*Archives Italiennes de Biologie, 145: 299-310, 2007.*

# A COMPUTATIONAL APPROACH TO THE STUDY OF AMPA RECEPTOR CLUSTERING AT PURKINJE CELL SYNAPSES

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## INTRODUCTION

The synapse between the granule cell axon and Purkinje cell dendritic spine, in the cerebellum is an attractive case study for synaptic physiology. First, the characterisation of cerebellar network connectivity is essentially complete and has revealed a relatively simple pattern of connections, repeating itself over the entire three-layered cortex. Second, each Purkinje cell receive only two types of excitatory inputs, a single climbing fibre axon from the inferior olive, but more than hundred thousand contacts from parallel fibre originating from granule cells (Reviewed in 15). Third, at both synapses, fast excitatory transmission is mediated solely by AMPA-type glutamate receptors, owing to the absence of functional NMDA-type receptor in mature Purkinje cells. Last but not least, plasticity of the granule-Purkinje cell synapse has been clearly established as the cellular substrate of some form of motor learning, especially for its most-studied flavour: the Long-Term Depression (LTD) resulting from repetitive paired stimulation of climbing and parallel fibres, as discovered by Ito and collaborators 20 years ago (19). Subsequent studies have shown that LTD can be induced by a variety of experimental protocols using either electrical stimulation or pharmacology, both *in vivo* and *in vitro* and is now established as a robust form of synaptic plasticity. LTD is expressed as a sustained reduction of AMPA-mediated synaptic transmission, lasting at least several hours in the lab and possibly a lifetime for individuals. Studies of the conditions and molecular mechanisms for LTD induction has revealed the involvement of various signalling pathways in LTD induction (30,28, Reviewed in 18). Convergent experimental evidences have suggested that persistent synaptic depression associated with cerebellar LTD is not associated with any changes in AMPA receptor desensitisation, kinetics, agonist affinity, or unitary conductance (22,29), but appears to be mediated by a decrease in the number of synaptic AMPA receptors, by declustering and/or endocytotic internalization (43,31). Among the five protein kinases known to be necessary for LTD induction, several lines of evidence suggests that PKC is responsible for the phosphorylation of GluR2 carboxy-terminal domain (6,7, but see 11). This region contains a conserved PDZ binding motif, which is a binding site for glutamate receptor interacting protein/AMPA receptor binding protein (GRIP/ABP). Phosphorylation of GluR2 at

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Ser880 (or GluR3 at the analogous residue Ser-885) drastically reduces the receptor's binding affinity for GRIP (32,4). Since the AMPA receptor seems to be stabilised at the synapse through interaction of its subunits carboxy-terminal domain with PSD scaffolding proteins, an attractive hypothesis actively explored by several groups is that this phosphorylation of the GluR2 cytoplasmic domain sub-unit results in gradual receptor declustering, thus reducing synaptic efficacy. Results from our lab and from others support the existence of such a mechanisms (32,31,4,45,3). Based on this scheme, it can be hypothesised that the GRIP-GluR2 interaction is the sole factor controlling AMPAR clustering at this synapse. Here we test this hypothesis by evaluating its consistency with other relevant experimental findings such as reported PDZ binding affinities or AMPA receptor density at synapse and within the extrasynaptic domain. We use a prototype model of cerebellar spine to explore GRIP-dependent AMPA receptor synaptic clustering. This model is a simplified version of a detailed description of cerebellar plasticity mechanisms, currently under development using the same formalism. It explores some of the basic theoretical and practical requirements for fusing heterogeneous experimental data from electrophysiology, imaging and biochemistry into a realistic spatial model.

## MATERIAL AND METHODS

The process that we attempted to simulate is the clustering of AMPA receptor at PF-PC synapse through interaction with scaffolding protein GRIP and binding of GRIP to an immobile synapse-anchored trans-synaptic (SATS) complex. This simplified model relies on two types of PDZ-ligand interactions: the GluR2/3 interaction with GRIP's PDZ4/5 and interaction of the cadherin complex with GRIP's PDZ6, respectively. The SATS complex is not described explicitly but may includes cadherin/liprin-a, or Ephrin/EphB complexes known to be present in AMPA receptor complexes isolated from brain tissue and necessary for AMPA receptor clusteringtative data are available concerning these interactions, we chose not to discriminate between these two trans-synaptic complexes and we will thereafter simply refer to the SATS complex. To build a realistic model of the cerebellar spine, we have used a stochastic reaction-diffusion framework, in a discretized space taking into account the three-dimensional geometry of the cytoplasm and plasma membrane as well as the diffusion-limited nature of the binding reaction between proteins in both environments. All parameters regarding spine geometry and protein diffusion were taken or derived from the literature and considered fixed. Here, we report in detail only the effects on AMPA receptor clustering of altering GRIP's affinity for its ligands, GluR2 and SATS.

