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Modeling spatial aspects of intracellular dopamine signaling

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Abstract

Dopamine binding to various dopamine receptors activates multiple intracellular signaling molecules, some of which interact with calcium activated signaling pathways. Many experiments measure agonist-stimulated elevations in signaling molecules using prolonged, diffuse application, whereas the response of neurons to transient and spatially localized stimuli is more important. Computational modeling is an approach for investigating the spatial extent, time course and interaction of post-synaptic signaling molecules activated by dopamine and other transmembrane receptors. NeuroRD is a simulation algorithm which can simulate large numbers of pathways and molecules in multiple spines attached to a dendrite. We explain how to gather the information needed to develop computational models, to implement such models in NeuroRD, to perform simulations and to analyze the simulated data from these models.

Keywords

computer model; signaling pathways; reactions; diffusion

Introduction

Dopamine binding to various dopamine receptors activates multiple intracellular signaling molecules. The importance of dopamine activated signaling pathways is evident from Parkinson's disease, a degenerative disease in which dopamine neurons of the substantia nigra pars compacta degenerate. The lack of dopamine input from the substantia nigra pars compacta to the basal ganglia produces abnormalities in voluntary motor activity(1). *In vivo* recordings have shown that the lack of dopamine alters the firing dynamics of these neurons and the striatum as a whole(2).

In vitro experiments have shown that dopamine modulates several voltage dependent and synaptic channels of striatal spiny projection neurons, and that dopamine activated signaling pathways interact with glutamate- and acetylcholine-activated pathways to produce neuromodulation(3). Most *in vitro* experiments demonstrating the effect of dopamine on signaling pathway molecules or ionic channels use prolonged, diffuse application of agonists. Because of adaptations such as receptor desensitization, inferences made from

prolonged, diffuse application might not accurately represent the effect of transient “*in vivo* like” inputs.

Computational modeling is an approach for investigating the spatial extent, time course and interaction between dopamine-activated and other signaling pathways(4). Computational modeling allows integration of the information obtained from biochemical, pharmacological, and molecular biology experiments with the information obtained from electrophysiology and calcium imaging experiments. The resulting integrative model provides insight into mechanisms underlying neuromodulatory effects in response to realistic synaptic inputs, and simulation experiments using the model generate testable hypotheses.

Several software packages are available for modeling signaling pathways(5-8). They differ in terms of user interface and simulation algorithm. The computational efficiency of the various algorithms depends both on the level of biophysical detail and the numerical approximations made. The computational efficiency then constrains the spatial scale and complexity of the signaling network which can be practically investigated.

The example provided here uses NeuroRD(5), which has a simple, text-based user interface, and is freely available (<http://krasnow.gmu.edu/CENlab/software.html>). The simulation algorithm can simulate large numbers of pathways and molecules in multiple spines attached to a dendrite. The model is defined in a set of files, each specifying a different attribute of the model: morphology, reaction, initial conditions, stimulation protocol, and output, and one additional file which specifies the name of the other files. The following procedure explains how to gather the information needed to create the model files, and how to run and analyze the resulting simulation output.

Methods

1. Signaling Pathways

1.1. Identify bimolecular and enzymatic reactions that form the signaling pathways of interest—The set of reaction pathways is typically determined from multiple experiments, some of which demonstrate that blocking specific molecules prevents a downstream effect, and some of which demonstrate that two substrate molecules interact to create a product. Perusal of published literature might be aided by consultation of online databases¹.

1.2. Find measures of rate constants from various biochemical experiments.—

a. Assays of bimolecular reactions typically provide $K_D = K_B/K_F$. If separate estimates of K_B or K_F are not provided, it is necessary to estimate the rates of forward and reverse reactions.

b. Enzyme reactions consist of a pair of reactions in which the enzyme and substrate form a complex in the first reaction and free enzyme is regenerated in the second reaction. Under

