Recent advances in understanding hypothalamic control of defensive responses to hypoglycaemia

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Abstract

Maintenance of normal blood glucose is important for survival. In particular, brain function is dependent on circulating glucose. In health, a series of powerful counterregulatory defences operate to prevent/ limit hypoglycaemia. These defences are altered to varying degrees in diabetes and in particular, a subset of people with diabetes can develop profound deficits in these defences placing them at increased risk of suffering episodes of severe hypoglycaemia. Brain is an important controller of glucose homeostasis and developments in molecular techniques have allowed the neurocircuitry of a number of important centrally-controlled homeostatic processes such as energy balance, thirst and thermoregulation to be defined. This review describes how some of these advances have allowed a better understanding of the neuronal/ brain ensembles which help protect against hypoglycaemia.

Keywords
Hypoglycaemia
Brain glucose-sensing
Hypothalamus
AgRP
Glucokinase

Introduction

After a century of treating diabetes with insulin, hypoglycaemia remains the main drawback of insulin therapy. Principally, hypoglycaemia is problematic because of the detrimental effects on brain which normally depends on a constant supply of glucose from the circulation. To maintain blood glucose, a series of physiological counterregulatory responses (CRR) comprising both neurohumoral and symptomatic responses are triggered by a falling blood glucose. The former includes a cessation of endogenous insulin secretion (in non-diabetes), followed by release of glucagon, catecholamines and other hormones which oppose insulin's actions to lower glucose. Hypoglycaemic symptoms are generally (although not always) unpleasant, carrying a negative valence which alerts the sufferer that something is amiss. In particular, hypoglycaemia leads to an intense glucoprivic hunger which specifically prompts gluco-corrective feeding.

The predominant site(s) triggering CRR are probably within brain, integrating information from specialised nutrient sensors within the brain and possibly also afferent input from peripheral sensors and perhaps circulating hormonal signals. CRR to rapid onset hypoglycaemia is likely mediated by the brain, whereas CRR to slower developing hypoglycaemia may be dependent on peripheral detection.[1] CRR thresholds are dynamic and can shift depending on prior prevailing glycaemic experience. A major problem in diabetes is that exposure to hypoglycaemia itself results in delayed and diminished counterregulatory responses to subsequent hypoglycaemic episodes.[2]

As well as the clear relevance to clinical diabetes, the study of hypoglycaemia and the brain is a physiological exemplar of nutritional homeostasis and the neurobiology of stress. Modern brain imaging techniques and increasingly powerful molecular tools in rodent studies are allowing the neurocircuitry of hypoglycaemia to be mapped out. In the article below we summarise recent advances in the understanding of the brain control of CRR including also feeding responses to low glucose. Experimental approaches used in rodents have included both insulin-induced hypoglycaemia and glucoprivation induced by non-metabolisable glucose analogues. These are not identical and we have tried to indicate in narrative below where gluprivation has been used as a model of hypoglycaemia.

Mechanisms of brain glucose sensing

To generate CRR, specialised brain areas integrate information both from local brain sensing of glucose and from peripheral glucose sensors. Some brain glucose-sensors may actually be broader nutrient sensors capable of responding to other substrates, with lactate being the predominant intermediate fuel available for neuronal metabolism.[3] In keeping with the literature, we have referred generically to these cells as "glucose-sensing". Broadly speaking, the electrical activity of specialised glucose-sensing cells either rises or falls during hypoglycaemia, termed glucose inhibited (GI) and glucose excited (GE) respectively.[4] It is important to note that brain glucose levels are lower than blood values with different sub-populations of GE and GI cells changing activity across the physiological glucose range (recently reviewed in depth by Garcia et al[5]). Sub populations may also detect changes

rather than absolute values, suggested by the identification of a population of GI neurones that adapt with prolonged hypoglycaemia.[6] Although much focus has been on neuronal glucose-sensing, non-neuronal cells may also be important for detection of hypoglycaemia. For example, astrocytes may act either as direct nutrient sensors and/or by interactions through metabolic coupling with neighbouring neuronal cells.[7],[8] Other non-neuronal brain cells such as tanycytes[9] and microglia [1011] have also been implicated in hypoglycaemia-sensing and glucose homeostasis.

