A Model of Nitric Oxide Diffusion Based in Compartmental Systems

Fernández López, P.[1], Suárez Araujo, C.P.[1], García Báez, P.[2]
and Simones Da Fonseca, J.[3]
[1]Institute for Cybernetics.
University of Las Palmas de Gran Canaria, Spain,
{pfernandez, cpsuarez}@dis.ulpgc.es
[2]Dept. of Statistics, Operations Research and Computation,
University of la Laguna, Spain.
pgarcia@ull.es
[3]Faculty of Medicine,
University of Lisboa, Portugal

Abstract The brain is a largely parallel system whose activity is induced by the functional coupling of many nerve cells, the cellular communication and learning. At present, a new type of process for signalling between cells seems to be emerging, the *Volume Transmission* (VT). Its underlying mechanism is the diffusion of neuroactive substances and diffusible signals, like nitric oxide (NO), in the extracellular space (ECS), extra-synaptic gap which affect the brain activity in a global way.
This paper is dedicated to the Theoretical Framework of the global study framework of NO diffusion (GSFNO). We present a new model of NO diffusion based in compartmental sytems and transport phenomena, which allows to understand how NO functions as a neural signalling molecule. We model the dynamic of NO diffusion from a molecular level where the spatial dimension is discrete. This model is highly powerful for studying and determining the dynamic of NO production and diffusion, both in brain and artificial neural systems, showing the capabilities of NO in cellular signalling and learning and its influence in the anticipatory behaviour. It has category of general formal tool with biological plausibility. We present a short analysis of the model in a three-dimensional environment for properties such as the dynamic of NO release, the self-regulation effect, the diffuse neighbourhoods (DNB), among others.
Keywords : Nitric Oxide diffusion, Volume transmission, Artificial neural networks, Compartmental systems, Cellular signalling.

1 Introduction

The brain is a largely parallel system whose activity is induced by the functional coupling of many nerve cells, the cellular communication and learning. The synaptic transmission, in the anterograde direction, is the process, for excellence, for nerve cells communication [1]. At the moment, it is accepted that nervous cells establish

their relationships not only by means of the classical synaptic connection system, but also over a complete set of cellular signals of diverse source that affect globally the brain activity. This phenomena includes a diffusion process in the nervous tissue that takes us to define a new type of cellular signalling, the volume transmission (VT). Its underlying mechanism is the diffusion of neuroactive substances and diffusible signals, like nitric oxide (NO), in the extracellular space (ECS), extra-synaptic gap which therefore serves not only as the microenvironment of nerve cells but also as an information channel [2], [3].

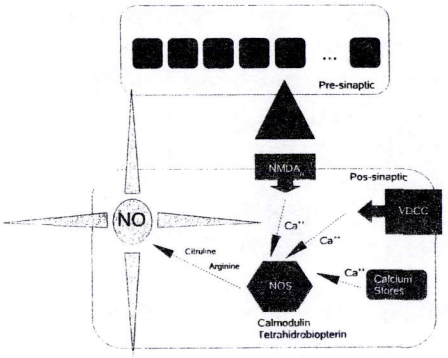


Fig. 1: Diagram of NO production stores at synapses. The calcium signal derived from the NMDA receptor-channel, voltage-dependent Ca^{++} channels (VDCC) or intracellular stores binds calmodulim and generates the nitric oxide synthase (NOS). Activated NOS produces NO from L-arginine. NO can difuse from its site of production to influence all neary synapses (Adapted from [5]).

Among all the main sources of volume transmission signals in the CNS such as neuropetides, local ion currents, paracrine transmission (diffuse catecholaminergic systems), reverse functioning of transmitter uptake carriers (the release of glutamate and GABA from astroglia and glutamate and dopamine from neurons), release of gaseous transmitters (nitric oxide release from neurons and endothelial cells) [3], we place our research and development in the NO. Unlike other neuroactive signals, the NO is one of the liposoluble molecules generated by the cells from the own tissue which allow a volumetric transmission little or no affected by the titular barriers.
A key property of NO that sets it apart from these other CNS signalling molecules is its extreme diffusibility in both aqueous and lipid environments, which allows a rapid three-dimensional spread of the signal irrespective of the presence of membranes [6]. NO is a free radical gas, which is synthesized from L-arginine by the NO synthase (NOS), nicotinamide adenine dinucleotide phosphate (NADPH), Fig. 1. NO regulates its own production by inactivating both the NOS and the NMDA receptor.

NO is considered an *atypical* cellular messenger, since it needs no receptor, does not accumulate in synaptic vesicles and once synthesized, it freely diffuses through membranes affecting all neighbouring cells, including the pre-synaptic one and the very cell that released it. NO action differs from some similar established modes of neural communication, e.g. feedback and paracrine secretion of neuropeptides. While NO may mediate feedback (defined as response directed to the cell which induced the initial signal), it may also have an *action in reverse* that modulates a presynaptic neuron even if the later did not cause NO release.