¹<http://www.wikipathways.org/> <http://www.genome.jp/kegg/regulation.html>

certain conditions (constant enzyme concentration, irreversible), which usually apply in enzyme assays, product formation is given by the following equation:

$$\frac{dP}{dt} = \frac{K_{cat} \cdot S}{S + K_M} \quad (1)$$

where $K_M = (K_B + K_{cat}) / K_F$. Assays typically measure enzyme activity at various substrate concentrations and provide K_M and K_{cat} values (9). When a separate estimate of K_B is not provided, it is assumed that $K_B = 4 \cdot K_{cat}$ (10).

c. One method of estimating rate constants is to create simulations of experimental assays and adjust the rate constants in the assay simulations until the output is a reasonable match to experimental assay data. Experimental assays that show dose response and/or time course relationships are the best. After choosing the experiment assays to simulate, a separate model is created to match the conditions of the assay. Enzyme assays are usually run for many minutes to provide time to accumulate sufficient substrate to measure. If conditions are such that product accumulation is a linear function of time, a few seconds of simulation will yield the same enzymatic rate as many minutes of simulation. In using results from simulations that run for a few seconds, one must be certain that the system is at steady state for all species except reaction substrate and product. Typically, there are many sets of rate constant values that will produce a simulation output that matches experimental data for a single assay. Although the same simulation output is obtained from these various sets of values, the time for various species in the system to reach steady state will be different. If one can find several different kinds of time course experiments to simulate, these can be used to constrain the final choice of rate constants.

d. Constants describing interactions of drugs with various components of the system can be estimated from functional data. When the concentration of agonist in an assay is low, the IC50 concentration for an antagonist is often a reasonable estimate of the dissociation constant for antagonist binding. If the concentration of agonist is higher and the agonist's dissociation constant is known, then the Cheng Prusoff equation (11, 12) is used to calculate the dissociation constant: $K_I = IC50 / (1 + (substrate/K_S))$, where K_S is the dissociation constant for the substrate. For agonists, the situation is more complex: because of signal amplification following interaction with receptor, EC50 values are often substantially lower than dissociation constants. Thus, EC50 values represent a lower boundary on the possible range of dissociation constants for an agonist.

Dissociation constants also can be obtained from radioligand binding data. The vast majority of the radioligand binding studies reported involve equilibrium binding methodology. These provide excellent data relative to affinity of interactions but do not provide information on rates of association and dissociation. Although association and dissociation studies can be done using radioligand binding methods, they are not commonly used.

e. Several databases² tabulate rate constants (and literature source of these rate constants). The modeler is always encouraged to look into the original references and protocols when trying to understand the source of possible discrepancies.

1.3. Find measures of diffusion constants—In some cases the diffusion constant of a molecule in the cytosol is available. In most cases, it is necessary to estimate diffusion constant from molecular weight and viscosity of cytosol using the following equation(5):

$$D = \frac{8.34e^{-8} \cdot T}{\eta \cdot M^{1/3}} \quad (2)$$

where the diffusion coefficient D is in cm²/s, T is temperature in K, the solution viscosity, η , is in cP (estimated at 1.2-1.4 for cytosol), and molecular weight M is in g/mol.

1.4 Create the ReactionScheme file which lists molecules, diffusion constants, and reactions—

The reaction file has two parts. The first part lists all molecular species. Each molecular species is defined by four attributes: *name*, *id*, *diffusion constant*, and *units* for the diffusion constant. The second part of the reaction file lists all reactions (Fig 1). Each reaction has six attributes: *reaction name*, *id*, *reactant*, *product*, forward reaction rate, and reverse reaction rate. Either one or two reactants and products can be specified. Enzyme reactions are specified as two bimolecular reactions, with the enzyme regenerated in the second step. Additional details are found in the README file available on the website.

For example, when the D1 type of dopamine receptor binds to dopamine, the receptor becomes an active enzyme that catalyzes the activation of the $G_{\alpha\text{olf}}$ variety of G protein. The $G_{\alpha\text{olf}}$ GTP activated subunit binds to and activates adenylyl cyclase, which catalyzes the production of cAMP from ATP. The following reactions describe the first steps of this pathway:

These reactions are represented by the following ReactionScheme file (Fig 1). Each enzymatic reaction requires specification of two reactions; thus forwardRate in the third reaction is turnover or catalytic rate for the enzymatic reaction.

