^{GFP} and fed^{ChR2} mice all demonstrated a high preference for the side of the chamber paired with the receptive female versus the chamber paired with an object, including a minimal duration of time spent in the designated object zone (Figure 5A–C). On the other hand, while fed^{GFP} controls continued to display a strong preference for the female-paired chamber in the presence of food, fasted^{GFP} and fed^{ChR2} animals reversed this initial preference, spending a significant amount of time in the allocated food zone, associated with enhanced food consumption (Figures 5A–C, S6B). Within-subject comparison established that chamber preference and zone duration in fed^{GFP} subjects was not altered in object versus food conditions compared to the statistical changes of these parameters observed in fasted^{GFP} and fed^{ChR2} mice (Figure S6D–F, H–J). To assess whether these shifts were specific to physiological and ARC^{AgRP}-mediated hunger, we tested the faculty of LH^{VGAT} neurons in mediating this condition-dependent transition. Importantly, we found that LH^{VGAT}-mediated hunger failed to modify either chamber preference or zone duration in a condition-dependent manner (Figure S6G, K).

Similar to the efficiency of natural or ARC^{AgRP}-evoked hunger to rival female social interactions, all groups revealed a selective preference for the juvenile male-paired side over the non-food-paired side in the object condition, spending a modest amount of time in the marked object zone (Figure 5D–F). While this bias toward the chamber paired with the juvenile male remained intact in the fed^{GFP} group in the food condition, fasted^{GFP} and fed^{ChR2} mice switched preference toward the food-paired side, spending significantly more time in the designated food zone, which is reflected in their elevated food intake levels (Figures 5D–F, S6C). Again, evaluation of within-subject data revealed fed^{GFP} subjects showed comparable levels of time spent in the male-paired side and zone regardless of

object or food, while fasted^{GFP} and fed^{ChR2} animals presented shifts toward the food-paired chamber and food zone in the presence of food versus object (Figure S6L–Q). In conclusion, when confronted with competing demands (hunger versus social interaction), calorically depleted mice modify their behavior from an innate preference to interact with a conspecific to the acquisition and consumption of food when it becomes accessible, an effect recapitulated at the ARC^{AgRP}, but not the LH^{VGAT}, neuron level.

ARC^{AgRP} neural activity responds to food and conspecifics

The experiments above would suggest that ARC^{AgRP} excitation is sufficient to overrule instinctive, motivational states, especially in the presence of food. However, the physiological activity of this cell-type in direct response to distinct, external stimuli is unknown. To probe these questions, fiber photometry was used to enable optical recording of real-time ARC^{AgRP} activity via selective targeting of the genetically-encoded calcium indicator GCaMP6s (Figure S7A–B) (Cui et al., 2013). This technique has convincingly shown that food exposure strongly and swiftly inhibited ARC^{AgRP} neurons in fasted mice compared to a non-food object and importantly this response was state-dependent as sated mice failed to show suppression of ARC^{AgRP} activity (Chen et al., 2015). We sought to examine the effects of a water, TMT, receptive female or juvenile male stimulus on ARC^{AgRP} activity compared to food or neutral object presentation in both hungry and sated animals. We employed 2 protocols to investigate these responses; In Protocol 1 (Figure S7A), after a 5 min baseline, a water, TMT, receptive female or juvenile male stimulus was introduced into the cage for 5 min (stimulus in), removed for 5 min (stimulus out), followed immediately by food introduction for 5 min (food in). This sequential presentation culminating in food introduction was chosen due to the sharp and persistent suppression of ARC^{AgRP} activity by food (Chen et al., 2015). Protocol 2 (Figure S7A) constituted a 5 min baseline signal followed by the introduction of a water, TMT, receptive female or juvenile male, food or non-salient object stimulus for 5 min allowing direct comparisons of these external cues on ARC^{AgRP} neural activity.

Supporting our behavioral data, the presentation of water to both fasted and sated animals had no significant effect on ARC^{AgRP} activity (respectively, $\Delta F/F = 0.87\% +/- 5.48\%$; $\Delta F/F = 0.63\% +/- 0.33\%$ comparing 5 min baseline average and 5 min water presentation average), while food presentation rapidly and robustly suppressed activity in the fasted condition ($\Delta F/F = -27.74\% +/- 5.66\%$) compared to both water and a non-salient object (Figure 6A–C). Congruent with previous findings this reduction in ARC^{AgRP} neural activity in response to food was state-dependent as it was absent in the sated condition ($\Delta F/F = -5.27\% +/- 2.54\%$; Figure S7C–E). Furthermore, the introduction of TMT failed to alter real-time ARC^{AgRP} neural dynamics in either hungry or sated animals (respectively, $\Delta F/F = 3.82\% +/- 5.62\%$; $\Delta F/F = -0.55\% +/- 0.74\%$ 5 min average during TMT vs 5 min baseline average), indicating that although predatory odors induce substantial behavioral adaptations, this is not encoded directly at the level of ARC^{AgRP} neurons (Figure 6D–F, S7F–H).

Given the strong social structure of mice, we next examined the effects of conspecific introduction on ARC^{AgRP} activity. Notably, conspecifics were food-deprived and kept in cages devoid of food before testing to assure no food-related sensory cues could affect the

recordings as just the smell of food has been shown to suppress ARC^{AgRP} activity (Chen et al., 2015). Conspecifics were isolated in grid enclosure sociability cages and introduced to a new cage housing the experimental animal. Surprisingly, we found that presentation of a receptive female or a juvenile male to fasted mice elevated ARC^{AgRP} neural activity (respectively, ΔF/F = 7.69% +/− 5.15%; ΔF/F = 7.12% +/− 7.9% 5 min average during stimulus,) although these changes in network dynamics over a 5 minute window were not significantly different compared to the introduction of a non-salient object (Figure 6G–L). Importantly, unlike the response to food, these ARC^{AgRP} response properties occurred regardless of the experimental animals appetite state, as conspecific presentation increased ARC^{AgRP} activity in the fed state (female ΔF/F = −2.70% +/− 2.50%; male ΔF/F = −1.60% +/− 3.51%; Figure S7I–N). Importantly, these dynamics were unrelated to mouse movement or changes in ambient light as they were absent from recordings of control mice expressing GFP in ARC^{AgRP} neurons, indicating that they represent calcium-dependent GCaMP6s signals (Figure S6M–X).

To further investigate this effect, we video-recorded the photometry sessions and compared the fluorescence signals in response to initial investigative contact with the receptive female or juvenile male mouse versus a non-salient object. Strikingly, we observed a significant increase in ARC^{AgRP} activity in response to first contact with either the receptive female or juvenile male, compared with a non-salient object in hungry mice (Figure 7A–C). This significantly heightened effect was found independent of the satiety state of the experimental animal (Figure 7D–F), although the response appeared to be dampened compared to the fasted condition. Next, to determine if ARC^{AgRP} neural response properties toward a conspecific was associated with novelty/familiarity, we ran another protocol (Protocol 3) whereby each experimental animal was exposed to either the empty, clean grid enclosure sociability cage or the same receptive female in four repeated sessions (Figure S7A). Reinforcing the authenticity of the GCaMP6 signals, we observed very little response to the empty cage over the four trials (Figure 7G–H). However, ARC^{AgRP} population dynamics transiently increased during the initial investigative contact with the receptive female, an effect that repeated itself over 3 trials before becoming extinguished on the final session, suggesting the involvement of a potential learning component to these responses (Figure 7G–H).