To detect changes in glucose, brain glucose-sensing may utilise some of the nutrient-sensing mechanisms which have been characterised elsewhere in the body. As an example, glucokinase (GCK) is a low affinity hexokinase utilised in pancreatic and hepatic glucose-sensing that regulates glycolytic flux through downstream ATP-gated potassium (KATP) channels. GCK has a discrete but widespread distribution in brain and has been demonstrated in both GE and GI neurones, including a sub-population of GI growth hormone releasing hormone (GHRH) neurones.[12] A number of studies have shown that (brain) GCK activity can affect responses to hypoglycaemia. CRR is increased in humans and mice with reduced GCK activity.[13] Employing a novel magnetic stimulation approach, Stanley, et al. demonstrated that activation of ventromedial hypothalamus (VMH) GCK neurones increased blood glucose and food intake, and that inhibition of these neurones blunted food intake.[14] In keeping with the latter, glucoprivic feeding in rats was triggered/ amplified by brain administration of the (non-specific) GCK inhibitor glucosamine.[15] Conversely, Hussain, et al used a viral vector approach to increase GCK activity in the basomedial hypothalamus of rats, finding increased food intake with a specific preference for glucose-rich foods.[16]

Another protein involved in pancreatic beta-cell glucose-sensing, glucose transporter 2 (GLUT2) is also found in brain, located in a number of discrete brain areas implicated in gluco-regulation. Electrophysiological studies show most GLUT2 neurones to be GI. In keeping with this, optogenetic activation of GLUT2 neurones in the nucleus of the solitary tract (NTS) increased glucagon secretion and autonomic activity through projections to the dorsal motor nucleus of the vagus nerve (DMX).[17] GI GLUT2 neurones have also been reported in the paraventricular thalamus (PVT). Optogenetic activation of PVT^{GLUT2} neurones increased brain blood flow analogous to the changes seen in hypoglycaemia. Conversely, inactivation of GLUT2 systemically or locally in the nervous system prevented this increase in response to hypoglycaemia.[18] Activation of the specific population of PVT^{GLUT2} neurones that project to the nucleus accumbens (NAc) stimulated sugar-seeking behaviours in mice.[19] Of interest, the same group recently identified a further group of hyperglycaemia-activated PVT^{GCK} which also project to the NAc exerting broadly opposite effects on feeding behaviour.

One model for brain glucose-sensing is that adaptations such as GCK and GLUT-2 are largely utilised by GE neurones with other mechanisms such as the fuel sensor AMP-activated protein kinase (AMPK) and neuronal NO synthase (nNOS) utilised by GI neurones, possibly linked to closure of cation/ chloride channels.[5] A recent report described amplification of CRR by peripheral administration of a brain-permeable AMPK activator to rats.[20] Further research is required to develop understanding of the AMPK pathway of glucose-sensing, including which brain nuclei and neurocircuitry are predominately involved.

Neurocircuitry of Hypoglycaemia Counterregulation

Brain areas exerting control over CRR may be involved directly in sensing low glucose and/or integrate information from other sensors. Additionally, efferent brain pathways effecting CRR may include both autonomic outflow, humoral signalling e.g. stimulation of the hypothalamus-pituitary adrenal axis and specific effector circuitry- for example leading to glucoprivic hunger. Human brain imaging or mapping genetic markers of activation in rodent brain show many brain areas responding to hypoglycaemia and/or glucoprivation.[21],[12] Although some of these may be directly glucose-sensing, other glucoregulatory areas may be not directly sensing but involved in control while others may be indirectly affected by changes in glucose. The predominant glucose-sensing areas and glucoregulatory areas described to date have been in hypothalamus and brain stem. For brevity, we have largely focused below on recent data on hypothalamus and connected neurocircuitry in this review.

Ultimately, hormonal CRR is delivered by a variety of efferent pathways from brain. Hypothalamic-pituitary-adrenocortical axis activation occurs via corticotrophin releasing hormone signalling from PVN to pituitary. Other CRR, including adrenomedullary responses, occur via autonomic outflow from PVN, PAG and brain stem nodes, such as the dorsal motor nucleus of vagus nerve.[22]

Ventromedial Hypothalamus (VMH)

Seminal work in rats at the end of the 20th century identified the importance of the VMH, an area containing the ventromedial nucleus (VMN) in responding to hypoglycaemia.[23] Building on this, Chan et al identified that a fall in VMH GABA (gamma-aminobutyric acid) during hypoglycaemia was permissive for CRR. Microdialysis delivery of norepinephrine or the adrenergic beta-2 agonist salbutamol for 3 sequential days into the VMH of rats resulted in a reduction in CRR (and higher extracellular lactate) during a subsequent hypoglycaemic challenge. The reduction in CRR was reversed by pharmacological blockade of lactate transport.[24] Taken together, these data suggest that VMH lactate may be acting as a fuel for nutrient-sensors to prevent detection of low glucose with lactate preventing the fall in GABAergic tone required for CRR.

