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Analysis of the network of feeding neuroregulators using the Allen Brain Atlas

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Abstract

The Allen Brain Atlas, the most comprehensive in situ hybridization database, covers over 21000 genes expressed in the mouse brain. Here we discuss the feasibility to utilize the ABA in research pertaining to the central regulation of feeding and we define advantages and vulnerabilities associated with the use of the atlas as a guidance tool. We searched for 57 feeding-related genes in the ABA, and of those 42 display distribution consistent with that described in previous reports. Detailed analyses of these 42 genes in the nucleus accumbens, ventral tegmental area, nucleus of the solitary tract, lateral hypothalamus, arcuate, paraventricular, ventromedial and dorsomedial nuclei suggests that molecules involved in feeding stimulation and termination are coexpressed in multiple consumption-related sites. Gene systems linked to energy needs, reward or satiation display a remarkably high level of overlap. This conclusion calls into question the classical concept of brain sites viewed as independent hunger or reward “centers” and favors the theory of a widespread feeding network comprising multiple neuroregulators affecting numerous aspects of consumption.

Keywords

CNS; food intake; obesity; anorexia; neuropeptides

The Allen Brain Atlas: Brief Overview

The human brain is undoubtedly the organ of the body that we have the least knowledge about in relationship to its complexity. It has been estimated that 99% of the neuroscience literature focuses on only 1% of the genes expressed in the brain (Gewin, 2005). Deciphering spatial gene expression patterns in the central nervous system is crucial for our understanding of functional neuronal networks. It has been speculated that “in the brain, more than any other organ, function follows form” (Gewin, 2005).

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There are now three large projects that have performed large-scale gene expression mapping of the brain. One such project conducted by the National Institutes of Health (NIH) is aimed at establishing a resource of targeted knockouts of brain-specific mouse genes or the Gene Expression Nervous System Atlas (GENSAT). This project provides in situ expression patterns in both several developmental stages as well as in the adult mouse brain [<http://www.gensat.org> and (Gong et al., 2003)]. Moreover, it also contains expression patterns from transgenic mouse lines that express green fluorescent protein (EGFP) reporter gene in cells that normally express a gene of interest. The GENSAT provides comprehensive information about the limited number of the mapped genes. Another project is the GenePaint atlas (www.genepaint.org), which displays in situ hybridization data of several thousand genes in selected sections of whole mouse embryos at embryonic day (E) 14.5 and as well as some genes in E10.5 embryos, E15.5 head, and in the P7 and adult brain (Visel et al., 2004). However, the largest project considering the number of genes is the Allen Brain Atlas (www.brain-map.org). The Allen Brain Institute was established in 2003 by one of the founders of Microsoft, Paul Allen, and aims to display expression of all known genes in the adult mouse brain. The first 2000 genes were uploaded in 2004 and the Atlas has continuously expanded since. The ABA uses high-throughput, semi-automated in situ hybridization methods developed by Eichele and colleagues, who were also behind the GenePaint project (Visel et al., 2004). The main publication of the ABA came out at the beginning of 2007 (Lein et al., 2007) and until today the website has compiled a database of expression of over 21,000 genes present in the adult mouse brain. Importantly, the in situ hybridization methodology is well standardized with quality control verification. The results are combined into an interactive map capable of presenting simultaneously the distribution of any number of genes of interest.

The ABA combines a web user interface with the Brain Explorer, a three-dimensional viewing program. The gene expression patterns are represented as in situ hybridization images, three-dimensional images and graphs. The data are anatomically correlated, thus, allowing easy identification of brain regions where each gene is expressed. Noteworthy, the Neuroblast function enables the user to search for genes with similar distribution throughout the brain or within particular central sites. The atlas works much like a standard search engine. The basic result list includes the gene name, gene info/abbreviated name, processing status (i.e. availability of quality control-passed data) and links to various databases. Images of coronal and sagittal sections are available. Each image series is linked to information about the mouse strain, the age and sex of animals and the number of images generated per series. Importantly for further studies, probe information is also available, showing the sequence of the probe, its length, orientation, GC content, type and the forward and backward primers.

Since the ABA presents data in a form of a three-dimensional digital brain map, for the ease of use, the results were converted into a multicolor scale based on fundamental interpretative expression parameters: expression level and density. The expression level reflects a classical approach to examining transcription intensity. It is calculated as the average pixel expressor intensity in a given site multiplied by the area of expression within this site and divided by the maximum possible area of expression of ubiquitous genes present therein. On the other hand, expression density is defined as the number of expressors divided by the total number of cells expressing ubiquitous genes in a particular site. These two parameters prove to be of profound importance as they enable the audience to analyze digital images without the necessity to study in detail actual hybridization photomicrographs.

The ABA project appears to be of paramount significance for those wishing to relate the distribution of a given gene with its potential function in the brain circuitry and, hence, its role in physiological and behavioral phenomena. In the current paper, we discuss the feasibility to utilize the ABA in research pertaining to the central regulation of food intake. Our goal is to define advantages as well as to pinpoint possible vulnerabilities associated with the use of this

atlas as a guidance tool. We focus on the structures and genes already known to be involved in food intake control and interpret data presented in the ABA based on evidence supplied by the current literature. Finally, we aimed to utilize the rich and standardized ABA data to characterize neural circuitry that governs consumption.

