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Logical modeling and steady state analysis of serotonin signaling pathway

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Abstract

Brain serotonin seems to have distinctive actions contributing to impulsive behavior and anxiety. Patients with evidence of low serotonin levels show mild to moderate depression, which can lead to symptoms like anxiety, apathy, fear, feelings of worthlessness, insomnia and fatigue. The signaling pathway responsible for the action of serotonin was drawn using pathway modeling methods from the literature. It was then used as an input in CellNetAnalyzer (CNA) for logical analysis. CNA is a graphical user interface for MATLAB, providing a comprehensive toolbox for structural and functional analysis of different types of cellular networks. CNA has been programmed with the MATLAB language enabling to use inbuilt functions of MATLAB. The pathway was scrutinized using the various tools in CNA and the results obtained were compared with the results published in previous reports. The various analyses include interaction matrix, dependencies of various species on each other, logical steady state analysis. As a result, a major compound responsible for mood enhancement was identified as cAMP-response element-binding protein (CREB), a transcription factor that activates brain derived neurotropic factor (BDNF) genes responsible for the propagation of neurons.

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Introduction

Serotonin (5-hydroxytryptamine or 5-HT) is a monoamine neurotransmitter synthesized in serotonergic neurons in the central nervous system (CNS) and enterochromaffin cells in the gastrointestinal tract of animals, including humans (Pithadia *et al.,* 2009).In the body, serotonin is synthesized from the amino acid tryptophan by a short metabolic pathway consisting of two enzymes: tryptophan hydroxylase (TPH) and amino acid decarboxylase (DDC) (Hare and Loer, 2004). The richest concentration of serotonin in the body can be found in the pineal body(Gunaratna *et al.,* 2006). Release of serotonin causes the other nerve to fire and continue the message along the neuron network (Dayan and Huys, 2009). Serotonin release is triggered by a carbohydrate load (sugar, etc.) and there are many who feel that eating carbohydrates under stress is aimed at this serotonin release (Singh, 2016). This is because carbohydrates increase tryptophan levels in the brain, hence increasing serotonin concentration. When the brain produces serotonin, tension is eased. Serotonin seems to have distinctive actions contributing to anxiety and impulsive behavior (Wurtman and Wurtman, 1995). Patients with evidence of low Serotonin levels show mild to moderate depression, which can lead to symptoms like anxiety, apathy, fear, feelings of worthlessness, insomnia and fatigue (Wurtman and Wurtman, 1995). Each of such biological effects of 5-HT is initiated by signaling through one or more of the multiple serotonin receptors. To develop a mechanistic understanding of these biological effects, it is imperative to analyze the signaling cascades stimulated by the serotonin receptors.

Serotonin Receptors

The 5-HT system is one of the main targets for the pharmacological treatment of several psychiatric disorders. In particular, the selective serotonin reuptake inhibitors (SSRIs) are used in the treatment of depression and a variety of other conditions, including obsessive compulsive and panic disorder (Nathan and Gorman, 2015). Knowledge of the detailed distribution of different 5-HT receptor subtypes in the human brain could be relevant for the understanding of the role of 5-HT in the pathophysiology of psychiatric disorders and for the development of psychoactive drugs targeting the 5-HT system (Lesch, 2001). To date, seven serotonin receptor families have been identified which are further subdivided into at least 14 distinct receptor subtypes based on pharmacological and structural characteristics, and transductional mechanisms asdepicted in Figure 1 and Table 1. Except for the 5-HT3 receptor, which is a ligand-gated ion channel, all known 5-HT receptors are G-protein coupled.

Role in mood enhancement

Serotonin plays a major role in mood enhancement. People with mood disorders like depression, etc. have fewer levels of serotonin and dopamine in their brains. Looking into how serotonin plays a role in mood enhancement we can analyze the various signaling pathways mediated by serotonin in the brain (Martinowich and Lu, 2008).

Components of cyclic AMP (cAMP) signaling were examined in postmortem cerebral cortex of a well characterized group of patients with mood disorders and nonpsychiatric control subjects (Dowlatshahi *et al.,* 1999). The G protein levels, adenylyl cyclase (AC) activity, and CREB levels in cerebral cortex of the subjects with respect to diagnosis, treatment, and suicide was analyzed. There was a trend towards decreased stimulated adenylyl cyclase activity in subjects with mood disorders relative to control subjects. A significant effect of suicide on temporal cortex CREB levels in subjects that died as a result of suicide relative to those that did not was detected, which was more evident in patients with major depressive disorder (Dowlatshahi *et al.,*1999).Serotonin through its signaling pathways increases the concentration of CREB in the brain, thus reducing major depressive disorders.

Signal Pathway Involved in Mood Enhancement

When serotonin binds to the 5-HT4 receptors on the post synaptic neuron, the Gs alpha subunit (or Gs protein), a heterotrimeric G protein subunit which

activates adenylate cyclase is released. The general function of G protein is to activate adenylate cyclase, which, in turn, produces cAMP, which, in turn activates cAMP-dependent protein kinase. cAMP dependent pathway, also known as Adenylyl Cyclase pathway, is a G proteinlinked receptor signaling pathway in cell communication (Sette and Conti, 1996). This pathway can activate enzymes and turn on genes. This pathway can lead to either the reactivation of existing proteins or gene expression.
Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor found in the brain and the periphery. It is a protein that acts on certain neurons of the central nervous system and the peripheral nervous system that helps to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses (Karege*et al.,* 2002). In the brain, it is active in the hippocampus, cortex, and basal forebrain-areas vital to learning, memory, and higher thinking. BDNF was the second neurotrophic factor to be characterized after nerve growth factor (NGF). Although, the vast majority of neurons in the mammalian brain is formed prenatally, parts of the adult brain retain the ability to grow new neurons from neural stem cells in a process known as neurogenesis. Neurotrophins are chemicals that help to stimulate and control neurogenesis, BDNF being one of the most active. Mice born without the ability to make BDNF, suffers developmental defects in the brain and sensory nervous system, and usually die soon after birth, suggesting that BDNF plays an important role in normal neural development.

In this study, we concentrate on the effects of serotonin in mood enhancement, especially depression and behavioral effects like suicidal tendencies. The effects of serotonin are determined by the distribution and number of the various serotonin receptors in the brain (Pazos *et al.,* 1985).