2. Morphology

2.1 Describe the morphology of the neuron to be modeled—At present, due to computational burden, only a part of the neuron can be simulated in a reasonable time, e.g., a dendrite with several spines, or several dendrites. The morphology of neurons is available from many publications and various databases. Neuromorpho.org provides morphology files of many different neuron types from many species and brain regions in a standard format in which a traced neuron is given as a set of points (x,y,z coordinates) with associated radii. Alternatively, the morphology of whole neurons or parts of neurons is often available from

²<http://doqcs.ncbs.res.in/>
<http://www.brenda-enzymes.info/>
<http://www.cyclic-nucleotides.org/>

immunocytochemistry figures in publications. Lengths and radii of discrete segments can be extracted from the figures. For simulations of experimental assays (section 1.3.c), it is advisable to use a single large compartment.

2.2 Create the Morphology file.—The NeuroRD morphology file (Fig 2) specifies the shape of the neuron by specifying the shape of all segments and how they are connected. Each segment has four attributes: *id*, *region*, *start* and *end*. The *id* attribute is required and must be unique whereas the optional *region* attribute does not have to be unique. Regions are used to group segments with the same initial conditions. The *start* and *end* attributes are specified using *x*, *y*, *z*, (*r*)adius, and *label*. The *label* is optional and can be used as a site where molecules are injected into the system. In general, segments are specified with the starting *x,y,z* coordinates and radius, and the ending *x,y,z* coordinates and radius. To connect the second segment to the first, it must start from the endpoint of the first compartment using the attribute *start on* (Fig 2). Note that subsequent segments which do not start on an existing segment will not be connected. Branching segments are made by creating two segments beginning at the same site but terminating at different points. The two branches need not have the same radius at the point of connection. In this case, a radius value must be specified.

2.3 Include spine specifications in the morphology file if desired—SpineType specifies a spine prototype and SpineAllocation (Fig 2) applies the spine prototype to the surface of a structure. This allows for random placement of spines according to a specified density in a constrained region or segment of the defined morphology. Multiple spine prototypes can be defined, e.g. to randomly distributed long, thin spines among short, stubby spines. SpineType has an *id* attribute and is defined using multiple *section* elements, which have four attributes: *width*, *at*, *regionClass*, and *label*. The *width* attribute specifies the radius of that section and the *at* attribute indicates the distance from the dendrite at which that radius begins to apply. The *regionClass* and *label* attributes are optional. SpineAllocation has four attributes: *id*, *spineType*, *region*, and *lengthDensity*. The *region* attribute indicates the region to which spines of type *spineType* will be added. The *lengthDensity* attribute is the average number of spines per micron. Alternatively, *areaDensity*, which is the number per unit area, can be used instead of *lengthDensity*. Fig 2 shows an example that creates two dendrites attached to the soma, with spines on both dendritic branches. Additional examples and documentation are available on the website.

3. Molecule quantities and initial conditions

3.1. Find measures or estimates of molecule quantities, and for non-diffusile molecules, their subcellular distribution—For example, in spiny neurons which have glutamatergic synapses on the spines, metabotropic glutamate receptors are located in the spine head. Plasma membrane pumps are located in the plasma membrane and do not diffuse through the cytosol. Once control conditions are selected, it is possible to determine changes in molecule quantities that will be used to run simulation experiments. For example, to simulate pharmacological block of a particular molecule, its value could be set to zero. Then, results of the control simulation and the blocked simulation can be compared to evaluate the role of that blocked molecule.

3.2. For quantities that are not available, use experiments that measure time course of a molecule in the signaling pathway as a constraint—For example, if the amount of substance produced by a stimulation is known and this substance is produced by an enzyme that has been purified and tested so that its K_{cat} is known but its concentration in the tissue of interest is not known, one can calculate the minimum concentration of enzyme that must be present to produce the response. Also, sufficient substrate must be present to generate the products of enzymatic reactions. The location of various molecules often is determined from immunogold labeling(13) or subcellular fractionation techniques(14).