Selection and interpretation of ABA data

We first selected a set of eight sites whose involvement in feeding control has been established beyond any reasonable doubt (Kelley, 2004; Levine and Billington, 1997; Petrovich and Gallagher, 2007). Areas classically thought of as governing energy- and reward-driven consummatory behavior were chosen (Levine and Billington, 1997). These regions include the nucleus accumbens (NAcb; a complete list of abbreviations included), ventral tegmental area (VTA), arcuate nucleus (ARC), lateral hypothalamic area (LHA; referred to as the lateral hypothalamic zone in the Brain Explorer), hypothalamic paraventricular nucleus (PVN), ventromedial nucleus (VMH), dorsomedial (DMH) nucleus and nucleus of the solitary tract (NTS).

We selected out 57 feeding-related genes and these are displayed in Tables 1 and 2. The genes were then searched for in the ABA, to see if the in situ hybridization process had been successfully performed and passed quality control verification. For all those genes, data files were downloaded, either directly from the Brain Explorer viewing program or from the Allen Brain Atlas.

Three-dimensional images and corresponding photomicrographs were analyzed visually and subjectively using the Brain Explorer by two scorers. The view was set so that all levels of expressions were visible using the Brain Explorer's threshold controls, which filters the minimum and maximum viewable expression levels. Multiple expressors were viewed simultaneously in order to study co-expression within each site.

First, apparent expression artifacts were identified, and the major ones were noted in Tables 1 and 2. In three-dimensional images, these artifacts were typically visible as round concentric rings of high gene expression or as illogically long strings of such. Artifacts were generally easily identifiable by analyzing the original in situ hybridization photomicrographs at a high magnification level. If no apparent artifact was detected, but suspicion still remained, an additional examining step was undertaken. The adjacent images in the image series were analyzed in the ABA, to see if any artifacts from the adjacent sections could have been a source of the problem for the automated computerized analysis system.

The entire brain expression pattern of all the investigated genes was also analyzed to search for any abnormalities, such as unusually low overall expression. When several three dimensional expression image series were available for one gene, a comparison was made in order to select the one with the lowest number of artifacts and most consistent expression distribution for further analysis. A preference was given to three-dimensional images produced from coronal image series which most often contained a higher number of original in situ hybridization images, which ensured a greater accuracy of expression analysis. In addition, even though only the left hemisphere was analyzed, coronal in situ hybridization images showed a full image of both hemispheres. Therefore, we confirmed visually that the distribution was bilaterally uniform.

Following exclusion of artifacts, gene expression patterns in the selected regions were analyzed and recorded using a system for recording both expression levels and densities. Gene expression levels are represented in the Brain Explorer by a complex multicolor scale with colors ranging from dark blue for the lowest level to red for the highest level of expression. We simplified the scale to enhance the overall readability of the data and enable better

comparison to previous literature. Therefore, the colors from the Brain Explorer's scale were converted into three main levels of expression, represented as a combination of plus signs. Thus, the "+" sign corresponds to low expression, which in the Brain Explorer is shown as a dark blue- to light blue-coded expression point. The "++" sign corresponds to the medium level of expression, which in the viewing program was shown as green to yellow expression points. The "+++" sign, corresponding to the high levels of expression, was represented as orange through red in the Brain Explorer.

When expression within a given site was predominantly uniform, i.e., low, medium or high, the appropriate sign was used. However, if expression throughout a brain region exhibited variability, a combination of the signs was then assigned: e.g. "++/+++" was used when a similar number of expression points represented medium and high expression levels. Finally, when no expression was found, this was noted with "0".

The expression density patterns were also recorded using a similar system consisting of signs. The lowest expression density sign, "0", was used when expression was very limited when compared to the entire three-dimensional space of the brain area. The "+" sign was used when expression was sparse. The "++" sign indicated expression occurring in most parts of the area. Finally, "+++" represented the highest expression density level throughout the entire area. A combination of these signs was used when the density was determined as intermediate between one of these four pre-defined levels.

Overall, we performed the analysis of 57 genes involved in stimulation or inhibition of consummatory behavior. The results are summarized in Tables 1 and 2. To visualize the distribution of feeding-related genes within the particular sites of the brain circuitry, we prepared the illustrations (Figs. 1 and 2) where our symbols for expression density and level were converted into the system utilizing circles of different diameters and colors. The advantage of such graphic representation is that it merges the two major expression parameters used in the atlas, density and level, to better define the topography and characteristics of the central network based on the presence of its molecular components. To visualize each gene's expression level in the various pertinent brain regions, the circles were color-coded, using colors sampled from the Brain Explorer program. The genes are shown in two different illustrations, each presenting genes which encode for peptides and receptors that participate in the process of termination or stimulation of food intake.

A subjective view on ABA images

As mentioned before, a particularly valuable function of the Brain Explorer is the possibility to study three-dimensional representation of expression patterns of several genes simultaneously. This serves as a very useful tool to define wider pathways and networks involved in a given mechanism, such as feeding regulation, where multiple neuroactive factors play a role. The two main parameters used to characterize the presence of these genes, namely expression density and level, provide adequate information that allows one to make a preliminary judgment on whether certain genes that show both a relatively high level/density of expression and, importantly, co-expression within a given site may be of similar functional significance.