Materials and methods

CellNetAnalyzer

CellNetAnalyzer (CNA) is a graphical user interface for MATLAB, providing a comprehensive toolbox for structural and functional analysis of different types of cellular networks (Klamt *et al.,* 2007). CNA extends its predecessor FluxAnalyzer, originally developed for metabolic network analysis, by new methods for signaling and regulatory networks, i.e. for networks where signal flows are dominating (in contrast to mass flows in metabolic networks). It is widely used for numerical computations and complex visualizations of numerical data. CNA has been programmed with the MATLAB language enabling to use built-in functions of MATLAB, in particular those for matrix operations. As, CNA runs in the MATLAB environment and because MATLAB is available for many operating systems, CNA itself is platform-independent (Klamt *et al.,* 2007). Upon starting CNA on MATLAB's command window, CNA runs virtually autonomously as a graphical user interface.

CAN provides a single suite to perform structural and qualitative analysis of both mass-flow and signal-flow based cellular networks in a user-friendly environment (Klamt *et al.,* 2007). It provides a large tool box with various, partially unique, functions and algorithms for functional network analysis. As a fundamental step, CNA facilitates the construction of network projects which can represent either a mass-flow (stoichiometric, metabolic) or a signal-flow (signal transduction/regulatory) network (Klamt *et al.,* 2007). For both types of networks, the abstract network model can be set-up and edited via a Network Composer.

The network description has comprised always the declaration of species and reactions and their respective attributes. The same nomenclature is used for both kinds of networks, but with a different meaning. In mass-flow networks (MFNs), reactions correspond to the stoichiometric conversions (Klamt *et al.,* 2007). Therefore, the reaction equation is interpreted as usual in MFNs, namely that the two reactants A and B are converted into C as shown below:

$$A + B \rightarrow C \quad (1)$$

A and B is consumed in this process representing the key characteristic of mass flows. MFNs are stored by the stoichiometric matrix and other variables such as capacity and the reversibility constraints of the reactions.

In contrast, a reaction (or interaction in this context) in signal flow networks (SFNs) express how the "product" (end node) of the reaction can be activated by the "reactants" (start nodes). Accordingly, equation (1) means in SFNs, C becomes activated if A AND B is active, i.e. "+" is interpreted as a logical AND. As a key characteristic of signal flows, the start nodes A and B on the left-hand side of the reaction equation are not necessarily consumed in this process. For example, eq. (1) can express that C becomes activated (e.g. phosphorylated at two phosphorylation sites) if the two kinases A and B are in an active state. Consequently, in CNA, an SFN is a logical (Boolean) network, where each reaction represents a Boolean AND clause expressing a condition under which the end node of this reaction becomes activated. If several reaction pointsare at one species S, then they are OR together, givingeventually rise to a Boolean function determining the activation state of S. In addition to AND and OR, representing arbitrary Boolean functions requires a NOT operator (indicated by "!"). Furthermore, multivalued logic is possible in CNA. Therefore, the equation depicted below:

$3A + !B \rightarrow 2C$ (2)

reveals that "C reaches level 2 if A is at level 3 AND B is inactive (level 0)". Using the formalism described above, CNA represents the logic of SFNs as logical interaction hypergraphs (strongly related with the sum-of-product or disjunctive normal form (DNF) representation of Boolean functions) which can be conveniently stored in two matrices, each having as rows the species and as columns the interactions: an interaction matrix captures the logical coefficients and a NOT-matrix stores where a NOT operation occurs.

In principle, network analysis with CNA can be done without any graphical representation of the network (Klamt *et al.,* 2007). However, for visualization purposes, each network project can be assigned one or several network maps. Network maps must be provided by the user. Like in this case, we made a diagrammatic representation of the serotonin signaling pathway.

This was fed into CNA for further analysis. CNA does not impose any restrictions on the design of these maps what is possible because the maps can be created with arbitrary (external) drawing tools.

The link between network maps and network model is realized in CNA by text boxes, which are small user interfaces, each associated with one network element. They can be positioned via drag-and-drop, at a proper place on the maps and enable input and output of context dependent data. In CNA, a network map with text boxes is called an interactive network map.

After loading a network the text boxes display the default values. The sign “#” indicates an unknown (undefined) value. Now, we can perform calculations provided by the menu “CellNetAnalyzer”. Once the entire network map is defined, the various species and interactions involved can be defined and edited illustrated in Figure 2.

The position of the text boxes can be fixed using Get x/y-Pos option. The visibility of the text boxes can also be altered so that complex and large network maps do not become crowded.

CNA provides a number of functions for studying signaling networks (Klamt et al., 2007). It is partitioned into several groups of functions and can be called via the menu item “CellNetAnalyzer” within the interactive maps. The menu is partitioned into the following groups, where (*) and (**) comprising the core functions for exploring the functional properties of the interaction network: Editing/Viewing the network structure and properties, Saving/Setting/Loading scenarios, Clipboard and arithmetic operations, Basic properties of the network structure, Tools for analyzing the underlying interaction graph (*), and Logical steady state analysis (**), Miscellaneous.

Results and Discussion

Pathway modeling

Thesignaling pathways responsible for the action of serotonin as an antidepressant were identified and drawn with reference shown in Figure 2.

Network composer

Network composer opens the main window for editing and saving the network structure described in Figure 3.

The equation defined in this e.g. is 5-HT binding to the 5-HT 2a receptor to activate the Gq protein. Using, this option we can define the various species and reactions occurring in the signal pathway.

Table 1. Various serotonin receptors and their functions.

Family	Type	Actions	Mechanism
5-HT_1	G_i/G_o coupled	neuronal inhibition, behavioural effects (sleep, thermoregulation, aggression, anxiety)	Decreasing cellular levels of cAMP
5-HT_2	G_q/G_{11} coupled	neuronal excitation, behavioural effects, learning, anxiety	Increasing cellular levels of inositol trisphosphate (IP_3) and diacylglycerol (DAG)
5-HT_3	Ligand-gated Na^+& K^+cation channel	neuronal excitation, anxiety, emesis	Depolarizing plasma membrane
5-HT_4	G_s coupled	Neuronal excitation, Gastrointestinal motility, behavioral effects	Increasing cellular levels of cAMP
5-HT_{5A}	Gi/o	unknown	Inhibiting adenylatecyclase activity.
5-HT_7	G_s coupled	unknown	Increasing cellular levels of cAMP

Basic properties of the network structure

Interaction matrix

The interaction matrix is displayed graphically in Figure 4, gives a quick overview of the network structure. The rows of the displayed matrix correspond to the species (names given on the left boundary of the window) and the columns to the reactions/interactions (names given to the lower boundary of the window). A red matrix element *eij* corresponds to a species *i* which has an inhibiting influence (NOT operation) in the j-th interaction. Analogously, a green matrix element *eij* corresponds to a species i which have an activating influence in interaction *j*. A blue matrix element *eij* corresponds to the species *i* which is activated in reaction*j*.