The presence of a molecule in the brain such as the nitric oxide, opens new perspectives in the study of the brain functioning, in the Perpherial Nervous System (PNS) as well as in the Central Nervous System (CNS). In the PNS, NO helps as an element of control for several systems, such as the cardiovascular system, the respiratory system, the digestive system, etc. In the CNS, NO acts as a retrograde neurotransmmiter in the brain and hippocampus. At this last one, with great impication in learning and the formation of memory, seeming to be indispensable for the LTP generation.

In the absence of determinant experimental data for understanding how NO functions as a neural signalling molecule, we have developed a NO diffusion computational model based on Compartmental Systems [8] and transportation phenomena. Our main objective is to proportion a formal framework which allows us to determine the generation of NO and diffusion dynamics in the brain. as well as the capabilities of NO in celullar signalling and formation or emerging of complex structures, in biological environments as well as in artificial ones. We will show that the proposed model in this paper is highly poweful to study these phenomena.

This compartmental model is different from the ones previously proposed. because of its formal frasmework category which allows to incorporae all the biological features as well as present restrictions in the NO emission and diffusion and in the environment where the NO diffusion processes take place. Thus, we will show that our proposal is neither limited to use a morphology of instantaneous NO production, of precise form, which is highly restrictive and not biologically plausible, nor to consider the diffusion in an isotropic and homogeneous environment, such as happens in the several NO diffusion models, analytical ones as well as discrete ones, that have been published, [9], [10], [11], [4], [12] y [13]. These models only allow to recreate the diffusion processes in a free environment where molecules move randomly, which does not reflect the biological reality, The presented model allows to consider the reality of diffusion of anisotropy, not homogeneity in the ECS, in the central nervous system (CNS) and in the peripheral one (PNS). The diffusion in the ECS is critically dependent on, and limited by, the structure and physico-chemical properties of the ECS the nerve cell microenvironment. The ECS is not homogeneous, since its properties vary not only around each cell, but also in different brain regions [3]. Moreover, the neural tissue and the cellular membranes which NO can spread through, also present non-homogeneity and anisotropy features, being able

the diffusion to be facilitated more in some directions than in other ones, [3].
All the modelling process can be divided into well differentiated stages, model construction, model analysis [14], and model validation. In the construction stage, all the especially relevant aspects that are wanted to be studied are extracted from the phenomena which is wanted to be modelled. This conditions the formal framework which should be employed. In the analysis stage, the capabilities, kindness, restrictions and manageability that the model presents are determined. At last, the validation stage allows to demonstrate the model kindness on the reproduction of the modelled phenomena as well as on the resolution of problems.
In this paper, we will centre, essentially, in the description of the built model and in its anlysis in a three-dimensional environment, over properties such as morphology of the generation of NO (emission), the self-regulation effect of NO diffusion, the diffusion neighbourhood, among others.
Finally, we can say our model represents an important tool for designing and interpreting biological experiments on NO behaviour and its effect on brain structure and function. The proposed model has been validated in the biological environment, concretely in the endotelio[15], [16], and applied to the study of NO influence in the learning phenomena, [1], [17], being its kindness confirmed.

2 Model of Nitric Oxide Diffusion Based in Compartmental Systems

In this section, we present a NO diffusion computational model which will build a formal framework that allows us to determine the dynamic of generation and diffusion of NO in the brain, as well as the NO capabilities in cellular signalling and formation or emerging of complex structures, in biological as well as artificial environments. One of its main powerful features is its biological plausibility, being able to reproduce certain biological reality aspects, such as that the morphology of generation of NO can be expressed by any variable function in time; that the environment where NO spreads can be non-homogeneous and anisotropic.
With the objective of contemplating, at least, the biological realities, we approach the problem from a microscopic description, modelling the dynamic of NO release and diffusion from a molecular level where the spatial dimension is discrete. We propose volume elements, as a theoretical abstraction, denominated compartments, where the production and diffusion process of NO have their expression. The compartment can have its biological counterpart in whatever neural level, from molecular to circuit level. In this way we are working with complex systems composed by many interconnected compartments using as formal tools the compartmental systems [8] and the transport phenomena.

Compartmental systems are a subclass of the linear dynamic systems. They consist of a finite number of subsystems, called compartments and considered cinematicly homogeneous, on the other hand, the transport phenomena takes place

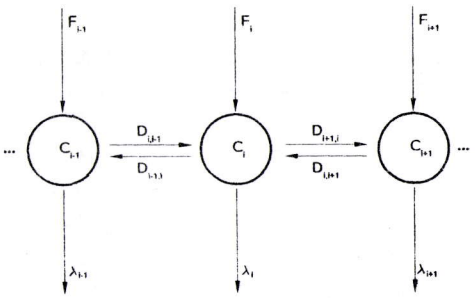


Fig. 2: Scheme of Compartmental System for NO diffusion in a monodimensional environment.

when, due to the gradient of a physical magnitude, another is displaced and invades and affects its setting in a structural and/or functional way. Its behaviour does not depend on any type of molecular theory and can be studied from a phenomenological point of view [9].