3.3 These values are used to create the InitialConditions file, which specifies the starting concentration or density for each molecule—The file must contain one general ConcentrationSet, which applies to everything unless overridden. Each *NanoMolarity* element names the species and provides a value for its concentration, entered in nanoMoles per liter. In addition, non-diffusing molecules can be assigned to a specific region using additional ConcentrationSets together with a *region* attribute. This should correspond to a specified region from the morphology file and indicates the parts of the structure to which the initial conditions apply. For membrane localized molecules, it is possible to specify initial conditions as a density (picomoles per square meter) using the SurfaceDensitySet which places these molecules only in the submembrane areas of the morphology. If the *region* attribute is specified, then that submembrane initial condition applies only to that region. If the *region* attribute is not specified, then the initial condition applies to all submembrane areas. In Fig 3, the dopamine receptor (D1R) density is zero in general, but non-zero in the submembrane part of the dendrite region.

4. Stimulation

4.1 Determine the pattern of stimulation for a particular experiment—For example, a brief electrical stimulation leads to release of dopamine from terminals (15), which then binds to post-synaptic dopamine receptors. Sometimes, a molecule downstream from the experimental stimulation can be used for the model stimulation, such as with NMDA type of glutamate receptors. In this case each glutamate release event and binding to receptors may be approximated as influx of calcium, and stimulation at high frequency would produce accumulation of individual calcium transients. Note that neither pre-synaptic terminal nor diffusion of dopamine in the extracellular space can be modeled presently.

4.2 The StimulationSet file specifies the time and location for (optional) injection of molecules during a simulation (Fig 4)—Each InjectionStim specifies the molecule injected and its site of injection. Additional required attributes include *onset* (in msec), *duration* (in msec) and *rate* (particles/msec). Optional attributes, *period* and *end*, can be used to specify a train of input. Thus, 100 Hz stimulation for 1 sec would be specified with a *period* of 10 ms, and *end* equal to *onset* plus 1000 msec. Multiple trains are possible with two more optional attributes: *interTrainInterval* and *numTrains*. Variations in stimulation protocols, e.g. brief, large amplitude (1000 particles during 1 msec) versus long, low amplitude (10 particles during 100 msec) dopamine release, are used to run simulation

experiments. Stimulation can be delivered to a single spine or to a range of spines as explained in more detail at the website.

5. Output

5.1 To evaluate the result of the simulation it is necessary to analyze the concentration or quantity of one or more molecules from one or more parts of the neuron morphology—For example, if studying synaptic plasticity, then quantity of activated kinase, calcium concentration, or phosphorylation state of particular target molecules may be of interest. To understand mechanisms underlying the simulation results, it may be necessary to evaluate the time course of several molecular species in the model. One advantage of simulations is the possibility to examine every molecular species.

5.2 The OutputScheme file specifies the file to which output is written and which molecules from which compartments are written to the file at which frequency (time interval)—Multiple output files are allowed: for example, information about some molecules, or information from some regions can be written more frequently than others. Every OutputSet specifies a single output file using two required attributes, *filename* and *dt* (for data output interval), and an optional attribute, *region*. If the *region* attribute is not specified, then the concentrations (or number of particles) for all subvolumes in the system are saved. Each *OutputSpecie* in the OutputSet indicates a molecule to be included in that output file.

6. Additional details on the simulation

One additional file, called the Model file (Fig 6), serves to identify all these other files and provides additional information such as discretization options, simulation seed(s) and various control parameters. This model file is input to the software to run the simulation, as explained in section 7.

6.1 Discretization options—For numerical calculations within the model, each of the morphology segments is subdivided into smaller compartments called subvolumes. The discretization element indicates the size of these subvolumes. Smaller sizes produce larger number of subvolumes which require more calculations and result in a longer run time. The defaultMaxElementSide specifies the largest size (in microns) for each side of the subvolume in each segment. This is the default, and can be overridden using MaxElementSide with the *region* attribute: the value supplied will control the size of subvolumes for that region. Similarly, spineDeltaX specifies the size of subvolumes in spines. Spines have a one-dimensional discretization along the spine axis. The geometry element is used to specify how the morphology is interpreted. 2D implies that there are multiple voxels in x and y directions, but only a single layer of subvolumes in the z dimension. Thus, the morphology is three-dimensional, but diffusion occurs in two dimensions only. For 2D, the user also specifies the depth of the subvolumes.