Table 2. Types of G-Proteins.

Family	Member	Action mediated	Functions
I	G_s	α	Activate adenylyl cylclase
II	G_i	α	Inhibit adnylylcyclase
		βγ	Activates K^+ channel
	G_o	βγ	Activates K^+ channel, inactivate Ca^{2+} channels
		α and βγ	Activates phospholipase C-β
	G_t	α	Activates cyclic GMP phosphodiesterase
III	G_q	α	Activates phospholipase C-β

A black matrix element *eij* indicates that the species *i* does not participate in reaction *j*. The connectivity number of each species (number of interactions where the species are involved) is given on the right boundary of the window (number in brackets, number of interactions where the species activates/inhibits/is activated). If the network is very large (more than 120 reactions or/and species) the display of this matrix might fail or become unreadable.

Tools for analyzing the underlying interaction graph

Shortest paths and species dependencies

Shortest paths and species dependencies has a similar function as "Graph-theoretical path lengths" in mass-flow networks, but it's more powerful. It enables the calculation of graphtheoretical (shortest) path lengths between all species, separately for negative and

positive paths from which then (by taking the minimum of both) the unsigned shortest path is also computed. Additionally, the average path length and the network diameter is computed (for the unsigned shortest path length).

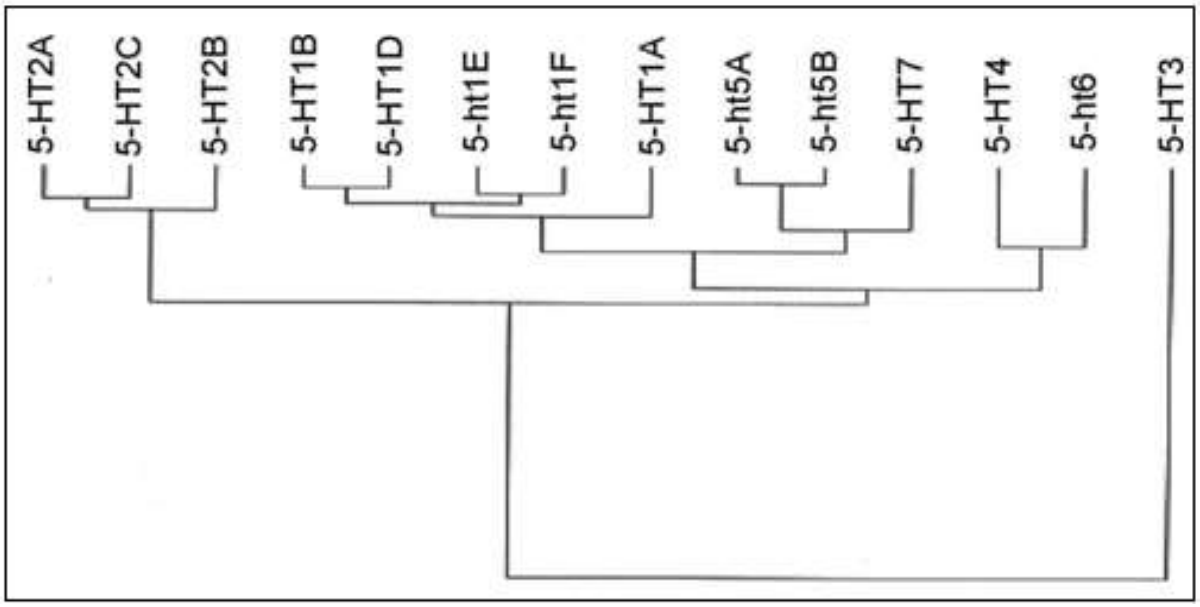

Fig. 1. Phylogenetic tree showing the relationship between human 5-HT receptor protein sequences (except 5-HT5 receptors, which are murine in origin). The length of each branch correlates to the evolutionary distance between receptor subtypes.

Furthermore, a dependency matrix is determined (see below) which is extremely helpful for detecting functional dependencies between each pair of species. Note that a signal-flow network in CNA is a Boolean network represented as a hypergraph.

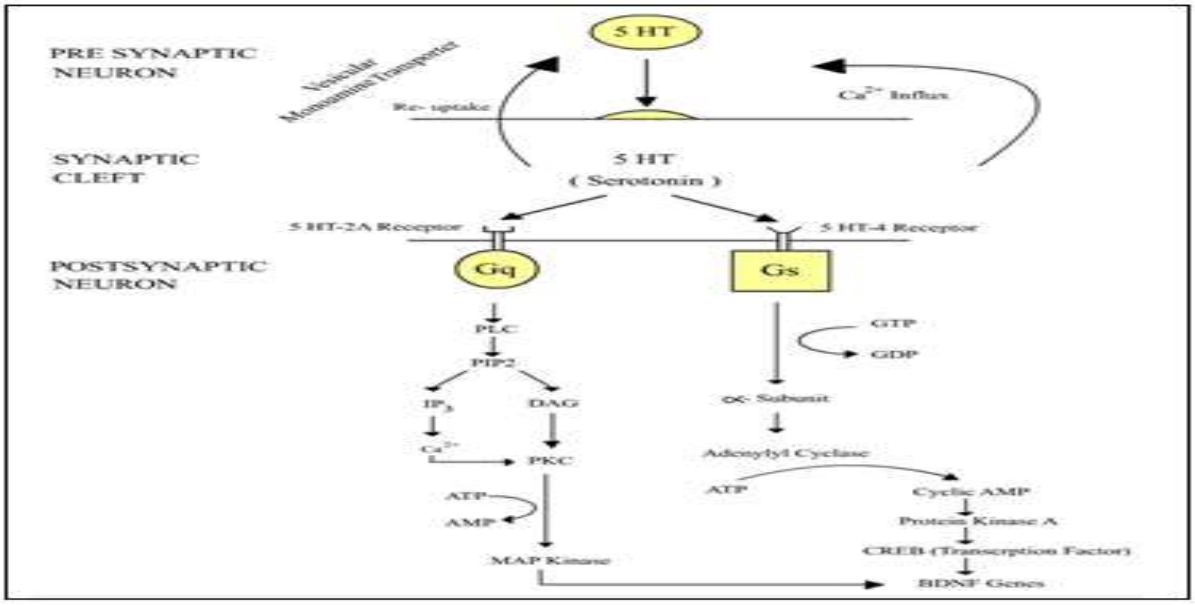

Fig. 2. Serotonin signaling pathway model as an antidepressant.

