Systems Biology Analysis of the Endocannabinoid System Reveals a Scale-free Network with Distinct Roles for Anandamide and 2-Arachidonoylglycerol

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Abstract

We represented the endocannabinoid system (ECS) as a biological network, where ECS molecules are the nodes (123) and their interactions the links (189). ECS network follows a scale-free topology, which confers robustness against random damage, easy navigability, and controllability. Network topological parameters, such as clustering coefficient (i.e., how the nodes form clusters) of 0.0009, network diameter (the longest shortest path among all pairs of nodes) of 12, averaged number of neighbors (the mean number of connections per node) of 3.073, and characteristic path length (the expected distance between two connected nodes) of 4.715, suggested that molecular messages are transferred through the ECS network quickly and specifically. Interestingly, ~75% of nodes are located on, or are active at the level of, the cell membrane. The hubs of ECS network are anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which have also the highest value of betweenness centrality, and their removal causes network collapse into multiple disconnected components. Importantly, AEA is a ubiquitous player while 2-AG plays more restricted actions. Instead, the product of their degradation, arachidonic acid, and their hydrolyzing enzyme, fatty acid amide hydrolase, FAAH, have a marginal impact on ECS network, indeed their removal did not significantly affect its topology.

Introduction

IN RECENT YEARS, THE AVAILABILITY of new high-throughput technologies has allowed an impressive advancement of our understanding of complex biological processes. Indeed, DNA microarrays, protein chips, 2D-electrophoresis, mass spectrometry, and in general all the “omics” techniques (e.g., genomics, transcriptomics, proteomics, and lipidomics) are able to deliver a huge amount of data that, on one hand, have the potential to disclose molecular details of multifactorial mechanisms underlying biological processes but, on the other hand, still make it difficult to appreciate the role of each factor and, ultimately, its actual relevance in physiological or pathological conditions. In addition, the interpretation of the data obtained through these techniques is made difficult by the intrinsic complexity of living systems, where nonlinear interactions occur among different components (i.e., molecules and cells) and new properties emerge when shifting from the single component to the whole system (i.e., from molecules to cells, and from cells to tissues). In an attempt to overcome these problems, systems biology has been developed as a new science, aimed at studying the complex interactions within

biological systems through a multidisciplinary approach based on the computational modeling of biological data (Van Regenmortel, 2004). In particular, systems biology is effective in studying the architecture of biochemical processes that involve a large number of molecules (from hundreds to thousands, and even more), that cross-talk with each other, such as metabolic networks or cell signaling pathways (Barabasi and Oltvai, 2004).

An emerging example of such a complex network is the so-called endocannabinoid system (ECS). Virtually every day in the last 20 years, new data on endocannabinoid ligands, receptors, and metabolic enzymes appear in the literature, and the number of molecular details about ECS regulation and interactions with other signaling systems is growing at an amazing rate. In particular, in response to the question of how could the compounds contained in *Cannabis sativa* extracts (such as marijuana and hashish) possess psychoactive and therapeutic properties; in 1964, Mechoulam and colleagues in Israel isolated delta-9-tetrahydrocannabinol (Δ^9 -THC) (Gaoni and Mechoulam, 1964). Twenty-four years later, in 1988, Howlett and colleagues (Devane et al., 1988) discovered the type-1 cannabinoid (CB₁) receptor in the brain, where it

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controls the coordination of movement, emotions, memory, pain sensation, reward, food intake, and reproductive events. It was found that CB₁ modulates the release of several neurotransmitters in a retrograde manner, that is, by going backwards from the postsynaptic to the presynaptic membrane (Dalton et al., 2009).