*Model geometry*

Model geometry was approximated from published (35) electron microscopy serial reconstruction (Figure 1A and 1B). Since membrane proteins distribute between spine and shaft, the area of the dendritic shaft compartment was chosen to match the spine-to-shaft surface ratio of Purkinje cell dendrite. This ratio for rat PC is close to unity, with 175,000 spines per PC and 1.38 $\mu m^2$ surface per spine (14,35), giving a cumulative surface of 241,500 $\mu m^2$. This is comparable to the dendrite shaft surface of 261,000 $\mu m^2$ measured by Rapp et al. (37).

*Complexes dissociation coefficients*

The present model includes only three individual species (AMPAR, GRIP, SATS) and three complexes (AMPAR_GRIP, GRIP_SATS, AMPAR_GRIP_SATS). All protein-protein interactions considered here involve binding to GRIP's PDZ domains; either PDZ 4/5 (AMPAR) or PDZ6

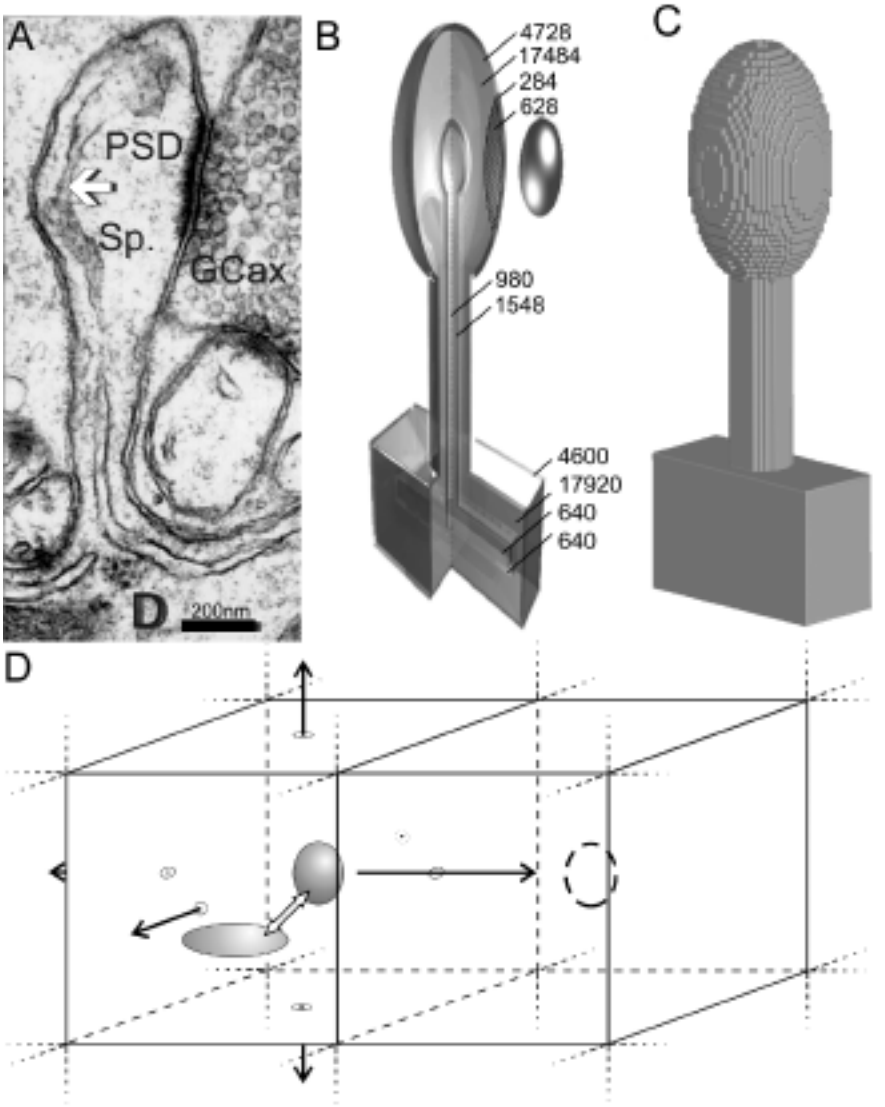


Fig. 1. - A: *Transmission electron microphotograph of a rat Purkinje cell spine, forming a synaptic contact with a parallel fibre varicosity*. Note the protrusion of endoplasmic reticulum (arrow) into the spine head (reproduced with permission from J. Spacek, Atlas of Ultrastructural Neurocytology. http://synapses.mcg.edu).
B: *Rendering of the model spine morphology.* The figures indicate the number of cubic compartments (203 nm3 voxels) used to model each structure. The ellipsoid facing the synaptic zone (hatched) indicates the position of the axonal bouton and is not part of the model.
*C: Rendering of the membrane surface following volume discretization by the simulation engine*. Note that the small voxel size adopted here (20nm) allows a good approximation of the model's geometry.
D: *Principle of the reaction-diffusion simulation implemented by MesoRD.* A molecule (sphere) within a voxel may either remain unchanged, bind (open arrow) to another molecule (ellipsoid) within the same voxel or move to any of the six adjacent voxels (black arrows), in accordance with the defined diffusion coefficient and binding rate constants.