Taking into account these two pillars, where the proposed model is sustained on, the expressions that define the NO diffusion dynamics in a determined environment are deducted, and therefore the model expressions in the discrete space.

Let us consider a set of volumetric elements or compartments in a monodimensional environment, Fig. 2, which the concentrations of NO (C_i) are associated to. In any of these compartments (i) can exist a generation of NO process (emission), which is represented in our model by the function of generation of NO, F_i. Because of transportation phenomena, the higher the concentration difference between two compartments, the faster the flow of NO from the higher concentration compartment to the lower one will be. Thus, the NO concentration gradient speed in a compartment will be proportional to the concentration difference between both compartments, taking into account the self-regulation process of NO [7]. This is mathematically expressed by the Eq. (1).

$$\frac{dC_i}{dt} = D_{i,i-1}(C_{i-1} - C_i) + D_{i,i+1}(C_{i+1} - C_i) - \lambda_i C_i + F_i \tag{1}$$

where $D_{i,i-1}$ and $D_{i,i+1}$ are the environmental coefficients of diffusion between the compartments i and $i-1$ and between i and $i+1$, respectively. λ_i is the self-regulation parameter of NO. It is being considered, for this case, a self-regulation of NO dynamics proportional to the quantity of concentration.

The global behaviour of the system, and thus, the model of the NO diffusion dynamics in such system, will be given by a system of N equations, such as (1), where N indicates the number of compartments considered in the system, and where there should be established contour conditions. Two typical conditions are:
1.- Non-cyclic contour conditions. The variation of the concentration in the limit compartments ($i = 1$ and $i = N$) only depends on the processes of generation of NO which are produced in it, on the self-regulation and on the difference of concentrations with its unique neighbour $(i - 1)$.
2.- Cyclic contour conditions. In this case, it is supposed that the limit compartments are connected to one each other, being the neighbours of compartment $i = 1$, compartments 2 and N.
This way, the model is defined by a system of first order differential equations, Eq. (2),

$$\frac{d\mathbf{C}}{dt} = \mathbf{HC} + \mathbf{F} \tag{2}$$

where if we employ cyclic contour conditions, we have $\mathbf{C} = (C_1, C_2, ..., C_N)^T$, $\mathbf{F} = (F_1, F_2, ..., F_N)^T$ and $\mathbf{H}$ according to the expression (3).
The model has been developed for a $\mathbf{N}$ compartments monodimensional linear environment, but it is extensible in direct form to bi- and tridimensional environments. The model based on Compartmental Systems presented allows to consider the non-homogeneity of the environment, being this able to cause different forms in the processes of generation of NO in different regions of the environment, as well as variations in the self-regulation of NO according to the treated region. This is represented in the model by all the F_i and λ_i functions.

$$\mathbf{H} = \begin{pmatrix} -(D_{1,N} + D_{1,2} + \lambda_1) & D_{1,2} & \dots \\ D_{2,1} & -(D_{2,1} + D_{2,3} + \lambda_2) & \dots \\ \vdots & \vdots & \ddots \end{pmatrix} \tag{3}$$

Also, it allows to establish the anisotropy of the environment by means of the use of specific diffusion coefficients for every intercompartmental environment, where the NO spreads by. This way, we can define different values for such coefficient, in the intercompartmental environment defined between the $i-1$ and i compartments, $D_{i-1,i}$ and the intercompartmental environment defined between compartments i and $i+1$, $D_{i+1,i}$.

All these features present in the proposed model have implications in the formation of diffusse neighbourhoods (DNB), in conjuction to the diffusion geometry. In a continuous model of diffusion, the DNB, due to the simetry of the process, will have a local character to the element, (neuron, dendritic spine, etc) where the NO is produced and its reach will be given by the diffusion radius. In our compartmental model, this limitation does not occur due to the diffusion process form depends on the geometry of diffusion, which is established in the expression of diffusion dynamics. This makes the DNB able to be not simmetric and not local and will be

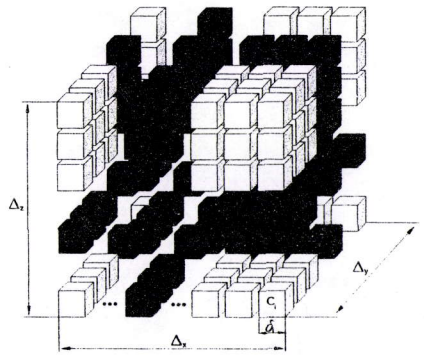


Fig. 3: 11x11x11 compartments environment where the proposed model will be analyzed.

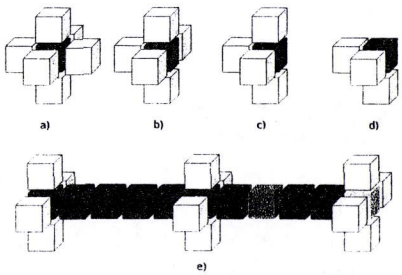


Fig. 4: Details of the diffusion geometry that establishes the adyacent compartments for the compartment $C_{i,j,k}$ when this is located inside the volume (a) and when it is part of one of the faces (b) an edge (c) or a corner of such volume (d). (e) Compartments, grey colour, where the concentration of NO profiles are studied for the different morphologies of NO generation.

closely related to the geometry of diffusion and to the NO concentration level that is established as sufficient, so the computing elements associated to a compartment are considered as influenced, being much more accorded to the biological reality.