In 1993, a novel type-2 cannabinoid (CB₂) receptor was discovered in the immune system, gastrointestinal tract, liver, kidney, bones, heart, as well as in the peripheral nervous system (Munro et al., 1993), where it was found to be engaged in a vast array of pathophysiological processes (Maccarrone, 2010). The existence of CB receptors in our body prompted research into their specific endogenous ligands. Thus, in 1992 arachidonylethanolamide (called “anandamide” [AEA] after the Sanskrit word for “joy”) was discovered as the first endocannabinoid (Devane et al., 1992), and 3 years later another major CB₁ and CB₂ ligand, 2-arachidonoylglycerol (2-AG), was demonstrated in mammals (Mechoulam et al., 1995). Since then, many molecules that bind to and activate CB₁ and CB₂ receptors have been discovered, along with additional target receptors, purported membrane transporters, and specific metabolic enzymes, making of the ECS an increasingly complex network of bioactive lipids and related proteins (Barna and Zelena, 2012; Luchicchi and Pistis, 2012). To date, solid evidence shows that the ECS plays key roles under physiological and pathological conditions, spanning from the control of memory and learning, mood, fear, food intake, immune response, and reproduction, to neuroinflammatory/neurodegenerative diseases, anxiety, depression, immune deficiency, obesity, skin disorders, and infertility (Battista et al., 2008; Downer, 2011; Gamage and Lichtman, 2012; Gorzalka and Dang, 2012; Kim et al., 2011; Riebe and Wotjak, 2011; Ruehle et al., 2012).

Here, we sought to use for the first time a systems biology-based approach to investigate the complexity of the ECS. To this aim, we built a computational model of ECS by using the graph theory formalism (Bollobas, 1979). Indeed, an ensemble of elements that interact and/or regulate each other can be represented by a mathematical object called “graph,” constituted of a variable number of nodes (the molecules) linked together by edges (interactions), thus originating a network (Albert, 2005). The edges can be undirected or directed, as in the case of metabolic and signal transduction pathways, when specifying a source (starting point) and a target (endpoint), and represent the mass or information flow (Albert, 2005). The systems biology approach was used with the aim of improving our understanding of the ECS architecture, and to disclose possible additional properties emerging from it: indeed, in complex systems such as the ECS, the whole ensemble is more than the sum of its single components.

Materials and Methods

Since a database with the molecular data concerning ECS is not currently available in deposited on-line archives, we built it up *de novo*, from the data present in recent peer-reviewed literature. In particular, the review articles retrievable from PubMed (www.ncbi.nlm.nih.gov/pubmed/) in the last 10 years were manually evaluated, using the following key words: endocannabinoids (819 articles), endocannabinoid system (1004), anandamide (349), and 2-arachidonoyl glycerol (100).

The following entries were also included in queries: AEA, 2-AG, CB₁, CB₂, G protein-coupled receptor 55 (GPR55 or CB₃), transient receptor potential cation channel subfamily V member 1 (TRPV1), peroxisome proliferator-activated receptors (PPARs), Ca²⁺, cAMP, G proteins, *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), fatty acid amide hydrolase (FAAH), diacylglycerol lipase (DAGL), monoacylglycerol lipase (MAGL), fatty acid binding protein (FABP), albumin, heat shock protein 70 (HSP70). No endocannabinoid-like compounds (palmitoylethanolamide, PEA; stearoylethanolamide, SEA, oleoylethanolamide, OEA) were included, because we chose to focus our analysis only on the AEA- and 2-AG-related elements of the ECS.

In particular, all the data regarding human and mammalian cells and tissues (including knockout mice) were considered only if confirmed by a large consensus and robust methods: the data were included in the database only when they were supported by multiple experimental evidence (at least three independent studies), possibly obtained with different experimental approaches (Bernabo et al., 2010; 2011). Freely available and diffusible molecules such as H₂O, CO₂, P_i, H⁺, and O₂ were omitted from the model, and in some cases the record represented complex events (e.g., cell adhesion or protein tyrosine phosphorylation) rather than a single molecule, because individual components of these ensembles are still unknown (Bernabo et al., 2010, 2011, 2012).