These studies largely employed regional pharmacology in rat brain with microdialysis/ microinjections etc to look at “pan-VMH” experimental alterations. The last decade has seen marked improvements in molecular tools largely in murine models that have allowed further identification of the neurochemical identity of brain cells involved in nutrient homeostasis and energy balance. An important caveat is that there is marked heterogeneity of neuronal and non-neuronal cell types within glucose-sensing brain regions which has been highlighted by recent large scale, unbiased RNA sequencing studies.[25],[26],[27] As described above for opposing actions of PVT^{GLUT2} and PVT^{GCK} circuitry, the VMN may also contain opposing glucoregulatory circuitry in close juxtaposition. Many but not all VMN neurones express the transcription factor steroidogenic factor 1 (Sf1). Optogenetic studies allowed identification of a specific subset of nitric oxide synthase VMN^{SF1} neurones projecting to the anterior bed nucleus of the stria terminalis (aBNST) and periaqueductal gray (PAG) involved in glucagon and behavioural (freezing in mice) responses respectively to

hypoglycaemia.[28],[29] In contrast, a recent study delineated aBNST-projecting VMN^{MC3R} neurones exerting opposite physiological effects to promote glucose disposal.[30]

Upstream of the VMN, a group of hypoglycaemia-responsive neurones in the superior part of the lateral parabrachial nucleus (LPBN) project to VMN^{SF1} neurones, acting via action of cholecystokinin (CCK) on CCKB receptors (CCKBR). Activation of this pathway increased blood glucose and consistent with this, silencing of VMN^{CCKBR} neurones inhibited CRR. [31],[32] Again there may be close overlap with parallel opposing circuitry with monosynaptic mapping showing that aBNST-projecting glucoregulatory VMN^{MC3R} neurones receive inputs from LPBN. The LPBN glucoregulatory projections to VMN^{SF1} neurones are largely to the dorsomedial/ central VMN.[30] The ventrolateral VMN contains abundant non-SF1 neurones containing estrogen receptor-α (ERα). A recent paper employed optogenetics, photometry and targeted CRISPR-Cas9 methodology to identify sub-populations of glucoregulatory GI and GE ventrolateral VMN^{ERα} neurones projecting to arcuate (ARC) and midline raphe nuclei respectively.[33]

Arcuate (ARC)

The ARC includes key cells important in energy and glucose homeostasis, raising the question of whether ARC circuitry is implicated in neurohumoral CRR and/or hunger responses to hypoglycaemia. The best characterised ARC cells are 2 groups expressing specific neuropeptides: neuropeptide Y (NPY)/Agouti-related protein (AgRP) neurones and pro-opiomelanocortin (POMC) neurones. Importantly though, there are other non-POMC/ non-AGRP neurones in the ARC. Stanley's group have described a specific sub-population of hypoglycaemia-responsive GABAergic GHRH neurones with evidence for adaption to recurrent low glucose exposure.[34]

POMC neurones are generally assumed to be responsive to "energy surplus" with some reporting a subset of POMC neurones to be GE[35] although notably others have reported a absence of POMC glucose-sensitivity.[36] Consistent with a glucoregulatory role, the aBNST-projecting glucoregulatory VMN^{MC3R} neurones described above receive input from POMC neurones.[30] There is heterogeneity within the POMC population though[37] and a recent paper showed hypoglycaemia-activation of POMC neurones suggesting that at least some may be GI. In keeping with this finding, CRR was impaired in both an ARC POMC knockout model and with knockdown of melanocortin 4 receptor in the paraventricular nucleus- a key downstream target for POMC neurones.[38]

NPY/ AGRP neurones are generally orexigenic and/or may act to signal negative valence,[39] although again with increasing realisation that distinct sub-populations exist, some of which may exert glucoregulatory actions.[40],[41] and at least some have been reported to be GI i.e. direct sensors of hypoglycaemia.[36] The importance of NPY/AGRP neurones for either feeding or CRR responses to hypoglycaemia remains uncertain. Ablation of NPY/AgRP neurones in neonatal mice had no apparent effect on feeding responses to either insulin-induced hypoglycaemia or glucoprivation, although early life changes likely allow compensatory mechanisms to develop.[42] To address this, Aklan et al. used chemogenetics to inhibit acutely AgRP neurones, finding glucoprivic feeding was attenuated, but not completely suppressed. It is unclear whether this partial reduction was because other non-

AgRP glucoprivic pathways exist or whether chemogenetic inhibition of AgRP neurones was incomplete.[43]