The proposed model constitutes a step forward in the development of the Theoretical Framework (TF) belonging to the global study framework of NO diffusion (GSFNO), for which we use elements of the experimental framework (EF). The TF and EF observe mutually reliance with the Natural-Artificial-Natural (NAN) methodology [7].

3 Model Analysis and Results

We perform the model analysis studying the dynamics of NO difussion, the formation of diffuse neighbourhoods and the emerging of complex structures, in a three-dimensional homogeneous, isotropic and non-isotropic environment. The used data set for this analysis are based in results of biological experiements. The diffusion coefficient value, in isotropic environment, is about 3.3 10^{-5} cm^2 s^{-1} [10]. The dynamics of generation of NO employed, punctual, step and trapezoidal, have been deducted from the researches performed by Balbatun et al. in endothelial cells of

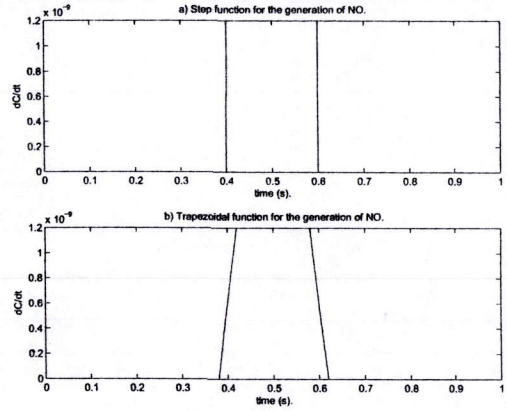


Fig. 5: a) Step function for the generation of NO. b) Trapezoidal function for the generation of NO.

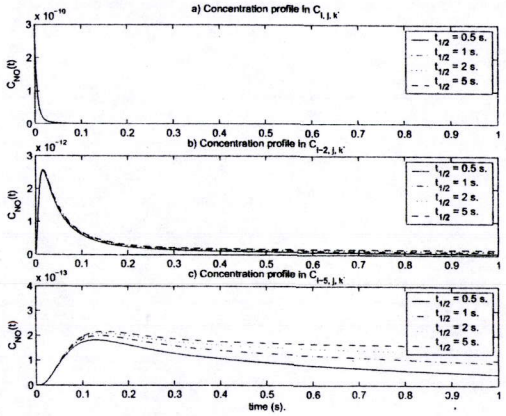


Fig. 6: Concentration profile in the compartments $C_{i,j,k}$, $C_{i+2,j,k}$ and $C_{i+5,j,k}$, when the generation in the compartment $C_{i,j,k}$. is instantaneous.

rats and rabbits [15]. Quantity of NO in $t = 0\ s$ will be of 0.24 $nmol\ cm^{-3}$, the time since the induction of the generation of NO process until the detection of diffusion was $400 + - 20\ ms$, the increasing rate has been $1.2 + - 0.05\ nmol\ cm^{-3}\ s^{-1}$, the maximum tip of concentration reached the value of $4.30 + - 0.15\ nmol\ cm^{-3}$ in a time of $600 + - 20\ ms$ and an average lifetime that varies between 0.5 s and 5 s. The environment constitutes a volume of 110x110x110 μm^3 , with a disposition of 11x11x11 compartments, Fig. 3, where $\Delta x = \Delta y = \Delta z = 110\ \mu m$ and $\delta_i = 10\ \mu m$. In this figure, we can see in black colour the different intermediate planes and compartments line, where it is going to be visualizated the dynamics of concentration of NO.

In the analysis of the model, the generation of NO processes take place in the central compartment of such volume. We will work with a diffusion geometry according to which each compartment is related to its six next neighbours, except the ones located in the faces, edges and corners of the volume, which will be with five, four and three compartments respectively, Fig. 4.

In the first part of the study, it will be analyzed the behaviour of the NO diffusion model as opposed to different dynamics of generation of NO and different values of average lifetime of NO, in a homogeneous and isotropic environment.

The morphology of instantaneous generation of NO, is gathered in the system by means of an initial condition in which we establish the value of the concentration of NO for $t = 0\ s$ in the $C_{i,j,k}$ compartment. This is not biologically plausible, even being the most used one in the studies of NO diffusion. Dynamics of generation nearer to the biological reality have been defined using the experimental data cited in

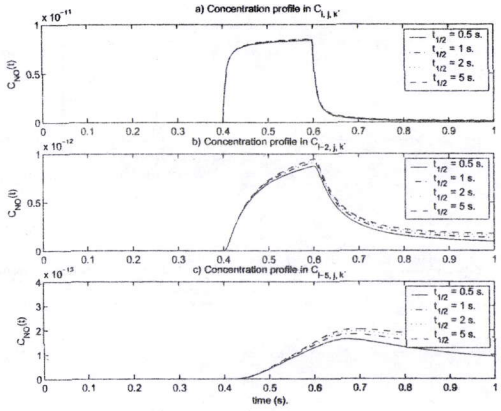


Fig. 7: Concentration profiles in the compartments $C_{i,j,k}$, $C_{i+2,j,k}$ and $C_{i+5,j,k}$, when the generation is in a step form (according to figure 6a) and takes place in the compartment $C_{i,j,k}$.