(Cadherin or EphB/Ephrin complex). The binding affinities of PDZ domains are moderate, with equilibrium dissociation constants ($K_D$) in the low nanomolar to high micromolar range (13), with typical value around 10 μM (reviewed in 20). Finally, although AMPA receptors are hetero-tetramers, and GRIP binds to both GluR2 and GluR3 subunits, here we considered only one binding site per AMPA receptor.

*Diffusion coefficient for membrane-anchored and cytoplasmic molecular species*

The diffusion of AMPA receptor tetramer within the membrane bilayer has been measured experimentally (40). The value of 0.5 $10^{-9}$ $cm^2/s$ that we use here corresponds to the high end of the measured range of apparent diffusion coefficient for extrasynaptic AMPA receptors. It probably corresponds to diffusion unhindered by cytoskeletal and transmembrane proteins. The cadherin transmembrane protein within the cadherin-catenin and the EphB/Ephrin complex was considered to form stable homophillic interactions with their presynaptic counterparts. Thus, for the sake of simplicity, the SATS complex was considered stable and its diffusion was restricted to the synaptic zone.

The only cytoplasmic protein in this model is GRIP1, a 7 PDZ domains scaffold protein that can bind to AMPA receptors through its PDZ 4/5 domains and to the SATS complex through PDZ6. GRIP diffusion coefficient has not been reported in the literature so we had to derive an estimate from its known physical properties, yielding an approximated diffusion coefficient of 100 $cm^2.s^{-1}$ (Details of this derivation will be reported elsewhere).

*Diffusion of protein complexes*

As proteins aggregate to form a complex, the resulting increase in molecular weight and hydrodynamic radius should reduce the diffusion coefficient. In the present model however, all complexes include at least one integral membrane protein. For such complexes, the corresponding diffusion is dominated by the mobility of the membrane protein, with little influence of the resulting size increase or shape alteration (23). Consequently, we used the diffusion estimate reported by (40), both for AMPAR and for the AMPAR-GRIP complex, without any adjustment for the increased size resulting from GRIP's binding to GluR2.

*Mesoscopic simulation engine*

All simulation were performed with MesoRD 0.2.0 (16, mesord.sourceforge.net). The engine exactly simulates the Markov process corresponding to the reaction-diffusion master equation (42), using the computationally efficient "next sub-volume" method (10). This approach relies on the discretization of space into sub-volumes (voxels). Here, MesoRD automatic mesh generator was set to divide the defined structure into 8,000 $nm^3$ cubic voxels (20nm side length). There was a total of 49452 voxels (0.39$\mu m^3$), including 25652 (0.205$\mu m^3$) voxels forming the spine compartment (Figure 1B&1C). Note that although a subcellular compartment corresponding to smooth endoplasmic reticulum was defined, no molecular species could enter this compartment (Figure 1B). Its only contribution to the present model was to restrict the cross-sectional area of the spine neck, thus reducing diffusion of the cytoplasmic protein GRIP. The plasma and ER membranes were each represented by a single layer of voxels. Initially all molecular species were distributed randomly, either in the "membrane" or "cytoplasm" voxels, unless otherwise stated. In this framework, the state of the system is entirely described by the number of each molecular species in each voxel. The number of molecules within a voxel changes probabilistically, resulting either from a binding/unbinding reaction or from diffusion of a species in/out of the voxels (Figure 1D).

## RESULTS

*Validation of model geometry*

In the present model of the spine three-dimensional structure, the plasma and ER membranes were each represented by a single layer of cubic voxels. Considering the

high membrane curvature at the apex of the spines, this discretization may create discontinuities. We modelled the diffusion of 10 membrane proteins for one hour of simulated time. This low particle count was chosen to avoid multiple occupancy within a single voxel. The occupancy probability distribution was normally distributed, with a coefficient of variation consistently below 0.3 with no unvisited voxels (data not shown). Thus, this preliminary simulation of particle diffusion within the membrane showed that every membrane voxels was contiguous with its neighbours, without gap or isolated membrane patch and no artifactual diffusion bias among membrane voxels.