Discussion

Recent work has begun to unravel the neural circuits fundamental for discrete behaviors including feeding, but these motivational systems are most often studied in behavioral isolation, with strict control over internal and external variables for simplification (Calhoon and Tye, 2015; Falkner and Lin, 2014; Liu et al., 2014; Oka et al., 2015; Sternson, 2013; Yang and Shah, 2014). In this way the animal is encouraged to express goal-seeking behavior directed toward one objective. From an ethological perspective, individual motivational systems probably rarely, if ever, operate in isolation untouched by competing factors. Hunger drive represents one of these motivation systems, and remarkably, despite an amalgamation of peripheral and central fluctuations coupled with varying satiety levels, ARC^{AgRP} neurons govern an increasingly well-defined behavioral program for the seeking and subsequent consumption of food (Aponte et al., 2011; Betley et al., 2015; Dietrich et al.,

2015; Krashes et al., 2011). However, the interaction between ARC^{AgRP}-mediated hunger with competing motivational systems has not been explicitly and rigorously tested to date. Here, through combinatorial behavioral and real-time physiological recording methods, we demonstrate that similar to starvation-induced hunger, ARC^{AgRP}, but not LH^{VGAT}, stimulation can drive motivated food-seeking and consumption behavior directed toward caloric sustenance over rival motivations. Furthermore, in addition to the state-dependent, prolonged suppression of ARC^{AgRP} activity by food, ARC^{AgRP} network dynamics are unaltered by water or TMT but display elevated activity in response to the investigation of conspecifics independent of satiety levels.

ARC^{AgRP} versus LH^{VGAT} neurons

Similar to ARC^{AgRP} neurons, activation of LH^{VGAT} neurons is sufficient to acutely promote voracious feeding behavior (Aponte et al., 2011; Jennings et al., 2015; Krashes et al., 2011; Navarro et al., 2015; Nieh et al., 2015). However, unlike photostimulation of ARC^{AgRP} neurons, photostimulation of LH^{VGAT} neurons failed to reproducibly suppress contesting drive systems in an appetite state-dependent manner. A number or reasons may explain for these observed behavioral disparities. Firstly, while ARC^{AgRP} activation selectively elicits caloric intake, chemogenetic stimulation of LH^{VGAT} neurons escalated wide-ranging consummatory behavior directed toward chow, sucrose, ethanol, saccharin, water, and even a block of wood (Navarro et al., 2015). Furthermore, photoactivation of a specific LH^{VGAT} projection to the ventral tegmental area (VTA) often resulted in repetitive gnawing and aberrant motor sequences (Nieh et al., 2015). Follow-up studies have demonstrated that optogenetic activation of the $LH^{VGAT}\rightarrow$VTA pathway promiscuously promoted the investigation of the most proximal salient object to the experimental animal (Nieh et al., 2016). In agreement with these prior findings, we found a significant increase in non-caloric pellet intake and excessive chewing on the inedible non-food object, bedding, social isolation cages and the plastic of the apparati used in our behavioral assays during LH^{VGAT} photoactivation, an effect we rarely if ever observed during ARC^{AgRP} photoactivation. However, it should be noted that high frequency, synchronous activation of these neural subsets might promote behavior beyond that of normal caloric deficiency.

Notably, activation of the $LH^{VGAT}\rightarrow$VTA circuit strongly supported positive reinforcement and place preference via the modulation of dopamine release in the nucleus accumbens (Nieh et al., 2016), subscribing to a more general change in the motivational state of the animal (Berridge and Robinson, 1998; Saunders and Robinson, 2012). Although the immediate effects on the mesolimbic system during ARC^{AgRP} activation are currently unknown, ARC^{AgRP} neurons have been shown to transmit a negative-valence teaching signal as mice learned to avoid locations or conditioned stimuli associated with ARC^{AgRP} photostimulation (Betley et al., 2015). However it should be noted that mice failed to perform instrumental responses leading to a reduction in ARC^{AgRP} neural activity, a hallmark of a negative reinforcement model, spawning alternative valence models such as increasing the positively rewarding properties of food (Chen and Knight, 2016). Moreover, elegant microendoscopic imaging studies uncovered that food rapidly inhibited the large majority (96%) of ARC^{AgRP} neural activity and that this suppression occurred prior to consumption (Betley et al., 2015) fortifying similar findings at the network level (Chen et

al., 2015). These findings would suggest ARC^{AgRP} activity encodes the appetitive, pre-consummatory phase of feeding behavior as both individual ARC^{AgRP} neurons and population level dynamics undergo quiescence time-locked to food-related cues before consumption ensues. Interestingly, this repressed activity steadily ramps back up if animals are prohibited from engaging in restoring caloric balance (Betley et al., 2015; Chen et al., 2015). Similar refined calcium imaging strategies were utilized to demonstrate that separate subsets of appetitive-coding and consumption-coding ensembles exist within the LH^{VGAT} neural network (Jennings et al., 2015), further highlighting the differences between these two sets of neurons.

ARC^{AgRP} neurons express the neuromodulators Agouti-related peptide and Neuropeptide Y along with the fast transmitter GABA, all of which influence feeding behavior (Clark et al., 1984; Krashes et al., 2013; Rossi et al., 1998; Stanley and Leibowitz, 1985; Stratford and Kelley, 1997). Furthermore, high-quality gene expression profiles of ARC^{AgRP} neurons have been carried out revealing tremendous fluctuations in transcript levels dependent on energy state (Henry et al., 2015). Far less is known about the molecular composition of LH^{VGAT} neurons. A complete transcriptional profiling of this subpopulation is currently lacking although it has been shown that LH^{VGAT} neurons are distinct from cells containing the feeding-related neuropeptides melanin-concentrating hormone and orexin (Jennings et al., 2015). Moreover, while several ARC^{AgRP} efferents, which are wired in a one-to-one fashion (Betley et al., 2013), have been assessed for their sufficiency to evoke acute food intake including projections to the bed nucleus of the stia terminalis (BNST), LH, parabrachial nucleus (PBN), amygdala, periaqueductal gray and both the paraventricular thalamus (PVT) and hypothalamus (PVH) (Atasoy et al., 2012; Betley et al., 2013; Padilla et al., 2016; Steculorum et al., 2016), only the $LH^{VGAT}\rightarrow$VTA circuit has been analyzed to date and the anatomical architecture including terminal targets and potential collateralization of axons has yet to be described.