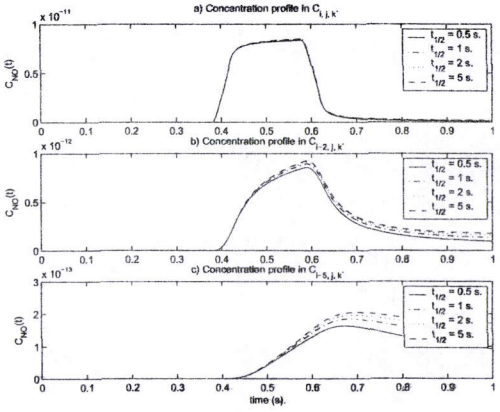


Fig. 8: Concentration profiles in the compartments $C_{i,j,k}$, $C_{i+2,j,k}$ and $C_{i+5,j,k}$, when the generation is in trapezoidal form (according to figure 6b) and takes place in the compartment $C_{i,j,k}$.

the above paragraphs, [15]. This dynamic is given by the function F_i, see expression (1), which basing on such data, have been defined as a step and trapezoidal function Fig. 5.

The dynamics of NO diffusion when a generation proces with punctual morphology takes places is the one shown in figure 6. In it, it is observed the concentration of NO profile in the generation compartment $C_{i,j,k}$, Fig. 6a, as well as in the compartments $C_{i+2,j,k}$ Fig. 6b and $C_{i+5,j,k}$ Fig. 6c. These compartments are shown in grey colour in figure 4e.

The dynamics of concentration for compartments $C_{i-2,j,k}$ and $C_{i-5,j,k}$ begins in zero and gradually increases to reach its corrresponding maximums in different times, but in an almost instantaneous form in compartments near to the generation one, (see figures 6b and 6c). It is observed how the influence of the self-regulation process becomes significant with the distance, becoming remarkable, in the studied case, in compartments where the quantity of NO that spreads is not significant.

Figure 7 shows the dynamics of the NO diffusion process when the generation corresponds to a started step in $t = 0.4$ s and a duration of 0.2 s. In figure 7a we can see the concentration of NO profile in the compartment where the generation is produced, with the established diffusion geometry, the concentration increases to converge asymptotically to the value of $C_{i,j,k} = (F_i + 6DC_{V(i,j,k)})/(1 + 6D)$, where $C_{V(i,j,k)}$ is corresponded to the concentration in the neighbour compartments, D with the diffusion coefficient and F_i with the function of generation of NO in such compartment. The dynamics of concentration for the compartments $C_{i-2,j,k}$ and $C_{i-5,j,k}$ present similar curves but of a much softer characterization where they reach their

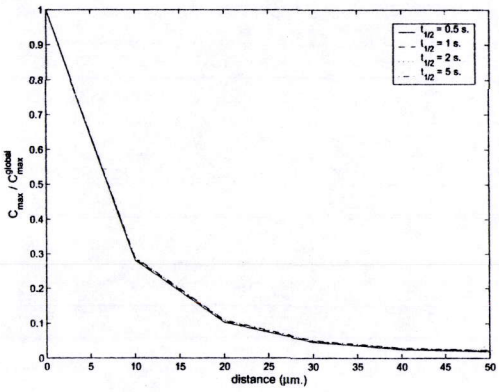


Fig. 9: C_{max}/C^{global}_{max}, as function of the distance for the generation of one unique compartment.

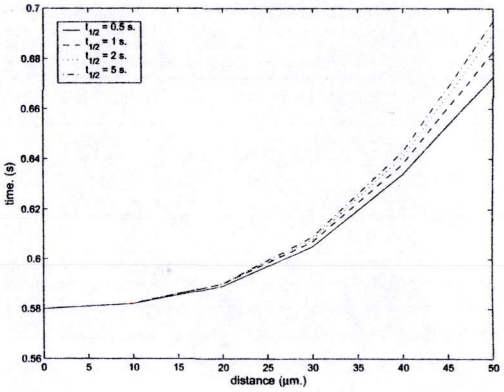


Fig. 10: Maximum concentration C_{max} reaching time as function of the distance for the generation of one unique compartment.

corresponding concentration maximums at different times (see figures 7b and 7c), being this already, in the compartment $C_{i-5,j,k}$ despicable. Similary, it can be observed that the influence of the self-regulation increases as we move away from the compartment $C_{i,j,k}$, this influence, as we will see for the trapezoidal generation, Fig. 8, presents more implication in the taken times in reaching such maximums than in the reached maximums.