*Simulation of GRIP-dependent AMPA receptor clustering*

We next examined whether the reported range of PDZ-ligand affinity would account for the clustering of AMPARs at synapse. As described above, the present model uses the simplifying assumption that AMPARs are anchored in the post-synaptic membrane solely though their interaction with GRIP and a SATS complex (Figure 2A). Since GRIP is assumed to bind the STAS complex through its PDZ6 domain, binding interactions in this model are all of PDZ type. Quantification of PDZ domain affinity are scarce and current estimates are within a four order of magnitude range, with 1-10μM generally considered to be the most reliable estimate (36,12, review in 20). We first examined the influence of the PDZ dissociation constant on AMPA receptor clustering at the synapse. All PDZ interactions in the model were assumed to have an identical dissociation constant. The experimental parameters that we attempted to reproduce were the absolute number of AMPAR at the synapse and the ratio of receptor density between synaptic and extrasynaptic domain. Electrophysiological studies of Purkinje cells in acute slice and in culture suggest that quantal post-synaptic current at PF-PC synapse is 15~35pA (2,33,25), corresponding to 75~140 AMPA receptors per synapse. This is in close agreement with quantification based on noise analysis or immuno-electron microscopy (33,39). The quantitative immuno-electron study of freeze-fractured Purkinje synapse by Tanaka et al. also provides an accurate estimate of receptor density in the extrasynaptic membrane (~19 receptors/$\mu m^2$). We ran simulations with PDZ-ligand dissociation constant ($k_D$) ranging from 0.05 μM up to 500μM (Figure 2). For each $k_D$ value we ran 5 to 12 simulations, each starting from different random distributions of molecular species in the voxels (see method). The number of molecules of each species reached steady-state level within 1000 seconds of simulated time (Figure 2B). For each simulation condition, we averaged the output time-course and considered all AMPAR-containing complex (ie: AMPAR, AMPAR-GRIP, AMPAR-GRIP-SATS) present within the PSD area (Figure 2B and 3C, "synaptic membrane") as contributing to synaptic transmission. Graphical rendering of molecular species distribution within the spine showed a gradual accumulation of AMPAR-containing complexes at the synapse, with non-synaptic species remaining randomly scattered (Figure 2C). The explored range of $k_D$ covered two extreme distributions: clustering of all AMPAR at the synapse for $k_D$<0.05μM and identical density in synaptic/extrasynap-

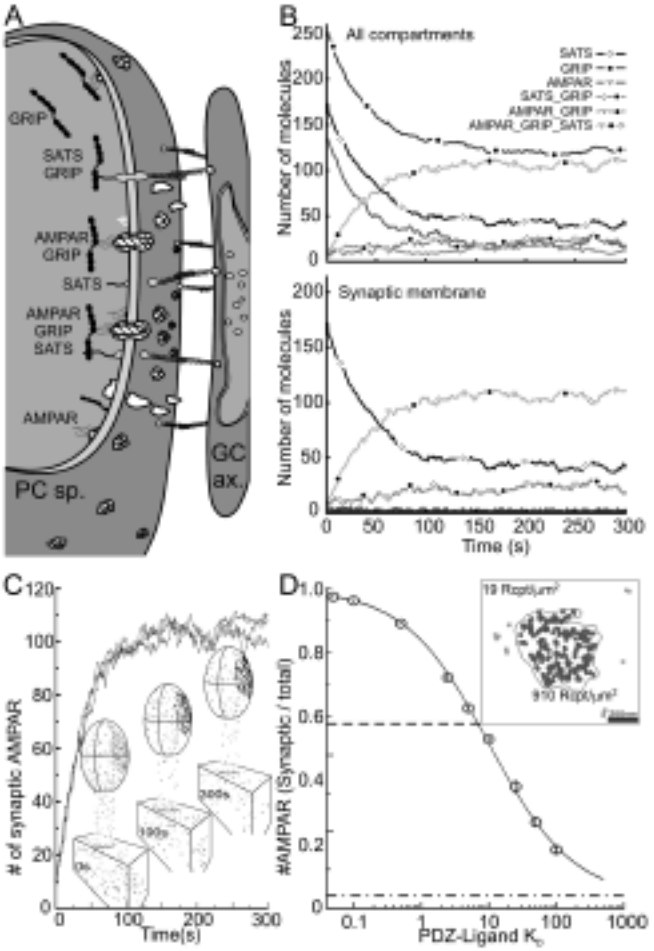


Fig. 2. - A: *Protein interaction model.* The simulation includes three individual species, two binary complexes and a ternary complex. SATS and complexes including this protein are confined to the post-synaptic membrane by virtue of SATS homophillic interaction with its counterpart in the axon terminal membrane. GRIP diffuse freely within the cytoplasm whereas AMPAR and the AMPAR-GRIP complex are confined to the plasma membrane (see text for details).
B: *Time-course of molecular species concentration change for the whole model (upper panel) and for the post-synaptic membrane (lower panel), for PDZ dissociation constant 5.0 μM.* Note that the total number of species decreases due to the formation of complexes but the total mass remains constant.
C: *Number of synaptic AMPA receptor (alone and in complex), in three independent runs of simulation for GRIP-AMPAR kD of 10μM.* Insert: 3D rendering of molecular species distribution at three time points during a simulation run. Initially, the synaptic membrane contains only isolated SATS. Note the gradual accumulation of AMPAR_GRIP_SATS ternary complex (black spheres) within the synaptic membrane.
D: *Ratio of synaptic and total AMPA receptor at steady-state as a function of GRIP-AMPAR kD.* A four-parameters Logistic function was used for interpolating data points. Insert: Scheme of freeze-fracture immuno-electron micrograph of AMPAR at an immature cerebellar synapse. The outline correspond to the contour of the synaptic membrane, with immuno-gold particle distribution indicated by grey and black dots (for the original figure see Tanaka et al. 2005, with kind permission from R. Shigemoto). The horizontal dashed line on the graph corresponds to this experimentally measured synaptic/extrasynatic density ratio (see text for detail). The dot-dash-dot line indicates the expected ratio in the absence of complex formation.