Facilitating food acquisition via inhibition of competing drives

Hunger is known to shift foraging behavior toward a higher risk-reward strategy to locate a food cache (Sih, 1980). Mice, like humans, have the ability to rapidly assess probabilities and risks and abruptly modify their behavior in sync with variable environmental fluxes (Kheifets and Gallistel, 2012). Given the inhibitory nature and widespread distribution of non-collateralizing ARC^{AgRP} projections throughout the mammalian brain, including axonal targeting to intrahypothalmic nuclei, limbic regions of the forebrain, midbrain and brainstem nuclei (Betley et al., 2013; Broberger et al., 1998), it is possible that these appetite-regulating neurons are capable of producing broad suppression signals which halt other motivated behaviors to prioritize the seeking and consumption of caloric food. Supporting this notion, both physiological and ARC^{AgRP}-mediated hunger dampened rival drive states such as thirst, anxiety-related behavior, innate fear and social interactions, which were often dependent on food accessibility. From a circuit perspective, GABAergic ARC^{AgRP} neurons densely innervate regions shown to play key roles in these competing behaviors (Calhoon and Tye, 2015; Kawano and Masuko, 2010; Oka et al., 2015; Silva et al., 2013; Stamatakis et al., 2014). In accordance with this model, a recent study found that photoactivation of the

ARC^{AgRP}→medial amygdala (MeA) terminal field curbed territorial aggression in the absence of food (Padilla et al., 2016).

Conspecific investigation transiently increases ARC^{AgRP} activity

Social interactions are a fundamental and adaptive biological component of numerous species, especially mice, in which recognition of conspecifics is imperative in maintaining social hierarchy and mate choice (Wang et al., 2014). Socially-isolated mice exhibit goal-directed behavior aimed toward the re-connection with conspecifics, investing time and energy when the opportunity presents (Niesink and van Ree, 1982). We explored the competitive relationship between hunger and the drive of socially isolated, naïve male mice to interact with either a receptive female or juvenile male. While our behavioral experiments concluded that both physiological and ARC^{AgRP}-mediated hunger, shifted preference from the conspecific-paired chamber to the food-paired side, photometry analyses revealed changes in ARC^{AgRP} activity time-locked to the investigation of a conspecific. Interestingly, these responses do not appear to be related to general arousal as no alterations in ARC^{AgRP} activity were detected in response to TMT, although it should be noted that conspecific interaction is largely associated with positive valence and approach (Niesink and van Ree, 1982), while TMT elicits freezing and avoidance (Fendt et al., 2005).

As previously reported, food presentation rapidly and persistently silenced ARC^{AgRP} network dynamics dependent on energy status (Chen et al., 2015), whereas the increased ARC^{AgRP} activity during conspecific investigation was transient and independent of satiety levels. These findings suggest the prospect of heterogeneity in ARC^{AgRP} response properties, however whether the same or distinct subpopulations respond to both food and/or conspecifics cannot be resolved using photometry targeted at soma. Notably, although deep-brain imaging revealed essentially all ARC^{AgRP} neurons were inhibited by food cues (Betley et al., 2015), optrode recordings found an ~65% reduction (Mandelblat-Cerf et al., 2015). Therefore it is plausible that a subset of ARC^{AgRP} neurons either respond independently to a conspecific or in concert with food cues. Further testing employing these individual cellular resolution approaches or alternatively, fiber photometry directed toward explicit terminal fields (Chen et al., 2015), given the one-to-one ARC^{AgRP} neural architecture (Betley et al., 2013), would begin to elucidate these questions. Importantly, projection-defined ARC^{AgRP} neurons target a number of downstream areas that fail to elicit acute feeding behavior giving rise to the possibility that these efferents modulate surrogate behaviors such as mating and territorial aggression (Falkner and Lin, 2014; Matthews et al., 2016; Padilla et al., 2016; Yang and Shah, 2014).

Why would ARC^{AgRP} activity be influenced by conspecifics? One interpretation may be another animal signals competition for resources enhancing the priority of procuring food. If food cues cause a pre-ingestive decrease in $AgRP^{ARC}$ activity due to the expectation of satiation, the addition of a competing mouse may increase activity as that satiety expectation dwindles. This decrease in expected food intake may stem from food contention but may also be related to the engagement of principal social behaviors such as mating and aggression. Further studies will be needed to address these possibilities.

Foraging, fighting, fleeing and mating decisions deeply impact evolutionary fitness and thus are likely to have played a vital role in molding the neural circuits that mediate decision-making. Here, we report how physiological and ARC^{AgRP}-mediated hunger coordinates food-seeking and vies for behavioral expression with other competing motivational drives such as thirst, anxiety, innate fear and social interaction. Future studies aimed at dissecting the explicit circuitry whereby ARC^{AgRP} neurons integrate with cell-types and structures encoding alternative motivational systems will be key to unlocking the complexity of hierarchical decision-making.

Experimental Procedures (See Supplemental Items for detailed methods)

Animals

All animal care and experimental procedures were approved by the National Institute of Health Animal Care and Use Committee. Mice were housed at 22–24 °C with a 12 h light: 12 h dark cycle with standard mouse chow (Teklad F6 Rodent Diet 8664; 4.05 kcal g^{-1}, 3.3 kcal g^{-1} metabolizable energy, 12.5% kcal from fat; Harlan Teklad) and water provided ad libitum, unless otherwise stated.

Viral injections

Stereotaxic injections were performed as previously described(Krashes et al., 2011). Mice were anaesthetised with isoflurane and placed into a stereotaxic apparatus (Stoelting Just for Mice). For postoperative care, mice were injected intraperitoneally with meloxicam (0.5 mg per kg). After exposing the skull via small incision, a small hole was drilled for injection. A pulled-glass pipette with 20–40 mm tip diameter was inserted into the brain and virus was injected by an air pressure system. A micromanipulator (Grass Technologies, Model S48 Stimulator) was used to control injection speed at 25 nl min^{-1} and the pipette was withdrawn 5 min after injection.

Behavioural testing testing (food intake screening, pellet and water intake studies) All animals were singly housed for at least 2.5 weeks following surgery and handled for 10 consecutive days before the assays to reduce stress response. Studies were conducted in a home-cage environment near the beginning of the light cycle (9 am) for food intake screening, pellet and water intake studies and in specialized environments for hole-board, Y-maze, open-field, elevated zero maze, TMT and social interaction assays. All Agrp-ires-Cre animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were photostimulated for 10 minutes in the homecage in the absence of food before being placed in the behavioral apparati.

In vivo fiber photometry

Unilateral optic-fiber cannulas (fiber: core=400μm; NA=0.48;M3 thread titanium receptacle; Doric Lenses Inc) were implanted in the ARC of each experimental mouse. Behavioral testing started 4 weeks later to allow for viral expression and recovery from surgery. Mice were then allowed to adapt to the experimental cages and the fiber patch cord for at least 3 days prior to experiments (core 400 uM; NA 0.53; M3 connector; Doric Lenses Inc). Continuous ~20 uW blue LED at 465 nm served as a light source delivered through optic fibers connected to a rotary joint (FRJ 1X1, Doric Lenses Inc.) to allow for movement.

GCaMP calcium GFP signals were collected through the same fibers via a dichroic port (FMC4 port, Doric Lenses Inc) into a femtowatt silicon photoreceiver (2151, Newport). Digital signals were then amplified and collected through Plexon softwares OmniPlex. Synchronized multi-angled videos were recorded via Cineplex for time-locked data analysis in NeuroExplorer.