The following fields were used to build up the database: a) source molecule, representing the molecule that is the source of interaction; b) interaction, representing the nature of the connection (i.e., activation, inhibition, binding, control, degradation); c) target molecule, representing the molecule that is the target of the interaction; d) species, representing the species where the interaction was described; e) reference, representing the bibliographic source of information; f) notes, representing all notations such as the presence of synonyms or the intracellular location (when appropriate), or the explanation of complex cellular events.

Next, an independent file was built up, in order to define the attributes of each molecule. In particular, this file described: a) cellular localization, that is the area where the molecule is located or where it exerts its biological activity; b) pathway, that is the chain of events to which the molecule belongs.

The data from the two databases were used to build up ECS networks through the Cytoscape 2.8.2 software (Smoot et al., 2011). The resulting networks were spatially represented by means of the Cytoscape Force Directed Layout. In the final representation, the node size was proportional to the connection number and the node color gradient was dependent on the betweenness centrality $C_b(n)$ (Brandes, 2001). It was computed as follows:

$$C_b(n) = \sum_{s \neq n \neq t} (\sigma_{st}(n) / \sigma_{st}) \quad (1)$$

“where s and t are nodes in the network different from n , σ_{st} denotes the number of shortest paths from s to t , and $\sigma_{st}(n)$ is the number of shortest paths from s to t that n lies on” (<http://med.bioinf.mpi-inf.mpg.de/netanalyzer/help/2.7/index.html>). This parameter ranges between 0 and 1 and quantifies the centrality on a node in a network, indeed it depends on the number of shortest paths present in the network that pass through that node.

In the representation, arrows indicate the direction of the interaction. The statistical and topological analyses were carried out assuming that networks were directed by the Cytoscape plugin Network Analyzer by assessing the topological parameters listed in Table 1.

The relevance of AEA, 2-AG, arachidonic acid, and FAAH within the ECS network was ascertained by removing the corresponding nodes from the graph, and re-assessing the topological parameters.

Results

Once the ECS network has been created (Fig. 1), a statistical analysis of ECS topology was performed, and the results are shown in Table 2 and Figure 2. In addition, ECS network was investigated after removal of the nodes of AEA and 2-AG (Fig. 3, Table 2) of their hydrolysis product arachidonic acid (Fig. 4, Table 2) (Bari et al., 2006), which is the precursor of a major family of bioactive lipids, collectively termed “eicosanoids,” that include prostaglandins, prostacyclines, thromboxanes, leukotrienes, and lipoxins (Haeggstrom et al., 2010), and of and the endocannabinoids hydrolyzing enzyme, FAAH (Fig. 5, Table 2).

From the statistical analysis of main topological parameters (see Table 1) of ECS networks, it was found that the number of nodes (i.e., the total number of molecules involved) was 123, giving rise to 189 interactions (edges). The averaged clustering coefficient (CC), which is a measure of how the nodes tend to form clusters, was 0.0009 and the network diameter (i.e., the largest distance between two nodes) was 12. The averaged number of neighbors (i.e., the mean number of connection of each node) was 3.073, and the characteristic path length (i.e., the expected distance between two connected nodes) was 4.715. As shown in Table 1, the exponential equations that describe the in- and out-degree distribution [the relationship between the number of nodes (y) and the number of in- and out-edges per node (x)] were computed and the respective correlation indexes were calculated. In both cases, it was evident that the fitting value of exponential laws, calculated from logarithmized data, had high values (Fig. 2A and B). Instead, the clustering coefficient had a very low value and its distribution did not follow an exponential law ($r=0.044$, $p>0.05$), thus excluding that ECS network can be hierarchical (Fig. 2C; see discussion).

In addition, it was found that the betweenness centrality value of each node was highly correlated with its connectivity ($r=0.717$) (Fig. 2D).

The calculation of node connectivity of ECS networks allowed identification of the nodes that were the most connected, and hence represented the hubs of the system. These hubs are summarized in Table 3, which shows how AEA was by far the most connected node, followed by 2-AG.