It seems that the ability to properly assess gene expression patterns in the Brain Explorer requires the use of both digitized images and photographs of corresponding sections. A failure to perform these two tasks may sometimes cause interpretation problems. The first reason to employ this dual approach stems from the presence of artifacts occasionally generated in the in situ hybridization process, though they can be easily identified visually and excluded from the analysis. The second issue is the assessment of distribution of cells that synthesize a given gene product: while digitized three-dimensional images provide visualization of expressor

points along with the excellent indication of expression level and density, the conclusions can be further enriched by histological analysis. For instance, the expression levels and densities for proopiomelanocortin (POMC) and Agouti-related protein (AgRP) genes in the ARC are the same based on the Brain Explorer-derived data (Table 1), yet a close examination of actual photomicrographs depicting ARC-containing sections reveals a different topography of the two respective neural systems.

In addition, relying solely on the digitized three-dimensional map may produce false-negative conclusions. A distribution of the glucagon gene, which gives rise to several molecules including satiety-related glucagon-like peptide (GLP)-1 and GLP-2, can provide an example of such possibility. As noted in Table 2, both expression level and density for the glucagon gene in the NTS, the major brainstem “relay” station for periphery-brain communication, appear negligible. However, the NTS is known to host neurons synthesizing GLP-1 and GLP-2 (Tang-Christensen et al., 2001). Although their number is relatively low, these cells project to the hypothalamus and act as the main source of intra-PVN GLP. GLP released in the PVN plays a key role in supporting termination of consumption not only due to satiety, but also due to other factors, including feeding-induced rise in plasma osmolality and ingestion of toxic substances (Tang-Christensen et al., 2001; Vrang et al., 2007). Therefore the NTS component of the glucagon gene family is crucial in mechanisms regulating food intake, hence the apparent lack of this gene’s expression in the NTS, according to Brain Explorer digital images, was surprising. However, the analysis of the actual sections that had undergone the in situ hybridization procedure revealed that a small population of cells expressing the glucagon gene indeed exists in that brainstem site.

Based on the above, we strongly believe that Brain Explorer’s digitized data should be verified against section images at least whenever suspicion arises as to whether some information was lost in the process of converting results into a computerized map or to control for possible artifacts. The ABA offers a tool of correlating the digital interpretation of gene expression with the database of tissue slices that served as the matrix for the expression map, because each expressor point is linked with an appropriate photomicrograph. This feature offers an additional advantage: a complete set of in situ hybridization images allows the reader to compare the results with data previously reported by other authors. Unlike classical publications which typically provide only selected images, this extensive project makes high-resolution photographs of all sections available for visual inspection, therefore additional analyses of, e.g., minute subdivisions of particular central sites, can also be performed.

As the ABA is an extremely vast undertaking and, to a large extent, it relies on an automated process, quality control mechanisms are of particular importance. Several genes of interest have not been successfully marked due to the probe failure. These include the growth hormone secretagogue (GHS) receptor ligand, ghrelin, that has been shown to act as one of the most potent orexigens known to date. Ghrelin mRNA has been detected in the brains of both mice and rats (Ghelardoni et al., 2006; Zigman et al., 2006). Zigman et al performed a thorough analysis of the ghrelin mRNA distribution in the central nervous system of mice and found that its particularly high levels are present in the ARC, but also in the PVN and in the ventrolateral and capsule portions of the VMH (Zigman et al., 2006). Certainly, the inclusion of ghrelin system’s description in the atlas would aid in understanding the topography of orexigens in the brain. However, one should note that the ABA is a dynamic and ongoing project, hence, data included therein will likely be updated and enriched.

Another missing piece of data is related to oxytocin, which is involved in termination of food intake due to a plethora of reasons, including stomach distention, rise in plasma osmolality or increased activity of the hypothalamic-pituitary-adrenal axis. In the hypothalamus, oxytocin mRNA expression is limited primarily to the PVN and supraoptic nucleus (SON) (Jing et al.,

1998; Martins et al., 2005). ABA images revealed that the probe hybridized also in the suprachiasmatic nucleus (SCN), whereas other sources disclose that the SCN does not contain oxytocin RNA, however, it displays a very high content of relatively homologous vasopressin mRNA. Finally, the galanin receptor 3 mRNA – which has been found by other authors in the VTA, PVN, VMH and DMH, to name a few (Hawes and Picciotto, 2004; Mennicken et al., 2002) - has not been detected due to method failure, although other components of this orexigenic system are well defined in the atlas.

The comparison of some quality control-approved in situ hybridization analyses shown in the ABA to those in other reports has revealed significant discrepancies, although one should note that it was a relatively rare circumstance. One questionable set of ABA data involves genes encoding for opioid receptors. The opioid system is known to propel hedonic aspects of consummatory behavior. Injections of agonists of kappa, mu and delta opioid receptors increase intake of palatable diets, such as those high in fat and/or sugar, whereas administration of antagonists particularly well blocks ingestions of these tastants (Cota et al., 2006; Olszewski and Levine, 2007). Importantly, modification of food intake can be achieved by infusing opioid ligands acting at particular receptor subtypes directly into discrete central sites, including the PVN, ARC, LHA, NAcb, VTA and NTS (Cota et al., 2006; Olszewski and Levine, 2007). Brain Explorer-assisted search showed that the mu and kappa receptors are virtually nonexistent in the sites included in the analysis, with an exception of the LHA and VTA where relatively low expression of the mu receptor mRNA was found. This outcome is in a striking contrast with the findings of other investigators. Kappa receptor mRNA was detected in large concentration in the PVN, ARC, VTA, NTS and NAcb, whereas the mu receptor, in the PVN, NAcb, VTA, NTS (Meng et al., 1993; Thompson et al., 1993). It is difficult to explain the nature of such profound discrepancies, therefore, certain in situ hybridization procedures may require additional attempts.

