The ‘Tyranny of Choices’ in the Ingestion-Controlling Network

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Key Words

Ingestion control · Obesity · Networks · Motifs · Maximal motifs · Polypharmacy

Summary

Background: Currently used antiobesity remedies offer only a modest weight reduction, and have untoward effects that can complicate treatment efforts. Motivated by the needs of the pharmacotherapy of obesity, the study explored the role of neuropeptide Y, leptin, and corticotrophin-releasing hormone. **Method:** The study used Ingenuity Pathway Analysis which is a tool for automated discovery and visualization of molecular interactions. **Results:** In ingestion-controlling networks, neuropeptide Y, leptin, and corticotrophin-releasing hormone molecules are commonly combined into the units designated as ‘maximal motifs’. The analysis of this triad allowed suggesting that maximal motifs are not more than a compendium of admission rules and transmission alternatives of their nodes catalogued in the dataset. Nonetheless, these options seem to endow them with the flexibility needed to respond dynamically as a functional unit to changing internal (metabolic) conditions or environmental challenges. **Conclusion:** Thus far, each peptide represents a separate target for pharmaceutical interventions (as judged by US patents scanned). The study concludes with predictions regarding designs of ‘multitargeted’ antiobesity agents since only by hitting a combination of targets can an appropriate therapeutic effect be achieved.

Introduction

The word ‘overweight’ appears on the web almost 10 times more often than the word ‘thinness’, and all attention to the topic seems justified. Economic costs attributable to obesity in the USA as of 1998, account for 9.1% of total annual US medical expenditures and may be as high as USD 78.5 billion (USD 92.6 billion in 2002) [1]. Therefore, more efficacious and safe agents than hitherto available for the regulation of adiposity are eagerly awaited.

Weight control hinges on development, differentiation, and interaction of a multitude of molecules. What is particularly perplexing about the latter is that they overlap with and are represented in intricately wired networks that are active in a host of nutrition-unrelated functions unless such functions are metaphorically expanded to include a vast array of processes supporting developments of individual cells or cognitive functions [2, 3]. Metabolic information these circuits receive and transmit for processing elsewhere does not appear as though traveling in linear sequences (formally defined as ‘walks’), but rather somehow is locked in repetitive loops that seem to permit a collective responding by the participants of these constructions. That is, unlike the ‘walks’, such items are wired for a quasi-synchronized recurrent messaging on the receiving end of communication that might sound – to borrow a musical metaphor – close to the intermittent ‘accords’. Such structures are now known under the name of ‘network motifs’ [4–7]. Arthur Koestler [8] coined the term ‘holon’ (i.e., ‘a part-whole’) as though in an anticipation of such architectures. So clustered, these few-node subgraphs or small ‘cliques’ embedded in the larger networks were suggested to function as elementary computational circuits with the diverse regulatory roles [5, 9]. Perhaps, that is why they are attracting attention in drug design and development, as well as in rehabilitation research [3, 10–13]. With the advent of bioinformatics, research

pharmacologists have become increasingly interested in probing such complex and distributed networks for therapeutically relevant motifs. The puzzle is that some molecular clusters have a rather redundant structure of bidirectional interconnections, thereby defying a simple input-output interpretation needed for making valid pharmacodynamic predictions. One might wonder what computations, if any, such an overrepresented assembly could provide.

In an attempt to answer this question, the present study singled out 3 well-studied ingestion peptides, such as neuropeptide Y (NPY), leptin (LEP), and corticotrophin-releasing hormone (CRH). The choice for selecting them for experimental portrayal is primarily justified by the fact that they are integrated into a recognizable recurring motif in diverse networks [2] and the fact that according to Zipf analysis [3], they are most frequent participants in the datasets explored via Ingenuity Pathway Analysis (IPA). In this regard, they might be the desirable targets for therapeutic intervention in the central nervous system and outside of it. In the following, IPA [reviewed in 14] was used as a tool for automated discovery and visualization of molecular interactions.