As evident from the analysis of nodes attributes, almost three out of four nodes were directly located on the cell membrane or acted at the level of this cell compartment (74%). However, other subcellular compartments were involved to different extents in ECS regulation (cytosol: 14%, nucleus: 9%, ubiquitous: 3%).

Discussion

The unprecedented analysis of ECS topology allowed classification of the architecture of this signaling network. Indeed, different classes of networks can be clearly classified as a function of their architecture (Barabasi and Oltvai, 2004). The simplest category is that of random networks, described by the Erdős-Rényi (ER) model (Erdos and Renyi, 1959), where N nodes are connected with probability p , thus creating a network with approximately $pN(N-1)/2$ randomly placed links. The node degree (i.e., the expected number of links per node) follows a Poisson distribution, and as a consequence most of the nodes have approximately the same number of links (close to the average degree), which will define the network scale. The tail (high k region) of the degree distribution $P(k)$ decreases exponentially, thus indicating that the nodes which significantly deviate from the average are extremely rare. The clustering coefficient is independent of the node degree, and the mean path length is proportional to the logarithm of the network size: $l \sim \log N$ (Erdos and Renyi, 1959).

Instead, the networks following the Barabási-Albert (BA) model (2002) are characterized by a power-law degree distribution. Therefore, the probability that a node has k links follows the equation $P(k) \sim k^{-\gamma}$, where γ is the degree exponent. In other words, the probability that a node is highly connected is statistically higher than in a random graph. This feature implies that the network properties are strongly determined by a relatively small number of highly connected

TABLE 1. MAIN TOPOLOGICAL PARAMETERS ASSESSED IN THE PRESENT STUDY

Parameter	Definition
Number of nodes	Total number of molecules involved
Number of edges	Total number of interactions found
Clustering coefficient	Calculated as $CI = 2nI / kI(kI-1)$, where nI is the number of links connecting the kI neighbors of node I to each other; a measure of how the nodes tend to form clusters
Averaged number of neighbors	Mean number of connections of each node
Characteristic path length	Expected distance between two connected nodes
Node degree	Number of interaction of each node
In- and out-degree distribution	Probability that a selected nodes has k links: the in-degree is the number of incoming edges, the out-degree is the number of outgoing edges
γ	Exponent of in- and out-degree equation
r	Pearson correlation coefficient of node degree vs. number of nodes, on logarithmized data
R^2	Coefficient of determination of node degree vs. number of nodes, on logarithmized data

FIG. 1. Diagram showing the structure of the ECS networks. The nodes diameter is proportional to the number of links, and the colors change depending on the betweenness centrality (see text for details). The spatial network arrangement was obtained by using the Cytoscape Hierarchical Layout.

nodes (the hubs), and that a “typical” node does not exist (scale-free topology). Furthermore, in these networks, $C(k)$ is independent of k and the average path length follows the equation $l \sim \log \log N$, which is significantly shorter than the $\log N$ typical of random networks (Albert and Barabasi, 2002).

Local clustering and scale-free topology coexist in hierarchical networks, which integrate a scale-free topology with an inherent modular structure (Barabasi and Oltvai, 2004). The most important feature of these networks is the scale-free distribution of the clustering coefficient, and a hierarchical architecture implies that sparsely connected nodes are part of highly clustered areas, with a few hubs maintaining communication between different, highly clustered neighbors.

Our present results clearly show that ECS follows the BA model, an observation that appears to bring some relevant biological implications. Indeed, these networks have the following features:

a) robustness against random damage: when a random perturbation affects the ECS network, it is very likely that only a scarcely linked node (i.e., a node belonging to the most frequent class of nodes) will be affected. Thus, the probability that a hub of the graph is affected remains very low, and in our model it can be estimated that is $\sim 6.5\%$ (8 out of 132);