The ABA displays images of also those sections that underwent unsuccessful in situ hybridization procedures, which is an asset of this tool, as it allows the reader to have some insight into the quality control and decision-making process and validates methodology employed in the development of the atlas. As mentioned above, to the contrary of data reported by other investigators, in situ hybridization of the ghrelin gene did not produce any proof of expression in the ARC, whereas oxytocin mRNA has been detected in the SCN. Both attempts were defined as failures, what reassures that the quality control mechanisms are sensitive to both false-positive and false-negative findings.

It should be emphasized that the majority of Brain Explorer data are similar to those obtained elsewhere. Among 57 genes searched for in the atlas, 42 or approximately 74% were found and appeared to be visualized properly (see Tables 1 and 2). The remaining genes included those that failed the quality control process, such as ghrelin, galanin receptor 3, oxytocin and vasopressin, as well as those that passed the quality check, yet the in situ hybridization outcome was questionable, especially in light of previously published work (Tables 1 and 2). The latter group included genes encoding for the melanocortin receptor 4, neuromedin U and its type-2 receptor, gastrin-releasing peptide receptor, neurotensin receptor 2, neuropeptide B, neuropeptide W and GLP-2 receptor. Finally, some data seem incomplete and likely require additional verification, such as those related to the distribution of opioid receptors or glucagon precursor. The observed discrepancies are quite unlikely to be attributable to potential species differences (i.e., rats versus mice) as in our literature search we relied primarily on data derived from murine studies. In addition, it should be noted that the sets of questionable gene data generally include mismatches in three or more analyzed brain areas compared to the findings of other authors, and the majority of disputed ABA analyses showed uniformly low expression throughout the network of sites and/or a high number of artifacts.

ABA perspective on feeding-related central network: System overlap

Studies examining central mechanisms of consummatory behavior typically focus on a particular gene, receptor or peptide. As a result of such approach, which relies on a vast array of techniques, including injection studies, gene expression analyses and behavioral manipulations, there is a tendency to “assign” genes and their products to specific roles in the regulation of feeding. For example, opioids have been dubbed as reward mediators, whereas NPY and ghrelin as hunger-related molecules (Levine and Billington, 1997). In line with this reasoning, transgenic animals lacking a given gene are examined for the presence of alterations of behaviors or physiological parameters that the deleted neural component is thought to affect. In fact, the absence of such alteration would sometimes raise concern as to whether the deletion was indeed successful, if there are adaptive (compensatory) changes caused by not having the gene expressed, and if the results truly serve as an argument against the proposed role of the gene product. There has been a tendency, as well, to define discrete brain structures as “centers” that govern a particular aspect of consumption; hence many authors refer to the PVN as the hunger/energy expenditure center and to the VTA as the site that serves as a relay station for pleasure-related gustatory stimulation (Cota et al., 2006; Levine and Billington, 1997). This, in fact, is in concert with the hypotheses behind early studies employing local lesions, which showed that, e.g., elimination of the entire PVN results in rapid body weight gain (Tokunaga et al., 1991) and increase in food intake, and with knife-cut studies that defined connections between, e.g., the NTS and PVN (Crawley et al., 1984) as essential in maintaining a proper pattern of consummatory behavior in animals.

However, quite frequently, published reports reveal data that, at least partially, contradict this narrow, single-track function of neuroactive components and sites. It has been shown for example that opioid receptor antagonists, naltrexone and naloxone, reduce hunger-driven feeding generated by deprivation (Hayward and Low, 2001; Koch et al., 1995), and the mu opioid receptor plays a greater role in motivation to seek foods than in hedonic processing of ingestive behavior (Papaleo et al., 2007). Aside from acting as an energy-related substance, AgRP, just like certain opioids, exhibits antiaversive properties and stimulates intake of palatable tastants (Olszewski et al., 2003; Wirth et al., 2002). NPY, which has been strongly linked with eating due to hunger, increases consumption of a non-caloric, palatable saccharin solution; conversely, saccharin intake elevates NPY mRNA levels in the brain (Furudono et al., 2006). There also has been a tendency to define brain areas as reward, motivation, hunger or satiety “centers” based on the expression of certain genes or observed consummatory responses upon injection of selected agents. While this desire to simplify the importance of sites in feeding control may be helpful in describing some basic mechanisms, the simplification does not reflect the true complexity of feeding-related circuitry. For example, ARC neurons give rise to POMC and NPY that have been linked with the consumption of both palatable and high-energy tastants (Levine and Billington, 1997; Olszewski and Levine, 2007). This hypothalamic structure also contains anorexigenic peptides and receptors, including CART and the leptin receptor (Elias et al., 2001). It is therefore impossible to refer to the ARC as a “satiety”, “orexigenic”, “reward” or “hunger” center. One of the attempts to escape from singling out sites or genes has been to delineate pathways of neurons located in different sites that are functionally and anatomically connected. Thus, for example, the GLP-oxytocin reciprocal innervation between the brainstem and hypothalamus has been described (Zueco et al., 1999). However, the pathway approach - although extremely helpful in visualizing “cascades” of neuronal events through topographic illustration - seems to be still relatively distant from the maze of neural-based interactions within the brain. In fact, if one accepts the conventional interpretation of data stemming from classical descriptions of “center”-based gene distribution in the CNS, then the seemingly contradictory data from feeding studies mentioned above represent a conundrum.