Material and Methods

Mining IPA-Generated Dataset

The term 'network' is used to describe a wide variety of real-world networks and processes [reviewed in 15–17]. The ingestion-controlling networks are knowledge-based products so that their data resources are constantly enriched and admit inferences. In this regard, molecular networks have features in common with semantic networks in the sense that they could document molecules together with their roles and locations; they could embrace each molecule or motif in their relationships with other networks. The IPA search engine generates lists of molecules on request. The latter can be further enriched by functional analyses as detailed elsewhere [2]. While the retrieved data are supported by the rich set of PubMed headings by which articles are indexed, the search was further supplemented by the AliBaba engine [18, 19] which can be queried just like PubMed, save for the opportunity it gives to visualize the data selected in the context of other findings. It allows extracting associations between cells, proteins, and tissues, and specifies filter options to manipulate the confidence value of visible associations, edge length, and degree on its graphs.

The dataset that provides the statistical basis of requested associations and functions is impossible to organize unaided by automated software. For hormonal activities of LEP alone, as well as for its roles in cells (activation, proliferation, hyperpolarization, inhibition, migration, and others) Ingenuity Knowledge Base lists over 4,000 findings. For CRH, with its functions in cell activation, proliferation, growth, neuroprotection, depolarization, infiltration, survival, binding, and others, Ingenuity Knowledge Base lists over 1,700 categorized literature findings. Finally, for NPY, Knowledge Base rests on over 1,600 findings. Some of this information is redundant and must be gated manually by filters or trimmed post hoc.

In keeping with conventions of the graph theory, each molecule in IPA is mapped to a node that is shown in its interactions with other entities (e.g., genes, metabolic products, or drugs), thereby creating directed graphs (termed digraphs). The connecting arcs symbolize the type and direction of their relationships or interactions, as will be shown below. By comparison, protein-protein interactions are represented by connections with no directionality that are commonly termed edges [17, 20]. To

be able to navigate in densely connected graphs, IPA provides macros for reducing 'entropy' or data filtering (e.g., removing molecules, such as chemical drugs, reactive groups, or chemical toxicants) that otherwise makes graphs unnecessarily cluttered. In special cases described below, the IPA allowed to adopt an opposite process of molecular acquisition by finding the shortest path (geodesic path) between two nodes in a network. The 'path' is a fundamental property of networks defined as a connection in which all nodes are visited only once. Since it is possible for one peptide or a group to be included in multiple occurrences of different sub-networks, the shortest-path computation estimates how many steps are required (and implemented) to go from one node to another along the shortest route. In this way, numerous other modules could be added to the graph of interest. The degree of their functional relevance is ascertained by the internal validation via functional analyses or an overview of the biological functions associated with a given network by a pull down menu that shows the molecule(s) that are known to support the relationship.

Primed with the influential studies by Milo et al. [5, 21, 22], motifs are commonly identified by the search algorithms based on their topology. The sheer scale of modern biological data collections involving thousands of genes or proteins makes such definition of motifs immensely useful for proving their recurrent nature [23], and it has been accepted in much of motif research ever since. However, in keeping with a precedence set in by Miller et al. [24] and continued by others [25], all nodes in the present study were labeled. The main benefit of labeling is in reducing uncertainty over the data sources, thereby sidestepping the need for proving their ubiquity by statistics-based computations. By contrast, each connection (arc, edge) linking two nodes may have a number of annotations (e.g., activation, binding, protein-protein interaction, metabolic transformation, catalysis, inhibition, phosphorylation/dephosphorylation, regulation of binding, transcription, and others).

Fischer's exact test was used to calculate a *p* value determining the probability that the association between the molecules in the dataset and the pathway isolated in the sub-network is not accounted by chance (a cut-off value here is *p* = 0.01). The Benjamini-Hochberg method is used as needed for multiple testing corrections [26].

IPA allows performing 'mental experiments' [27] posed by questions of interest when probing for available information in the process of literature-mining routine. In such unconventional experiments, each succeeding question can only be legitimately formulated when the answer to the previous one was attempted. That is why in the current presentation the format of 'Results and Discussion' was preferred.