Statistical analysis

Statistical analyses were performed using Prism 6.0 (GraphPad) software. In all statistical tests normal distribution and equal variance was established. The data presented met the assumptions of the statistical test employed. Exclusion criteria for experimental fed^{ChR2} animals were <0.40 grams of food intake during the screening period. This criterion was established prior to data collection. N-numbers represent final number of animals used per assay.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Aponte Y, Atasoy D, Sternson SM. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. Nature neuroscience. 2011; 14:351–355. [PubMed: 21209617]

Atasoy D, Betley JN, Su HH, Sternson SM. Deconstruction of a neural circuit for hunger. Nature. 2012; 488:172–177. [PubMed: 22801496]

Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev. 1998; 28:309–369. [PubMed: 9858756]

Betley JN, Cao ZF, Ritola KD, Sternson SM. Parallel, redundant circuit organization for homeostatic control of feeding behavior. Cell. 2013; 155:1337–1350. [PubMed: 24315102]

Betley JN, Xu S, Cao ZF, Gong R, Magnus CJ, Yu Y, Sternson SM. Neurons for hunger and thirst transmit a negative-valence teaching signal. Nature. 2015; 521:180–185. [PubMed: 25915020]

Boissier JR, Simon P. The exploration reaction in the mouse. Preliminary note. Therapie. 1962; 17:1225–1232. [PubMed: 13968530]

Broberger C, Johansen J, Johansson C, Schalling M, Hokfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95:15043–15048. [PubMed: 9844012]

Calhoon GG, Tye KM. Resolving the neural circuits of anxiety. Nature neuroscience. 2015; 18:1394–1404. [PubMed: 26404714]

Chen Y, Knight ZA. Making sense of the sensory regulation of hunger neurons. Bioessays. 2016; 38:316–324. [PubMed: 26898524]

Chen Y, Lin YC, Kuo TW, Knight ZA. Sensory detection of food rapidly modulates arcuate feeding circuits. Cell. 2015; 160:829–841. [PubMed: 25703096]

Clark JT, Kalra PS, Crowley WR, Kalra SP. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology. 1984; 115:427–429. [PubMed: 6547387]

Cui G, Jun SB, Jin X, Pham MD, Vogel SS, Lovinger DM, Costa RM. Concurrent activation of striatal direct and indirect pathways during action initiation. Nature. 2013; 494:238–242. [PubMed: 23354054]

Dietrich MO, Zimmer MR, Bober J, Horvath TL. Hypothalamic Agrp neurons drive stereotypic behaviors beyond feeding. Cell. 2015; 160:1222–1232. [PubMed: 25748653]

Falkner AL, Lin D. Recent advances in understanding the role of the hypothalamic circuit during aggression. Frontiers in systems neuroscience. 2014; 8:168. [PubMed: 25309351]

Fendt M, Endres T, Lowry CA, Apfelbach R, McGregor IS. TMT-induced autonomic and behavioral changes and the neural basis of its processing. Neuroscience and biobehavioral reviews. 2005; 29:1145–1156. [PubMed: 16099043]

Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, Plum L, Balthasar N, Hampel B, Waisman A, et al. Agouti-related peptide-expressing neurons are mandatory for feeding. Nature neuroscience. 2005; 8:1289–1291. [PubMed: 16158063]

Henry FE, Sugino K, Tozer A, Branco T, Sternson SM. Cell type-specific transcriptomics of hypothalamic energy-sensing neuron responses to weight-loss. Elife. 2015; 4

Jennings JH, Ung RL, Resendez SL, Stamatakis AM, Taylor JG, Huang J, Veleta K, Kantak PA, Aita M, Shilling-Scrivo K, et al. Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. Cell. 2015; 160:516–527. [PubMed: 25635459]

Kawano H, Masuko S. Region-specific projections from the subfornical organ to the paraventricular hypothalamic nucleus in the rat. Neuroscience. 2010; 169:1227–1234. [PubMed: 20678996]

Keys A. Human starvation and its consequences. Journal of the American Dietetic Association. 1946; 22:582–587. [PubMed: 20991087]

Kheifets A, Gallistel CR. Mice take calculated risks. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:8776–8779. [PubMed: 22592792]

Krashes MJ, Koda S, Ye C, Rogan SC, Adams AC, Cusher DS, Maratos-Flier E, Roth BL, Lowell BB. Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. The Journal of clinical investigation. 2011; 121:1424–1428. [PubMed: 21364278]

Krashes MJ, Shah BP, Koda S, Lowell BB. Rapid versus delayed stimulation of feeding by the endogenously released AgRP neuron mediators GABA, NPY, and AgRP. Cell metabolism. 2013; 18:588–595. [PubMed: 24093681]

Kutscher CL. Interaction of food and water deprivation on drinking: effect of body water losses and characteristics of solution offered. Physiology & behavior. 1972; 9:753–758. [PubMed: 4655168]

Liu X, Ramirez S, Redondo RL, Tonegawa S. Identification and Manipulation of Memory Engram Cells. Cold Spring Harbor symposia on quantitative biology. 2014; 79:59–65. [PubMed: 25637263]

Luquet S, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science. 2005; 310:683–685. [PubMed: 16254186]

Mandelblat-Cerf Y, Ramesh RN, Burgess CR, Patella P, Yang Z, Lowell BB, Andermann ML. Arcuate hypothalamic AgRP and putative POMC neurons show opposite changes in spiking across multiple timescales. Elife. 2015; 4

Matthews GA, Nieh EH, Vander Weele CM, Halbert SA, Pradhan RV, Yosafat AS, Glober GF, Izadmehr EM, Thomas RE, Lacy GD, et al. Dorsal Raphe Dopamine Neurons Represent the Experience of Social Isolation. Cell. 2016; 164:617–631. [PubMed: 26871628]

Montgomery KC. The relation between fear induced by novel stimulation and exploratory behavior. Journal of comparative and physiological psychology. 1955; 48:254–260. [PubMed: 13252152]

Navarro M, Olney JJ, Burnham NW, Mazzone CM, Lowery-Gionta EG, Pleil KE, Kash TL, Thiele TE. Lateral Hypothalamus GABAergic Neurons Modulate Consummatory Behaviors Regardless of the Caloric Content or Biological Relevance of the Consumed Stimuli. Neuropsychopharmacology. 2015

Nieh EH, Matthews GA, Allsop SA, Presbrey KN, Leppla CA, Wichmann R, Neve R, Wildes CP, Tye KM. Decoding Neural Circuits that Control Compulsive Sucrose Seeking. Cell. 2015; 160:528–541. [PubMed: 25635460]

Nieh EH, Vander Weele CM, Matthews GA, Presbrey KN, Wichmann R, Leppla CA, Izadmehr EM, Tye KM. Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation. Neuron. 2016; 90:1286–1298. [PubMed: 27238864]

Niesink RJ, van Ree JM. Short-term isolation increases social interactions of male rats: a parametric analysis. Physiology & behavior. 1982; 29:819–825. [PubMed: 7156221]

Oka Y, Ye M, Zuker CS. Thirst driving and suppressing signals encoded by distinct neural populations in the brain. Nature. 2015; 520:349–352. [PubMed: 25624099]

Padilla SL, Qiu J, Soden ME, Sanz E, Nestor CC, Barker FD, Quintana A, Zweifel LS, Ronnekleiv OK, Kelly MJ, et al. Agouti-related peptide neural circuits mediate adaptive behaviors in the starved state. Nature neuroscience. 2016; 19:734–741. [PubMed: 27019015]

Rossi M, Kim MS, Morgan DG, Small CJ, Edwards CM, Sunter D, Abusnana S, Goldstone AP, Russell SH, Stanley SA, et al. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. Endocrinology. 1998; 139:4428–4431. [PubMed: 9751529]

Saunders BT, Robinson TE. The role of dopamine in the accumbens core in the expression of Pavlovian-conditioned responses. Eur J Neurosci. 2012; 36:2521–2532. [PubMed: 22780554]

Sherington, C. The Integrative Action of the Nervous System. New Haven, Conn: Yale Univ. Press; 1906.