Analysis of the distribution of feeding-related genes in the ABA strongly supports the hypothesis stating that the central regulation of consummatory behavior is governed by a widespread central network. This network consists of genes ubiquitous throughout the structural elements of this circuitry. One should note that every single region included in our analysis encompasses genes that support initiation or termination of consumption. Furthermore, these genes are classically viewed as mediators of different aspects of feeding, such as hunger and reward. For example, genes expressed in the VTA, which is dubbed as the “reward center” (Levine and Billington, 1997), include those that give rise to hypophagia-linked CRH, CRH1R, VP, CART, MC3R, leptin receptor and CCKAR, energy/metabolism-related feeding stimulators, such as NPY and ghrelin’s GHS receptor, alertness- and ingestion-promoting orexin and its receptor and, finally, reward mediators: dynorphin, endorphin and the delta opioid receptor. A similarly diverse list could be made for each of the sites (see Table 1 and Fig. 1 for comparison). Importantly, almost every single gene is present in more than just one area, therefore, it affects multiple components of the circuitry. The degree of colocalization of specific genes involved in food intake control within these areas appears very high; hence, the probability of interplay between the gene-based neural systems seems to be parallel with it. In fact, this regional coexpression of multiple genes can provide explanation of diverse actions of specific neuropeptides injected in discrete sites. It certainly contradicts the notion that food intake is governed by “centers” which support a particular direction of a feeding response. These “centers” should be rather viewed as components of a dynamic, widespread network, whose overall and site-specific activity determines the feeding status of the animal.

Furthermore, the question arises whether we should attempt to define the role of specific genes so precisely to single out energy, palatability, motivation and – on the other side of the spectrum - satiety or aversion aspects of their action or should we rather generalize their roles to either orexigens or anorexigens. This may be particularly the case when such overall conclusions are drawn based on a relatively limited spectrum of experimental models and paradigms. The exact function of a given gene and, thus, of a derived peptide or receptor, seems to be affected and modified by its anatomical localization and, in particular, by colocalization with other genes involved in consumption. One can hypothesize that the outcome of consummatory activity resulting from the expression of a given gene (such as feeding for reward or energy) is modulated by dynamic changes of expression of other genes. Hence, the involvement of gene products present in neural circuits in a given aspect of consummatory response may be viewed not only as an inherent “quality” of a given molecule, but the outcome of complex interactions with other neural systems at both anatomical and functional level. This concept can also explain “plasticity” of consumption-related reward - that is, when one is hungry, bland food is rewarding, whereas when the need to replenish calories is low, typically sweet and/or fat tastants are perceived as “attractive” (Levine and Billington, 2004). The lack of clear demarcations between hunger and reward is reflected by the lack of such mechanisms driving various aspects of consumption.

It should be noted that the analysis of the ABA as well as of available literature shows that genes involved in feeding regulation are expressed also outside the circuitry classically viewed as one that governs consummatory behavior and energy homeostasis. Therefore, one should not assume that a given feeding-related gene expressed in, e.g., the hippocampus, does not affect consumption at all. For example, Carlini et al. have recently shown that the growth hormone secretagogue (GHS) receptor expressed in the dorsal raphe nucleus and hippocampus mediates consummatory responses to ghrelin (Carlini et al., 2004). Hence, expression level of the gene encoding for the GHS receptor outside the brainstem-hypothalamic network may certainly influence ingestive behavior.

Perspectives

One of the greatest challenges in medical research is to develop effective treatment methods to combat obesity and other disorders largely derived from improper consumption patterns, such as anorexia and bulimia. The majority of currently available medications fail to produce significant benefits. However, the scope of action of these drugs, i.e., targeting a narrow subset of the feeding network components, tends to neglect the complexity of the feeding network and falls into the trap of the single center/single gene product notion. The clear image of the widespread central circuit of genes provided by the ABA offers an insight into the mechanisms of eating behavior being a result of combination of factors related to energy needs, reward, motivation and alertness, to name a few. In fact, processes affected by these neural factors mirror the reasons for humans to begin, continue or end a meal. Hence, the ABA presenting the complexity of the feeding-related network may serve as a tool in conceptualizing novel treatment methods that would simultaneously target several components of this circuitry and, hence, several aspects of eating behavior. From the functional perspective, the localization and density of expression of genes encoding for feeding-related peptides is probably less important or has a different meaning than the localization and level of expression of genes giving rise to receptors for these signaling molecules within the neural circuit. It may be particularly true considering that many of these receptors – typically G-protein-coupled ones (GPCRs) – are membrane-bound (Schiöth, 2006), whereas peptides synthesized at a given site act via autocrine, paracrine and endocrine (thus, “dispersed”) mechanisms. Therefore, the topography and expression characteristics of receptors should be a particular focus in studies aimed at defining spatial organization of feeding-related neuronal network as well as in finding clinical solutions to eating disorders.

In addition, one should note that the Atlas provides expression maps of genes whose physiological roles have not been defined thus far. Considering a remarkably high degree of overlap between multiple feeding-related systems, it can be speculated that widespread coexpression of a given gene of unknown function with molecules involved in consumption control may warrant undertaking studies to examine this gene’s possible role in consummatory behavior. Therefore, the atlas should be also viewed as a potential tool in search for novel molecules that govern feeding.

