Review

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# Hypothalamic GABAergic neurocircuitry in the regulation of energy homeostasis and sleep/wake control

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**Abstract:** Gamma-aminobutyric acid (GABAergic) neuron, as one of important cell types in synaptic transmission, has been widely involved in central nervous system (CNS) regulation of organismal physiologies including cognition, emotion, arousal and reward. However, upon their distribution in various brain regions, effects of GABAergic neurons in the brain are very diverse. In current report, we will present an overview of the role of GABAergic mediated inhibitory neurocircuitry in the hypothalamus, underlying mechanism of feeding and sleep homeostasis as well as the characteristics of latest transcriptome profile in order to call attention to the GABAergic system as potentially a promising pharmaceutical intervention or a deep brain stimulation target in eating and sleep disorders.

**Keywords:** energy homeostasis; feeding; gamma-aminobutyric acid; hypothalamus; neurocircuitry; sleep; wakefulness.

## Introduction

Sleep and eating are two basic and essential behaviors for organism survival. Increasing evidence supports that sleep has influenced on feeding and vise vera. In fact, previous meta-analyses have showed that sleep deprivation, night shift circadian, and other lifestyles are linked to obesity, most likely caused by encouraging excessive eating, which involved in incentive salience and inhibitory control [1]. In contrast, dietary patterns (low or high carbohydrate combine with high fat or low carbohydrate) affect sleep architecture including both rapid eye movement (REM) sleep and non-REM (NREM) sleep. However, causal relationship between sleep, obesity, diet, and energy balance remains uncertain. One of the perspectives to address this gap appears the investigation of the overlapping neuronal circuit as well as the mechanistic explanation of sleep and feeding behaviors. The roles of hypothalamus in the control of feeding behavior and energy balance are well established in past decades and, currently, its roles in sleep/wake cycle are also emerging. While gamma-aminobutyric acid (GABAergic) neurons, as important and wide efferent neurons, could be key point to unlock the node between sleep and feeding related energy homeostasis. The objective of this review, thus, is to provide an overview of up-to-date studies that accentuate the importance of hypothalamic GABAergic neurons in the regulations of diet and sleep.

## The characteristic of GABA and its signaling pathway in the CNS

Gamma-aminobutyric acid (GABA) is one of the principle neuronal transmistters released from neurons named GABAergic neurons, which typically express GABA synthesized enyzem-glutamate decarboxylase 1/2 (GAD1 or GAD2) and vesicular GABA transporter (VGAT). Its amount level and distribution determine crucially the excitability of the neuronal networks.

Its expression has been reported in rodent, primate to human. GABAergic neurons are widely located in the brain regions such as cerebral cortex, striatum, hippocampus, globus pallidus, amgydala as well as hypothalamus [2–7]. The basic working principle of GABAergic neurons is through releasing GABA effecting on presynaptic autoreceptors or postsynaptic GABA receptors and acting as an inhibitory role or excitatory role (only in certain circumstances, for example, during developmental stage).

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Moreover, there are two types of GABA receptors, which bind to GABA mediating downstream effect of GABAergic synapses transmission. One is $GABA_A$ and $GABA_C$ receptors; it is ion channels gated by GABA with similar reversal potentials close to $Cl^-$equilibrium [8]. The effect of ionotropic GABA receptors opening is the increase in chloride and consequently inhibition of corresponding neurons in the most of cases. However, during neuronal developmental stages or chronic epilepy, GABA acts as an excitatory role [9, 10]. The second type of GABA receptors are $GABA_B$ receptors. Different from $GABA_A$ and $GABA_C$ receptors, $GABA_B$ receptors are G-protein-coupled proteins. Through binding to GABA, $GABA_B$ receptors have been described to negatively couple to adenylyl cyclase, stimulate phospholipase $A_2$, inactivate voltage gated $Ca^{2+}$–channels, increase in $K^+$ conductance and so on. The downstream process of $GABA_B$ receptors can decrease probabilities of synaptic transmitter release, therefore regulate efficacy of synapses [11]. More detailed molecular, cellular and network level of GABA inhibition are beyond the scope of this review, since the related mechanisms had intensively been discussed in previous studies.