Therefore, for applying graph-theoretical methods, this hypergraph are temporarily transformed into its underlying interaction graph (see also "Convert to interaction graph"). Regarding the interpretation, it is important to realize that the shortest paths as computed by this function to indicate whether there is a positive/negative/any influence of a species A to B, without a guarantee that the path is active under a given scenario in the logical model. Note that shortest path algorithm work well also in large networks.

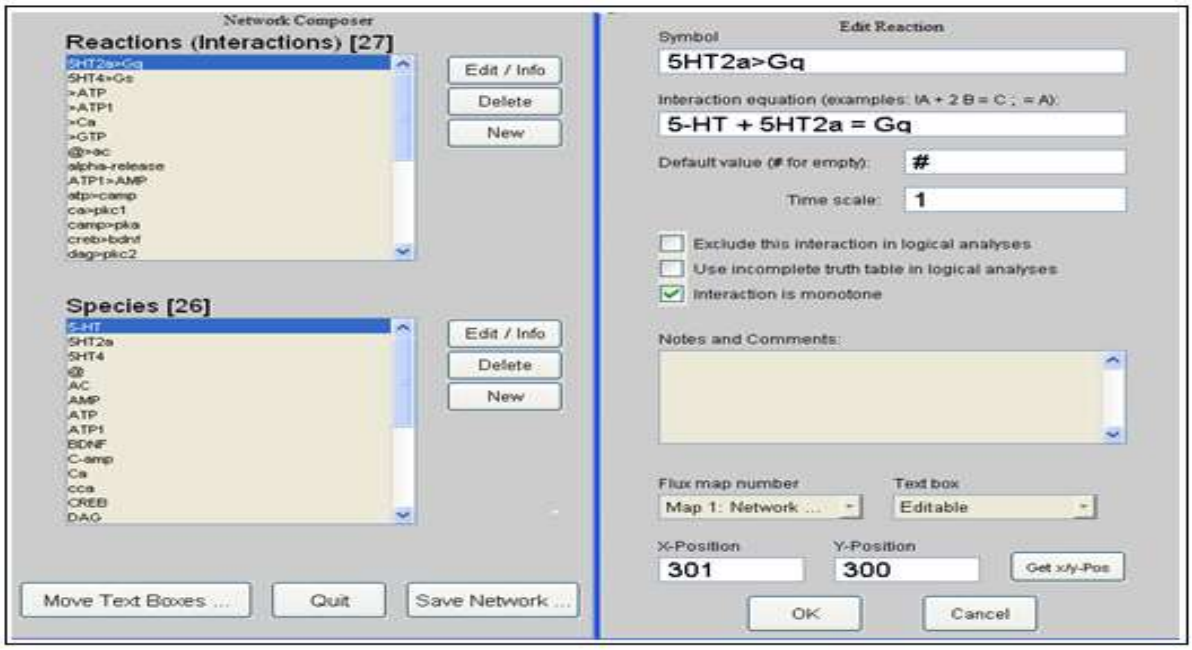

Fig. 3. Paradigm of network construction for species and reactions in the serotonin pathway.

Basic topological properties

This function is particularly useful to detect errors in the network structure that might have been inserted during network construction.

a) Species participating in none of the interactions are detected.

b) Sources and Sinks: Species that are not influenced by others (=sources: no predecessor) orthat do not influence others (=sinks: no successor) are detected.

c) Input Arcs and Output Arcs: Interactions with an empty left-hand side (INPUT) or with anempty right-hand side (OUTPUT) in their interaction equations are identified. These arcstypically represent signal flows across system boundaries.

d) Parallel Reactions: Reactions with the same participating species are detected.

Shortest paths and species dependencies

It enables the calculation of graph-theoretical (shortest) path lengths between all species, separately for negative and positive paths from which then (by taking the minimum of both) the unsigned shortest path is also computed.

Additionally, the average path length and the network diameter are computed (for the unsigned shortest path length).

A dependency matrix is determined which is extremely helpful for detecting functional dependencies between each pair of species.

The classification is displayed in the species text boxes, also illustrated by different box colors.

In the second case, the converse relationships are determined (for which compounds is the specified species an (total) activator, (total) inhibitor, ambivalent factor or non-influencing) and then displayed in the species text boxes.

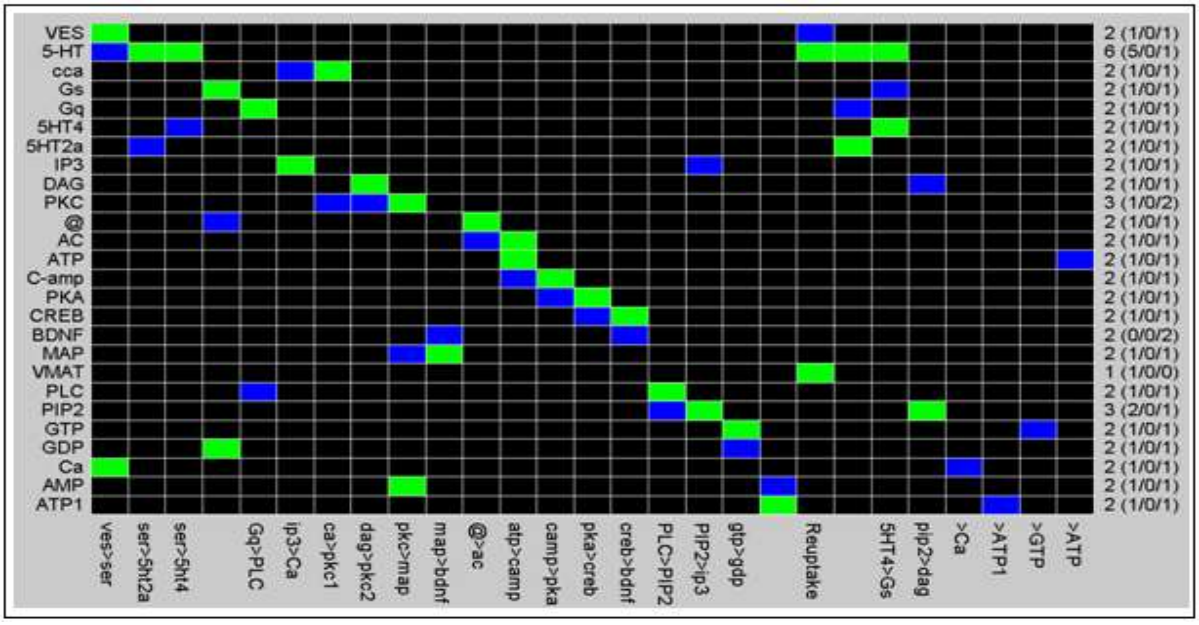

Fig. 4. Graphical representation of Interaction matrix.