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List of abbreviations

AgRP	Agouti-related protein
ARC	Arcuate nucleus
CART	Cocaine and amphetamine-regulated transcript
CCKBR	Cholecystokinin receptor B
CB	Cerebellum

CRH	Corticotropin-releasing hormone
CRHR1	Corticotropin-releasing hormone receptor 1
CRHR2	Corticotropin-releasing hormone receptor 2
CTX	Cortex
DMH	Dorsomedial nucleus
GAL	Galanin
GALR1	Galanin receptor 1
GALR2	Galanin receptor 2
GALR3	Galanin receptor 3
Gcg	Glucagon
GHSR	Growth hormone secretagogue receptor
Glp1r	Glucagon-like peptide receptor 1
Glp2r	Glucagon-like peptide receptor 2
GRP	Gastrin-releasing peptide
GRPR	Gastrin-releasing peptide receptor
Lepr	Leptin receptor
LHA	Lateral hypothalamic area
MB	Midbrain
MC3R	Melanocortin receptor 3
MC4R	Melanocortin receptor 4

MCHR1	Melanin concentrating hormone receptor 1
MY	Medulla
NAcb	Nucleus accumbens
Nmb	Neuromedin B
NMBR	Neuromedin B receptor
Nmu	Neuromedin U
Nmur1	Neuromedin U receptor 1
Nmur2	Neuromedin U receptor 2
Npb	Neuropeptide B
Npw	Neuropeptide W
NPY	Neuropeptide Y
NPY1R	Neuropeptide Y receptor 1
NPY2R	Neuropeptide Y receptor 2
NPY5R	Neuropeptide Y receptor 5
NTS	Nucleus of the solitary tract
Nts	Neurotensin
Ntsr1	Neurotensin receptor 1
Ntsr2	Neurotensin receptor 2
OLF	Olfactory
Oprd	Opioid receptor delta

Oprk	Opioid receptor kappa
Oprm	Opioid receptor mu
ORX	Orexinl
ORXR1	Orexin receptor 1
ORXR2	Orexin receptor 2
Oxy	Oxytocin
P	Pons
Pdyn	Prodynorphin
PMCH	Pre-melanin-concentrating hormone
POMC	Proopiomelanocortin
PVN	Hypothalamic paraventricular nucleus
Pyy	Peptide YY
SCN	Suprachiasmatic nucleus
SON	Supraoptic nucleus
Tac2	Tachykinin
Ubl5	Beacon gene
Ucn	Urocortin
VMH	Ventromedial nucleus
VP	Vasopressin
VPR1A	Vasopressin receptor 1A

VPR1B

Vasopressin receptor 1B

VPR2

Vasopressin receptor 2

MTA

Ventral tegmental area

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Figure 1. Expression of orexigenic genes in the central circuitry

Data have been derived from the Allen Brain Atlas, using the Brain Explorer v1.4, and were interpreted based on the visual assessment. Each box represents a brain region. The circles in each box indicate expression levels and densities of genes in a specific brain area. Gene symbols without corresponding circles are mentioned when only expression density or level was above 0. The size of the circle corresponds to one of six levels of expression density. The expression density assigned the “+” sign means that expression is sparse, “++” should be understood as expression occurring in most parts of the area, and “+++” as expression occurring throughout the entire area. For the expression levels, a similar system was used for registration, however expression levels are featured as colors. Dark blue corresponds to the lowest expression level featured in the figure, “0/+”. Light blue corresponds to “+”, turquoise to “+/+”, light green to “++”, orange to “++/+++” and finally, red corresponds to the highest expression level, “+++”.

+

. The location of each gene symbol in boxes representing brain areas does not correlate with this gene's distribution within a site. It should be noted that POMC is a precursor for an orexigen, beta-endorphin, and for an anorexigen, alpha-melanocyte stimulating hormone.

Figure 2. Expression of anorexic genes in the central circuitry

Data have been derived from the Allen Brain Atlas, using the Brain Explorer v1.4, and were interpreted based on the visual assessment. Each box represents a brain region. The circles in each box indicate expression levels and densities of genes in a specific brain area. Gene symbols without corresponding circles are mentioned when only expression density or level was above 0. The size of the circle corresponds to one of six levels of expression density. The expression density assigned the “+” sign means that expression is sparse, “++” should be understood as expression occurring in most parts of the area, and “+++” as expression occurring throughout the entire area. For the expression levels, a similar system was used for registration, however expression levels are featured as colors. Dark blue corresponds to the lowest expression level featured in the figure, “0/+”. Light blue corresponds to “+”, turquoise to “+/+”, light green to “++”, orange to “++/+” and finally, red corresponds to the highest expression level, “+++”. The location of each gene symbol in boxes representing brain areas does not correlate with this gene’s distribution within a site. It should be noted that POMC is a precursor for an orexin, beta-endorphin, and for an anorexin, alpha-melanocyte stimulating hormone.

Expression densities (ED) and levels (EL) of orexigenic genes in feeding-related brain sites. The expression scale ranges from 0 (none) to +++. Dark cells indicate genes whose distribution was unsuccessful due to probe failure as indicated in the ABA or it was considered unsuccessful because of questionable artifact/gene distribution and discrepancies between ABA data and other reports (in the latter case, reports showing contradicting data quoted in the table).