Due to its broad effects and comparatively clear mechanisms, it has been reported that GABA as promising pharmaceutical targets play a crucial role in anti-sleeplessness, anti-diabetes, anti-memory loss, anti-depression and other neurological or metabolic disorders [12]. On the contrary to most pharmaceutical drugs, GABA is vastly distributed in nature and food (e.g. natural fermentation, apocynum venetum, green tea, soybean and so on) [12, 13]. Therefore, as a major inhibitory neurotransmitter in the CNS and a wide target in various physiology phenomenon, GABA contribution in the hypothalamus mediated behaviors seem to a promising entry point to better understand how sleep and feeding behaviors interact.

Similarly, it should be noted that, although typically GABAergic neurons are types of interneurons that are well investigated in local cortex inhibition, they do not only balance the excitability of the local circuit, but they also play as a role as projecting cells with specific afferent and efferent [14–16]. The latter will be the main focus in the following discussion.

## ARC GABAergic neurons in feeding regulation

Two decades ago, researchers had ascertained leptin as a key player against the development of obesity and, subsequently, leptin responsive neurons were directly pointed to the Arcuate area, in which exist a bidirectional regulatory role of agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons on feeding and glucose metabolism [17–22]. However, when deletion of leptin separately or combined in AgRP and POMC neurons, mice have only shown very minor effect than expected. Through Cre-expressing mice, Vong and her colleagues have discovered majority of leptin effect against obesity highly rely on GABAergic neurons [23]. Therefore, for the first time, GABAergic neurons contribution in energy homeostasis has captured greatly attention and many questions immediately were raised regarding the location of those leptin-responsive GABAergic neurons and through what mechanisms they regulate energy balance.

As it is well established in last decades, arcuate nucleus (ARC) in the hypothalamus, considered as the first order region of sensing energy fluctuations-contains several key neuronal populations such as Agouti gene-related protein (AgRP), the rat insulin-2 promoter (RIP) drivers dependent expressing neurons latest recognized Prepronociceptin (PNOC) neurons and so on. One of the key factors is that they all express GABA and through partly GABAergic transmission regulate metabolic or hedonic feeding. Interestingly, those pivotal peptide expressing neurons may rely on neuronal transmitters like GABA or Glutamate as fast response, while, as slow response, they mediate long-term adjusting of energy homeostasis through peptide release. For instance, Michael Krashes et al. have found that acute activation of the AgRP neurons can robustly increase feeding. Similarly, they have separately observed that central injection of AgRP or co-release neuropeptide Y (NPY) or transmitter-GABA has different feeding response compared to the activation of AgRP in a whole [24]. Therefore, it seems underline the idea that the heterogenous expressing peptide or transmitter may have temporal and functional effect on the regulation of responsible behavior like feeding. In fact, they have employed chemogenetics to control AgRP neurons acutely in different conditions including either melanocortin-4-receptor (MC4R) deletion, GABA knockout or NPY knock out as well as three whole knockouts. With completed knockout of MC4R, GABA and NPY, acute stimulating feeding caused by AgRP neuron activation is dramatically impaired, but the food promoting effect becames comparable at the later phase (24 h after Clozapine N-oxide [CNO]). The team has also done separated deletion of single peptide or transmitter, and two-two combination strategies. In sum, GABA and NPY together are required for the acute effect and AgRP alone is enough for long term food promoting effect. Also, it is important to note that GABA is not only key mediator of the acute function of AgRP

neurons. Many studies have substantively demonstrated that AgRP neurons, through presynaptic GABA afferent, mediate acute leptin inhibition of feeding [25–27].