The more adjusted case to the biological reality will be that one where the ratio of variation of substance concentration during the generation process increases gradually during an arbitrary time interval, to remain constant during a considerable interval of time and them decrease constantly until reaching its null value. Such generation process displays certain similarity to the one defined by the step, but the changes do not present abruptness on it. Figure 8 shows the concentration profiles for the compartments number $C_{i,j,k}$, $C_{i-2,j,k}$ and $C_{i-5,j,k}$. The behaviour of such profiles, although similar to the one obtained by the step-type generation, displays a soft behaviour in those points where the dynamics presented higher change and where the maximums are reached before in time. This can be because of the appearance of more graduated generation of NO in time than the one presented by the morphology in step.

Figures 9 and 10 show the maximum reached tips of concentration of NO in function to the distance to the unique compartment , $C_{i,j,k}$, that generates NO in the enviroment and the time that takes to reach such maximums, respectively, for different values of the self-regulation constant, corresponding to $t_{1/2}$ = 0.5, 1, 2 y 5 s. The morphology of generation of NO is the trapezoidal one. In figure 9, it is observed how the maximum tip of concentration at 30 μm does not surpass the 5% of the

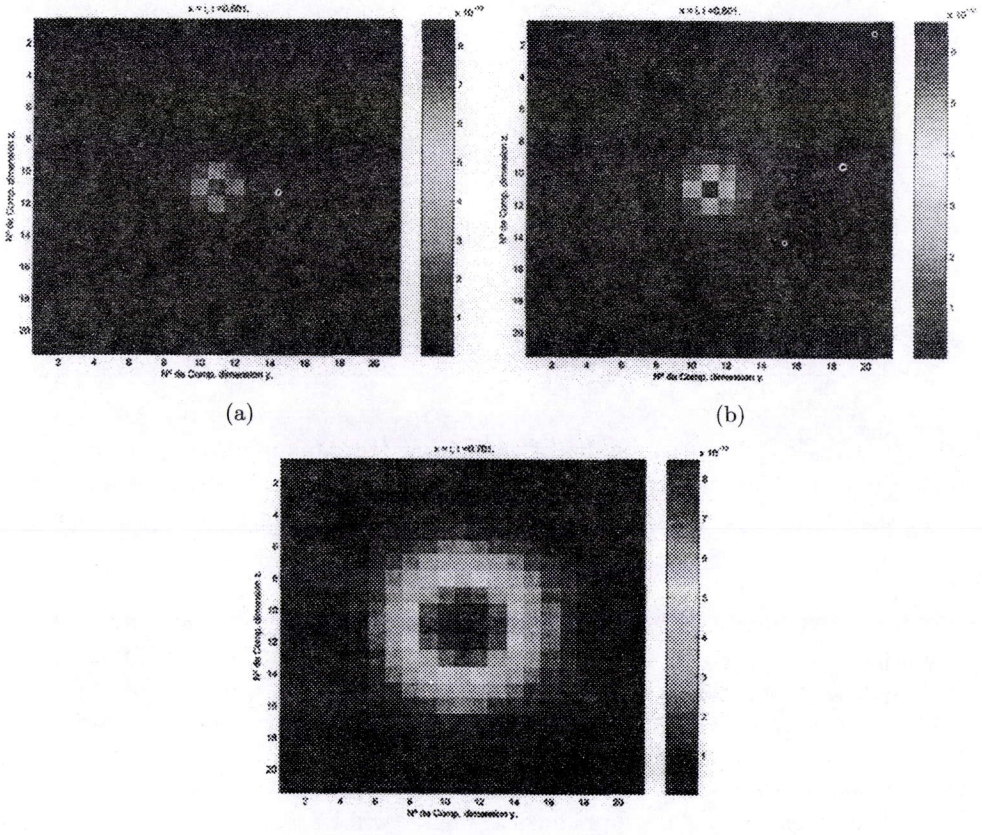
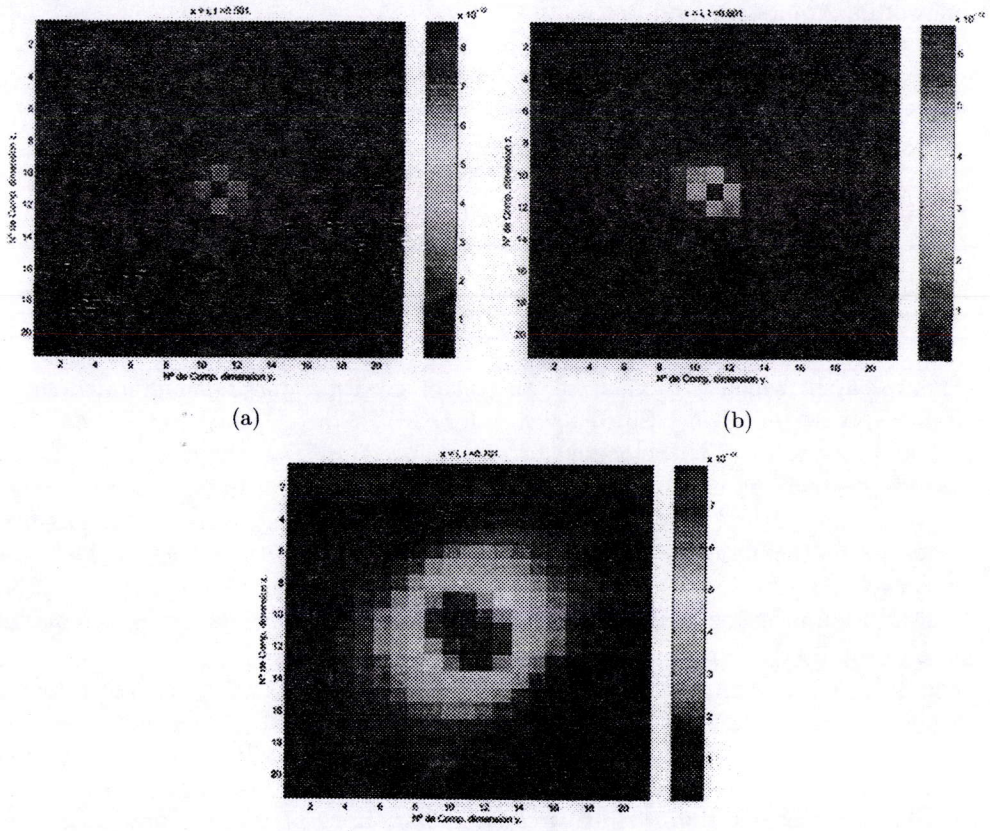