b) controllability: the small number of highly linked nodes implies that the whole system can be modulated with high efficiency by acting just on a few molecules, thus reducing the energetic cost and facilitating/accelerating the cell response. In comparison, if the same network had a “democratic” structure with all nodes showing the same number of links, and hence the same relevance in controlling the network, cell behavior would be controlled simultaneously by tens (or even hundreds) of different molecules (i.e., 1 to 2 orders of magnitude more than in scale-free networks). The latter condition would require an exponential increase in system complexity, and a higher energetic cost to coordinate the different signaling pathways. Incidentally, a scale-free topology offers the advantage of being more prone to external manipulations, for instance, through biotechnological or pharmacological interventions. Indeed, the identification of a limited number of hubs could be helpful in controlling the whole system, and it could be exploited to develop diagnostic or therapeutic strategies;

c) easy navigability: the virtual absence of clustering, together with the low values of the characteristic path length and of the averaged number of neighbors, confers to ECS a

TABLE 2. MAIN TOPOLOGICAL PARAMETERS OF ECS, ECS WITHOUT AEA AND 2-AG NODES, AND ECS WITHOUT ARACHIDONIC ACID NODE (SEE TEXT FOR DETAILS)

Topological parameter	ECS	ECS minus AEA and 2-AG	ECS minus arachidonic acid	ECS minus FAAH
N° nodes	123	121	122	122
N° edges	189	120	185	184
Connected components	1	19	1	1
Diameter	12	8	12	12
Clustering coefficient	0.0009	0.0130	0.0009	0.0009
Averaged n° neighbors	3.073	1.983	3.033	3.016
Characteristic path length	4.715	3.014	4.723	4.748
IN				
γ	-2.188	-1.928	-2.191	-2.190
r	0.976	0.999	0.976	0.980
R^2	0.915	0.917	0.903	0.904
OUT				
γ	-1.078	-2.084	-1.059	-1.082
r	0.965	0.999	0.953	0.963
R^2	0.694	0.985	0.684	0.691

AEA, arachidonylethanolamide; 2-AG, 2-arachidonoylglycerol.

TABLE 3. MOST CONNECTED NODES (HUBS) OF ECS NETWORK (SEE TEXT FOR DETAILS)

Node	Number of links
AEA	45
2-AG	22
Ca^{2+}_i	12
CB ₁	9
cAMP	8
Gs protein	8
CB ₂	6
TNF- α	6

AEA, arachidonylethanolamide; 2-AG, 2-arachidonoylglycerol; Ca^{2+}_i , intracellular calcium concentration; cAMP, cyclic AMP; CB₁, type-1 cannabinoid receptor; CB₂, type-2 cannabinoid receptor; Gs protein, G stimulatory proteins; TNF- α , tumor necrosis factor alpha.

FIG. 2. Graphs showing the in- (A) and out-degree distribution (B), the number of edges and the correlation between clustering coefficient and number of edges (C), and the correlation between betweenness centrality (D).

FIG. 3. Diagram showing the structure of the ECS networks after AEA and 2-AG removal. The nodes diameter is proportional to the number of links, and the colors change depending on the betweenness centrality (see text for details). The spatial network arrangement was obtained by using the Cytoscape Hierarchical Layout.

typical structure of signaling networks. Interestingly, this low value of clustering coefficient implies also a little redundancy of network, indeed randomly picking two nodes into the network there is a high probability that only a single path between them exists. This could determine a little redundancy in ECS function and could expose it to external perturbation. In addition, each molecule interacts with the others through a small number of steps, then the loss of information due to signal decrease is minimized and, consequently, the signaling efficiency is maximized. In other words, any local network perturbation will affect the whole system in a very short time, thus increasing the responsiveness to intracellular and extracellular stimuli. This condition is in keeping with the classical “small world” concept, originally observed in social studies and subsequently extended to several systems, from neural networks to the world wide web (Barabasi and Oltvai, 2004). As demonstrated by Latora and Marchiori (2001), the analysis of real data indicates that networks characterized by the small world behavior are both globally and locally highly efficient. This implies that ECS is able to elaborate and to transfer molecular messages from different cellular systems in a fast and specific way. This finding strengthens the idea that ECS acts as a virtually ubiquitous controller of integrated responses against homeostatic perturbation of the organism, as it has been documented within the central nervous system (CNS) or the immune system (Downer, 2011; Frazier, 2011; Tanaescu et al., 2010).