GABA mediates acute effect from the ARC, which also takes place in the RIP neurons. One of the cell types in the ARC, distinct from AgRP and POMC neurons, is responsible for regulating energy balance upon GABA release [28–30]. Dong Kong and his colleagues have found that deletion of GABA in RIP neurons of ARC can dramatically increase body weight by reducing energy expenditure, while deletion of Glutamate in RIP neurons does not cause metabolism changes. In addition, a major population of ARC neurons is Tyrosine Hydroxylase (TH) positive dopamine neurons. It has been demonstrated that dopamine/TH neurons in the ARC by releasing GABA communicate with other cell types in the ARC. For instance, ARC TH cells reduce energy expenditure by inhibiting POMC neurons to increase food intake as well as inhibiting outside ARC, paraventricular neurons (PVH) within the hypothalamus [31, 32].

Newly expound neuronal population in the ARC-PNOC neurons particularly responds to the energy dense recognized by unbiased phosphoribotrap approach [33]. By employing *in situ* hybridization and electrophysiology methods, several authors have found that this type of neuron is rarely overlapped with well-defined feeding related neurons-AgRP and POMC neurons but strongly co-expresses Slc32a-vgat marker for approximately 80%, and displays inward rectification electrophysiological property. Optogenetic stimulation of PNOC neuron shows increased food intake in high fat diet feeding paradigm. In this vein, Alexander Jais and his colleagues have also demonstrated that PNOC neurons in the ARC, has more arborization, which can be used to communicate with other neurons in the ARC. Moreover, it is known that ARC-PNOC neurons through projections to bed nucleus of the stria terminalis (BNST), remains main descending pathway for hyperphagia. From the cited articles, it is not clearly stated that GABAergic neurons (e.g. PNOC) act as interneurons or projecting cells. However, observations from the anatomical neurocircuitry elaboration, soma morphology as well as functional connection within and outside hypothalamus, indicate that GABAergic neurons is not only modulating excitability balance.

Alongside of the perspectives from neuronal population, many findings discuss the roles of the GABAergic neurons. From the metabolism regulation itself, GABAergic cells have great importance. For example, the function of leptin-hormone released from white adipose tissue-is well established in energy regulation. Indeed, it may suppress the food intake, which can be the main reason for body weight reduction. However, previous findings have illustrated that leptin, mainly through GABA release from critical neurons, reduces body weight. It has been also observed that with deletion GABA expression in leptin receptor-expressing neurons, mice develop mild body weight gain in chow diet but were very sensitive to high fat diet [34]. Moreover, during development time, feeding behavior is also critical for establishing energy homeostasis. Accordingly, Tong's team has shown again using cre-line, which is not expressed neither in AgRP and POMC neurons, deletion of GABA attenuates feeding in post-weaning period and slows the growth as well the sensitivity to NPY [35]. In brief, arcuate neurons, as the first-order energy sensing region, is dominantly under GABAergic regulation.

## LHA GABAergic neurons in feeding regulation

Although arcuate nucleus is recognized as the first energy sensing entry of the brain, the lateral hypothalamus area (LHA)-the second sensing site, as well as integrator in the hypothalamus, are responsible for following feeding regulation and other distinct physiological adaptions such as aggression, sleeping and thermoregulation. Recent state-of-the-art methodologies in molecular characterization and neurocircuit tracing confirm to a greater extent the sophisticated LHA interconnections.

This latest functional distinction of GABAergic neurons in the LHA ($LHA^{GABA}$) has demonstrated the fundamental roles that activation of $LHA^{GABA}$ play in stimulating robust consummatory and rewarding behaviors [36]. Indeed, Jennings and his colleagues have identified LHA-GABAergic neurons distinct from MCH and Orexin-expressing neurons encode both appetitive and consummatory behaviors, employing two-photon *in vivo* calcium imaging. The study indicates these types of neuron are two distinct GABAergic populations. One population is excited when mice approached to food related location, by contrast the other is inhibited. Furthermore, they have discovered that the former has responded to nose-poker indicating appetite, while the latter has reacted to consumption. As a result, the downstream projections of $LHA^{GABA}$ further elaborate the involvement of GABA in feeding and underlying mechanisms. For instance, it has been shown that LHA sends one of the most steady inputs to ventral tegmental area (VTA) to promote motivation behaviors and its effect is further found to be mediated by