Siegel PS, Talantis BS. Water intake as a function of privation interval when food is withheld. Journal of comparative and physiological psychology. 1950; 43:62–65. [PubMed: 15404696]

Sih A. Optimal behavior: can foragers balance two conflicting demands? Science. 1980; 210:1041–1043. [PubMed: 17797495]

Silva BA, Mattucci C, Krzywkowski P, Murana E, Illarionova A, Grinevich V, Canteras NS, Ragozzino D, Gross CT. Independent hypothalamic circuits for social and predator fear. Nature neuroscience. 2013; 16:1731–1733. [PubMed: 24212674]

Stamatakis AM, Sparta DR, Jennings JH, McElligott ZA, Decot H, Stuber GD. Amygdala and bed nucleus of the stria terminalis circuitry: Implications for addiction-related behaviors. Neuropharmacology. 2014; 76(Pt B):320–328. [PubMed: 23752096]

Stanley BG, Leibowitz SF. Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. Proceedings of the National Academy of Sciences of the United States of America. 1985; 82:3940–3943. [PubMed: 3858854]

Steculorum SM, Ruud J, Karakasilioti I, Backes H, Engstrom Ruud L, Timper K, Hess ME, Tsaousidou E, Mauer J, Vogt MC, et al. AgRP Neurons Control Systemic Insulin Sensitivity via Myostatin Expression in Brown Adipose Tissue. Cell. 2016; 165:125–138. [PubMed: 27015310]

Sternson SM. Hypothalamic survival circuits: blueprints for purposive behaviors. Neuron. 2013; 77:810–824. [PubMed: 23473313]

Stratford TR, Kelley AE. GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. The Journal of neuroscience: the official journal of the Society for Neuroscience. 1997; 17:4434–4440. [PubMed: 9151760]

Takahashi KA, Cone RD. Fasting induces a large, leptin-dependent increase in the intrinsic action potential frequency of orexigenic arcuate nucleus neuropeptide Y/Agouti-related protein neurons. Endocrinology. 2005; 146:1043–1047. [PubMed: 15591135]

Van Loo PL, Van de Weerd HA, Van Zutphen LF, Baumans V. Preference for social contact versus environmental enrichment in male laboratory mice. Lab Anim. 2004; 38:178–188. [PubMed: 15070458]

Verplanck WS, Hayes JR. Eating and drinking as a function of maintenance schedule. Journal of comparative and physiological psychology. 1953; 46:327–333. [PubMed: 13109046]

Wang F, Kessels HW, Hu H. The mouse that roared: neural mechanisms of social hierarchy. Trends Neurosci. 2014; 37:674–682. [PubMed: 25160682]

Wu Q, Lemus MB, Stark R, Bayliss JA, Reichenbach A, Lockie SH, Andrews ZB. The temporal pattern of cfos activation in hypothalamic, cortical, and brainstem nuclei in response to fasting and refeeding in male mice. Endocrinology. 2014; 155:840–853. [PubMed: 24424063]

Yang CF, Shah NM. Representing sex in the brain, one module at a time. Neuron. 2014; 82:261–278. [PubMed: 24742456]

Yang H, Yang J, Xi W, Hao S, Luo B, He X, Zhu L, Lou H, Yu YQ, Xu F, et al. Laterodorsal tegmentum interneuron subtypes oppositely regulate olfactory cue-induced innate fear. Nature neuroscience. 2016; 19:283–289. [PubMed: 26727549]

Yapici N, Zimmer M, Domingos AI. Cellular and molecular basis of decision-making. EMBO Rep. 2014; 15:1023–1035. [PubMed: 25239948]

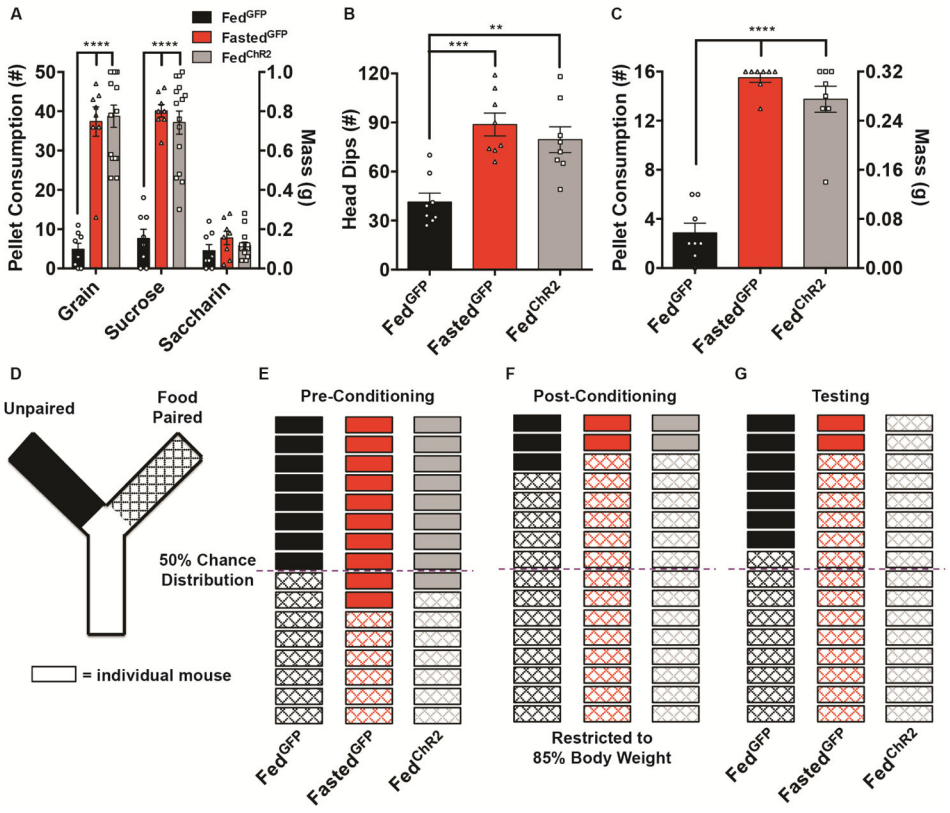

Figure 1. ARC^{AgRP} neural activation directs biased caloric consumption (See also Figure S1)

Physiological or ARC^{AgRP}-mediated hunger significantly enhanced (A) homecage consumption of grain and sucrose, but not saccharin pellets, (B) total number of exploratory head dips and (C) foraging-based consumption of grain pellets in a hole-board apparatus, compared to sated controls. (D) Schematic of Y-maze used to condition mice to associate paired arm with a food reward. (E) All groups of mice revealed unbiased distribution comparable to chance pre-conditioning. (F) All groups of mice revealed biased distribution toward the paired arm statistically different to chance post-conditioning. (G) Physiological or ARC^{AgRP}-mediated hunger revealed biased distribution toward the conditioned (paired) arm statistically different to chance while sated mice revealed unbiased distribution comparable to chance during testing. Error bars represent mean +/− SEM. $^{\bullet}p < 0.05$. $^{\bullet\bullet}p < 0.01$,$^{\bullet\bullet\bullet}p < 0.001$,$^{\bullet\bullet\bullet\bullet}p < 0.0001$.