Distance Matrix

Distance Matrix displays in three separate windows the shortest positive/ negative/unsigned path lengths (distances) as colored arrays. The array element eAB represents the shortest pathlength from A to B. The larger the path length,the brighter will be array element. A blue cell marks species pairs, between which no path exists.

Shortest Positive Paths

Shortest positive path is represented in Figure 5.

Dependency matrix

Dependency matrix is illustrated in Figure 6. The color of the matrix element eAB indicates one of 6 possible types of dependency: (1) black: no influence of A to B, (2) yellow: A has activating and inhibiting effect on B, (3) light red: A is pure inhibitor of B, (4) light green: A is pure activator of B, (5) dark red: A is a total inhibitor of B), (6) dark green: A is a total activator of B.

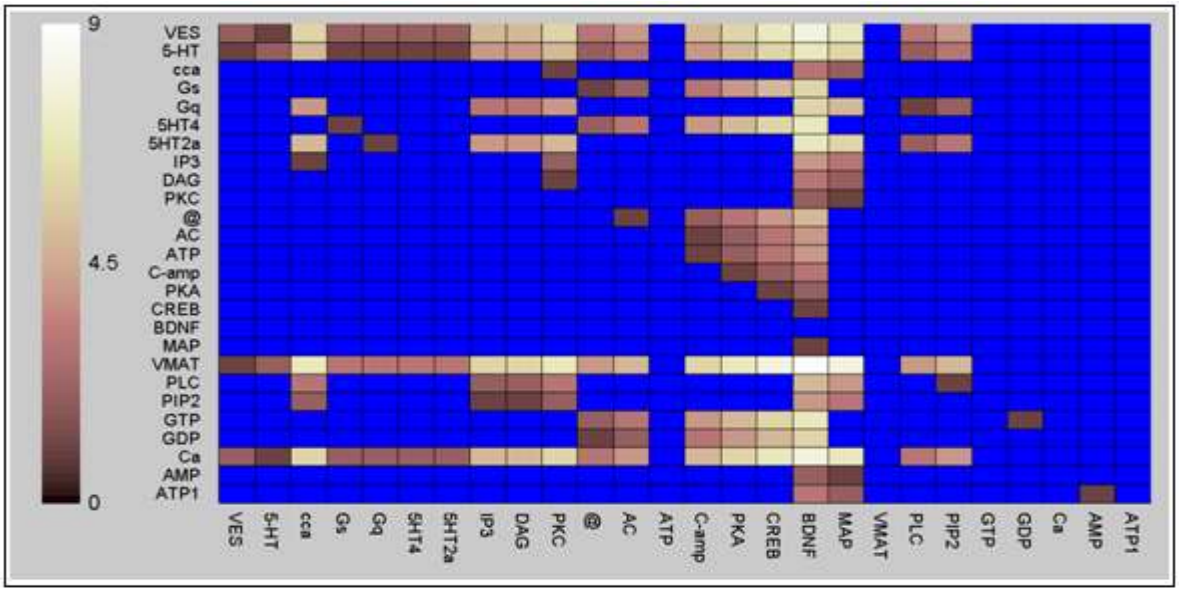

Fig. 5. Shortest positive path length of the network structure.

Since there isno inhibitor in the pathway shown in Figure 6, it is inferred that all the compounds present in the pathways are activators. The green color signifies that all species present is total activators for BDNF.

Dependency of BDNF gene expression on all species

Using CNA, the dependency of BDNF on all the compounds present in the signal pathways is calculated. Figure 7(a) represents all of the compounds as total activators (TA) of BDNF.

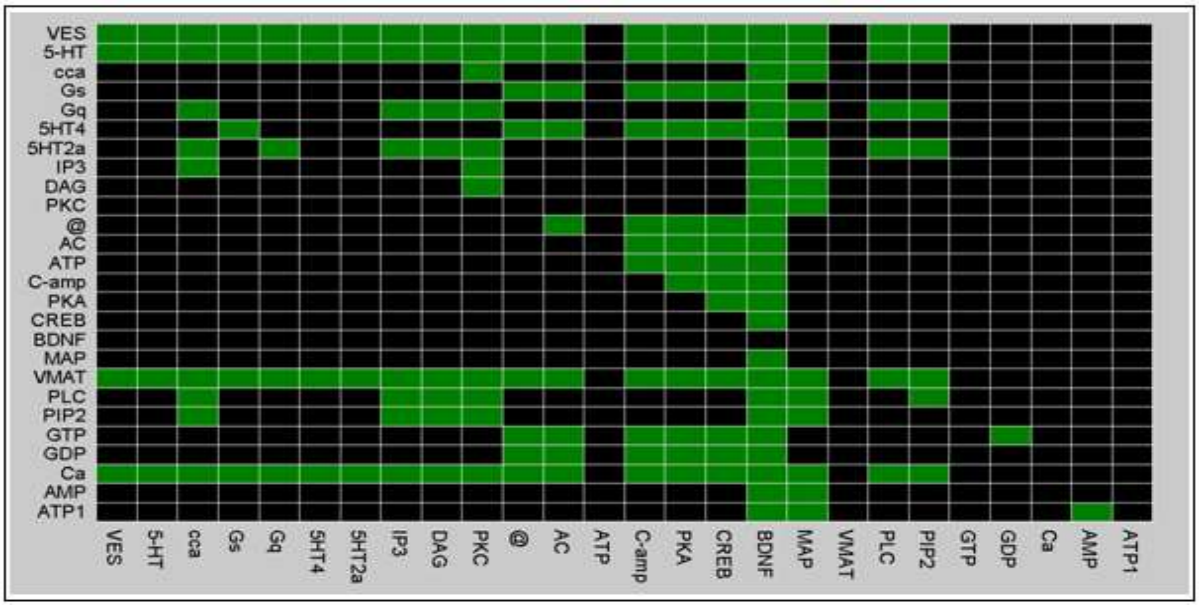

Fig. 6. Depiction of dependency matrix pathway.