GABAergic neurons from the LHA and through disinhibiting GABAergic cells in the VTA and consequently this pathway enhances dopamine (DA) release [37]. This direct relationship between $LHA^{GABA}$ and VTA may be the neural circuit basis for hedonic feeding behaviors. Another downstream of $LHA^{GABA}$ work has shown that the long projections from $LHA^{GABA}$ cells to ventrolateral periaqueductal gray (vlPAG). Similar to wise VTA projections, $LHA^{GABA}$ also drives disinhibition of GABAergic cells in vlPAG to significantly promote food intake as well as related feeding behaviors [38]. Qingchun Tong and his team, too, have reported alike inhibitory control of LHA in feeding. In fact, they have discovered that the disruption of GABA released from LHA attenuates feeding and optogenetic stimulation of $LHA^{GABA}$ to PVH through $GABA_A$ receptors promotes feeding [39].

In order to extensively unravel the complexity of LHA structure and neuronal characteristics, recent studies employ single-cell transcriptomic analysis of the LHA and reveal molecular distinct GABAergic and Glutamatergic neuronal cell types [40]. Taken advantage of the anatomical isolation and the systematic validation of cell clusters in the LHA, authors have reported 15 GABAergic clusters with robust expression of *Slc32a1*, which encodes vesicular GABA transporter. Importantly, the VGAT marked GABAergic clusters also match with a co-expression of synthetic enzyme of GABA encoding genes GAD1 and GAD2. Moreover, the further identification unravels that conventional GABAergic phenotype may be caused by the subpopulation of neurotension (Nts) and Cocaine- and amphetamine-regulated transcript protein (Cartpt)-expression neurons. It has, indeed, been shown that Nts expressing LHA neurons significantly co-express long isoform of the leptin receptor (LepRb) and MC4R, both of which play critical roles in energy homeostasis. In addition, single-cell transcriptome analysis confirms the unique characteristics of GABAergic in the LHA: its expression has no overlap with MCH and Orexin neurons, meanwhile no direct connection with the formers. In the same vein, subcluster validation has proved that Hcrt+ (Orexin) neurons exhibit largely expression of *Slc17a6* (Glutamatergic, accounted for 93%) while only sparsely express *Slc32a1* (GABAergic, accounted for 4%). By contrast, detailed analysis of MCH neurons shows 100% expression of *Slc17a6* but are incapable of detecting the *Slc32a1*.

Doubtlessly, single-cell level transcriptome and *in vivo* calcium imaging together with conventional cell-specific neuronal manipulation may well unravel the heterogeneity of GABAergic cells in the LHA and help to understand how the LHA position in the hypothalamus integrates information and delivers information to the intra-hypothalamus and the extra-hypothalamus for the regulation of energy homeostasis.