Fig. 11: (a,b,c) Sequence of snapshots taken from the plane $x = i$ when we are in an isotropic environment of a simple generation process of NO and the formation of DNB. These snapshots are shown in false colour.

maximum NO generated concentration. Nevertheless, we can see the little influence that the self-regulation of NO presents when the generation process is performed in an unique compartment and the generated quantity of NO is low, as it has been shown in the concentration profiles, Fig. 6, 7 and 8. Figure 10 shows the time that takes to reach the maximum concentration of NO in each distance. In it, we can see how there is no delay to reach this maximum value for a distance of 10 μm in relation to the generation process and how, as we increase the distance to the generating compartment, the self-regulation takes a greater role, causing a delay in reaching such maximum value.

The behaviour of the model shown in figures 9 and 10 allows to see the possibility of obtaining not simmetric and not local DNBs, being able to define the DNB as



(a) (b)

(c)

Fig. 12: (a,b,c) Sequence of snapshots taken from the plane $x = i$ when we are in a non-isotropic environment of a simple generation process of NO and the formation of DNB. These snapshots are shown in false colour.

a function of the spread concentration of NO considered as relevant and/or of the time that takes to reach a defined concentration maximum. In the case at study, if the significative concentration of NO is just the one over the 5% of the generated one, the DNB will be made up just of the two nearer compartments to the generator in each space direction.

We finish the analysis of the model presenting a sequence of results taken from the plane $x = i$, when a generation process with trapezoidal morphology occurs in the central compartment of such plane in a homogeneous and isotropic environment Fig. 11a,b,c and in a non-isotropic one Fig. 12a,b,c of 21x21x21 compartments. The non-isotropy has been established by quandrants, being the diffusion coefficients of the quadrants one and three equal, and at the same time, these ones different to the

ones of quadrants two and four.
The snapshots have been taken by identical time intervals ($t = 0.501\ s$, $t = 0.601\ s$, $t = 0.701\ s$). In figure 11a,b,c it can be perceived the total simmetry existing in the diffusion neighbourhood that such process is generating, as well as its reach, while in figure 12a,b,c it can be observed the no simmetry and the higher complexity of the formed DNB and the reach of the diffusion process of the generated NO.
Finally, this model allows to observe the emergency and formation of complex structures when there exist simultaneous generation processes of NO in time.

4 Conclusions

This research, which is framed into the set of scientific works denominated as NO and ANNs Series [7], constitutes a step forward in the development of the Theoretical Framework (TF) belonging to the global study framework of NO diffusion (GSFNO). In it, it is proposed a new NO diffusion model in the discrete, which allows to understand how NO works as a neural signalling molecule. It has been introduced a theoretical abstraction for the development of the model from a molecular level, *the compartment* which is a volume element where the generation of NO and diffusion process takes place. The compartment can have its biological counterpart in whatever neural level, from molecular to circuit level.
This model has been categorized as a generalized formal tool with high power to study and determine the dynamics in the NO production and diffusion, in the brain as well as in artificial neural systems, showing the capabilities of NO in cellular signalling and learning and its influence in the anticipatory behaviour.
It allow to consider the own features and restrictions of the NO production and diffusion and of the environment where it spreads. So it does not present limitations in what relates to the possible morphology of the NO production dynamics, being proposed in this work, according to the existent biological experiments, a dynamics of production with trapezoidal morphology.This implies an important advance in respect to previously proposed models where it is only possible to consider a morphology of instant production. On the other hand, it gathers the real features of the diffusion environment such as the no homogeneity and the no-isotropy. This has made able the use of the model to study which are the implications presented by such aspects in the creation of diffusse neighbourhoods and in the formation of complex structures when there exist a multitude of active processes of generation in a simultaneous way, being presented the results obtained in a tridimensional homogeneous isotropic and anisotropic environment in this work. The emerging structures in non-isotropic environments are of higher complexity than the ones formed when there is not isotropy present. Furthermore, it is presented a study in the formation of diffusse neighbourhoods where the non-isotropy generates asymmetries in such neighbourhoods. We demonstrate, thus, the possibility of non local and non simmetric diffusse neighbourhoods generation, aspects that are real but that the

