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REVIEW

# Neonatal development and central appetite regulation

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**Abstract:** Appetite serves to regulate adequate energy intake to maintain metabolic needs. It is regulated by a close interplay between the digestive tract, adipose tissue and the brain. The role of hypothalamus, as part of the brain, in preserving energy homeostasis should be stressed. The hypothalamus can be subdivided into nuclei consisting of collections of neurones with discrete functions (e.g. arcuate nucleus, known as the infundibular nucleus in humans, paraventricular nucleus, ventromedial nucleus, dorsomedial hypothalamic nucleus, lateral hypothalamic area, etc.). Neuronal projections between these nuclei, as well as to and from other areas in the brain, enable the hypothalamus to integrate signals from the brain, the peripheral circulation and the gastrointestinal tract. What are these signaling substances and when do they appear? How and when do these projections develop? This review focuses on development of brain mechanisms regulating appetite in neonates, mainly rats and mice.

**Key words:** neonatal development, food intake regulation, energy homeostasis, stimulatory factors, food intake, inhibitory factors

**Abbreviations:** AgRP – agouti gene–related protein; ARH – arcuate nucleus; CART – cocaine- and amphetamine-regulated transcript; CRH – corticotropin-releasing hormone; DBH – dopamine β hydroksylase; DMH – dorsomedial hypothalamic nucleus; GABA – γ-aminobutyric acid; GAL – galanin; GALP – galanin-like peptide; GHRH – growth hormone-releasing hormone; GIP – gastric inhibitory peptide; GLP-1 – glukagon-like peptide 1; GLP-2 – glukagon-like peptide 2; GRP – gastrin-releasing peptide; i.c.v. – intracerebroventricularly; LC – locus coeruleus; LHA – lateral hypothalamic area; MC – melanocortin; MCH – melanin-concentrating hormone; NHP – non-human primates; NPY – neuropeptide Y; NTS – nucleus of the solitary tract; OT – oxytocin; PFR – perifornical region; PVN – paraventricular nucleus; TRH – thyrotropin-releasing hormone; vLGN – ventral lateral geniculate nucleus; VMN – ventromedial hypothalamus nucleus; ZI – zona incerta; αMSH – α-melanocyte-stimulating-hormone

## INTRODUCTION

The traditional division into hunger and satiety centres in the brain was first created in the 1940s, based on electrophysiological observations and lesions of various hypothalamic structures [1, 2]. The "hunger centre" was claimed to be localized to the lateral hypothalamic area (LHA) because its stimulation resulted in increased food intake. On the contrary, lesion in this region was responsible for decreased food intake and weight loss [3, 4]. The "satiety centre" appeared to be the ventromedial hypothalamus nucleus (VMN), which was shown to be activated by nervous, hormonal and metabolic inputs from the gastrointestinal tract after eating a meal. Lesions in this region caused increased food intake and weight gain [5]. Today, we know that appetite regulation is much more complex and that other regions in the hypothalamus, brain stem and signals from the periphery are involved in feeding behavior.

To make evident the entire complexity of the food intake phenomenon, it is sufficient to mention that only if it comes to signaling substances, more than 50 various factors were discovered that stimulate or inhibit food intake, including neuropeptide Y (NPY), agouti gene–related protein (AgRP), orexins, galanin (GAL), noradrenaline, serotonin, ghrelin, cocaine- and amphetamine-regulated transcript (CART), oxytocin, neurotensin, leptin, insulin, cytokines, etc. to name but a few [6-9] (Table 1). Some of these factors, e.g. ghrelin or leptin, have an influence on the gastrointestinal tract development. The function of other neuropepeptides is not confined soley to central regulation since many other orexigenic and anorectic neuropeptides found in the hypothalamus, including orexins, NPY, GAL and CART, have neurons and/or epithelial endocrine cells in the gut [10].

The aim of this review is to highlight the developmental aspect of factors impacting the appetite. The data concerning feeding regulatory pathways in the neonatal development are derived mainly from research on rodents: rats or mice. Taking into account species differences, availability of material and possible research application to human, the question arises whether pigs may be a better animal model. Since, to date, little is known about central appetite regulation in pigs, this warrants starting investigation in this field.