## DMH GABAergic neurons in feeding regulation

As mentioned above, AgRP neurons receive strong GABAergic afferent to mediate feeding behaviors. One of the input sources is from dorsalmedial nucleus of the hypothalamus (DMH). Previously through monosynaptic retrograde mapping from Cre recombinase specific marked AgRP-Cre mice, it has been shown that DMH has the strongest proportion of input to AgRP neurons, followed by PVH and Ventralmedial Hypothalamus (VMH) regions [41]. To evaluate how much is the inhibitory contribution from DMH, Bradford Lowell and his team have employed channelrhodopsin-assisted circuit mapping (CRACM) to determine presynaptic GABAergic from DMH to AgRP neurons. They have found that VGAT marked GABAergic cells from DMH has inhibitory synaptic input on AgRP neurons, displayed by picrotoxin (GABAA receptor antagonist) sensitive-light evoked synaptic current. Subsequently, they have identified the leptin receptor marker as GABAergic input from DMH afferents to AgRP neurons and have found 100% connection by CRACM technique and LepR-DMH neurons > AgRP neurons are sufficient to inhibit feeding [42]. This finding has established clearly that the leptin from peripheral circulation may act on DMH leptin receptor neurons and further through inhibitory regulation from DMH to Arcuate, consequently, suppress feeding. Similarly, another study has shown that distinct GABAergic cell type in the DMH, tropomysin related tyrosine kinase B (TrkB) receptor expressing neurons, can be activated by either fasting or refeeding status [43]. Chemogenetically, the activation of TrkB-DMH neurons robustly suppresses homeostatic feeding during dark cycle and, by contrast, the inhibition of those neurons promotes feeding in the light cycle. Further neurotracing works have conveyed that GABAergic TrkB neurons do not innervate AgRP but do POMC neurons, which is distinct from LepR-DMH neurons [43]. Functional connection from GABAergic afferents from DMH to POMC neurons in the ARC implies that frequency of inhibitory postsynaptic current (IPSC) as well as GABA vesicles release increase at the DMH-POMC synapse upon overnight fasting [44]. However, since inhibitory input from DMH to Arcuate potentially exerts opposite effects on feeding behaviors, the puzzling question is how the neurocircuitry between DMH and Arcuate coordinates to finely tune the feeding response upon energy state.

In sum, GABAergic neurons in the hypothalamus are mainly distributed in the ARC, LHA, and DMH but to less extent in VMH and PVH. From functional connection perspectives between GABAergic neuron and others discussed above, these neurons are partly as the primary driving cells for feeding regulation and partly may participate as interneurons to connect local circuits. During the development time, GABAergic maturation also has gone through a critical function switch. Within four appetite related centers in the hypothalamus, namely ARC, LHA, PVH and VMH, Kobayashi and his colleagues have demonstrated that upon $K^+$-$Cl^-$-cotransporter 2 (KCC2)- which is responsible for GABA action shifts from excitatory to inhibitory, GABAergic neurons mature first in the LHA, followed by PVH before birth, while VMH and ARC GABA neurons do after birth [45]. In preclinical studies, it has observed that diet can lead to alteration in reward signaling and in inhibitory neurotransmission control by GABA [46, 47]. The practical issue is whether, at the adolescent age, children should have junk food, which may affect brain development [48]. Therefore, should the GABAergic neurons play an essential role in this maturation process of brain regions responsible for diet habit, energy expending rate and regular circadian cycle for healthy sleep.

## Hypothalamic GABAergic neurons in sleep homeostasis

Unlike feeding regulatory mechanisms by GABAergic neurons in each subnucleus of hypothalamus, the role of inhibitory tone in sleep control is less addressed in the field of innate behaviors.

As essential as feeding for life, sleep is regarded to be reciprocally interconnected with feeding and a good quality of life. Yet, the mechanisms on how feeding regulates sleep, and vice versa, have not been adequately addressed. We know that when animals are in the sleep state they should not eat and if they so do, they will not be able to sleep. This is, indeed, two conflict physiological needs for mammalian [49, 50]. So, how do the organisms adjust sleep based on food availability and prioritize feeding or sleep over one another? Although the sleep control mechanisms in the hypothalamus are not so well studied, there are extensive interactions between feeding (or energy sensing neurons) intermingled with sleep active or promoting neurons) and even related descending pathways [51–53]. It has been revealed that hypothalamic neurons responsible for fast and feeding must be aligned with the sleep/wake cycle. The temporal control is known to be related to activity-dependent gene repertoires in NPY/AgRP neurons to synaptogenesis, neurotransmitter and peptidergic signaling [54]. This work highlights the link between energy-sensing neurons in the hypothalamus and sleep/wake state.