Influence of 5-HT on all Species

Since, ATP, Ca and GTP are not dependent on 5-HT. The symbol (N) representing that there is no influence is displayed in the text box shown in Figure 7(b).

Logical steady state analysis

Compute logical steady state

Compute logical steady state takes the currently defined reaction values (=fixed signal flows) and species values (= fixed states) and computes from this information,as far as uniquely possible resulting logical steady state of the species as it follows from the Boolean network structure. This function is in a certain sense similar to metabolic flux analysis. First, the user may fix some states of species (which is particularly relevant to INPUT species and for indicating knock-outs or knock-ins). Additionally, the user may enter values for signal flows along some interactions. This is particularly relevant for INPUT, but it might also be useful to deactivate certain interactions by setting the signal flow along this interaction to zero. Note that only INPUT arcs are allowed to have a zero or non-zero value, whereas all other interactions may only be assigned a zero (non-zero value will be ignored in the latter case).

So the evaluation order of a given species/reaction values is as follows:

a) If a species value has been defined, then the logical state of this species will be fixed to that value; any given value for a reaction that points into that species will be ignored.

b) A reaction will be considered to be off (i.e. temporarily removed) if a signal flow of zero has been defined for that reaction (note: if all reactions pointing into a species have been set to zero, this species will be assigned a zero in steady state, i.e. this is analogous to set the respective species value to zero).

c) If a non-zero value has been defined for an INPUT arc, then the state of the node connected to this INPUT arc will be fixed to that value. If non-zero values for several INPUT arcs pointing into one and the same species have been defined than the one with

the highest activation level will be taken to fix the state of the species. If a non-zero value has been defined for at least one INPUT arc all other signals flows pointing into that reaction will have no effect on the logical steady state of this species.

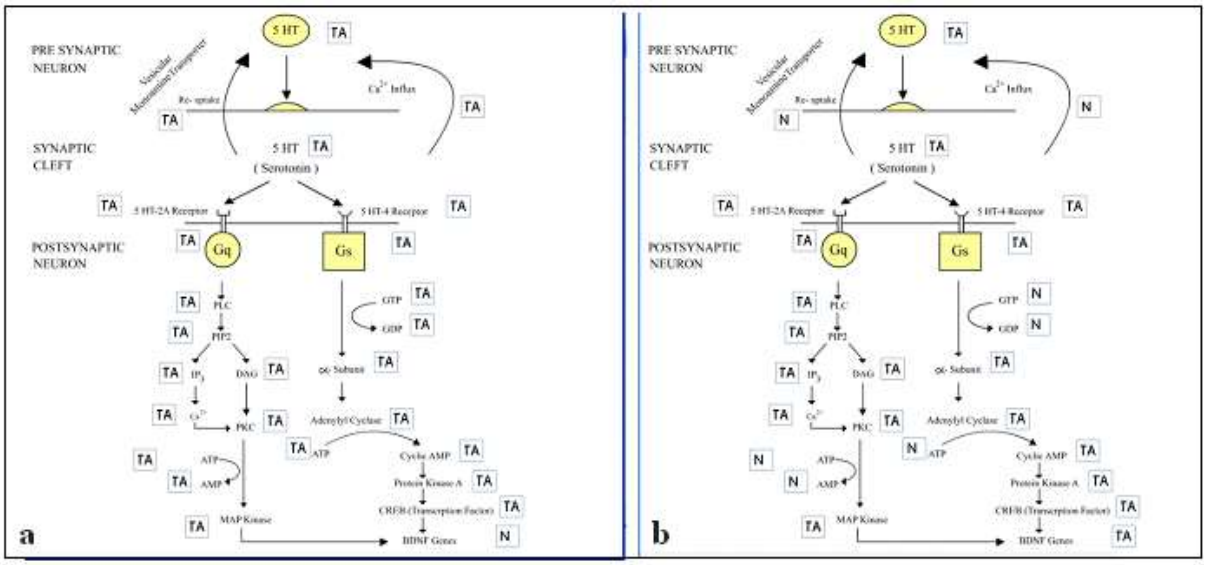

Fig. 7. Illustrative pathway of BDNF compounds.

Thus, if you have two interactions "= A" and "B = 2 A" then setting a "1" for the first interaction and letting the second undefined means that A will be fixed to 1 irrespective whether B will be assigned a value of 1 or not. Furthermore, for an INPUT arc of type " = 2 A", setting any value different to "2" will be automatically converted to "2" in order to be consistent with the level of the signal level flowing along this reaction.

a) Setting a non-zero value for reactions that are not INPUT arcs will be ignored.

Upon defining fixed species and reaction values and starting this function, the logical steady states resulting from the given scenario are computed. The algorithm tries iteratively to derive (partial) logical steady states for the species until no further update is possible.

Important remarks

a) The logical steady state resulting from a scenario depends heavily on the fixed signal flows and species states.

b) Note, that a (global) logical steady state might not exist at all for a given scenario or that it cannot be resolved completely. For example, setting only the signal flow of input arc at I2 to one and everything else as unknown, nothing could be concluded. Setting instead the incoming signal flow at I1 to 1, we could derive at least some partial logical steady states.

a) There are a few cases where CNA does not identify existing logical steady state values. CNA does not (yet) check whether positive feedback loops are self-sustaining by initial state values of participating species (for example, in a dynamic Boolean network model, assuming initial values of "1" for F and G would result in partial logical steady states F=G=1 due to the positive feedback loop between F and G). Positive feedback loops can, however, be identified by the algorithms described above and therefore be checked by the user himself. There might be some other, for cellular networks very unlikely situations where existing logical steady states are not identified. Certainly, all steady state values that have been found by CNA are definitely correct.

A logical steady state network map shown in Figure 8, calculated with values of Ca^{2+}, 5-HT, GTP and ATP defined as 1. The whole pathway gets activated and the end product BDNF is at level 1. This is because all compounds are total activators of BDNF thus resulting in its activation.