Moreover, as a consequence of the scale-free topology, it is possible to identify the nodes of ECS whose connectivity has a higher value, shown in Table 2. Remarkably, the two most connected nodes are the two most biologically active endocannabinoids, AEA and 2-AG, having, respectively, 45 and 22 links. On the one hand, it is not surprising that these two prototypical CB₁/CB₂ ligands play a central role in determining ECS network architecture. On the other hand, it seems

remarkable that AEA has twice the links of 2-AG, a finding that could have at least two different explanations. First, the higher number of links of AEA could partly reflect the fact that the discovery of 2-AG (1995) is more recent than that of AEA (1992). Second and more interestingly, the analysis of ECS architecture seems to suggest a different role for the two endocannabinoids, AEA acting as an ubiquitous player at multiple target receptors within the ECS network, and 2-AG playing a role as CB₁/CB₂ agonist in more specialized contexts. This unprecedented finding suggests that AEA and 2-AG are only apparently overlapping molecules, which are able to activate the same receptors and trigger the same signaling pathways (Ligresti et al., 2005), but possibly under different patho-physiological conditions. Recent experimental evidence strongly supports this hypothesis, showing that indeed AEA and 2-AG can have distinct biological actions (Luchicchi and Pistis, 2012).

The importance of AEA and 2-AG nodes within the ECS graph is pointed out also by the finding that they express the highest value of betweenness centrality (i.e., they are the ones that exert a higher control on information flow through the network, and act as a bottle neck in information transduction): all the messages to be transferred from the input terminal of the network to the output must pass through one or both of them. Together, AEA and 2-AG support more than one third (67 out of 182) of all the network links.

Other nodes appear to play a major role in controlling ECS architecture. Intracellular calcium, a well-known second messenger of signal transduction pathways, is central also in the ECS network. In this context, it is noteworthy that in many cell types, the so-called “excitable cells,” the intracellular calcium concentration directly controls cell functionality, for instance, in neurons where neurotransmitter release is a calcium-dependent process (Gleichmann and Mattson, 2011), or in sperm cells where the increase of calcium concentration

FIG. 4. Diagram showing the structure of the ECS networks after arachidonic acid removal. The nodes diameter is proportional to the number of links, and the colors change depending on the betweenness centrality (see text for details). The spatial network arrangement was obtained by using the Cytoscape Hierarchical Layout.

FIG. 5. Diagram showing the structure of the ECS network after FAAH removal. The nodes diameter is proportional to the number of links, and the colors change depending on the betweenness centrality (see text for details). The spatial network arrangement was obtained by using the Cytoscape Hierarchical Layout.

causes acrosome exocytosis during the acrosome reaction (Darszon et al., 2011). In both neurons and sperm the ECS has been shown to have impressive biological effects (Maccarrone, 2009; Maccarrone et al., 2005; Schuel and Burkman, 2005).

Another universal second messenger like cAMP, that is downstream CB₁, CB₂ and type 1 vanilloid receptors, is also an important hub of the ECS graph, and so are the classical cannabinoid receptors, CB₁ and CB₂. The latter act as message transducers, which receive the input message (the ligand) and produce an output response, usually by inducing a modification in the concentration of second messengers, like cAMP (Clapham et al., 2003).

An additional interesting observation is the central role in the ECS network of tumor necrosis factor alpha (TNF- α), a cytokine involved in inflammation and in promoting the acute phase reaction of immune cells. Recent findings demonstrate that TNF- α is engaged by endocannabinoid signaling, for instance, in neuroinflammation, cell adhesion, and immune cells function (Cencioni et al., 2010; Nilsson et al., 2006; Rossi et al., 2011; Rubio-Araiz et al., 2008; Yang et al., 2009), thus pointing out the important role of ECS in modulating these cytokine-mediated events.