Results and Discussion

The starting point of the study is a figure (fig. 1) showing that LEP, NPY, and CRH have bidirectional connections between them, thereby creating a 'fully connected triad' [5], a union with 'maximal structure', i.e., the one that has more edges than are necessary to tie all of its nodes [28, 29]. Both definitions suggest that each node of the triad is excessively occupied by interactions with its neighbors. This hunch was submitted to a formal testing using IPA 'growth' and 'connect' macros that expanded the triad into a network with a number of relevant molecules. It showed that each of the nodes is a hub in the network of its own. In the next step, IPA permitted to compute the degree to which the same molecules of the triad or all three of them are engaged in diverse roles. These roles appear to be numerous, ranging from behavior to cardiovascular functions. Only several of them were exemplified

Fig. 1. Maximal motif along with the procedure of its disarticulation and reconnections of nodes. **A** Ancestral maximal motif, LEP-CRH-NPY with edge labels. **B** The sequence of generating a family of novel triads derived from the ancestral one. It opens with a removal of all arcs between the nodes. In the next step, they were submitted for reconnection by using path explorer or a 'connect' macro. When one or two nodes were submitted for connection sequentially (e.g., CRH, NPY + LEP; LEP + CRH, NPY + LEP, and so on), the ensuing motif was not identical to the primary one. By comparison, when all nodes were processed at once by a 'connect' macro, the ancestral (initial) topography (such as in A) was readily regained. **C** A set of de novo ('hardwired') motifs based on the same nodes (1–12) that differ merely in arcs and edges. For clarity, their molecule names are communicated by shading and shape conforming to that in (A). Motif becomes 'maximal' when the filter was set to include all compartments, brain sites, and all experimental animals for node interactions available in Ingenuity data bases. Their enumeration does not follow the standard [5] patterns. Note differences in edge labels for the maximal (A) as compared to derived motifs (B). Edge labels: E – expression (includes metabolism); I – inhibition; LO – localization; PP – protein-protein binding; T – transcription (© 2002–2009 Ingenuity Systems, Inc.; all rights reserved).

in figure 2 A merely to emphasize their variety. Some of them could not be easily predicted from the studies on isolated peptides, their receptors, or their knockouts. Teleologically, the redundant connectivity might simply reflect the property of pluripotency of the clique assuming that their numerous interactions ought to be temporally segregated, as they would be in different evolutionary scenarios [30].

The meaning of pluripotency of maximal motifs is further illustrated in two computationally simple directed graphs of about identical size and connectivity in figure 2 B, C. Submitted to IPA functional analysis, molecules of both graphs appear to be implicated in a number of functions (e.g., cell signaling, cellular growth and proliferation, as well as molecular transport) that only tangentially could be related to appetite and ingestion. They reflect the basic roots of the system in the development of energy homeostasis and the trajectories of multipotent adipogenic precursor cells capable to evolve into diverse cell types, including endothelial cells, myoblasts, osteoblasts, and others [31]. A number of three-node cliques (triads) can be seen in each graph, including those created by NPY, LEP, and CRH nodes.

A drawback of the IPA-supervised search is that at this stage, it is not supported by the mathematical analysis of the constructed networks and the manual alterations of its layout. This shortcoming notwithstanding, the picture that emerges suggests that molecules shown in the graphs were recruited repeatedly from early ontogeny to adulthood. Consequently, the edges of the ancestral triad are not realistic descriptors of the actions taking place in both directions synchronously, inasmuch as the nodes they connect must also be able to act in different circuits. If there is a biological benefit of their overlapping engagement, what is it? In the following, the functionality of these redundant options, dubbed below as 'tyranny of choices' is explored by several maneuvers. One of them uses deletions of edges and then a submission of the disconnected nodes to the reassembly by IPA macros. That is done to overcome the static nature of graphs. Even adhesion bonds that hold molecules to each other or to tissue matrix or the cell cytostructure, eventually spontaneously dissociate under thermal activation [32]. Therefore, disassembly and reassembly of networks have important implications for the mechanisms of ligand-receptor interactions in diverse systems in general.

Fig. 2. Maximal motif (LEP-NPY-CRH) with functional interactions predicted by IPA functional analysis. Graph **A** is ordered to show motif molecules as hubs revealed by 'growth' macro connected to 49 molecules with 157 direct and indirect relationships (shading of the triad as in figure 1). Superimposed functional labels the triad molecules may be recruited for dissimilar functions. Statistical significance values computed using a Fisher's exact test based on IPA knowledge bases. Graphs **B** and **C** are predicted by IPA functional analysis. Their molecules are involved in diverse tasks (as marked in the legends). These functions exceed the random expectation of their probability as shown in p values based on Fisher's exact test. Arrowheads signify directions of interaction between nodes (e.g., binding, phosphorylation, dephosphorylation, ligand-receptor activation, or a destination of metabolic outcome for metabolic pathways). Lines with a terminal bar indicate negative regulation. In the interest of reading ease, the labels of arcs and edges, as well as self-connections were removed.