Table 1

Gene	EL	ED	ARC	PVN	DMH	LHA	NTS	NAcb	VTA	VMH	Major artifacts in / additional notes
GAL	EL	++/+++	++/+++	++/+++	++/+++	++/+++	+++	0	0	++	CB, CTX, P, MY
	ED	+/++	+/++	++	++	++	++	0	0	+	
GALR1	EL	0	++/+++	++	++	++	++	++	++	++	OLF
	ED	0	+	+	0/+	0/+	+/++	0/+	0	0	
GALR2	EL	+++	0	0	0	++	++	++	0	0	CB, PAL, OLF
	ED	0	0	0	0	0	0	0	0	0	
GALR3	EL	++/+++	++	++/+++	++/+++	++/+++	+++	++	++/+++	++/+++	
ORX	EL	++/+++	++	++	++	++	+++	++	++/+++	++/+++	
	ED	++/+++	++	+	+	++	++	++	+/++	++	
ORXR1	EL	0	0	0	0	++	++	0	0	0	MB, P
	ED	0	0	0	0	0	0	0	0	0	
ORXR2	EL	++	0	++	++	++	++	++	++	++	CBX
	ED	+/+++	0	0	0/+	0/+	0/+	0	0	+	
PMCH	EL	0	++	++/+++	++	++	0	0	0	++	
	ED	0	0	+	+	+	0	0	0	0	
MCHR1	EL	0	0	0	0	0	++	++	0	0	CB, P
	ED	0	0	0	0	0	0	+/++	0	0	
POMC	EL	+++	0	++	++/+++	++/+++	++	0	++	++	
	ED	++/+++	0	0/+	0/+	0/+	0	0	0	+/++	
AgRP	EL	+++	0	0	0	0	++	0	0	0	CTX, MY, OLF, P
	ED	++/+++	0	0	0	0	0	0	0	0	
NPY	EL	+++	++	0	0	++	+/++	++/+++	++	+++	
	ED	+++	0	0	0	0	0	0/+	0	0	
NPY1R	EL	++/+++	++	0	0	++/+++	++	++/+++	0	++	CB, OLF
	ED	++	++	0	0	0/+	0	0/+	0	0/+	
NPY2R	EL	0	0	0	0	++	++/+++	++	0	++/+++	LHA and NAcb artifacts excluded from the analysis
	ED	0	0	0	0	0	0	0/+	0	0/+	
NPY5R	EL	0	0	0	0	++	++	++/+++	0	++	Probably an artifact in the lower portion of the NAcb
	ED	0	0	0	0	0/+	+	+	0	0	
GHSR	EL	++/+++	0	++	++	++	++	0	++	++	
	ED	++	0	0	0	0/+	0/+	0	0/+	0	
Ghrelin	EL	0	0	0	0	0	0	0	0	0	CTX / Almost undetected; failed QC
	ED	0	0	0	0	0	0	0	0	0	
Pdyn	EL	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++	++/+++	
	ED	++/+++	++/+++	++/+++	++/+++	+	++	++	0	++/+++	
Ubl5 (Beacon)	EL	++/+++	++	++	++	++	++	++	++	++	CB, HY, MB, MY
	ED	++/+++	+	+	+	+/++	+/++	+/++	+	+	
Oprk	EL	0	0	0	0	0	0	0	0	0	OLF / Likely probe failure: no expression except for artifacts (Meng et al., 1993)
	ED	0	0	0	0	0	0	0	0	0	Very high expression throughout the brain
Oprd	EL	++	++	+/++	+/++	+/++	+/+++	++	+/++	+/++	
	ED	0	0	0	0	0	++	0	++	0	HIP / Uniformly very low expression may indicate probe failure(Thompson et al., 1993)
Oprm	EL	0	0	0	0	0	0/+	0	0	0	
	ED	0	0	0	0	0	0	0	0	0	

Expression densities (ED) and levels (EL) of anorexigenic genes in feeding-related brain sites. The expression scale ranges from 0 (none) to +++. Dark cells indicate genes whose distribution was unsuccessful due to probe failure as indicated in the ABA or it was considered unsuccessful because of questionable artifact/gene distribution and discrepancies between ABA data and other reports (in the latter case, reports showing contradicting data quoted in the table).