tic plasma membrane for $k_D$>1000μM (~5 uncomplexed synaptic AMPARs, Figure 2D). The $k_D$ values satisfying our requirement in terms of number of receptor and relative synaptic/extra-synaptic density were in the range 6-8 μM. Note that the synaptic membrane is represented by 284 voxels (3.0% of the total plasma membrane area) thus with ~100 synaptic AMPAR at steady-state, only a fraction of the synaptic voxels were occupied. We also verified that the probability of receptor colocalisation within one voxel was <15% (see discussion). Changing voxel size or diffusion parameters within a 20% range did not affect significantly this estimate ($p>0.5$ Mann-Whitney U Test), suggesting that this estimate is robust.

## DISCUSSION

We chose to simulate the GRIP-mediated clustering of AMPA receptor to the synapse since the alteration of GRIP-GluR2 is a key event in LTD induction and one of the best experimentally characterised aspects of this synapse (32,31,45,3). One of the basic assumptions in this model is that AMPA receptors are stabilised within the synaptic area solely by their interaction with GRIP, GRIP itself being anchored by its binding to other PSD proteins. Here we have designed a very simple model to examine whether this model is compatible with our present knowledge of PDZ-type binding affinities and receptor distribution. A second motive was to explore the potential of realistic stochastic simulation to correlate heterogeneous experimental data under a common formalism.

For the construction of this simple model, we allowed several major approximations. First, we considered Ephrin/EphB receptor and cadherin as a single monomeric entity, providing attachment to the pre-synaptic axon terminal and thus defining the post-synaptic zone. Cadherin however interact with GRIP and other cytoplasmic proteins indirectly, through a stable complex formed with liprin-α and with catenins β or δ (44,34). Furthermore, the trans-synaptic interactions are themselves labile. For the sake of simplicity, we kept the number of molecular species low for this simple model by recognising that these binding affinities are several order of magnitudes higher than the micromolar range measured for PDZ interactions. Thus, we chose to describe the cadherin-catenin-liprin complex and the EphB-Ephrin as “SATS”.

Second, we considered neither the interaction of GRIP with other PSD proteins or even the possibility for GRIP to form homomultimers through interactions of its PDZ4, PDZ5 and PDZ6 domains (38,8). The main expected influence would be that fewer SATS complex would be require in the model since a single SATS would be able to anchor a GRIP multimer at the synapse, thus providing multiple attachment sites for AMPA receptors. Since our simulation is mostly influenced by the GRIP-AMPAR rather than by the GRIP-SATS interaction, we do not expect this modification to fundamentally affect the model behaviour and the estimate that we derived for GRIP-AMPAR interaction.

Third, AMPAR were also modelled as monomeric entity with a single PDZ- binding domain. AMPA receptors are tetramers, mostly composed at this synapse of GluR2 and GluR3 subunits. Although the exact sub-unit stochiometry is not established, both can bind PDZ domains. Steric hindrance may prevent all subunits from binding GRIP simultaneously but future refinement of the model will have to take this hypothesis in consideration.

Finally, protein translocation in the model are the result of random diffusion while active transport along microtubules and actin filaments is a well-described mechanism used by cells to address proteins and vesicle to specific regions of the cytoplasm or membrane. We adopted the view that AMPA receptor distribution result from a dynamic equilibrium between a synapse with limited number of binding "slots" and a shaft plasma where the receptor is freely diffusing. Recent studies of AMPA receptor movement lend support to this theory (40,1). In this context, receptor diffusion is relevant as determinant of reaction kinetic, without denying the important of active long-range transport and internalisation/recycling.

*Relevance of GRIP-AMPA affinity for synaptic plasticity*

Our simulation suggest that a GRIP-AMPAR dissociation constant of ~7μM would be compatible with both the absolute number of AMPAR at PF-PC synapse (~100) and with the relative synaptic/extrasynaptic receptor densities (33,39) previously described for synapse on PC. This estimate is robust even when the total number of GRIP protein is varied by ±15% (data not shown) and as long as there is an excess of GRIP compared to AMPAR. This seems to be the case in neurones since GRIP is abundant in the dendritic shaft of neurones and has been detected by immuno-electron microscopy (8). Furthermore, long-lasting potentiation has been described at the PF-PC synapse (27,5,21), suggesting that there is a pool of readily available GRIP that could be recruited at the synapse to stabilise the newly inserted AMPA receptors. This relatively low binding affinity in the 1-10 μM range is common for PDZ-ligand interaction (reviewed in 20) and is considered to be suitable for regulatory function since it is easily reversible following changes of intracellular conditions, of ligand conformation or of phosphorylation.