When concluded, these trials yielded a series of simplified 'deterministic' motifs that reduced to the minimum the range of functional 'choices' each connection has. In discussing the deterministic motifs in the final section, the utility of the latter as 'rehabilitation patches' of damaged network was explored.

'Tyranny of Choices' in Molecular Networks

In social psychology, such increment of individual opportunities is not considered a desirable outcome since some people become increasingly unhappy when their options expand. The tradeoffs between their various 'motivations' could not be simply accomplished by maximization of benefits. On the first glance, maximal motifs too, are likely to malfunction due to what Barry Schwartz [33] designated as the 'tyranny of choices' since the flow logic within the triad was uncertain. One cannot easily predict which connection is potentially relevant, and when or what is the temporal succession of their utilization. Since the social metaphor tells us that in society,

the 'maximizers' are the least happy soles with the fruit of their efforts, the 'misery' of flow choices might be remedied by simply removing some edges (i.e., some 'choices'). In so doing, we expect to learn what is gained after motif's connectivity is minimized and then see what happens when it is restored.

In the first step of the test shown in figure 1 A, all bidirectional arcs were deleted thereby reducing the connectivity to zero, i.e., leaving just 3 bare nodes. Subsequently, the nodes were reconnected using the IPA 'connect' or 'path exploration' macros. The latter procedures permit to probe numerous potential combinations by examining path availability, say from node A to B and then from B to A, then both of them to C, and so on (fig. 2 B). There is no way of programming to run all combinations at once. Therefore, each node had to be reconnected to the other one sequentially by brute force alone. By randomly combining nodes for reconnection (e.g., LEP → CRH; CRH → LEP; LEP, CRH → NPY; NPY → CRH, LEP, etc.), the pre-existing set of double pathways that distin-

guished the ancestral motif was not recovered. Instead, a family of derived novel structures was obtained (or edge-disjoint sets) with different connections between the old nodes in each trial and different edge functions (fig. 1 B, C). In addition to changing the direction and type of functional interactions, this procedure revealed some protein-protein connections that were not graphically identified in the ‘packaged’ maximal subgraph without scrutinizing the edge labels. By comparison, when all nodes were fed into the macro engine synchronously, the ancestral (‘maximal’) topography was readily restored as is shown in figure 1 A.

Twelve such de novo motifs that were obtained with this procedure are exemplified in figure 1 C (placed under the ancestral type). They minimize the directions and types of interaction of the nodes. In this regard, they compare favorably to the topology of the classical categories [5] in the sense that the whole group is more ‘deterministic’, save for the fact that these are original nodes that gained dissimilar connections and thus, presumably acquired another dimension to the functionality of maximal subgraphs from which they originated. In retrospect, the LEP-NPY-CRH triad proved to be an attractive choice since its topography appears to be identical to one of the subgraphs (#13) that is exemplified as a standard one among other three-node connected subgraphs [5].

Are Motifs Guardians of Functionality?

The ‘tyranny’ is a provisional term that captures the necessity of choosing a path among numerous alternative bonds in the ligand-protein network. The in-flow and out-flow traffic within the maximal formation prompts to rethink the role of maximal motifs by suggesting that in the artificial situation of ‘injury and repair,’ they represent a useful design needed to salvage the nodes by providing the alternative arcs/edges as ‘patches’. Assuming at least 10 interactions for LEP, NPY, and CRH (that are stored in the IPA knowledge base as activation, inhibition, biochemical modification, transcription, and others), as shown in arc labels (fig. 1 B, C), the maximal triad with pluripotent job scripts could be connected with any subgraph with no particular role assignment. By contrast, each novel connection is presumably performing after ‘recovery’ a different ‘analysis’ on the data provided by the neighboring node. An acceptable metaphor might be that by monopolizing its services, the nodes partially enslave one another by establishing a committed duo. In a word, by abandoning maximal choices, maximal motifs evolve into more dedicated, ‘causal’, or ‘hardwired’ cliques (e.g., [30]) that fit a single-mode operation as pictured by Milo et al. [5]. Although we concede to have no direct evidence of a causal interaction of these motifs in the present networks for they cannot have temporal segregation mode, such modulation would be consistent with the data on interaction of these peptides.