**Development of hypothalamic projections important for appetite regulation.** Among several nuclei in the

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**Table 1** [7, 9, 83, 84]

| Factors stimulating food intake | | Factors inhibiting food intake | |
|---|---|---|---|
| central | peripheral | central | peripheral |
| AgRP | ghrelin | α-MSH | adiponectin |
| β-endorphin | | CART | amylin |
| dynorphin | | CRH | cholecystokinin |
| GABA | | dopamine (in hypothalamus) | cytokines (some) |
| GAL | | GALP | enterostatin |
| GHRH | | GRP | estrogens |
| MCH | | GLP-1 | GIP |
| noradrenaline | | nesfatin | GLP-2 |
| NPY | | neuromedin B and U | insulin |
| orexin A and B | | neuropeptide B and W | leptin |
| | | neurotensin | obestatin (?) |
| | | oxytocin | peptide YY |
| | | prolactin | |
| | | serotonin | |
| | | somatostatin | |
| | | TRH | |
| | | urokortins | |

hypothalamus, the arcuate nucleus (ARH) seems to play a pivotal role in the central regulation of food intake and for the integration of peripheral signals. The ARH is an "arc-shaped" group of neuronal cell bodies localized around the base of the third ventricle, just above the median eminence (Fig. 1). In the adult brain, ARH possesses dense reciprocal connections with other hypothalamic areas, including the paraventricular nucleus (PVN), the dorsomedial hypothalamic nucleus (DMH), VMN and LHA [11]. The ARH also shares connections with the subfornical organ and the vascular organ of the lamina terminalis [12]. Neurons in ARH that project into PVN form a particular orexigenic pathway, which is considered a key for the regulation of food intake in order to meet the energy demands of day-to-day activity [13]. The PVN is also an integrating centre on which converge many neural pathways that influence energy homeostasis [11].

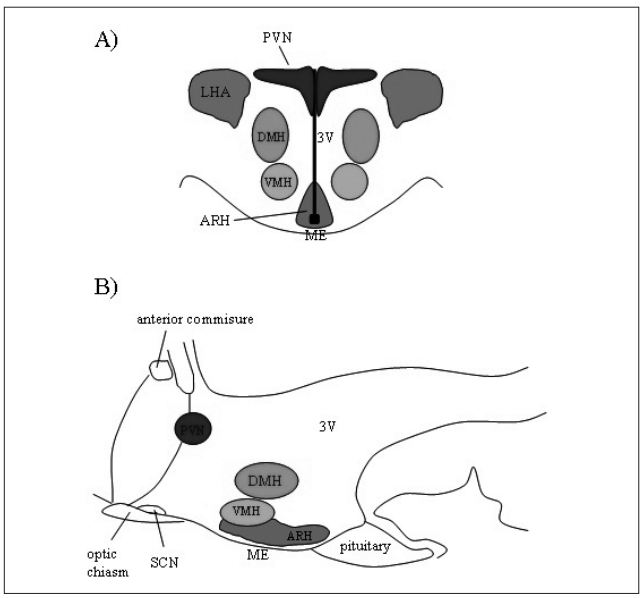


**Figure 1** Schematic drawings of the rat hypothalamus. A – coronal view, B – saggital view. ARH – arcuate nucleus, DMH– dorsomedial nucleus, LHA – lateral hypothalamic area, ME – median eminence, PVN – paraventricular nucleus, SCN – suprachiasmatic nucleus, 3V – third ventricle.

It seems that the adult pattern of ARH projections is not fully developed early in the life of rodents. Using DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) implants to visualized axons in the hypothalamus, Bouret et al. [14] have proposed the developmental pattern of ARH projections in mice and proved that these pathways develop entirely postnatally. At the earliest, projections develop from ARH to DMH, as early as at P6. Then, the axons from ARH reach the PVH (at P10) and the LHA (at P12). The mature pattern of projections from ARH to the medial preoptic nucleus is observed by P14. The projections from ARH to the anteroventral periventricular nucleus achieve their mature distribution by P18, the time point when the pattern of projections from ARH resembles that of an adult.