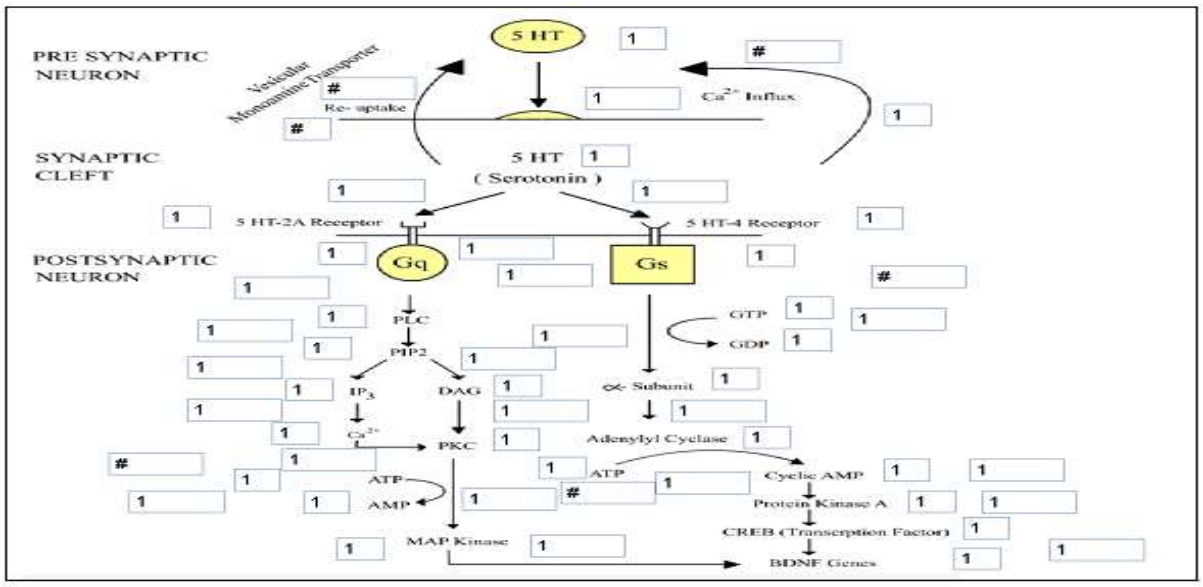

Fig. 8. A logical steady state network map using CAN.

Elementary modes

A mode is a complete set of independent reactions from substrate to product in a pathway. The elementary mode menu shows us the number of modes in the signaling pathway. Paths and loops can be displayed subsequently in the network by using the "Next" and "Previous" button. Involved reactions on a path are indicated by a value "1".

No of Modes cal: 57

No of Positive Modes: 1

No of Negative Modes: 0

No of Feedback Loops: 1

Positive Mode

A positive mode is one which has an activating influence on the following compound in the pathway depicted in Figure 9(a).

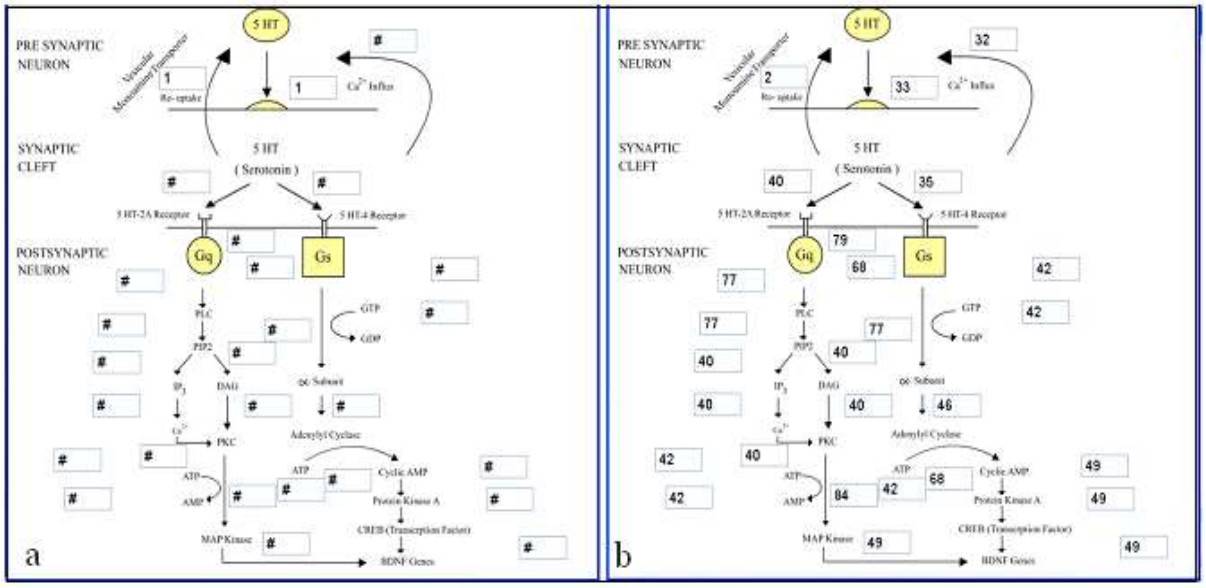

Fig. 9. Network map pathway of BDNF compounds (a) Positive mode (b) Relative participation.

Relative participation

The relative participation of each species in the pathway of both receptors are activated at a time is shown in Figure 9(b). This is expressed in terms of signal strength.

Pathway length

Pathway length represented in the histogram in Figure 10, depicts the frequency distribution of the number of participating reactions in the elementary modes. This option is useful to assess whether there are many/few small/large pathways.

The pathway length graph shows the length or the number of reactions in each of the 57 modes calculated.

Structural coupling

Determines enzyme subsets [= groups of pathways which operate in the current selection of flux modes always together (if one reaction of an enzyme subset has a rate unequal to zero in a certain flux mode, then all other member of this enzyme subset have a rate unequal to zero, too)] and mutually excluding reaction pairs [= two reactions which never occur together (with rate unequal to zero) in any mode].

Enzyme subsets (reactions occuring always together):

Found reaction subset: {ca>pkc1, ip3>Ca, PIP2>ip3}

Found reaction subset: {Gq>PLC, PLC>PIP2}

Found reaction subset: {dag>pkc2, pip2>dag}

Found reaction subset: {camp>pka, pka>creb, creb>bdnf, map>bdnf}

Found reaction subset: {gtp>gdp, >GTP}

Found reaction subset: {ATP1>AMP, >ATP1}

Result: 6 reaction subsets found.