Formally, by apprising the number of edges entering each node and leaving it could describe a flow in the network. The flow could also be read from the directionality of arcs. How-

ever, it is not necessarily accurate in view of the fact that the arcs identify specific biological instructions that may not exist on the same time scale as other signals of interest in the pathway. In pharmacology, this limitation is likely to be acutely felt since drug efficacy balances time and accuracy of their delivery to the protein receptors of the cellular membrane in order to create the needed spatial summation for its signals to be securely transmitted to the needed target.

The paradox of maximal triads (if they are a ‘creation of nature’) might seem to be in generating rather than solving the difficulty in that they represent a visual example of spatial restrictions in the networks, akin to an ‘absorbing system’, i.e., a state from which the probability of exiting approaches zero. Such ‘whorls’ ought to be better avoided if signals are to progress though each node in a limited time. The arrangements of information flow through such whorls are determined not only by various types of molecules they comprise, but also by a diverse process as codified by their connections, i.e., the specific action of one node on the other. In addition, an option available for one path may also be selected for other signals. Thus, ‘maximal whorls’ could easily decelerate transmission or halt it temporarily on the model of ‘tyranny of choices’ if all options are allowed in disregard of timekeeping. What then, if anything, can be concluded about the functions of such whorls? What function should they serve, and what could plausibly gate information passing through the whorl? An answer has yet to be provided since a flow of information through it hinges on time at each of its nodes that may not perform in isolation and is theoretically bidirectional. To a point, the mission of such constellations of molecules might be compared with the recognition of real-life patterns, such as handwritten characters formulated by Oliver Selfridge 50 years ago in a whimsically named feature-analysis approach, the ‘Pandemonium model’ [34, 35].

Pandemonium in Whorls

Briefly, in the Pandemonium model, a set of narrowly tuned input feature detectors (designated as ‘demons’) recognizes each input letter [reviewed in 36]. A demon is programmed to shriek with loudness determined by the degree of fit identified. A probabilistic pandemonium of the shrieks destined to scan each icon for the presence of specific patterns, is hierarchically organized in layers of minor ‘demons’ that would ultimately reach a ‘decision-making demon’ that selects the weighted sequence to signify the best solution. A template-matching pandemonium may achieve high accuracy [37], yet matching ligands in metabolic networks is still hindered by a number of ways any two molecules could be involved with each other as mentioned above [38]. One problem is that all demons in a bi-directionally wired triad may act simultaneously and would communicate the same value signal by recognizing one another rather than a stray ligand in the network. Consequently, the binding/transmission options of their interactions may not dominate one another so that a flow would

Fig. 3. Outcome of ‘rehabilitation’ of percolated graphs. **A** Original and percolated (Aa) graphs after deletion of LEP-NPY-CRH nodes. **B** Percolated graph reconnected by IPA macro with triads #1, 3, and 6 (consult fig. 1 C). Note that some edges may change following reconnection (#1, 3), or remain unaltered (#6). Nonetheless, the bidirectional connections of the ancestral motif (as in A) were never restored. For clarity, only bold connections supposed to be activated are shown. Nodes engaged in the hypothetical changes of traffic are gradient-shaded. Abbreviations of labels and symbols as in figures 1 and 2 (© 2002–2009 Ingenuity Systems, Inc.; all rights reserved).

stall in the asset-protection performance. Still, that could be a minor difficulty if assumed that such motifs work in a context-dependent manner, so as to permit – through some form of molecular memory – to determine a preferred direction by learning new responses under nutritional pressures [39], or by taking into account the patterns of physical and/or psychological stressors [40]. However, as was pointed out, the present network was already available according to IPA functional analysis to perform very early in ontogeny for a diversity of functions. Therefore, what the ‘demons’ located somewhere in the adipose tissue, muscle, and liver, as well as those in the gastrointestinal system signal to the central controllers in the brain, might be unrelated to food or metabolic rates. Rather their messages could reflect the needs of plasticity, memory, and excitability of neuronal sites and behaviors. If the central controllers integrate all the signals based solely on their intensity, they would channel energy depositions into storage compartments inaccurately, that is, in disregard of other overlapping functions served by the major network.