6.2 Additional parameters—Runtime is used to specify run time in milliseconds. fixedStepDt specifies the time step, in milliseconds, which must be smaller than the fastest reactions or diffusion processes. The required simulationSeed specifies the seed for the

random number generator. In morphologies with spines, a separate seed, spineSeed, is used to randomly place spines within the specified region. outputQuantity specifies whether quantity of molecules in the output is in number of molecules or concentration.

7. Run simulations and evaluate output

7.1 Experimental design—The key to meaningful experiments is asking good questions that can be answered using data from the simulations. Simulations are excellent tools for addressing questions such as the effect of morphology and spatial constraints, or the conditions which would be required to produce an experimentally observed response. Similar to experiments, to address particular effects, it is necessary to run two or more simulations which differ only in the desired parameter. For example, the effect of morphology on output, may be evaluated by performing two simulations which differ only in their morphologies. To evaluate the effect of an antagonist requires a control simulation (the default simulation with no antagonist) and a simulation either with antagonist added (simulating competitive or allosteric binding) or, alternatively, with the antagonist target concentration close to zero.

7.2 Running simulations—To run a simulation from the command line in Unix, the following command should be issued:

```
java -jar NeuroRD.jar DopamineModel.xml Dopamine.out >> Dopamine.log
```

To run a simulation in Windows, type 'cmd' in the run command from the start menu to obtain a command line window. Then, type the following:

```
java -jar NeuroRD.jar DopamineModel.xml Dopamine.out
```

where NeuroRD.jar file is the simulation software downloaded from the website (<http://krasnow.gmu.edu/CENlab/software.html>), DopamineModel.xml is the model file (“master” file that specifies the other files), and Dopamine.out is the main output file. The name of additional output files are composed of the main output file name plus the filename specified in OutputScheme file.

7.3 Evaluating simulation output—The first set of simulations typically is used to validate the model; thus simulation output is compared to experimental data. Due to stochastic variability, multiple simulation runs must be performed to generate several observations, analogous to experiments which require multiple trials or samples. Then, conventional statistical tests are used to compare levels of relevant molecules from simulations to experimental data. For more quantitative comparison to imaging data, simulations can include fluorescent indicator molecules and calculate the change in fluorescence.

Two additional types of simulations are typically performed. One set of simulations implements the experimental design, e.g., evaluating the role of a particular molecule, or the effect of stimulation pattern. Again, multiple simulation runs coupled with statistical analysis are required for hypothesis testing. The other set of simulations addresses the

crucial question of how robust the conclusions are. It is important to verify that biological conclusions do not change with small changes in parameter values or different stimulation protocols. The ideal parameters for robustness simulations are those whose experimental measurements span a wide range (i.e., molecule quantities and diffusion constants) and those to which the results are more sensitive. The ability to evaluate dynamics of many different molecules simultaneously, and to perform manipulations that are not possible experimentally such as changing diffusion coefficients, allows evaluating the biochemical mechanisms underlying experimental observations.

Evaluating simulation output is challenging due to the amount of numerical data that is generated. In principle, the quantity of every molecular species in the system in every subvolume is available in the numerical experiments. This abundance necessitates additional processing of the data for efficient presentation of these vast numerical results generated from computational modeling. For example, numerical results can be converted to multi-dimensional visualization (movies) to facilitate understanding and scientific intuitions, e.g., using VNRD which is available on the website (<http://krasnow.gmu.edu/CENlab/software.html>). Fig 7 shows average concentrations in the dendrite and soma for the model presented in Figures 1-6, and illustrates that the concentration of $G_{olf}GTP$ (diffusible solely for the sake of illustration) increases first in the dendrite (the location of the dopamine receptors), and subsequently in the soma. The average concentrations were generated by VNRD.