Table 2

Gene	EL	ED	ARC	PVN	DMH	LHA	NTS	NAcB	VTA	VMH	Major artifacts in / additional notes
CRH	EL	++	++	++/+++	++/+++	++	++	++/+++	+/++	+/++	
	ED	++	+	+	0/+	+	0	0/+	0	++	
CRHR1	EL	++	++	0	++	++/+++	++	++	++	0	
	ED	+/++	0	0	0	+	0/+	0/+	0/+	0	
CRHR2	EL	++/+++	0	0	0	0	++	0	0	0	CBX
	ED	0/+	0	0	0	0	0	0	0	0	
VP	EL	++/+++	++/+++	++/+++	++/+++	++/+++	0	0	++	0	CB, CBX, HIP, HY, MY, P / Failed QC
	ED	+	0/+	0	0	0	0	0	0	0	
VPRIA	EL	++	++	0	++	0	++	0	0	++	CBX, MY
	ED	0	0	0	0	0	0	0	0	0	
VPRIB	EL	0	0	0	0	++	++	++	0	0	
	ED	0	0	0	0	0	0	0	0	0	
VPR2	EL	0	0	0	0	0	0	0	0	0	MY
	ED	0	0	0	0	0	0	0	0	0	
CART	EL	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++	++/+++	P
	ED	++	+	+	0/+	0	+/++	++	0	+	
POMC	EL	+++	0	++	++	++/+++	++	0	++	++	
	ED	++/+++	0	0/+	0/+	0/+	0	0	0	+/++	
MC3R	EL	++	0	0	0	++	++	++	++	++	CB
	ED	+	0	0	0	0/+	0/+	0/+	0	0/+	
MC4R	EL	0	0	0	0	0	0	0	0	0	CB, CBX / Likely failed probe as expression uniformly low throughout the brain. Other reports contradict ABA findings (Huang et al., 2003; Mountjoy et al., 1994)
	ED	0	0	0	0	0	0	0	0	0	High in ventricles, except for the 3V: might indicate that it is not the functional splice form of the receptor (OB-Rb), but a different variant that is involved in transport into the brain and clearance from CSF.
Lepr	EL	++	0	0	++/+++	++	++	0	++	++	CBX, MB, MY P; artifact in the VTA excluded from the analysis / Unlike in previous reports, no expression in the brainstem (Tang-Christensen et al., 2001; Vrang et al., 2007)
	ED	++	0/+	0/+	0	0/+	0	0/+	0	0/+	CB, OLF
Geg	EL	0	0	0	0	0	0	++	0	0	CB, CBX, OLF, MY, RHP / Other reports contradict ABA findings (Lovshin et al., 2001)
	ED	0	0	0	0	0	0	0	0	0	
Glp1r	EL	++	++	++/+++	++	++	0	++	0	0	
	ED	+	+	+	0	0	0	0	0	0	
Glp2r	EL	0	0	0	0	0	0	0	0	0	
	ED	0	0	0	0	0	0	0	0	0	
CCK	EL	++	++	++	++/+++	++	++/+++	++	++	0	
	ED	0	0	0	0	0	0/+	0	0	0	
CCKAR	EL	++	++	++	++	+/++	+/++	+/++	++	++	OLF
	ED	+/++	+	+	0	0/+	+/++	+/++	0/+	+/++	
CCKBR	EL	++	++	0	0	0	++	++	0	++	
	ED	0/+	0	0	0	0	0	0/+	0	+	
Nmu	EL	0	0	0	0	0	0	0	0	0	CTX, OLF / likely probe failure: expression uniformly low in the brain, unlike in previous reports (Graham et al., 2003)
	ED	0	0	0	0	0	0	0	0	0	CB, CTX, MB, MY
Nmur1	EL	0	0	0	0	++	++	++	++	0	
	ED	0	0	0	0	0/+	0	0/+	0/+	0	

Gene	EL	ED	ARC	PVN	DMH	LHA	NTS	NAcB	VTA	VMH	Major artifacts in / additional notes
Nmur2	EL	0	0	0	0	0	++	0	0	0	CB / Other reports contradict ABA findings (Funes et al., 2002; Graham et al., 2003)
	ED	0	0	0	0	0	0	0	0	0	
Nmb	EL	++	++	++	++	++	++	++	++	++	CTX
	ED	0	0	0	0	0/+	0/+	0/+	0/+	0	
NMBR	EL	0	0	0	0	++	0	0	0	0	CB, CTX, OLF, STR
	ED	0	0	0	0	++	0	0	0	0	
GRP	EL	++	++	++	++	++	++	0	0	0	Artifact in LHA excluded from the analysis
	ED	0/+	0	0/+	0/+	0	0/+	0	0	0	
GRPR	EL	0	0	0	0	0	0	0	0	0	CTX / Probe failure likely as it should be expressed in the hypothalamus(Wada et al., 1991)
	ED	0	0	0	0	0	0	0	0	0	
Nts	EL	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	++/+++	CB
	ED	0/+	0/+	0/+	0	+/+	+	+/+	0/+	0	
Ntsr1	EL	0	0	0	0	0	++	++	++/+++	0	CTX, MY
	ED	0	0	0	0	0	0	0	0/+	0	
Ntsr2	EL	0	0	0	0	0	0	0	0	0	CB, CTX, OLF,STR / Mainly artifacts: QC questionable.Other reports contradict ABA findings (Yamauchi et al., 2007)
	ED	0	0	0	0	0	0	0	0	0	
Ucn	EL	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	CB, CTX, HY, MB, MY, P / Artifact in the ARC excluded from the analysis
	ED	++	++	0	0/+	0/+	0/+	0/+	0/+	0	
Adipoq	EL	++	++	++	++	++	++/+++	++	++	++	CB, CTX, TH
	ED	+	0/+	0/+	+/+	+/+	+/+	++	+	+/+	
Adipor1	EL	++/+++	++	++	++/+++	++/+++	++/+++	++/+++	++/+++	++	Missing several continuous forebrain sections, however all sites investigated herein were included
	ED	++	++	++	++	++	++	++/+++	++/+++	+/+	
Adipor2	EL	++	++	0	++	++	++	0	++	++	
	ED	0	0	0	0	0	+	0	+	0	
Pyy	EL	0	0	0	0	++	++	++	++	0	CB, CTX, HY / artifact in HY excluded from the analysis
	ED	0	0	0	0	++	0	0	0/+	0	
Tac2	EL	+++	++	++	++/+++	++/+++	++	++/+++	0	++/+++	CTX, HIP, MY,TH /Neuropeptide K alias
	ED	+	0	0	0/+	0/+	0	+	0	0/+	
Npb	EL	0	0	0	0	0	0	0	0	0	No expression – only artifacts
	ED	0	0	0	0	0	0	0	0	0	
Npw	EL	0	0	0	0	0	0	0	0	0	No expression – only artifacts
	ED	0	0	0	0	0	0	0	0	0	
Oxy	EL	0	0	0	0	0	0	0	0	0	----- did not pass QC; probe failure-----