In previous studies, researchers-manipulating hunger and satiety center neurons, AgRP, and POMC respectively, have found that AgRP stimulation promotes wakefulness while attenuates sleep quantitively and qualitatively, reflected by increased wakefulness duration and sleep counts [55]. By contrast, POMC stimulation furthers sleep and reduces sleep fragmentation caused by food deprivation. This is one of the examples of how feeding related neurons bidirectionally regulates sleep. Since we have demonstrated above how pivotal is the GABAergic neurocircuitry in the regulation of feeding, in the following paragraphs, we intend to look into the role, if any, of the same type of inhibitory population in the sleep/wakefulness control. First, in the ventral preoptic neurons in the hypothalamus (VLPO), recognized as a sleep and thermoregulation center, there are large population-GABAergic cells sending long projections to other brain regions to regulate sleep [56, 57]. Seventy five per cent (75%) of these neurons are glucose excited. This gives us the hint that sleep regulatory neurons, on the one hand, are influenced by energy homeostasis and, perhaps on the other hand, are tank for energy maintaining. For instance, GABAergic neurons, in this region, induce large-amplitude and slow electroencephalogram (EEG) oscillation-typical characteristics of NREM [52, 58]. Subsequently, local factor which modulates NREM results in brain energy saving and promotes memory consolidation. Not only in the NREM, the GABAergic neurons, by controlling glutamatergic neurons in the brain stem, cause REM sleep stage reflecting as muscle atonia, REM movement and dreaming. GABAergic neurons in preoptic area (PO) projecting to tuberomammillary nucleus (TMN) are both sleep active and sleep promoting. This team has used optogenetic stimulation of GAD2 neurons in PO-TMN pathway and has observed an immediate increase in the NREM as well as a delayed increase in the REM [57, 59]. However, when they stimulate general GAD2 expressing neurons in PO, the final net effect of GABAergic activation is consisted of wakefulness increase, which underlines the fact that the wake-promoting neurons of GABAergic in PO are dominant.

Interactions between wake promoting neurons in anterior hypothalamus and sleep promoting neurons in posterior hypothalamus play an important role in the maintenance of the sleep/wake cycle, as exemplified by

Orexin neurons and MCH neurons [60]. Juxtacellular electrophysiology recording of VGAT neurons in the LHA of rats has shown that spike activity is more excited either in the wake or in the REM sleep, and to a less extent in the NREM sleep [52]. It has been reported that chemogenetics or optogenetics activation promotes wake or reduce NREM and REM sleep. Nevertheless, the REM stage specific inhibition of VGAT neurons does not have any effect on sleep/wake cycle and REM sleep. By contrast, in another independent study, optogenetics silencing VGAT neurons in the LHA in REM stage can reduce food intake but does not in wakefulness stage [61]. These finding indicates that GABAergic neurons in the LHA may serve as an arousal signal for wakefulness, while they may play different roles during REM sleep.

Furthermore, there is a lack of research on GABAergic neurons in the LHA with regards to sleep control. Nevertheless, it appears that they send projections to dorsal raphe, which can consequently inhibit GABAergic neurons locally to promote arousal system [62]. It means that they may be important mediator for the inter-talk between hypothalamus and brain stem/cortex. Indeed, the disruption of GABAergic neurons in the LHA is likely to cause severe impairment of sleep problems. Meanwhile, same type of GABAergic neurons found in the LHA induce vigorous eating habits. In fact, underlying mechanisms lie in disinhibition of GABAergic cells in VTA and locus coeruleus (LC) areas [15, 62]. In respect to GABAergic neurons in LHA, they has two characteristics: first, projection-specific regulation of sleep and eating; second, subclusters of GABAergic neuron may play distinct roles in regulation of sleep and feeding behavior in order to adapt internal state of arousal and energy. In summary, hypothalamus inhibitory control in sleep circuit is not well defined, with the exception of VLPO area. While, other regions, such as VTA, PAG, and Dorsal Raphe outside hypothalamus have more clear roles in sleep regulation [63, 64].