The second limitation is that the pandemonium model assumes, even if implicitly, that the demons are similar to the audio-visually savvy sight-singers in the sense that they are

trained to mentally produce the frequency and duration of sounds by merely looking at the score. That specificity assumption is hardly a realistic one in a multimolecular environment where ligands are ‘hunting for features’. Although ‘spelled’ identically, each drug or a molecular signal could carry different meaning, much as ‘homographs’ do [41]. The now classical studies of David Koshland alert to the presence of a degree of binding promiscuity that violates an antiquated lock-and-key view of ligand-receptor interaction [42, 43]. The ligands may be as ‘avid’ to combine with receptors as receptors are to accommodate the stray ligands. Therefore, one protein (receptor) cannot be expected to selectively respond to a single ligand and thus resolve ambiguity. Allosteric proteins are capable of propagating conformational charges over considerable distances. That means that substrates at active sites would generate analogous changes of sufficient amplitude such as to be read by a number of unintended recipients [44]. Said differently, any design intended to recognize a set of molecules in networks should anticipate the pandemonium of the promiscuous demons assembled in motifs. Their tendency for ‘induced fit’, as well as the bind and slide processes in clustered transmembrane receptors [45], suggest a paradigm shift

in expecting a more complex scenario whereby ligands search for their places when interacting with neuronal and immunological synapses. These limitations would defy regulatory plausibility of maximal motifs. Although the IPA-based paradigm offered an automated way of determining the role of nodes in such constellations, without a special aid in plotting the directionality flow in network, this multiplicity of options can be among the most confusing.

Network Breakdown and Treatment Options

IPA permits to develop a more specific view of changes in network by removing manually the motif (LEP-NPY-CRH) from one of the graphs, as shown in figure 3, thereby causing the lattice to undergo ‘percolation’ [15, 16]. In a second step, a novel triad from the family of those exemplified in figure 1 C was used as a ‘patch’ and submitted into a ‘connect’ macro with a percolated lattice, without prior expectations of the interconnectivity pattern to emerge. It was not expected that each reintroduced node would be connected according to the Barabasi-Albert model [46] to another one with large degree. The rehabilitation connectivity logic for selected molecules is limited by the information on molecular interrelations available in the Ingenuity Knowledge Base. Therefore, the percolated graph was easily reconnected and nodes acquired their old locations (fig. 3). Yet, no motif regained the ancestral maximal topography when the nodes were introduced sequentially regardless of the order. In some cases, merely one of the arcs/edges of the novel motif was amended (e.g., #1, #3). Otherwise, no topography changes were obtained at all (e.g., #6). Only when all 3 nodes were added as patches at once, their reconnection with the native network regained the ancestral maximal topography.

It was interesting to examine how the IPA database interpreted the flow of information in the network altered by patching. Searching the path of a message requires knowing precisely where to begin. One could arbitrarily select a node as though originating a signal, but it would be difficult to guess for how long the message wanders when generated without mathematical analysis that is yet unavailable in IPA. In a small graph examined here, it was still possible to view the ‘recovered’ lattice by comparing the directionality of edges of the reconnected ‘patch’ with those of the original graph (fig. 3 A). It appears as though the rehabilitated network acquires transmission capacity that is not identical to that of the original network. That is, in one case, adding patches #1 and #6 (fig. 3 B) could be interpreted as though causing CRH to increase the OXT and insulin (INS) proteins. By comparison, adding a path #3 (fig. 3 B), LEP appeared with only inhibitory input to NPY (decreasing its release from the arcuate nucleus) as though causing disinhibition of arginine vasopressin (AVP) peptide. At present, the neurobiological significance of the changes cannot be specified other than using this new connectivity as a heuristic guide when planning to explore the molecular, biophysical, and biochemical meaning of patches

for fear-related, or appetitive situations. Such polar options may be of interest since even temporary malfunction could potentially spread through the network by re-routing information into its other sections (i.e., other part of the brain) that would conceivably cause either acute or lasting changes of some functions of interest. For example, at the cellular level, LEP was shown to influence hippocampal synaptic plasticity by enhancing NMDA (N-methyl-D-aspartate, GRIN1) receptor function [47]. Direct administration of LEP into the hippocampus can facilitate the hippocampal long-term potentiation in vivo and improve memory processing in mice thereby suggesting that LEP is a potential cognitive enhancer. A prolonged GRIN1 activation would be capable of leading to long-term plasticity changes [48, 49]. Yet, the latter may also appear as unexpected cardiovascular responses [50] or alterations of social behaviors via AVP [51]. Alternatively, NPY-expressing neurons are nearly exclusively GABAergic [52], and their activation could limit excitatory neurotransmission by decreased glutamate release in excitatory synapses and thus reduce synaptic plasticity and learning in neuronal networks [53]. These alternatives of the novel traffic are only approximations, but they may cause unexpected consequences. In the future, they would require rigorous simulations and computations such as based on Hidden Markov Models [54].