continuous models cannot gather.
We have also presented studies in the behaviour of NO diffusion concerning with the self-regulation processes, showing its influence in the generation of no local DNB. The self-regulation presents its higher effect in the distance where the maximum NO concentration is reached, $25\mu m$ from the compartment of NO generation.
Finally, we can say that our model represents an important tool for designing and interpretation of biological experiments on NO behaviour and its effect on brain structure and function.

References

[1] C.P. Suárez Araujo, P. Fernández López, J. Regidor García and J. Simoes da Fonseca; The Retrograde Neuromessenger Nitric Oxide (NO) Effect in the Hebbian Learning in both Artificial and Biological Neural Networks: A New Mathematical Formulation for the Hebb's Learning Law; 4 Forum of European Neurosciences. FENS 2004. Lisbon.

[2] Syková, E.; The extracellular space in the CNS: its regulation, volume and geometry in normal and pathological neural function; The Neuroscientist 3 28-41, 1997.

[3] Syková, E.; Extrasynaptic Volume Transmission and Diffusion parameters of the Extracellular Space: Neuroscience 129 2004 861-876.

[4] Krekelberg, B.; Modelling cortical seft-orgnization by volumen learning; Doctoral Dissertation, King's College London, 1997.

[5] Schuman, E.M. and Madison D.V.; Nitric Oxide and Synaptic Function; Annu. Rev. Neurosci. 1994. 17:153-183.

[6] Garthwaite, J. and Boulton, C.L.; Nitric Oxide Signalling in the Central Nervous System; Annu. Rev. Physiol. 57 pp. 683-706, 1995.

[7] Suárez Araujo, C.P.; Study and Reflections on the Functional and Organisational Role of Neuromessenger Nitric Oxide in Learning: An Artificial and Biological approach; Computer Anticipatory Systems, AIP 517, 296-307, 2000.

[8] Jacquez, J.A. and Simon C.P.; Qualitative Theory of Compartmental Systems; SIAM Review Vol. 35, No. 1, pp. 43-79, March 1993.

[9] Suárez Araujo, C.P., Fernández López, P. and García Báez, P.; Towards a Model of Volume Transmission in Biological and Artificial Neural Networks: A CAST Approach; R. Moreno-Daz et al. (Eds.): EUROCAST 2001, LNCS 2178, pp 328-342, 2001.

[10] Wood, J. and Garthwaite, J.; Models of the Diffusional Spread of Nitric Oxide: Implications for Neural Nitric Oxide Signalling and its Pharmacological Properties; Neuropharmacology Vol. 33, N. 11, pp. 1235-1244, 1994.

[11] Vaughn, M.W., Kuo, L., and Liao J.C.; Effective diffusion distance of nitric oxide in the microcirculation; Am J Physiol, 1705-1714, 1998.

[12] Philippides, A, Husbands, P. And O'shea M.; Four-Dimensional Neuronal Signaling by Nitric Oxide: A Computational Analysis; The Journal of Neuroscience, February 1, 2000, 20(3); 1199-1207.

[13] Lancaster, J.R (JR.); Simulation of the diffusion and reaction of endogenously produced nitric oxide; Proc. Natl. Acad. Sci. USA, Vol. 91, pp 8137-8141, 1994.

[14] Pichler, F.; Heinz Schwaertzel (Eds): CAST Methods in Modelling. Springer Verlag, Berlin Heidelberg New York. ISBN 3-540-55405-X,1992

[15] Aleh Balbatun, Febee Rophail Louka and Tadeusz Malinski. Dynamics of nitric oxide release in the cardiovascular system. Biochemistry Research Laboratories, Onio University, Athens, USA, 2003.

[16] Malinski, T. Taha Z., Grunfeld, S, Patton, S., Kapturczak, M. and Tomboulian, P.; Difusion of nitric oxide in the aorta wall monitored in situ by porphyrinic microsensors; Biochem Biophys Res Commun, 193, 1076-1082, 1993.

[17] C.P. Suárez Araujo, P. Fernández López, P. García Báez and J. Regidor García; Study of Nitric Oxide Effect in the Heabbian Learning: Towards a Diffuse Hebb's Law; ICANN 2005; in Press.