In adults, ARH is a region where receptors for leptin are highly expressed [15]. Contrary to adults, in the neonatal rodent, leptin is not effective in inhibiting food intake and does not downregulate hypothalamic NPY mRNA. Neither intraperitoneal nor central leptin injection inhibits growth, acute ingestion, or energy expenditure during the first 2-3 postnatal weeks [16-18]. Lack of sensitivity of leptin early after birth may be due to immaturity of the ARH circuits. Here is worth mentioning that a surge of leptin occurring in the early postnatal period [19] appears to be critical for establishing the ARH circuits [20]. The development of leptin-induced immunoreactivity in the nuclei in the hypothalamus coincides with the development of innervation of these nuclei by the ARH axons. It was shown that peripheral leptin injection induces cFos– immunoreactivity (marker for neural activity) in ARH as early as at P6, but in PVN not before P10, and in LHA not before P16 [14].

**NPY system.** NPY is a 36-amino-acid member of the pancreatic polypeptide family synthesized in the adult rodent, mainly in ARH, with efferent projections to several hypothalamic regions, including PVN [21]. NPY acts as a powerful appetite stimulant [22]. Moreover, NPY is implicated in several regulatory functions, including memory [23], regulation of blood pressure [24] and changes in hormonal release [25]. In the rat brain, NPY acts through Y1-Y6 receptors [26]; however, there is evidence indicating the existence of more subtypes [27]. It was shown that Y1 and Y5 receptors are primarily involved in NPY-induced feeding [28, 29].

**Development of NPY neurons.** The early postnatal period is a time of dynamic change in the hypothalamic NPY system. In the adult rodent, NPY neurons are present throughout the brain, but the major site of NPY production in the hypothalamus is ARH [21]. In contrast, in the newborn rodent, NPY mRNA and NPY proteins are also transiently expressed in several hypothalamic regions, including DMH, the perifornical region (PFR), PVH and LHA [30-32]. NPY expression in ARH is relatively abundant throughout the postnatal period. In all other regions, NPY expression is low to undetectable early in the postnatal period, rapidly increases to a peak around P15-16, and subsequently declines to adult levels by P30 [30]. The physiological significance of the presence of NPY neurons in these different hypothalamic regions early in life, as well as the significance of the peak expression of NPY mRNA, is unknown. However, it has been speculated that the increase in NPY may reflect changes in dietary needs prior to weaning. Around this age (P15-16), the initiation of independent ingestion of solid food occurs in rats. Moreover,

the same authors hypothesized that these changes in NPY system may be due to the development of ARH-α-melanocyte-stimulating-hormone (αMSH) projections [33].

**Development of NPY fibres.** NPY-immunoreactive fibres are present throughout the hypothalamus as early as at birth in rat [34, 35]. The question arises about the source of single-labeled NPY fibres since they do not appear to originate only from ARH, as indicated by the lack of AgRP colocalization. AgRP mRNA and protein expression is present exclusively in cell bodies in ARH throughout postnatal development starting at P2, and is colocalized in the vast majority of ARH-NPY neurons [36, 37]. This co-localization of AgRP and ARH-NPY neurons makes it a good marker for these neurons and neuronal fibre projections. Postnatal ontogeny of ARH-NPY fibres into PVN and DMH were determined in rats by Grove et al. [36]. Although NPY fibers are abundant in the DMH and PVH as early as P2, ARH-NPY-AgRP labeling is not present in these regions until P5-6 and P10-11, respectively. Similar observation were noted in mice; numerous NPY-immunoreactive fibres were detected in PVN at P0, but only some of which were AgRP-positive [37]. A portion of NPY fibres within PVN and ARH at P2 originate from the brainstem, as indicated by their co-localization with dopamine β hydroksylase (DBH). According to the authors' suggestion, the rest of NPY fibres could represent brainstem projections, anyway, i.e. projections that do not express DBH. The source of single-labeled NPY fibres could be the neurons within the hypothalamus that transiently express NPY during the postnatal period. Another source of NPY neurons could be the geniculate nucleus as its projections are known to innervate the hypothalamus [38, 39]. However, one has to be careful and state that their postnatal development is not known. Another source of NPY fibres within PVN could be the noradrenergic neurons of the medulla oblongata [40, 41].

As far DMH is concerned, the first projections from ARH to DMH in rats seem to start developing at P5-6, where a relatively low concentration of NPY/AgRP-immunoreactive fibres is present within dorsal part of DMH. These projections are almost fully developed at postnatal days 10-11, although there are still numerous single-labeled NPY-immunoreactive fibres [36]. It should be noted that ARH-DMH projections develop earlier than ARH-PVN projections, the reason for which could be simply the longer distance that ARH projections have to travel.