Brain Stem Input to ARC/ Hypothalamus

In addition to potential direct sensing of changes in glucose, $ARC^{AgRP/\ NPY}$ neurones may receive information about nutrient status from periphery (particularly from the portal-mesenteric vein)[1] and/or other brain nutrient-sensing areas.[44] There are data suggesting a role for brain stem hypoglycaemia-sensing in modulating $ARC^{AgRP/\ NPY}$ activity. Local delivery of norepinephrine into the hypothalamus stimulated feeding and immune-toxic destruction of medial hypothalamic norepinephrine or epinephrine terminals, or hindbrain catecholaminergic neurones, reduced glucoprivic feeding in rats.[45] Building on this, Aklan, et al also used mice expressing Cre-recombinase under the control of the tyrosine hydroxylase (TH) promoter (TH-Cre) to examine catecholaminergic projections from the Nucleus Tractus Solitarius (NTS) to ARC. Optogenetic activation of ARC projecting NTS^{TH} neurones stimulated glucoprivic feeding whereas chemogenetic silencing reduced glucoprivic feeding. Feeding appeared to be mediated by direct noradrenergic alpha-1 stimulation of $ARC^{AgRP/\ NPY}$ by NTS^{TH} neurones.[43]

Two other recent publications have employed contemporary neuroscientific approaches to implicate catecholaminergic projections from another hindbrain catecholaminergic area, the ventrolateral medulla (VLM) in glucoprivic feeding. Using transgenic TH-Cre rats, chemogenetic stimulation of C1 (but not A1) areas of VLM stimulated feeding.[46] This was associated with activation of neurones in the paraventricular hypothalamus (PVN) and increased corticosterone levels. More recently, using TH-cre mice, projections were identified from VLM^{TH} to the PVN, aBNST and the paraventricular nucleus of the thalamus (PVT), with stimulation of latter projections stimulating feeding. The PVT is anatomically connected to the nucleus accumbens (NAc), an area implicated in reward including feeding behaviour. The authors found that photostimulation of VLM^{TH} axonal inputs to the PVT increased the activity of NAc neurones. Activity of NAc-projecting PVT neurones increased during food-seeking behaviour in hungry mice and decreased with feeding. Finally, glucoprivic feeding was suppressed by either optogenetic silencing of PVT-projecting VLM^{TH} neurones or chemogenetic silencing of NAc-projecting PVT neurones.[47]

Taken together, these data suggest that the activation of hunger during hypoglycaemia may involve integrated responses of both hindbrain and diencephalic areas of hypothalamus and thalamus. Involvement of reward circuitry areas such as NAc is intriguing and suggests that analogous to non-glucoprivic feeding, there may be considerable overlap between homeostatic and hedonic circuitry for glucoprivic feeding.

Dorsomedial hypothalamus (DMH)

DMH has long been recognised as containing glucose-sensitive neurones. Using a combination of optogenetics and electrophysiology, Otgun-Uul *et al* found that DMH GABAergic neurons are depolarized by low glucose (GI). Optogenetic activation of PVN projecting DMH^{GABA} neurones promoted food intake.[48] Using a data driven approach (quantitative trait loci mapping in a panel of 36 different mouse strains), Picard et al identified that the DMH transcription factor *Fgf15* was associated with glucagon responses

to glucopenia.[49] Recent work characterised these as glutamatergic GE and GI neurones, with chemogenetic activation reducing glucagon secretion to insulin-induced hypoglycaemia but increasing hepatic glucose production.[50] Although still uncertain why apparently paradoxical effects were seen, this study demonstrates the power of using unbiased screening methodology followed by in depth characterisation of pathways of interest to mapping the complex neurocircuitry of hypoglycaemia.

Conclusions

The development of sophisticated tools largely utilising molecular techniques in mice (optogenetics and chemogenetics) and advances in microscopy have allowed the molecular identity and neurocircuitry of the central control of glucose homeostasis (including hypoglycaemia) to start to be mapped out. Increasingly, this has allowed "unbiased" approaches to be applied. Transgenic approaches are possible in rats also, as exemplified by the TH-Cre rat work summarised above. Mice and rats do not always faithfully reflect human physiology, let alone the pathological changes seen in diabetes. The important future challenge will be applying this knowledge gained about brain homeostatic mechanisms to relevant disease models of diabetes in rodents and translating into clinical advances for human medicine.