Mutually excluding reaction pairs (never occurring together):

Found mutually excluding reaction pair: {ser>5ht2a, Reuptake}

Found mutually excluding reaction pair: {ser>5ht4, Reuptake}

Found mutually excluding reaction pair: {Reuptake, 5HT2a>Gq}

Found mutually excluding reaction pair: {Reuptake, 5HT4>Gs}

Found mutually excluding reaction pair: {Reuptake, ca>pkc1}

Found mutually excluding reaction pair: {Reuptake, alpha-release}

Found mutually excluding reaction pair: {Reuptake, Gq>PLC}

Found mutually excluding reaction pair: {Reuptake, ip3>Ca}

Found mutually excluding reaction pair: {Reuptake, dag>pkc2}

Found mutually excluding reaction pair: {Reuptake, pkc>map}

Found mutually excluding reaction pair: {Reuptake, @>ac}

Found mutually excluding reaction pair: {Reuptake, atp>camp}

Found mutually excluding reaction pair: {Reuptake, camp>pka}

Found mutually excluding reaction pair: {Reuptake, pka>creb}

Found mutually excluding reaction pair: {Reuptake, creb>bdnf}

Found mutually excluding reaction pair: {Reuptake, map>bdnf}

Found mutually excluding reaction pair: {Reuptake, PLC>PIP2}

Found mutually excluding reaction pair: {Reuptake, PIP2>ip3}

Found mutually excluding reaction pair: {Reuptake, pip2>dag}

Found mutually excluding reaction pair: {Reuptake, gtp>gdp}

Found mutually excluding reaction pair: {Reuptake, ATP1>AMP}

Found mutually excluding reaction pair: {Reuptake, >Ca}

Found mutually excluding reaction pair: {Reuptake, >ATP1}

Found mutually excluding reaction pair: {Reuptake, >GTP}

Found mutually excluding reaction pair: {Reuptake, >ATP}

Result: 25 mutually excluding reaction pairs found.

Hence, it has been inferred that for treating mood related disorders we need to sustain CREB levels at normal in order to prevent the stress induced atrophy of neurons. During stress, the BDNF genes that help in the propagation of neurons are inhibited,

thus leading to the destruction of neurons and thus depression. Hence the importance of serotonin as a signaling molecule for expression of BDNF genes is required for reducing the physiological effects of stress.

Regulation of BDNF gene expression is purported as a major component in the long-term action of antidepressants (Martinowich and Lu, 2008). The transcription factor cAMP-response element-binding protein (CREB) is activated by chronic antidepressant treatments. Treatment with antidepressant like fluoxetine, which act as a serotonin reuptake inhibitors (SSRI) help in the treatment of mood disorders. These selective serotonin reuptake inhibitors function by blocking the breakdown of monoamine neurotransmitters (serotonin and norepinephrine) by inhibiting the enzymes, which oxidize them, thus leaving higher levels still active in the brain. They inhibit VMAT thus maintaining the levels of serotonin in the synaptic cleft. This logical analysis suggests many important molecules in the cascade of signaling reactions like Ca, PKC and PKA. So, deregulation of these compounds also produces a negative effect on the normal function of BDNF genes in time of stress.

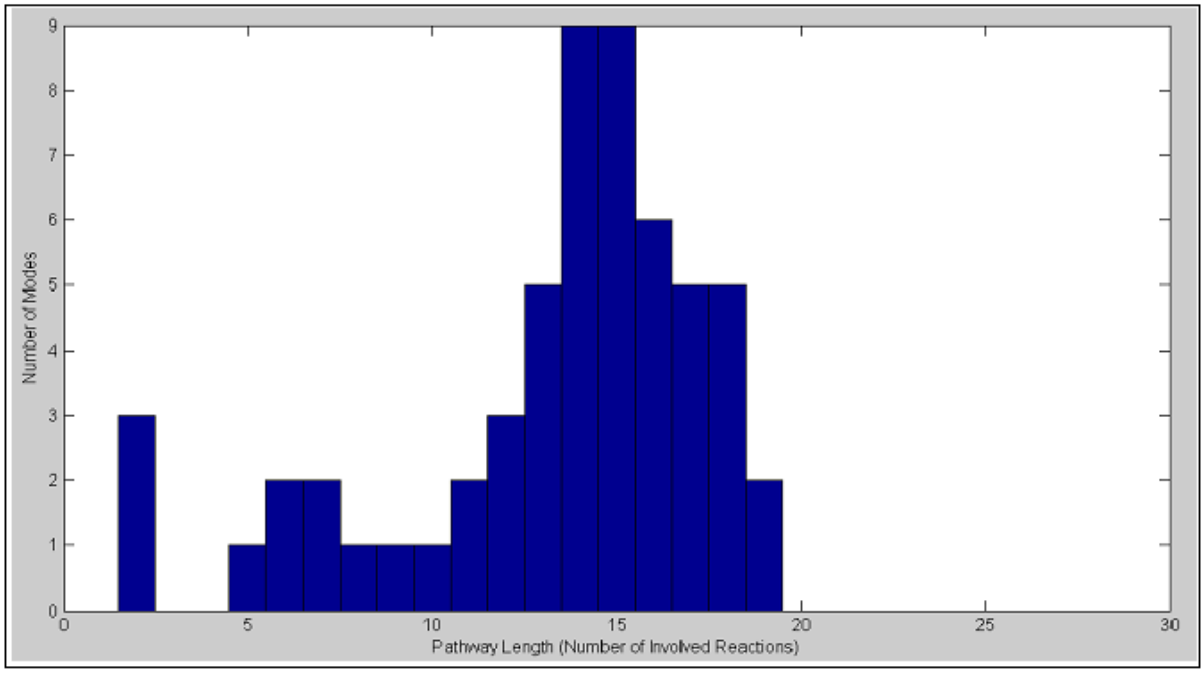

Fig. 10. Frequency distribution of pathway length.

Conclusion

The results obtained by analyzing the serotonin signaling pathway produced are incoherent with the available literature particularly, the Ca influx which triggers the release of serotonin from presynaptic vesicles are seen.

Thus, it tells us that serotonin pathway model building works for further logical analysis at steady state. It also interprets major biochemical signaling molecules responsible for antidepressant activity are BDNF, CREB and 5-HT. By inferring the dependency matrix it is clear that the above three molecules take part in many important reactions than any other molecules in this pathway. This clearly shows that these are the primary or the most necessary compounds involved in the entire serotonin signaling cascade. Furthermore, logical steady analysis also shows that the required output for the activation of BDNF genes will not occur if 5-HT or CREB are at zero level.

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