**Development of Y1 receptors.** Both Y1-immunoreactive neurons and Y1-immunoreactive fibres are abundant in the hypothalamus, including ARH, DMH, PVH, PFR, LHA and the preoptic area in the adult rat [42, 43, 44]. Several data demonstrate that the Y receptors system is functional early after birth. Non-direct rationale originates from the work of Capuano et al. [45] work, who have shown that micro-injection of NPY into PVN at P2 can elicit an increase in milk intake. This suggests that NPY receptors involved in the regulation of food intake are present and functional in rats at this time. Indeed, other findings have also confirmed this statement. In newborn rats, light Y1-immunoreactivity staining is present in neuronal cell bodies within ARH, DMH and PVH as early as P2 [36]. However, at this time, the pattern of immunoreactivity is immature. The staining intensity steadily increases at P5-6 and P10-11, peaking around P15-16 in all of these regions. It appears that in the latter time point the pattern of Y1-immunoreactivity matched the adult level. These data are in agreement with the experiment of Tong et al. [46], where a low level of NPY Y1 receptor mRNA and a low level of receptor binding in the rat hypothalamus at birth was found. As determined by *in situ* hybridization NPY Y1 receptor mRNA displays the adult distribution by the end of the 3rd postnatal week. Similarly, receptor binding constantly increases during brain maturation, and the plateau is reached also by the end of 3rd postnatal week. The above data indicate that although Y receptors system is immature at birth, it is sufficient to mediate NPY signal.

The ontogeny of other NPY receptors has not been reported until now.

**Melanocortin system.** The main populations of melanocortin neurons, i.e. POMC and AgRP neurons, are located within ARH. The second region, where POMC is synthesized, is the nucleus of the solitary tract (NTS). POMC is a precursor of peptides, of which αMSH is considered to be the most important regulator of feeding. The αMSH or POMC neurons are located predominantly in the ventro-lateral portion of the nucleus. αMSH induces an anorexic response (satiety signal) through the actions on MC receptors. αMSH stimulates specifically MC3 and MC4 receptors [47]. Additionally, AgRP probably mediates its reverse agonist effects on MC4 receptors, thus inhibiting αMSH binding [48]. Therefore, the physiological outcome depends on the balance between αMSH and AgRP release.

The early postnatal period in rat is a time of change in the melanocortin system, although many of its components are already present at birth, including POMC, AgRP and melanocortin (MC)-4 receptor mRNA [36, 49, 50]. In pigs, the signal for POMC mRNA is present as early as in the foetus [51]. In the anterior lobe, it progressively increases from E30 to E80, and then remains at a relatively constant level. In contrast, POMC transcripts in the intermediate lobe first appear at E40, and steadily increase during development [51].

αMSH-immunoreactive neurons are evident in ARH as early as at P2 in rats (unpublished observation) [36]. αMSH-immunoreactive fibres, likely originating from the brainstem, are present at PVN and also at P2 [36].

There are two endogenous antagonists of MC receptors: agouti peptide and AgRP. A short history about their discovery: initiallyt, Miller et al. [52] cloned *agouti* gene and identified a protein (agouti) that functions as an antagonist of cutaneous MC1 receptors, and normally is expressed by hair follicles. Reduced MC1 signaling by agouti protein inhibits production of black melanin, thus causing lightening of the coat colour. Agouti mice (with an autosomal dominant mutation within a promoter region of the *agouti* gene) express agouti in tissues throughout the body, including the hypothalamus. They develop both a yellow coat colour and obesity. Subsequently, Shutter et al. [53] cloned *agrp* gene and identified a peptide – AgRP, with homology to *agouti,* that is an antagonist of MC3 and MC4 receptors [54]. AgRP was found to be an orexigenic signaling molecule which causes hyperphagia when administered intracerebroventricularly (i.c.v.). What is unusual is that the increase of food intake following a single i.c.v. injection of AgRP lasts for up to a week [55].