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```

<ReactionScheme>
<!--List of all molecular species -->
  <Specie name="Dopamine" id="Dopamine" kdiff="100" kdiffunit = "mu2/s"/>
  <Specie name="D1R" id="D1R" kdiff="0" kdiffunit = "mu2/s"/>
  <Specie name="G" id="G" kdiff="0" kdiffunit = "mu2/s"/>
  <Specie name="DaD1R" id="DaD1R" kdiff="0" kdiffunit = "mu2/s"/>
  <Specie name="GDaD1R" id="GDaD1R" kdiff="0" kdiffunit = "mu2/s"/>
  <Specie name="GolfGTP" id="GolfGTP" kdiff="10" kdiffunit = "mu2/s"/>
  <Specie name="GolfGDP" id="GolfGDP" kdiff="10" kdiffunit = "mu2/s"/>

<!--List of all reactions -->
<!-- Bimolecular Reaction: Dopamine + D1R <=> Dopamine-D1R -->
  <Reaction name = "Da+D1R--DaD1R reac" id="DaD1Rreac">
    <Reactant specieID="Dopamine" />
    <Reactant specieID="D1R" />
    <Product specieID="DaD1R" />
    <forwardRate> 0.0011e-03 </forwardRate>
    <reverseRate> 10e-03 </reverseRate>
    <Q10> 0.2 </Q10>
  </Reaction>

<!-- 2 step Enzyme reaction: DaD1R + G <=> GDaD1R -> DaD1R + GolfGTP -->
  <Reaction name = "DaD1R+G--G-DaD1R reac" id="DaD1R-Enz1">
    <Reactant specieID="DaD1R" />
    <Reactant specieID="G" />
    <Product specieID="GDaD1R" />
    <forwardRate> 0.06e-03 </forwardRate>
    <reverseRate> 0.01e-03 </reverseRate>
    <Q10> 0.2 </Q10>
  </Reaction>

  <Reaction name = "G-DaD1R--DaD1R+GolfGTP reac" id="DaD1R-Enz2">
    <Reactant specieID="GDaD1R" />
    <Product specieID="GolfGTP" />
    <Product specieID="DaD1R" />
    <forwardRate> 20e-03 </forwardRate>
    <reverseRate> 0e-03 </reverseRate>
    <Q10> 0.2 </Q10>
  </Reaction>

<!-- 1st order hydrolysis: GaolfGTP <=> GolfGDP -->
  <Reaction name = "GolfGTP--GolfGDP reac" id="GolfGTP2GolfGDPreac">
    <Reactant specieID="GolfGTP" />
    <Product specieID="GolfGDP" />
    <forwardRate> 10e-03 </forwardRate>
    <reverseRate> 0e-03 </reverseRate>
    <Q10> 0.2 </Q10>
  </Reaction>
</ReactionScheme>

```

Figure 1.
DopamineReac.xml

```

<Morphology>

  <!-- spine morphologies: width for sections along the spine -->
  <SpineType id="spineA">
    <Section width="0.2" at="0.0" regionClass="neck"/>
    <Section width="0.2" at="0.2" regionClass="neck"/>
    <Section width="0.2" at="0.3" regionClass="head"/>
    <Section width="0.6" at="0.4" regionClass="head"/>
    <Section width="0.6" at="0.5" regionClass="PSD"/>
    <Section width="0.2" at="0.6" label="pointA"/>
  </SpineType>

  <SpineAllocation id="sa1" spineType="spineA" region="dendrite" lengthDensity="0.4"/>

  <!--Three compartments -->
  <Segment id="soma" >
    <start x="-1.0" y="1.0" z="0.0" r="1.5"/>
    <end x="5.0" y="1.0" z="0.0" r="1.5"/>
  </Segment>

  <Segment id="dendrite1" region="dendrite">
    <start on="soma" at="end" r="1.0" />
    <end x="8.0" y="3.0" z="0.0" r="0.5" label="pointB" />
  </Segment>

  <Segment id="dendrite2" region="dendrite">
    <start on="soma" at="end" r="1.0" />
    <end x="12.0" y="-2.0" z="0.0" r="0.5" />
  </Segment>