Using a very simple model of AMPA receptor synaptic clustering, based on its interaction with GRIP and cadherin, we have explored several strategies to extract estimates of model parameters from diverse source of data, including electronic and photonic imagery, electrophysiology and molecular biology. While the resulting model has no pretension of being a faithful and exhaustive representation of the mechanisms controlling receptor clustering, our results show reasonable agreement with an array of experimental evidences.

Our knowledge of the signalling proteins and cellular mechanisms involved in the maintenance and plasticity of granule-Purkinje cell synapse has increased markedly in recent years with more than 30 proteins identified as playing important roles in AMPAR removal following LTD induction (30,28, Reviewed in 18). At the "input

stage" of the signalling network, at least seven membrane receptors and channels are known to be implicated in LTD induction, together with several cytoplasmic targets of membrane-diffusible messengers. The downstream signalling cascades involve no less than five different families of protein kinases as well as several phosphatases. The issue is further confounded by the interdependencies and cross-talks between these cascades, some of which have already been clearly established by several studies (26, 24, 11). Thus, even if we restrict ourselves to the molecular events controlling AMPA receptor clustering, designing experiments addressing exhaustively the multiple aspects of this regulation and generating quantitative measurements has become increasingly difficult.

There have been very few attempts so far to model this cellular processes; Hernjak and collaborators (17) restricted their model to a detailed description of the calcium dynamic in spine in relation to LTD induction while the Kawato group recently developed a deterministic kinetic model of LTD signalling cascade leading to AMPAR phosphorylation (24). As our knowledge of the intricacies of synaptic plasticity increases, such quantitative modelling of the underlying molecular and cellular mechanisms are becoming increasingly important in reducing the apparent complexity of experimental findings. To date, most of our knowledge of this plasticity comes from studies using electrophysiology/pharmacology since LTD is in essence an electrophysiological phenomenon. The recent advent of innovative approaches to directly measure protein displacement and interaction *in situ* offers hope that combining data from different experimental modalities will allow to test model predictions not only against a single index (i.e.: EPSC amplitude) but against an array of correlated observations, including protein post-translational modifications, complex alteration or protein translocation within and between cellular compartments. To this end, realistic simulation approaches incorporating multimodal data fusion will not only be helpful, they will be indispensable.

## SUMMARY

Synapses between parallel fibres and Purkinje cells in the cerebellum exhibit unique forms of synaptic plasticity thought to be associated with the refinement of motor skills. Since the discovery of Long Term Depression (LTD), more than twenty years ago, many molecular signalling pathways potentially underlying LTD have been explored. These have revealed a surprisingly diverse array of cellular and molecular mechanisms. Foremost has been the now well-established discovery that LTD is the electrophysiological manifestation of a reduced density of AMPA receptors at the synapse, following induction. Although LTD is primarily an electrophysiologically defined phenomenon, recent studies have increasingly combined electrophysiological, imaging, proteomic and biochemical approaches to probe its mechanisms. The challenge is now to integrate data from different modalities into a unified formalism that can deal with the complexity of the system, as well as generate exper-

imental predictions. Here, we use particle-based stochastic modelling as a prototype to explore the feasibility of building realistic model of synaptic plasticity, at the molecular level.

*Acknowledgements*. - We are grateful to M. Ito for his advice and constant support. We thank R. Shigemoto and J. Spacek for allowing us to use some of their microphotographs. Discussions with S.M. Korogod and C. Rockland inspired some of the studies presented here. We gratefully thank J. Elf, J. Hattne and D. Fange for the creation, public release and active support of MesoRD.