The development of the arcuate AgRP system has been studied both in rats and mice, and has shown that it is not mature until the third postnatal week [36, 56, 37]. Thus, at P0, only very low AgRP mRNA levels are present in ARH,

and only a few AgRP fibres can be detected in hypothalamic (e.g. PVN, LHA, DMN) and extrahypothalamic (amygdala, the paraventricular nucleus of the thalamus, the dorsal raphe, parabrachial nuclei) target regions [37]. The number and intensity of AgRP-immunoreactive fibres, as well as AgRP mRNA signal increases gradually with age, and reaches adult levels between P15 and P21, with regional variations. Adult pattern of AgRP innervation is first achieved in the DMN and LHA at P15. In more rostral hypothalamic regions, like PVN, the medial preoptic area and the bed nucleus of the stria terminalis (BNST), the adult pattern of AgRP innervation is registered later, at P21. In general, the pattern of AgRP system maturation is in agreement with the DiI tracing study of ARH neurons [14].

**Melanin-concentrating hormone (MCH) and orexins systems.** Evidence indicates that both MCH and orexins may be involved in the regulation of feeding behaviour. These neuromodulators are localized in separate populations of neurons in LHA. MCH is a cyclic orexigenic hypothalamic peptide originally isolated from the pituitary gland of teleost fish where it controls skin pigmentation [57]. In rats, MCH is found in neurons of the zona incerta (ZI) and LHA, with a few MCH-expressing cells found in the olfactory tubercle and pontine tegmentum [58, 59]. The orexins are two related peptides derived from a single prepropeptide. Orexins A and B are expressed solely in neurons within the perifornical LHA and ZI [60, 61]. Locus coeruleus (LC) is a major target of orexins innervation. Orexins, as their name suggests, are thought to be orexigenic. It has been shown that they increase feed intake in rats [62, 61], pigs [65] and sheep [64]. Orexins knockout mice are of normal weight; however, they may be hypophagic [65]. Data concerning MCH are incnsistant since central injections of MCH acutely increase [66, 67] or decrease [68] food intake. However, MCH knockout mice are lean and hypophagic [69], and fasting increases MCH gene expression [68, 66].

**Development of orexins system.** In animal species born in a very immature state (e.g. the eastern grey kangaroo) the presence of orexin A and B in the hypothalamic nuclei develops postnatally [70]. In rodents, the orexin system begins to develop during late embryogenesis and matures in the postnatal period. An *in situ* hybridization study has identified orexins mRNA at E18 [71]. However, orexins-immunoreactivity in cell bodies is similar to that observed in adult animals, and does not have extensive dendritic branches until P16. Orexin-immunoreactive fibres have been found in hypothalamus at E20; however, the density of innervation in LC progressively increased throughout the early postnatal period, and peak levels not reached in that region until P21. Then, the innervation remains at the same level into adulthood. On the other hand, in precocial species (e.g. sheep or human), the brain largely develops *in utero*, and this may be the reason that orexin-positive fibres were observed throughout the hypothalamus, both in the foetus and in newborns [10]. There are some regional variations about the developmental pattern of orexin-A fibres density in lambs. In some of the hypothalamic nuclei, such as PVN and LHA, the density of orexin-A fibres does not change between the foetal stage and the 15-day-old lamb. However, for the VMH, a striking increase in the density of orexin-A positive fibres has been reported in such lambs compared to foetuses [10]. The developmental pattern of orexin-B remains to be elucidated.

**Development of MCH system.** In the rat, MCH neurons are born in the embryonic phase between E10 and E16, with peak genesis at E12 [72]. Morphology of MCH-immunoreactive neurons alters with time in neonates: the cell body reaches adult size by P10, but density of the dendritic tree is attained not earlier than at P21, which is similar to that observed in the adult. A major target for MCH innervation is the diagonal band of Broca (DBB). MCH-immunoreactive axon density in that region progressively increases throughout the early postnatal period, reaches peak density at P21, and then declines by about approximately 25% to adult levels [71]. Development of MCH system is also characterized by particular fleeting expressions. Transient MCH-immunoreactive cells were identified in the ventral lateral geniculate nucleus (vLGN), lateral ZI, PVN and in the lateral septum. The first appearance of MCH-immunoreactivity in the ZI and vLGN is at E16, and the disappearance of this immunoreactivity, takes place gradually until P21 or P31. The first appearance of MCH-immunoreactivity in PVN is later, between E20 and P2, and this immunoreactivity is virtually absent by P16 [71].

**Development of GAL system.** GAL, a 29-amino acid peptide, is extensively distributed in both brain and intestine, and is involved in numerous functions including the control of feeding, growth and reproduction. GAL stimulates food intake in normal rats when injected into different hypothalamic areas involved in feeding, such as PVH, VMN and LHA [73].