Conclusions: Between Individuality and Polypharmacy

The present case study is driven by the needs of the pharmacotherapy of obesity. A search for a therapeutically useful agent is based on the classical requirement of drug specificity. The pharmaceutical industry invests considerable resources in producing the pharmaceuticals manipulating a single target rather than searching for agents that could hit several targets at once. US patents issued between January 2001 and March 2004 with ‘obesity’ in the abstract include 1 for CRH ligands (Neurogen), 8 for LEP/Ob receptor (Millenium, SKB), and 20 for NPY, NPY1, NPY5 antagonists/modulators (Amgen, Bayer, Hoffman-La Roche, Lilly, Merck, Neurogen, Pfizer, Schering Plough, SKB) [55]. These molecules are genuinely multifunctional and as such, are implicated in a range of psychopathological conditions other than control of appetite. The same molecules also modulate the functionality of diverse brain circuits related to reproduction, sleep, memory, and cognitive functions that become aberrant in numerous ‘nonfocal disorders’ [56]. The dilemma of manipulating their state becomes even more relevant when their interactions are exposed in all of its complexity. The example of LEP-NPY-CRH triad is instructive. There is a massive literature on each of these peptides, but they are never discussed together as a therapeutically relevant functional unit. The present approach is in keeping with the need to engage multiple docking sites of molecules of interest by a single formulation since only by hitting a combination of targets can an appropriate effect be achieved [57].

An intriguing but thus far unexplored question is whether using motifs as therapeutic units is easily achievable. Such novel drug designs might be too expensive to produce in the current climate of dwindling resources [58]. Any further practical step would require making inferences and predictions based on drug parameters and formulations for their bio-availability, metabolism, and clearance. Two recent articles provided an exciting new perspective on research investigating multitargeting. Pawson and Linding [59] envisaged two ways of ligand targeting: either i) re-wiring networks with small molecules; introducing novel proteins with ligand-binding-site specificity; or ii) applying small molecules against several nodes simultaneously. The last option is the one where network analysis could aid in providing a heuristic platform. Hopkins [13] pointed out that the development of network pharmacology promises pinpointing a combination of nodes in a graph whose perturbation assures a desired therapeutic outcome without causing misbehavior of bystanders in connected but nosologically irrelevant networks. The question is whether we could define the collection of such targets and the flow of signals within a complex system, some of which may need to be synchronized on the millisecond time scale. The functionality of such drug targeting is a topic of an ongoing discussion [59]. The present study shows that formally, maximal motifs are not more than a catalogue of admission rules for nodes to function in diverse networks and transmission possibilities of their arcs as saved in IPA dataset. In this regard, as one of my readers cautioned, the methodology use is 'prone of imposing structure on data rather than finding structure in data'. Nevertheless, the redundancy of molecular connections (creating the 'tyranny of choices') exposes an intrinsic problem to contend with when designing multitargeted therapeutic agents: the need to face the scheduling of diverse interface and binding options and to overcome the predicament of protein promiscuity.

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Another advantage of 'imposing (hypothetical) structure' on network is that such formations of biomolecules could be used for testing and forecasting network resilience against damage – pharmacological, structural, or metabolic. Damaging the motif and then patching the graph with original nodes might be conceived of as a sentinel of integrity of the standard molecular groups when they are 'adopted' into a percolated lattice. The result provided thus far is that the functionality of networks so rehabilitated seems questionable since biomolecules introduced sequentially produced simplified motifs with more limited (deterministic) properties. Yet similar deterministic motifs may be pharmacologically generated 'on demand' by inactivating previously active binding sites thereby limiting the number of targets contributing to untoward effects. Maximal motifs offer an interesting testing ground for these ideas since they may be converted into reversible traps, sort of network 'traffic lights' that offer the passage of signals at the right time and under particular conditions. These possibilities have yet to be explored for obesity pharmacotherapy.

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