## REFERENCES

1. Ashby, M.C., Maier, S.R., Nishimune, A., and Henley, J.M. Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology. *J. Neurosci*., **26**: 7046-7055, 2006.
2. Barbour, B. Synaptic currents evoked in Purkinje cells by stimulating individual granule cells. *Neuron*, **11**: 759-769, 1993.
3. Chung, H.J., Steinberg, J.P., Huganir, R.L., and Linden, D.J. Requirement of AMPA receptor GluR2 phosphorylation for cerebellar long-term depression. *Science*, **300**: 1751-1755, 2003.
4. Chung, H.J., Xia, J., Scannevin, R.H., Zhang, X., and Huganir, R.L. Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. *J. Neurosci*., **20**: 7258-7267, 2000.
5. Coesmans, M., Weber, J.T., De Zeeuw, C.I., and Hansel, C. Bidirectional parallel fiber plasticity in the cerebellum under climbing fiber control. *Neuron*, **44**: 691-700, 2004.
6. Crepel, F. and Krupa, M. Activation of protein kinase C induces a long-term depression of glutamate sensitivity of cerebellar Purkinje cells. An in vitro study. *Brain Res*., **458**: 397-401, 1988.
7. De Zeeuw, C.I., Hansel, C., Bian, F., Koekkoek, S.K., van Alphen, A.M., Linden, D.J., and Oberdick, J. Expression of a protein kinase C inhibitor in Purkinje cells blocks cerebellar LTD and adaptation of the vestibulo-ocular reflex. *Neuron*, **20**: 495-508, 1998.
8. Dong, H., Zhang, P., Song, I., Petralia, R.S., Liao, D., and Huganir, R.L. Characterization of the glutamate receptor-interacting proteins GRIP1 and GRIP2. *J. Neurosci*., **19**: 6930-6941, 1999.
9. Dunah, A.W., Hueske, E., Wyszynski, M., Hoogenraad, C.C., Jaworski, J., Pak, D.T., Simonetta, A., Liu, G., and Sheng, M. LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses. *Nat. Neurosci.*, **8**: 458-467, 2005.
10. Elf, J. and Ehrenberg, M. Spontaneous separation of bi-stable biochemical systems into spatial domains of opposite phases. *IEE Syst. Biol.*, **1**: 230-236, 2004.
11. Endo, S. and Launey, T. ERKs regulate PKC-dependent synaptic depression and declustering of glutamate receptors in cerebellar Purkinje cells. *Neuropharmacology*, **45**: 863-872, 2003.
12. Harris, B.Z., Hillier, B.J., and Lim, W.A. Energetic determinants of internal motif recognition by PDZ domains. *Biochemistry*, **40**: 5921-5930, 2001.
13. Harris, B.Z. and Lim, W.A. Mechanism and role of PDZ domains in signaling complex assembly. *J. Cell Sci*., **114**: 3219-3231, 2001.
14. Harris, K.M. and Stevens, J.K. Dendritic spines of rat cerebellar Purkinje cells: serial electron microscopy with reference to their biophysical characteristics. *J. Neurosci*., **8**: 4455-4469, 1988.

15. HARVEY, R.J. and NAPPER, R.M. Quantitative studies on the mammalian cerebellum. *Prog. Neurobiol.*, **36**: 437-463, 1991.
16. HATTNE, J., FANGE, D., and ELF, J. Stochastic reaction-diffusion simulation with MesoRD. *Bioinformatics.*, **21**: 2923-2924, 2005.
17. HERNJAK, N., SLEPCHENKO, B.M., FERNALD, K., FINK, C.C., FORTIN, D., MORARU, I.I., WATRAS, J., and LOEW, L.M. Modeling and analysis of calcium signaling events leading to long-term depression in cerebellar Purkinje cells. *Biophys. J.*, **89**: 3790-3806, 2005.
18. ITO, M. The molecular organization of cerebellar long-term depression. *Nat. Rev. Neurosci.*, **3**: 896-902, 2002.
19. ITO, M., SAKURAI, M., and TONGROACH, P. Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. *J. Physiol. Lond.*, **324**: 113-134, 1982.
20. JELEN, F., OLEKSY, A., SMIETANA, K., and OTLEWSKI, J. PDZ domains - common players in the cell signaling. *Acta Biochim. Pol.*, **50**: 985-1017, 2003.
21. KAKEGAWA, W. and YUZAKI, M. A mechanism underlying AMPA receptor trafficking during cerebellar long-term potentiation. *Proc. Natl. Acad. Sci. U.S.A*, **102**: 17846-17851, 2005.
22. KOJIMA, H., ICHIKAWA, K., ILEVA, L.V., TRAYNELIS, S.F., and YOSHIOKA, T. Properties of AMPA receptor channel during long-term depression in rat cerebellar Purkinje cells, in KUBA K. and BROWN D.A. (Eds.), *Slow Synaptic Responses and Modulation*, Tokyo, Springes, 1999, pp. 307-314.
23. KUCIK, D.F., ELSON, E.L., and SHEETZ, M.P. Weak dependence of mobility of membrane protein aggregates on aggregate size supports a viscous model of retardation of diffusion. *Biophys. J.*, **76**: 314-322, 1999.
24. KURODA, S., SCHWEIGHOFER, N., and KAWATO, M. Exploration of signal transduction pathways in cerebellar long-term depression by kinetic simulation. *J. Neurosci.*, **21**: 5693-5702, 2001.
25. LAUNEY, T., ENDO, S., SAKAI, R., HARANO, J., and ITO, M. Protein phosphatase 2A inhibition induces cerebellar long-term depression and declustering of synaptic AMPA receptor. *Proc. Natl. Acad. Sci. U.S.A*, **101**: 676-681, 2004.
26. LEV-RAM, V., JIANG, T., WOOD, J., LAWRENCE, D.S., and TSIEN, R.Y. Synergies and coincidence requirements between NO, cGMP, and Ca2+ in the induction of cerebellar long-term depression. *Neuron*, **18**: 1025-1038, 1997.
27. LEV-RAM, V., WONG, S.T., STORM, D.R., and TSIEN, R.Y. A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. *Proc. Natl. Acad. Sci. U.S.A*, **99**: 8389-8393, 2002.
28. LEVENES, C., DANIEL, H., and CREPEL, F. Long-term depression of synaptic transmission in the cerebellum: cellular and molecular mechanisms revisited. *Prog. Neurobiol.*, **55**: 79-91, 1998.
29. LINDEN, D. J. The expression of cerebellar LTD in culture is not associated with changes in AMPA-receptor kinetics, agonist affinity, or unitary conductance. *Proc. Natl. Acad. Sci. U.S.A*, **98**: 14066-14071, 2001.
30. LINDEN, D.J. and CONNOR, J.A. Long-term synaptic depression. *Annu. Rev. Neurosci.*, **18**: 319-357, 1995.
31. MATSUDA, S., LAUNEY, T., MIKAWA, S., and HIRAI, H. Disruption of AMPA receptor GluR2 clusters following long-term depression induction in cerebellar Purkinje neurons. *EMBO J.*, **19**: 2765-2774, 2000.
32. MATSUDA, S., MIKAWA, S., and HIRAI, H. Phosphorylation of serine-880 in GluR2 by protein kinase C prevents its C terminus from binding with glutamate receptor-interacting protein. *J. Neurochem.*, **73**: 1765-1768, 1999.