Declarations of Interest:

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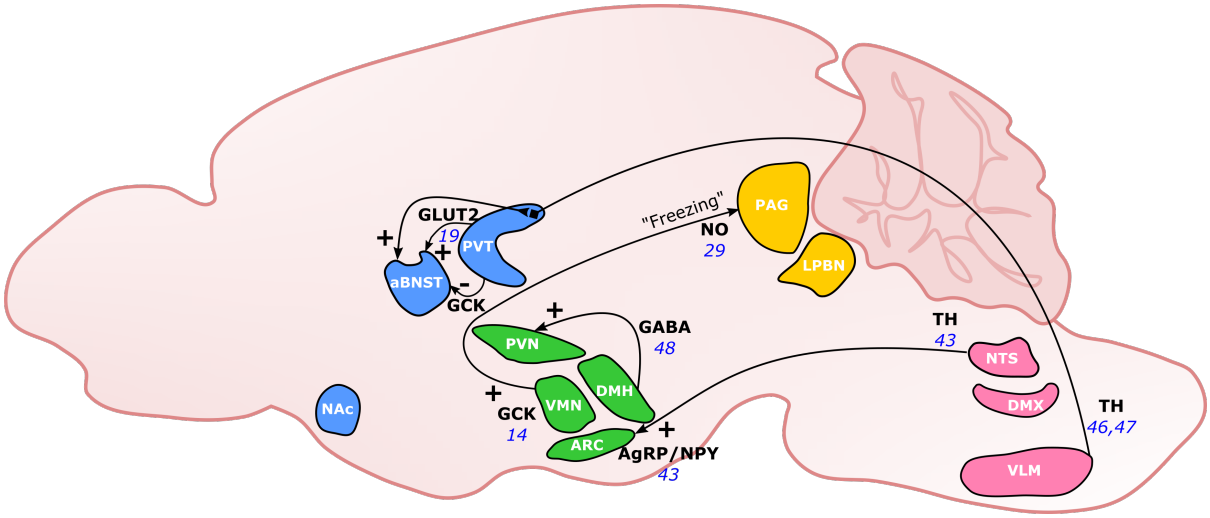
Figure 1 a. Circuitry described in Manuscript for Brain Control of Feeding/ Behavioural responses to Hypoglycaemia.

Figure 1 b. Circuitry described in Manuscript for Brain Control of CRR responses to Hypoglycaemia

Numbers in blue italics indicate paper referenced. + OR – indicates stimulation or diminution of responses to low glucose or glucoprivation respectively.
Brain areas: ABNST: anterior bed nucleus of stria terminalis. NAC: nucleus accumbens. PVT: paraventricular thalamus. PVN: paraventricular nucleus of hypothalamus. DMN: dorsomedial nucleus. VMN: ventromedial nucleus. ARC: arcuate. PAG: periaqueductal grey. LPBN: lateral parabrachial nucleus. NTX: nucleus tractus solitarius. DMX: dorsal motor nucleus of vagus. VLMN: ventrolateral medulla.
Molecular mechanisms: CCK: cholecystokinin. GCK: glucokinase. NO: nitric oxide. SF1 steroidogenic factor 1. GLUT2: glucose transporter 2. GABA: gamma aminobutyric acid. TH: tyrosine hydroxylase. AGRP/ NPY: agouti related peptide/ neuropeptide Y. FGF15: fibroblast growth factor 15. MC4R melanocortin 4 receptor. POMC: proopiomelanocortin.


GLUT2
19
+
PVT
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aBNST
-
GCK
"Freezing"
NO
29
PAG
LPBN
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PVN
GABA
48
+
GCK
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VMN
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AgRP/NPY
43
NAc
TH
43
NTS
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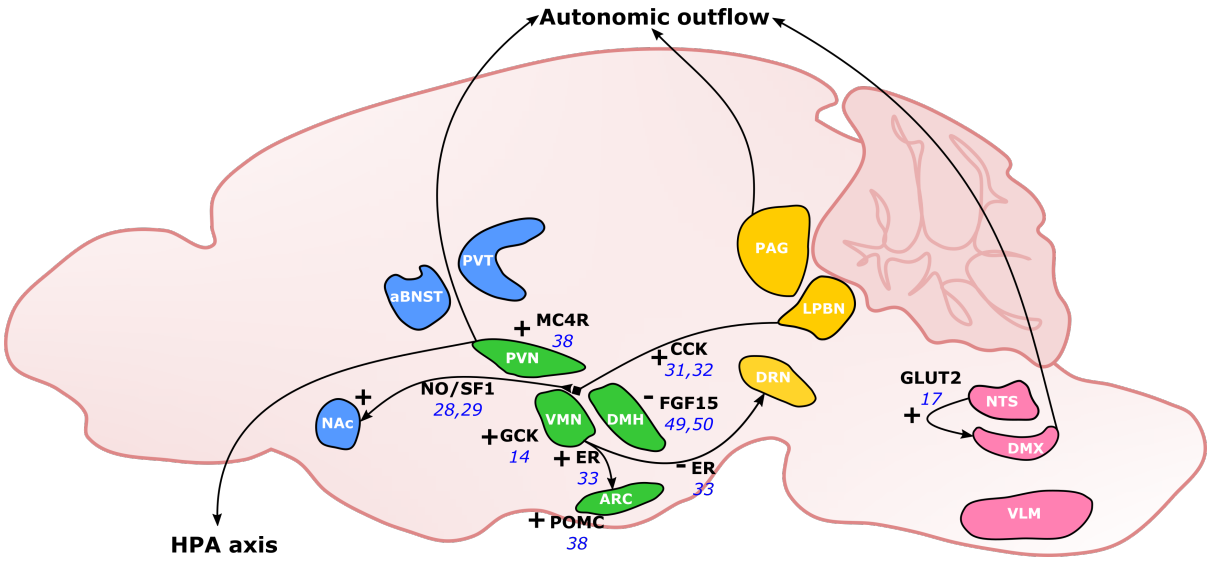
46,47
VLM