Remarkably, ECS topology was not significantly affected by the removal of arachidonic acid (Table I), supporting accumulated experimental evidence that the biological activity of endocannabinoids is distinct from that of this fatty acid (Basavarajappa, 2007; Di Marzo, 2008). Instead, network integrity was completely destroyed by the removal of AEA and 2-AG, in that the network was reduced from 1 connected component to 19 (Figure 1 and Table 2).

Analogously, an important element of ECS like fatty acid amide hydrolase (FAAH), which controls the endogenous tone of AEA *in vivo* (Cravatt et al., 2004) and also cleaves 2-AG (Fowler et al., 2001), was not a hub, on the basis of its connectivity. Indeed, the threshold limit to identify a hub was set at 6 links per node (Vallabhajosyula et al., 2009), and FAAH had only 5 much alike other important molecules (phospholipase C, PLC; N-methyl-D-aspartate receptors, K⁺, N-arachidonoyl-phosphatidylethanolamine, PPAR α and γ). This observation is consistent with the finding that removal of the FAAH node from the ECS network did not significantly alter its topology (Table 2). Interestingly, even if in our model, FAAH is not a hub of ECS networks (i.e., it is not a super-connected node), it seems to exert a strong control on network

function by directly modulating the concentration of AEA, which is the most connected node.

Importantly we think that, on one hand, the availability of molecular tools (such as specific inhibitors) and of animal models (particularly KO mice) could justify the research effort on a specific topic and, as a consequence, the amount of available information; yet, on the other hand, we are confident that the number of publications on each topic should only have a limited influence on the number of links per node. Indeed, in a recent, independent study, we have assessed the correlation of the number of articles on different topics (i.e., the smooth and striated muscle contraction, the neurotransmitter release cycle of six neurotransmitters [norepinephrine, acetylcholine, γ -aminobutyric acid (GABA), serotonin, glutamate, and dopamine], the visual phototransduction (rods), the sperm capacitation, the insulin signaling pathway, the p53 pathway, the regulation of retinoblastoma protein (pRb), the mitochondrial ATP metabolism, the glucose metabolism, the signaling events mediated by stem cell factor receptor c-Kit (CD117), and the circadian clock) with the topological parameters of corresponding networks, and we have found no statistically significant correlation (Bernabo et al., 2013).

Another major outcome of the systems biology analysis of the ECS is that it is a largely membrane-related network: ~75% of nodes are directly located on the cell membrane, or exert their activity on this cell compartment. This seems noteworthy when considering ECS regulation, that is likely to depend on the membrane environment, besides depending on control proteins. In line with this, very recently the role of membrane lipid composition as a key determinant in driving endocannabinoid signaling has attracted attention, leading to the finding that cholesterol controls CB₁ activity in membrane subdomains known as “lipid rafts” (Bari et al., 2008; Botto et al., 2010; Dainese et al., 2007; Grimaldi and Capasso, 2012; Maccarrone et al., 2009; Rimmerman et al., 2012).

As a perspective, we believe that the use of a dynamic networks-based approach (He et al., 2011; Zhu et al., 2007) and the study of ECS networks in different cell types and tissues will provide additional important insights into the complexity of their biological activity.

Conclusions

In conclusion, our unprecedented systems biology analysis of the ECS shows that: a) it is possible to build up a biological

network that represents the ECS complexity, thus opening the perspective of the use of such a computational approach to study a lipid signaling system of pivotal importance in several physiological and pathological processes; b) the ECS network architecture has the features of the classical scale-free model, that confers to ECS a high robustness against random failures, a high controllability, and an easy navigability; c) AEA and 2-AG are the true core of ECS. They act as a funnel in filtering and transferring the information within the network, and cooperate in different contexts. Apparently, the ECS galaxy of components turns around these two major stars.

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Author Disclosure Statement

The authors declare that there are no conflicting financial interests.

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