Role of Arcuate AgRP Neurons in Ghrelin Action on Food Reward

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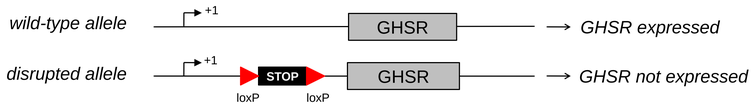
1. STRESS-BASED EATING AND GHRELIN

- Stress is a major cause of increased intake of highly palatable, calorie-dense 'comfort foods' in many people.
- This behavior may partially explain the increased incidence in obesity in individuals with chronic stress.
- Previous work demonstrates that the orexigenic gut hormone ghrelin is one molecular link between stress and eating.
  - Ghrelin is the only known hormone that stimulates appetite.
  - Chronic stress increases ghrelin.
  - Ghrelin receptor-deficient mice lack stress-induced food reward behavior.
- The ghrelin receptor (GHSR) is highly expressed in several brain regions including the Arc, a key site for both homeostatic and hedonic feeding.
- The current study aims to identify the role of ghrelin-responsive neurons in the hypothalamic arcuate nucleus (Arc) in mediating stress-based eating.

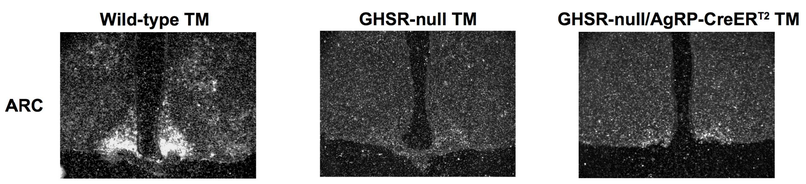
2. PRELIMINARY DATA: GENERATION OF THE MOUSE MODEL

- GHSR-null mice contain a loxP-flanked transcriptional blocker inserted into their endogenous GHSR alleles:

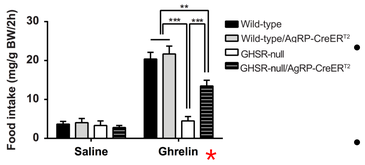


- To study the role of the Arc in ghrelin action, we generated a novel mouse model in which GHSRs are selectively reactivated by a tamoxifen-inducible Cre-recombinase mechanism only in Arc AgRP neurons.
- Exposure of the GHSR-null allele to Cre recombinase results in removal of the transcriptional blocker and reactivation of GHSR expression:

- In our AgRP-CreER^{T2} model, Cre recombinase, and in turn, GHSR, are expressed in a tamoxifen-dependent manner only in AgRP neurons of the Arc.
- In situ hybridization histochemistry for GHSR mRNA:



3. PRELIMINARY DATA: THE ROLE OF THE ARC IN GHRELIN-INDUCED EATING

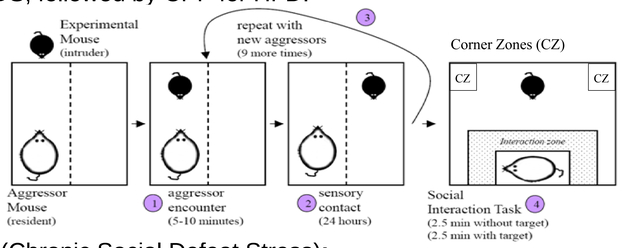


*Mice with GHSRs only in Arc

- Mice were given saline or ghrelin s.c. and food intake was measured for 2 hrs.
- Ghrelin increased food intake in the wild-type groups but not in the GHSR-null mice.
- In mice with GHSRs only in the Arc AgRP neurons, ghrelin's orexigenic effect was partially rescued.

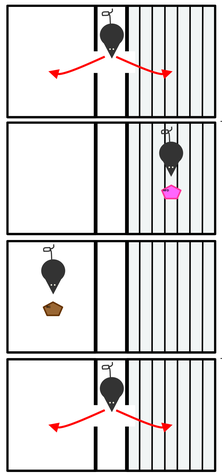
5. STRESS-INDUCED FOOD REWARD BEHAVIOR

Our stress-induced food reward behavioral model involves exposing mice to CSDS, followed by CPP for HFD.