Autonomic outflow
PVT
aBNST
PAG
LPBN
+ MC4R
38
PVN
+ CCK
31,32
DRN
GLUT2
17
NTS
+
NAc
+
NO/SF1
28,29
VMN
DMH
- FGF15
49,50
+ GCK
14
+ ER
33
- ER
33
DMX
ARC
VLM
+ POMC
38
HPA axis

References

1. Donovan CM, Watts AG. Peripheral and central glucose sensing in hypoglycemic detection. Physiology (Bethesda). 2014;29(5):314-324. doi:10.1152/physiol.00069.2013
2. Iqbal A, Heller S. Managing hypoglycaemia. Best Pract Res Clin Endocrinol Metab. 2016;30(3):413-430. doi:10.1016/j.beem.2016.06.004
3. Kokorovic A, Cheung GWC, Rossetti L, Lam TKT. Hypothalamic sensing of circulating lactate regulates glucose production. Journal of Cellular and Molecular Medicine. 2009;13(11-12):4403-4408. doi:10.1111/j.1582-4934.2008.00596.x
4. Oomura Y, Yoshimatsu H. Neural network of glucose monitoring system. J Auton Nerv Syst. 1984;10(3-4):359-372. doi:10.1016/0165-1838(84)90033-x
5. Garcia SM, Hirschberg PR, Sarkar P, et al. Insulin actions on hypothalamic glucose-sensing neurones. J Neuroendocrinol. 2021;33(4). doi:10.1111/jne.12937
6. Santiago AM, Clegg DJ, Routh VH. Estrogens modulate ventrolateral ventromedial hypothalamic glucose-inhibited neurons. Molecular Metabolism. 2016;5(10):823-833. doi:10.1016/j.molmet.2016.08.002
7. Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A. 1994;91(22):10625-10629. doi:10.1073/pnas.91.22.10625
8. Marty N, Dallaporta M, Foretz M, et al. Regulation of glucagon secretion by glucose transporter type 2 (glut2) and astrocyte-dependent glucose sensors. J Clin Invest. 2005;115(12):3545-3553. doi:10.1172/JCI26309
9. Elizondo-Vega R, Cortes-Campos C, Barahona MJ, Oyarce KA, Carril CA, García-Robles MA. The role of tanycytes in hypothalamic glucosensing. J Cell Mol Med. 2015;19(7):1471-1482. doi:10.1111/jcmm.12590
10. Winkler Z, Kuti D, Polyák Á, et al. Hypoglycemia-activated Hypothalamic Microglia Impairs Glucose Counterregulatory Responses. Sci Rep. 2019;9(1):6224. doi:10.1038/s41598-019-42728-3
11. Valdearcos M, Myers MG, Koliwad SK. Hypothalamic microglia as potential regulators of metabolic physiology. Nat Metab. 2019;1(3):314-320. doi:10.1038/s42255-019-0040-0
12. Stanley S, Domingos AI, Kelly L, et al. Profiling of Glucose-Sensing Neurons Reveals that GHRH Neurons Are Activated by Hypoglycemia. Cell Metabolism. 2013;18(4):596-607. doi:10.1016/j.cmet.2013.09.002
13. Chakera AJ, Hurst PS, Spyer G, et al. Molecular reductions in glucokinase activity increase counter-regulatory responses to hypoglycemia in mice and humans with diabetes. Molecular Metabolism. 2018;17:17-27. doi:10.1016/j.molmet.2018.08.001
14. Stanley SA, Kelly L, Latcha KN, et al. Bidirectional electromagnetic control of the hypothalamus regulates feeding and metabolism. Nature. Published online March 23, 2016. doi:10.1038/nature17183
15. Osundiji MA, Zhou L, Shaw J, et al. Brain glucosamine boosts protective glucoprivic feeding. Endocrinology. 2010;151(4):1499-1508. doi:10.1210/en.2009-0989
16. Hussain S, Richardson E, Ma Y, et al. Glucokinase activity in the arcuate nucleus regulates glucose intake. J Clin Invest. 2015;125(1):337-349. doi:10.1172/JCI77172