33. MOMIYAMA, A., SILVER, R.A., HAUSSER, M., NOTOMI, T., WU, Y., SHIGEMOTO, R., and CULL-CANDY, S.G. The density of AMPA receptors activated by a transmitter quantum at the climbing fibre-Purkinje cell synapse in immature rats. *J. Physiol., Lond.*, **549**: 75-92, 2003.
34. MONEA, S., JORDAN, B.A., SRIVASTAVA, S., DESOUZA, S., and ZIFF, E.B. Membrane localization of membrane type 5 matrix metalloproteinase by AMPA receptor binding protein and cleavage of cadherins. *J. Neurosci*., **26**: 2300-2312, 2006.
35. NAPPER, R. M. and HARVEY, R. J. Quantitative study of the Purkinje cell dendritic spines in the rat cerebellum. *J. Comp Neurol*., **274**: 158-167, 1988.
36. NIETHAMMER, M., VALTSCHANOFF, J.G., KAPOOR, T.M., ALLISON, D.W., WEINBERG, T.M., CRAIG, A.M., and SHENG, M. CRIPT, a novel postsynaptic protein that binds to the third PDZ domain of PSD-95/SAP90. *Neuron*, **20**: 693-707, 1998.
37. RAPP, M., SEGEV, I., and YAROM, Y. Physiology, Morphology and Detailed Passive Models of Guinea Pig Cerebellar Purkinje Cells. *J. Physiol. Lond.*, **474**: 101-118, 1994.
38. SRIVASTAVA, S., OSTEN, P., VILIM, F.S., KHATRI, L., INMAN, G., STATES, B., DALY, C., DESOUZA, S., ABAGYAN, R., VALTSCHANOFF, J.G., WEINBERG, R.J., AND ZIFF, E.B. Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP. *Neuron*, **21**: 581-591, 1998.
39. TANAKA, J., MATSUZAKI, M., TARUSAWA, E., MOMIYAMA, A., MOLNAR, E., KASAI, H., and SHIGEMOTO, R. Number and density of AMPA receptors in single synapses in immature cerebellum. *J. Neurosci*., **25**: 799-807, 2005.
40. TARDIN, C., COGNET, L., BATS, C., LOUNIS, B., and CHOQUET, D. Direct imaging of lateral movements of AMPA receptors inside synapses. *EMBO J.*, **22**: 4656-4665, 2003.
41. TORRES, R., FIRESTEIN, B.L., DONG, H., STAUDINGER, J., OLSON, E.N., HUGANIR, R.L., BREDT, D.S., GALE, N.W., AND YANCOPOULOS, G.D. PDZ proteins bind, cluster, and synaptically colocalize with Eph receptors and their ephrin ligands. *Neuron*, **21**: 1453-1463, 1998.
42. VAN KAMPEN, N.G. *Stochastic Processes in Physics and Chemistry*. 2nd Edition North-Holland, Amsterdam 1997.
43. WANG, Y.T. and LINDEN, D.J. Expression of cerebellar long-term depression requires postsynaptic clathrin-mediated endocytosis. *Neuron*, **25**: 635-647, 2000.
44. WYSZYNSKI, M., KIM, E., DUNAH, A.W., PASSAFARO, M., VALTSCHANOFF, J.G., SERRA-PAGES, C., STREULI, M., WEINBERG, R.J., and SHENG, M. Interaction between GRIP and liprin-alpha/SYD2 is required for AMPA receptor targeting. *Neuron*, **34**: 39-52, 2002.
45. XIA, J., CHUNG, H.J., WIHLER, C., HUGANIR, R.L., and LINDEN, D.J. Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. *Neuron*, **28**: 499-510, 2000.