Also, researchers have found that stimulating GABAergic neurons in the VTA can elicit long-lasting non-REM sleep like, for example, sleep resembling sedation. They have discovered that GABAergic neurons in the VTA may, through inhibiting glutamatergic/nitrergic (NOS1) neurons, limit wakefulness. Lesion of GABAergic neurons is able to increase wakefulness for 4 month long [15]. This area is critically related to goal-and reward-directed behaviors. Again, these findings make it clear that GABAergic neurons play integrator roles for the inner state homeostasis.

In other brain stem region, the GABAergic neurons are also involved in sleep control. For instance, S. Valencia Garcia and her team have shown that inactivation of ventralmedial medulla (vmM) VGAT expression neurons can induce REM sleep without atonia [65]. Specifically in their study, they have systematically revealed that inhibitory neurons are circumscribed within the vmM, and also through short hairpin RNA virus inhibition of them impaires the neurotransmission from vmM. They have subsequently demonstrated that REM sleep displays robust REM characteristics without muscle atonia, meaning GABAergic neurons here do not interfere with projections to the spinal cord.

As well, the brain region-ventrallateral periaqueductal gray (vlPAG) is proven to be important for REM sleep. Yang Dan and her colleagues have shown that optogenetic activation of vlPAG GABAergic neurons suppresses REM sleep, while it enhances NREM sleep [64]. They have used cell specific recording illustrating that the majority of GABAergic neurons in vlPAG change rapidly during the brain state transitions, decrease gradually between REM sleep, and reset by each REM episode. This functional study underscores that vlPAG GABAergic neurons strongly gate REM sleep and contribute to REM and NREM transition.

## Future perspectives

GABA is the widespread and the major inhibitory neurotransmitter of the CNS. As stated above, GABAergic neurons in the hypothalamus contribute greatly to feeding and sleep/wake control, particularly the region-specific anatomical expression and related mechanisms (See the Figure 1).

Their mediated inhibitory or disinhibitory processes favor feeding and sleep. Since modern lifestyles brings more and more sleep problems and eating disorders, the link between sleep disturbance and feeding/glucose imbalance becomes further evident. In line with this observation, this review brings together potential utilities of GABAergic system as possible future therapeutic targets for symptoms such as obstructive sleep apnea, most evident phenotype of obesity-related sleep disorder [66]. Indeed, clinically, GABA receptor agonists-including barbiturates, benzodiazepines, chloral hydrate, ethanol, and gaseous anesthetics, have been used for insomnia and sleep apnea [67, 68]. However, researchers have found drug effects are region- and receptor subunit-specific manners. Growing concerns emerge in relation with the fact that sleep, obesity, adolescents' development, and age-related comorbidities are to large extent correlated [69–72]. Therefore, whether GABA system can constitute a promising tank for pharmaceutically or invasively therapeutical device stimulation remains to test.