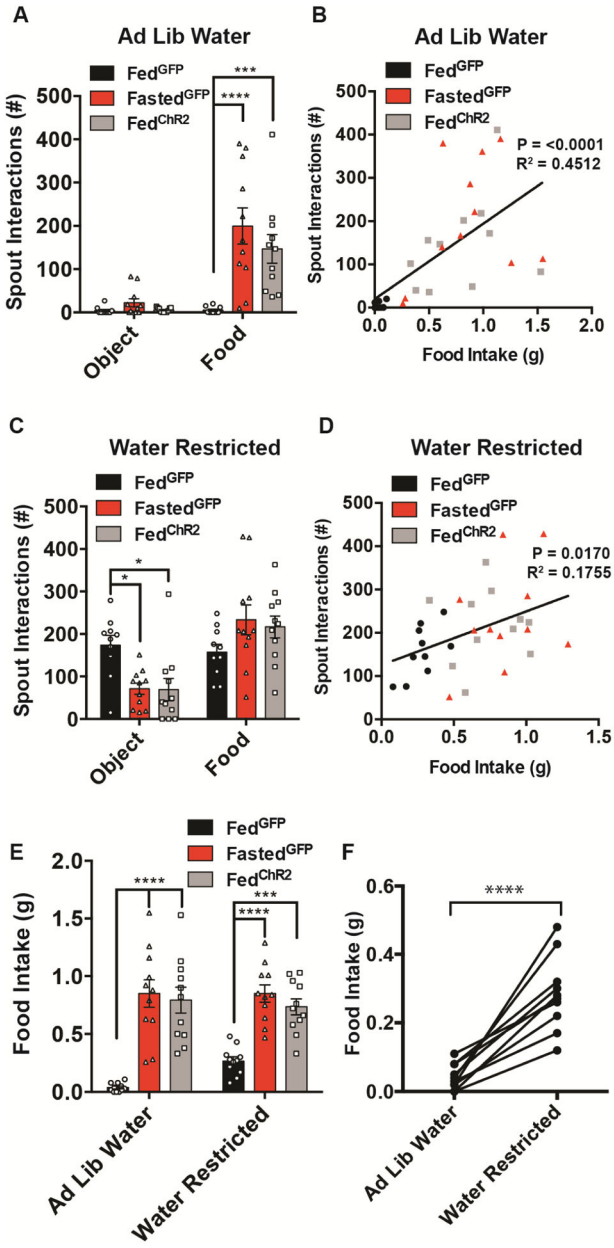

Figure 2. ARC^{AgRP} neural activation influences water intake (See also Figure S2)

(A) Physiological or ARC^{AgRP}-mediated hunger failed to elicit water intake in the object condition but significantly elicited water intake in the food condition compared to sated controls when animals had *ad lib* access to water pre-experiment, (B) an effect correlated to total food intake. (C) Physiological or ARC^{AgRP}-mediated hunger significantly decreased water intake in the object condition compared to sated controls when animals were water restricted pre-experiment. All groups of water-restricted animals exhibited comparable water intake in the food condition, (D) an effect correlated to total food intake. (E–F) Physiological or ARC^{AgRP}-mediated hunger enhanced food intake in both *ad lib* and water

restricted conditions compared to sated controls. Error bars represent mean +/− SEM. ⁃p < 0.05. ⁃⁃p < 0.01,⁃⁃⁃p < 0.001,⁃⁃⁃⁃p < 0.0001.

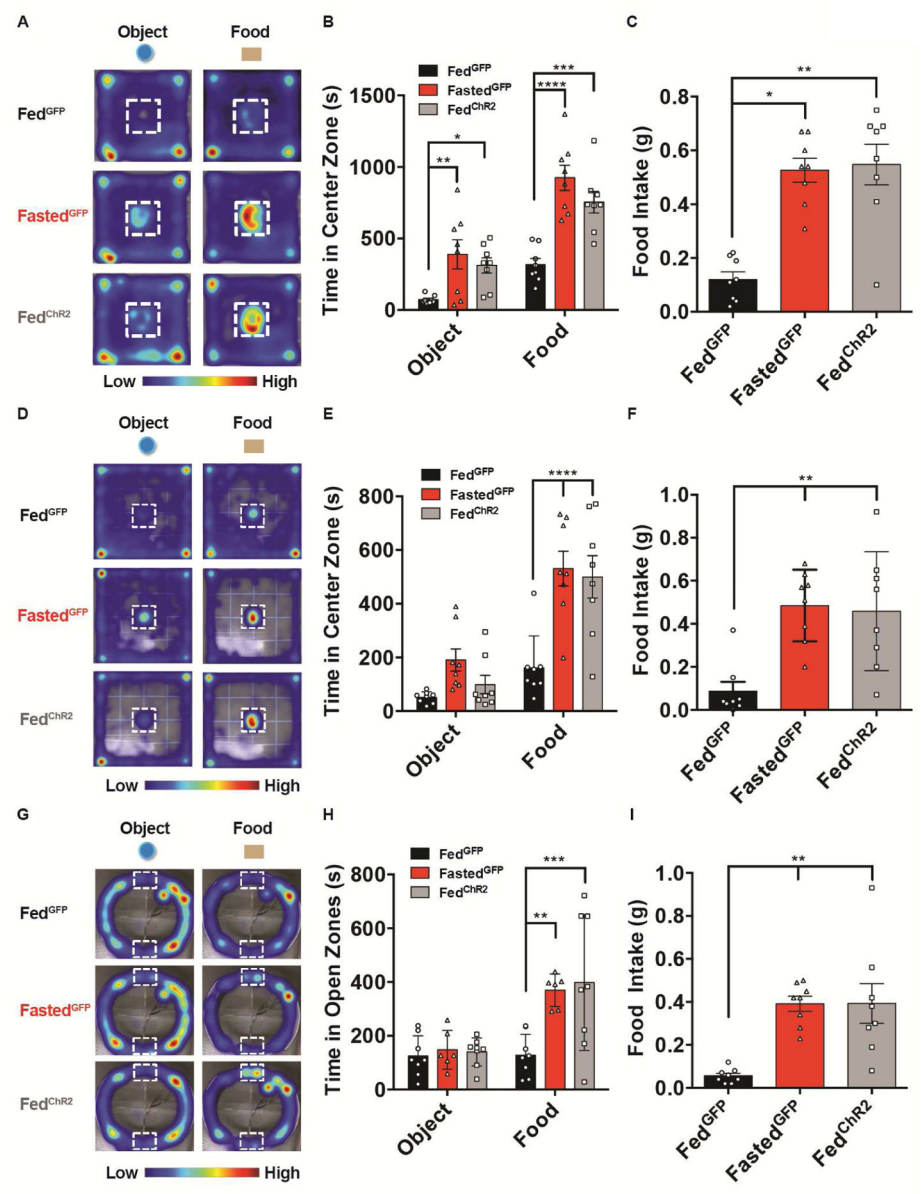

Figure 3. ARC^{AgRP} neural stimulation suppresses anxiety-like behavior toward food (See also Figures S3–4)