17. Lamy CM, Sanno H, Labouèbe G, et al. Hypoglycemia-activated GLUT2 neurons of the nucleus tractus solitarius stimulate vagal activity and glucagon secretion. Cell Metab. 2014;19(3):527-538. doi:10.1016/j.cmet.2014.02.003
18. Lei H, Preitner F, Labouèbe G, Gruetter R, Thorens B. Glucose transporter 2 mediates the hypoglycemia-induced increase in cerebral blood flow. J Cereb Blood Flow Metab. 2019;39(9):1725-1736. doi:10.1177/0271678X18766743
19. Labouèbe G, Boutrel B, Tarussio D, Thorens B. Glucose-responsive neurons of the paraventricular thalamus control sucrose-seeking behavior. Nat Neurosci. 2016;19(8):999-1002. doi:10.1038/nn.4331
20. Cruz AM, Partridge KM, Malekizadeh Y, et al. Brain Permeable AMP-Activated Protein Kinase Activator R481 Raises Glycaemia by Autonomic Nervous System Activation and Amplifies the Counterregulatory Response to Hypoglycaemia in Rats. Front Endocrinol. 2021;12:697445. doi:10.3389/fendo.2021.697445
21. Nwokolo M, Amiel SA, O'Daly O, Macdonald IA, Zelaya FO, Choudhary P. Restoration of Hypoglycemia Awareness Alters Brain Activity in Type 1 Diabetes. Diabetes Care. 2021;44(2):533-540. doi:10.2337/dc20-1250
22. Rosario W, Singh I, Wautlet A, et al. The Brain–to–Pancreatic Islet Neuronal Map Reveals Differential Glucose Regulation From Distinct Hypothalamic Regions. Diabetes. 2016;65(9):2711-2723. doi:10.2337/db15-0629
23. Borg MA, Sherwin RS, Borg WP, Tamborlane WV, Shulman GI. Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycemia in awake rats. J Clin Invest. 1997;99(2):361-365. doi:10.1172/JCI119165
24. Sejling AS, Wang P, Zhu W, et al. Repeated Activation of Noradrenergic Receptors in the Ventromedial Hypothalamus Suppresses the Response to Hypoglycemia. Endocrinology. 2021;162(3):bqaa241. doi:10.1210/endocr/bqaa241
25. Campbell JN, Macosko EZ, Fenselau H, et al. A molecular census of arcuate hypothalamus and median eminence cell types. Nat Neurosci. 2017;20(3):484-496. doi:10.1038/nn.4495
26. Dowsett GKC, Lam BYH, Tadross JA, et al. A survey of the mouse hindbrain in the fed and fasted states using single-nucleus RNA sequencing. Mol Metab. 2021;53:101240. doi:10.1016/j.molmet.2021.101240
27. Ludwig MQ, Cheng W, Gordian D, et al. A genetic map of the mouse dorsal vagal complex and its role in obesity. Nat Metab. Published online March 25, 2021. doi:10.1038/s42255-021-00363-1
28. Meek TH, Nelson JT, Matsen ME, et al. Functional identification of a neurocircuit regulating blood glucose. Proc Natl Acad Sci USA. 2016;113(14):E2073-E2082. doi:10.1073/pnas.1521160113
29. Faber CL, Matsen ME, Velasco KR, et al. Distinct Neuronal Projections From the Hypothalamic Ventromedial Nucleus Mediate Glycemic and Behavioral Effects. Diabetes. 2018;67(12):2518-2529. doi:10.2337/db18-0380
30. Sutton AK, Goforth PB, Gonzalez IE, et al. Melanocortin 3 receptor-expressing neurons in the ventromedial hypothalamus promote glucose disposal. Proc Natl Acad Sci USA. 2021;118(15):e2103090118. doi:10.1073/pnas.2103090118
31. Garfield AS, Shah BP, Madara JC, et al. A Parabrachial-Hypothalamic Cholecystokinin Neurocircuit Controls Counterregulatory Responses to Hypoglycemia. Cell Metabolism. 2014;20(6):1030-1037. doi:10.1016/j.cmet.2014.11.006