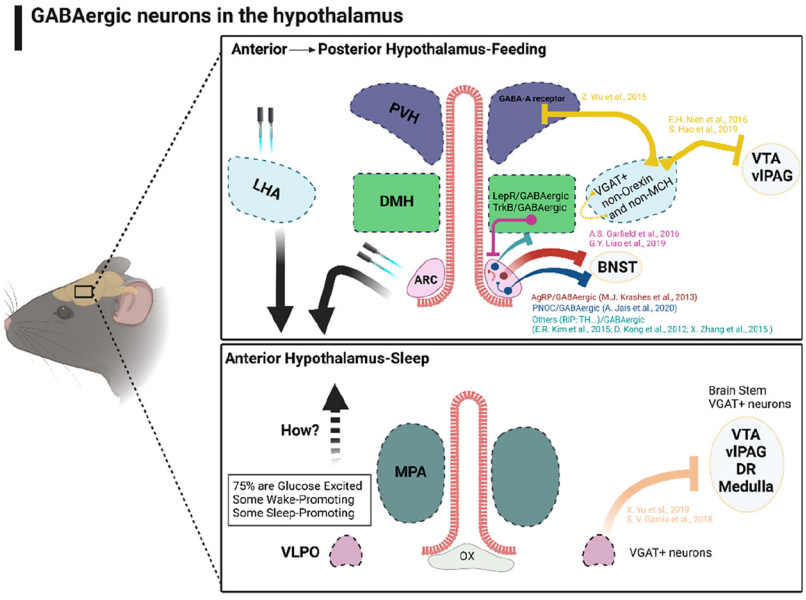


**Figure 1:** GABAergic neurons anatomical distribution in the hypothalamus and their roles in feeding or sleep regulation. Upper figure: As illustrated, well reported GABAergic neurons in feeding regulation are located in the ARC area, where GABAergic neurons are co-expressed with AgRP, PNOC and others like RIP and TH positive neurons. Those neurons, through brain area-BNST, contribute to feeding behaviors. In the LHA, where GABAergic cells marked by VGAT and are distinct from Orexin and MCH neurons. By sending projections to brain stem (VTA and vlPAG) as well as to the PVH, LHA-GABAergic neurons regulate homeostatic and hedonic feeding behaviors. In the DMH, there are two inhibitory mechanisms regulating food intake: One does it through leptin receptor-expressing neurons releasing GABA and innervate AgRP neurons; the other emphasizes it through TrkB receptor-expressing neurons released GABA targeting POMC neurons in the ARC. Lower figure: This panel describes the most studied sleep center-VLPO located in the anterior hypothalamus. There are several different GABAergic neurons. Upon their downstream target, they either promote wakefulness or sleep. However, 75% of those neurons are glucose excited, which highlights the link between sleep and feeding behavior or energy homeostasis. As the laser logo pointed it, when optogenetic stimulating LHA or ARC, sleep counts and duration changes in fasting or refeeding state. LHA, lateral hypothalamus area; ARC, arcuate; PVH, paraventricular hypothalamus; DMH, dorsal medial hypothalamus; MPA, medial preoptic area; VLPO, ventral lateral preoptic nucleus; VTA, ventral tegmental area; vlPAG, ventral lateral periaqueductal gray; DR, dorsal raphie; BNST, bed nucleus of the StriaTerminalis; OX, optic chiasm.

Apart from GABA, other neurotransmitters have been studies in several findings. For instance, serotonin is also one of oldest neurotransmitters related to sleep treatment since 1950. Based on working mechanisms of serotonin, selective serotonin reuptake inhibitors (SSRI) are used to treat binge-eating disorder and insomnia [72]. Controversially, its injection into the brain had biphasic effects with initial waking promoting and then sleep. More studies have shown that SSRI reduced REM sleep, regardless of its anti-depression effect. In addition, another neurotransmitter in the CNS-dopamine, is widely distributed as well. Latest research group has exhibited dopamine signaling in the basolateral amygdala initiates rapid eye movement [73]. This mechanism may explain how mood affect sleep, particularly deep sleep or dream [74]. Dopamine system has been discussed in hedonic behaviors intra- and extra-hypothalamus and has control of hypothalamic information output [75, 76]. Despite that dopamine pathway correlated with sleep and hypothalamus related innate behaviors, dopamine has been discussed more in non-feeding related behaviors and or hedonic feeding through VTA dopamine neurons [77].

In conclusion, progress can be slow and challenges remain hard at present. Despite significant advancement in neuroscience over the last two decades, discoveries made have not shred sufficient light on the brain architecture as well as its underlying mechanisms related to feeding, sleeping and their corresponding disorders. However, multidisciplinary research and initiative seem to lead to an optimistic outlook for the future. We can envision-due to the modern system neuroscience progress, from anatomical and functional circuit mapping, activity-dependent transcriptional characterization, bulk or single-cell level of calcium imaging as well as cell manipulation by chemogenetics/optogenetics combining with sleep *in vivo* recording, recording – a greater comprehension of the impact of sleep

and metabolism on each other and, possibly, uncover therapeutic interventions against insomnia, obesity and their related disorders.

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