(A–B) Physiological or ARC^{AgRP}-mediated hunger significantly enhanced open field center zone duration time in both object and food conditions compared to sated controls. (C) Increased center zone duration time during the food condition was related to levels of food intake. (D–E) Physiological or ARC^{AgRP}-mediated hunger significantly enhanced big open field center zone duration time in the food condition, but failed to do so in the object condition, compared to sated controls. (F) Increased center zone duration time during the food condition was related to levels of food intake. (G–H) Physiological or ARC^{AgRP}-mediated hunger significantly enhanced zero maze open arm center zone duration time in the food condition, but failed to do so in the object condition, compared to sated controls. (I) Increased open arm center zone duration time during the food condition was related to levels

of food intake. Error bars represent mean +/− SEM. ˙p < 0.05. ¨p < 0.01,˙˙˙p < 0.001,˙˙˙˙p < 0.0001.

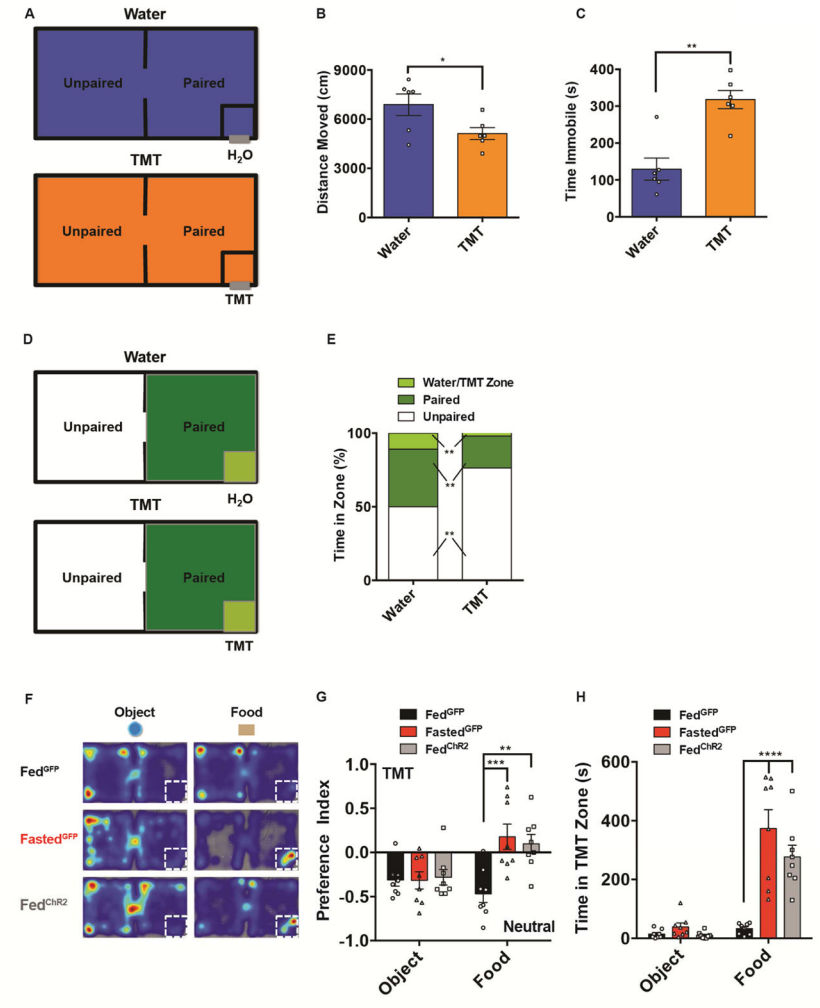

Figure 4. ARC^{AgRP} neural activation suppresses TMT-induced fear responses toward food (See also Figure S5)

(A, D) Schematic of two-chamber apparatus (water or TMT). Within-subject analyses revealed that TMT odor significantly (B) decreased total distance traveled, (C) increased total amount of time spent immobile and (E) reduced time spent in both the paired chamber and designated water/TMT zone, compared to a neutral water stimulus. (F–H) Physiological or ARC^{AgRP}-mediated hunger significantly shifted chamber preference from the neutral side toward the TMT side and enhanced TMT zone duration in the food condition, but failed to do so in the object condition, compared to sated controls. Error bars represent mean +/− SEM. ⁃$p < 0.05$. ⁃⁃$p < 0.01$,⁃⁃⁃$p < 0.001$,⁃⁃⁃⁃$p < 0.0001$.

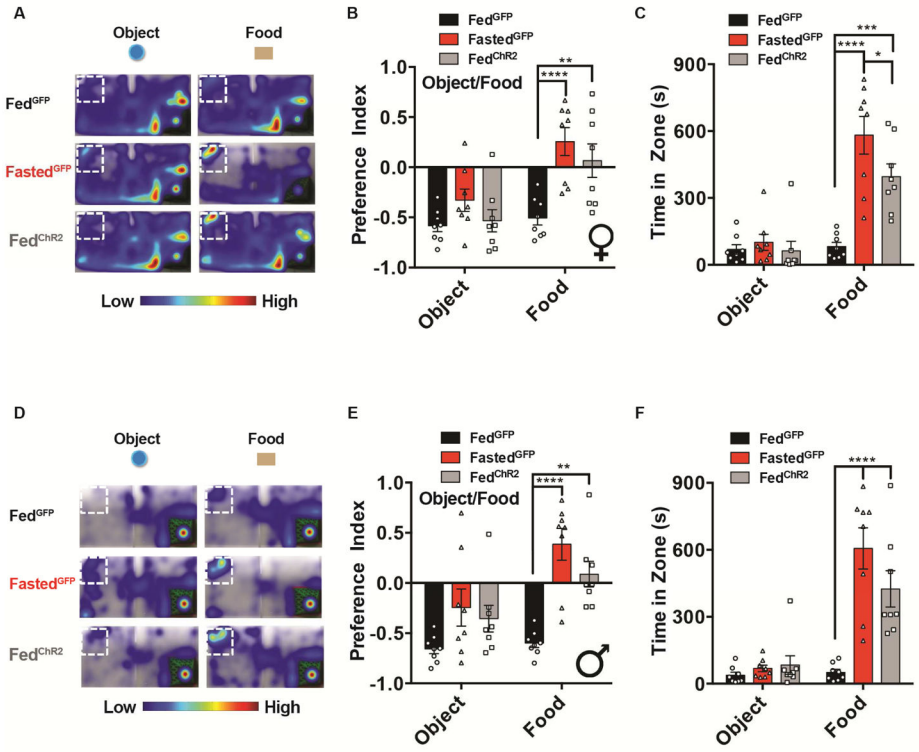

Figure 5. ARC^{AgRP} neural activation competes with innate social drive in the presence of food (See also Figure S6)

(A–C). Physiological or ARC^{AgRP}-mediated hunger significantly shifted chamber preference from the receptive female side toward the food side and enhanced food zone duration time in the food condition, but failed to do so in the object condition, compared to sated controls. (D–F). Physiological or ARC^{AgRP}-mediated hunger significantly shifted chamber preference from the juvenile male side toward the food side and enhanced food zone duration time in the food condition, but failed to do so in the object condition, compared to sated controls. Error bars represent mean +/− SEM. ⁃p < 0.05. ⁃⁃p < 0.01,⁃⁃⁃p < 0.001,⁃⁃⁃⁃p < 0.0001.