32. Flak JN, Goforth PB, Dell'Orco J, et al. Ventromedial hypothalamic nucleus neuronal subset regulates blood glucose independently of insulin. Journal of Clinical Investigation. 2020;130(6):2943-2952. doi:10.1172/JCI134135
33. He Y, Xu P, Wang C, et al. Estrogen receptor-α expressing neurons in the ventrolateral VMH regulate glucose balance. Nat Commun. 2020;11(1):2165. doi:10.1038/s41467-020-15982-7
34. Bayne M, Alvarsson A, Devarakonda K, et al. Repeated hypoglycemia remodels neural inputs and disrupts mitochondrial function to blunt glucose-inhibited GHRH neuron responsiveness. JCI Insight. 2020;5(21):e133488. doi:10.1172/jci.insight.133488
35. Ibrahim N, Bosch MA, Smart JL, et al. Hypothalamic Proopiomelanocortin Neurons Are Glucose Responsive and Express K ATP Channels. Endocrinology. 2003;144(4):1331-1340. doi:10.1210/en.2002-221033
36. Fioramonti X, Contie S, Song Z, Routh VH, Lorsignol A, Penicaud L. Characterization of Glucosensing Neuron Subpopulations in the Arcuate Nucleus: Integration in Neuropeptide Y and Pro-Opio Melanocortin Networks? Diabetes. 2007;56(5):1219-1227. doi:10.2337/db06-0567
37. Biglari N, Gaziano I, Schumacher J, et al. Functionally distinct POMC-expressing neuron subpopulations in hypothalamus revealed by intersectional targeting. Nat Neurosci. Published online May 17, 2021. doi:10.1038/s41593-021-00854-0
38. Tooke BP, Yu H, Adams JM, et al. Hypothalamic POMC or MC4R deficiency impairs counterregulatory responses to hypoglycemia in mice. Molecular Metabolism. 2019;20:194-204. doi:10.1016/j.molmet.2018.11.004
39. Betley JN, Xu S, Cao ZFH, et al. Neurons for hunger and thirst transmit a negative-valence teaching signal. Nature. 2015;521(7551):180-185. doi:10.1038/nature14416
40. Betley JN, Cao ZFH, Ritola KD, Sternson SM. Parallel, Redundant Circuit Organization for Homeostatic Control of Feeding Behavior. Cell. 2013;155(6):1337-1350. doi:10.1016/j.cell.2013.11.002
41. Engström Ruud L, Pereira MMA, de Solis AJ, Fenselau H, Brüning JC. NPY mediates the rapid feeding and glucose metabolism regulatory functions of AgRP neurons. Nat Commun. 2020;11(1):442. doi:10.1038/s41467-020-14291-3
42. Luquet S, Phillips CT, Palmiter RD. NPY/AgRP neurons are not essential for feeding responses to glucoprivation. Peptides. 2007;28(2):214-225. doi:10.1016/j.peptides.2006.08.036
43. Aklan I, Sayar Atasoy N, Yavuz Y, et al. NTS Catecholamine Neurons Mediate Hypoglycemic Hunger via Medial Hypothalamic Feeding Pathways. Cell Metabolism. 2020;31(2):313-326.e5. doi:10.1016/j.cmet.2019.11.016
44. Goldstein N, McKnight AD, Carty JRE, Arnold M, Betley JN, Alhadeff AL. Hypothalamic detection of macronutrients via multiple gut-brain pathways. Cell Metabolism. 2021;33(3):676-687.e5. doi:10.1016/j.cmet.2020.12.018
45. Ritter S, Bugarith K, Dinh TT. Immunotoxic destruction of distinct catecholamine subgroups produces selective impairment of glucoregulatory responses and neuronal activation. J Comp Neurol. 2001;432(2):197-216. doi:10.1002/cne.1097
46. Li AJ, Wang Q, Ritter S. Selective Pharmacogenetic Activation of Catecholamine Subgroups in the Ventrolateral Medulla Elicits Key Glucoregulatory Responses. Endocrinology. 2018;159(1):341-355. doi:10.1210/en.2017-00630
47. Sofia Beas B, Gu X, Leng Y, et al. A ventrolateral medulla-midline thalamic circuit for hypoglycemic feeding. Nat Commun. 2020;11(1):6218. doi:10.1038/s41467-020-19980-7

48. Otgon-Uul Z, Suyama S, Onodera H, Yada T. Optogenetic activation of leptin- and glucose-regulated GABAergic neurons in dorsomedial hypothalamus promotes food intake via inhibitory synaptic transmission to paraventricular nucleus of hypothalamus. Mol Metab. 2016;5(8):709-715. doi:10.1016/j.molmet.2016.06.010
49. Picard A, Soyer J, Berney X, et al. A Genetic Screen Identifies Hypothalamic Fgf15 as a Regulator of Glucagon Secretion. Cell Rep. 2016;17(7):1795-1806. doi:10.1016/j.celrep.2016.10.041
50. Picard A, Metref S, Tarussio D, et al. Fgf15 Neurons of the Dorsomedial Hypothalamus Control Glucagon Secretion and Hepatic Gluconeogenesis. Diabetes. 2021;70(7):1443-1457. doi:10.2337/db20-1121