CSDS (Chronic Social Defeat Stress)**:**

- A test mouse is placed into the home cage of a different aggressor mouse for five minutes each day for Days 1-10.
- Afterward, the two mice remain in the same cage but are physically separated with a perforated barrier, allowing sensory contact only.
- On Day 11, the Social Interaction Test is performed to assess depressive-like behavior.



Pre-test Session.
Free access to both chambers in absence of food.

Conditioning sessions.
Restriction to one chamber with high fat diet (HFD) on even days and to the other chamber with regular Chow (RC) on odd days

Test session.
Free access to both chambers in absence of HFD and RC.

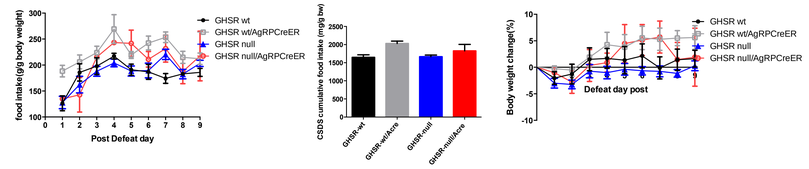
1	2	3	4	5	6	7	8	9	10	11	12
HFD	RC	HFD	RC	HFD	RC	HFD	RC	HFD	RC	HFD	RC

CPP for HFD (Conditioned Place Preference for HFD)**:**

- The CPP apparatus has a central shuttle chamber in between 2 conditioning chambers that differ in wall pattern (solid and striped as shown) and floor texture.
- A pretest assesses initial biases for a particular side.
- Mice are conditioned to associate one chamber with HFD and the other chamber with regular chow, over a period of 12 days.
- On Day 13, preference for the chamber paired to HFD is assessed.

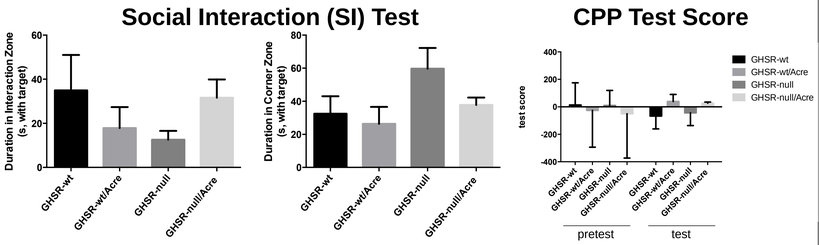
6. ROLE OF ARC IN STRESS-INDUCED FOOD REWARD

CSDS-induced effects on food intake and body weight



- Over a 9 day period after CSDS, daily food intake and body weight were measured.
- Additional cohorts of these four study groups will be needed to determine stress-induced eating's effects on daily food intake, total food intake, and % body weight change.

CSDS-induced effects on depressive-like behavior and stress-based eating



- In the SI Test, more time spent in the Interaction Zone near the target mouse indicates a lack of depressive-like behavior.
- Conversely, more time spent in the Corner Zones indicates depressive-like behavior.
- The trend suggests ghrelin activity protects against depressive-like behavior, but more animals are needed to determine significance.
- Using these small cohorts, we did not observe CPP for HFD in any of the four genotypes tested.

6. CONCLUSIONS

- Intact ghrelin signaling in AgRP neurons only can partially restore ghrelin's orexigenic action.
- Our findings regarding the role of AgRP neurons in stress-based food reward behavior are inconclusive at the present time.
- Experiments with more animals will be needed to confirm the role of ghrelin-responsive Arc AgRP neurons in stress-based hedonic eating.

7. REFERENCES

Wang, Q et al. Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin. Molecular Metabolism. 21 October 2013 (10.1016/j.molmet.2013.10.001)