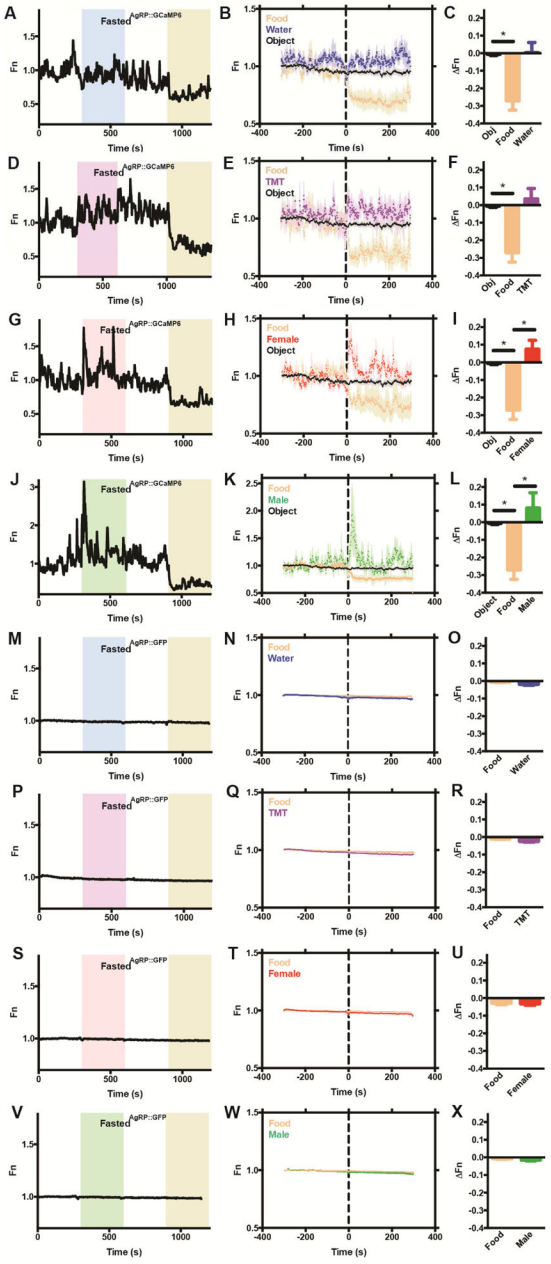

Figure 6. ARC^{AgRP} neural activity responds to food and conspecifics in hungry mice (See also Figure S7)

Normalized representative *in vivo* calcium imaging traces showing GCaMP (A, D, G, J) or GFP (M, P, S, V) fluorescent signal fluctuations through the presentation of baseline→stimuli→removal of stimuli→food. Plots showing calcium signal (B, E, H, K) of GFP (N, Q, T, W) changes between object, stimuli (water, TMT, female, or juvenile male), and food. (C, F, I, L) Calcium levels were significantly decreased upon the presentation of food, and increased upon the presentation of conspecifics, but not water or TMT. No changes were detected in GFP controls (O, R, U, X). Unpaired t-test with equal SD revealed

significant differences between GCaMP and GFP signals in response to food (p=0.0034), female (p=0.0423) and male (p=0.0486). Error bars represent mean +/− SEM. $^{*}p < 0.05$.

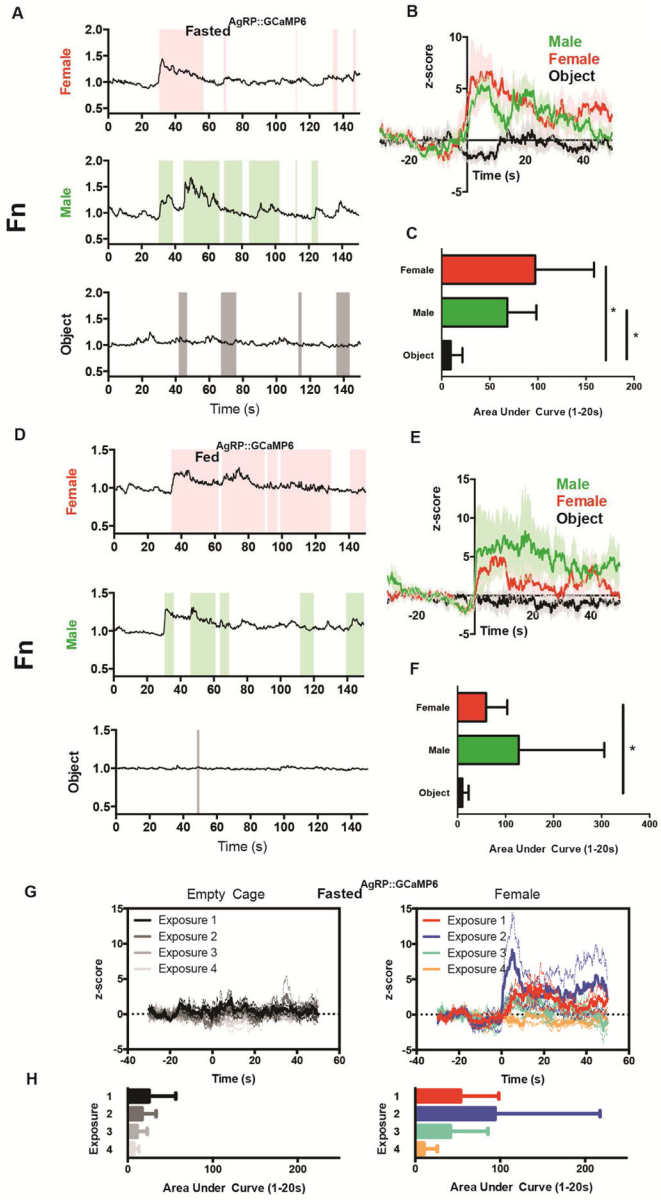

Figure 7. ARC^{AgRP} neural activity increases in response to initial social contact

(A, D) Normalized sample traces of calcium signals during bouts of interaction with different stimuli in hungry and sated mice, respectively. (B, E) Population z-score plots showing the averaged response to the first interaction bout in hungry and sated mice, respectively. (C, F) ARC^{AgRP} neurons showed a significantly greater increase in activity upon first contact with conspecifics, compared with response to a non-salient object in hungry and sated mice, respectively (G) Representative traces of 4 repeated exposure to an empty grid isolation cage or receptive female, respectively. (H) Comparison of area under the curve of z-score between each exposure. Error bars represent mean +/− SEM. $^{*}p < 0.05$.