</Morphology>

```

Figure 2.
DopamineMorph.xml

```
<InitialConditions>

  <!-- these apply to everything unless overridden -->
  <ConcentrationSet>
    <NanoMolarity specieID="Dopamine" value="0" />
    <NanoMolarity specieID="D1R" value="0" />
    <NanoMolarity specieID="G" value="1000" />
    <NanoMolarity specieID="DaD1R" value="0" />
    <NanoMolarity specieID="GDaD1R" value="0" />
    <NanoMolarity specieID="GolfGTP" value="0" />
    <NanoMolarity specieID="GolfGDP" value="0" />
  </ConcentrationSet>

  <!-- these apply to surface voxels in dendrite -->
  <SurfaceDensitySet region="dendrite">
    <PicoSD specieID="D1R" value="100" />
  </SurfaceDensitySet>
</InitialConditions>
```

Figure 3.
DopamineIC.xml

```
<StimulationSet>

  <InjectionStim specieID="Dopamine" injectionSite="sal[0].pointA">
    <onset>      40      </onset>
    <duration>    5      </duration>
    <rate>       100.0   </rate>
    <period>     20     </period>
    <end>        200    </end>
    <!-- <interTrainInterval> 10000 </interTrainInterval> -->
    <!-- <numTrains> 1 </numTrains> -->
  </InjectionStim>

</StimulationSet>
```

Figure 4.
DopamineStim.xml

```
<OutputScheme>

  <OutputSet filename = "dt1dend" region="dendrite" dt="1.0">
    <OutputSpecie name="DaD1R" />
    <OutputSpecie name="GDaD1R" />
  </OutputSet>

  <OutputSet filename = "dt1all" dt="5.0">
    <OutputSpecie name="Dopamine" />
    <OutputSpecie name="GolfGTP" />
    <OutputSpecie name="GolfGDP" />
  </OutputSet>
</OutputScheme>
```

Figure 5.
DopamineIO.xml


```

<SDRun>
  <!-- this file defines a single run or trial of the simulation -->

  <reactionSchemeFile> DopamineReac </reactionSchemeFile>
  <morphologyFile> DopamineMorph </morphologyFile>
  <stimulationFile> DopamineStim </stimulationFile>
  <initialConditionsFile> DopamineIC </initialConditionsFile>
  <outputSchemeFile> DopamineIO </outputSchemeFile>

  <!--2D means only 1 layer of subvolumes, 3D gives multiple layers -->
  <geometry> 2D </geometry>
  <depth2D> 0.5 </depth2D>
  <distribution> BINOMIAL </distribution>
  <algorithm> INDEPENDENT </algorithm>
  <simulationSeed> 971263 </simulationSeed>

  <runtime> 400 </runtime>

  <spineSeed> 9897623 </spineSeed>

  <discretization>
    <defaultMaxElementSide> 1.0 </defaultMaxElementSide>

    <!-- discretization for spines, microns -->
    <spineDeltaX> 0.1 </spineDeltaX>

    <!-- override the default for a particular region. -->
    <!-- Matches against id or regionClass in the morphology file -->
    <!-- <MaxElementSide region="soma">4.0</MaxElementSide> -->
  </discretization>

  <fixedStepDt> 0.01 </fixedStepDt>

  <outputInterval>1</outputInterval>

  <!-- save NUMBER of particles in each element or the CONCENTRATION. -->
  <outputQuantity> CONCENTRATION </outputQuantity>

  <!-- the tolerance is not used yet -->
  <tolerance> 0.001 </tolerance>

  <!-- GRID_STEPPED_STOCHASTIC or GRID_STEPPED_CONTINUOUS -->
  <calculation> GRID_STEPPED_STOCHASTIC </calculation>
</SDRun>

```

Figure 6.
DopamineModel.xml

Figure 7.

Concentration of Dopamine bound D1 receptor and $G_{\text{olf}}\text{GTP}$ in the dendrite, where it is produced, and in the soma, to which it diffuses. Fluctuations are due to stochastic nature of simulation.