In the Meishan pig – a Chinese breed known for its superior reproductive characteristics, but slow growth rate and abundant adipose tissue – GAL-like immunoreactivity in cell bodies and fibres is first evident in the brain at gestational day 30, primarily in the hypothalamus [74]. During postnatal development, the number of cell bodies displaying GAL-immunoreactivity decreases comparing with the prenatal period, again particularly in hypothalamic areas. The distribution pattern of GAL-immunoreactivity fibres becomes mature by P1, after which it continues unchanged during later postnatal ages. Some additional increases in GAL-immunoreactivity occur postnatally, especially in the periventricular hypothalamus [74]. GAL-immunoreactivity is first detectable in the rat brain by day 15 of gestation [75]. Concentrations of GAL-immunoreactivity increase after birth in the hypothalamus. Kawagoe et al. [76] have reported that GAL mRNA is first detected in ARH at P8. GAL mRNA gradually increases between P8 and P14, and then again markedly increases between P14 and P40, which are the weaning and pubertal periods in rats. After P40, there were no significant differences in GAL mRNA level.

**Hypothalamic – dorsal vagal complex (DVC) projections.** In considering the central control of ingestive behaviour, one cannot fail to take into account of the role of the hindbrain DVC, where modulating of hypothalamic neural signaling occurs. DVC comprises the dorsal motor nucleus of the vagus, NTS, and area postrema. Neurons within the DVC receive direct input from neurons in hypothalamic regions involved in appetite regulation, such as PVN, LHA, DMH and ARH [77]. However, compared with NPY or AgRP systems in ARH, relatively less in known about the ontogeny of hypothalamus inputs to the DVC.

In rats, projections from the DVC to the hypothalamus develop prior to those from ARH, and are already present at birth. However, some data show chemical immaturity of these projections until the 3rd postnatal week. Although the number of brain stem neurons projecting to PVN is similar between newborn and adults, the catecholaminergic fibres undergo significant changes [78]. Thus, the density of DBH-immunoreactive fibres innervating PVN is relatively low at birth, but thereafter gradually increases until P21, when it reaches the adult level. The opposite direction of fibres density maturation has been reported for phenyletanolamine *N*-methyl-transferase projections. The density of these projections is relatively high in PVN in the newborn rats, but then gradually decreases to reach the adult level by P21.

Another example of chemical immaturity can be oxytocin (OT)-immunoreactive reciprocal descending projections from PVN to the brain stem [79]. OT acts centrally to inhibit food intake in adult rats [80]. The PVN is the only source of OT-immunopositive fibres and terminals in the DVC, providing an anatomical marker for this projection pathway [81]. OT-immunoreactivity increases from birth until P20, when they reach the adult level. More detailed information about central inputs to the DVC can be found in the reviews by Rinaman [81, 77].

Taken together, it seems that both projections from the DVC to the hypothalamus and reciprocal projections from the hypothalamus to the brain stem are largely formed at birth in rats; however, they continue to mature during the first weeks of life.

**Importance of species differences.** Investigations concerning feeding regulatory pathways in the neonatal development were conducted in majority on rodents. Although many of the same neuropeptide and hormonal signals were reported to be important both in rodents and primates (e.g. NPY, AgRP, αMSH, leptin), development of the central neural circuitry involved in the regulation of feeding differs among species. In the rodent hypothalamus, NPY or AgRP efferent projections do not completely develop until the 2nd or 3rd postnatal week, whereas in non-human primates (NHP) these projections to the PVH appear to be nearly completely developed at birth [82]; only their density is less than that observed in the adult. NPY system is much more complex in NHP than in rodents. While in adult rodents, the majority of NPY neurons are localized in ARH, in NHP there are several populations of NPY containing neurons including ARH, the supraoptic nucleus and the PVH [83]. Species differences also encapsulate brainstem projections. Data indicate that in NHP, the NPY inputs into the PVH may originate from sources other than ARH, more so than in the case of rodents. This means that the concentration of NPY/AgRP fibres originating from ARH to NPY fibres originating from other sources, such as the brainstem, is low in NHP compared with that observed in the rat [82, 36]. These and other differences, especially the temporal pattern of projection development, may have important consequences. It is likely that in primates, the factors operating in the prenatal period may be more significant for the correct establishment of hypothalamic feeding circuits, whereas in rodents the postnatal cues could be more crucial.

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