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Phys. Rev. Lett. **106**, 238104 — Published 8 June 2011

DOI: [10.1103/PhysRevLett.106.238104](https://doi.org/10.1103/PhysRevLett.106.238104)

# Formation and Stability of Synaptic Receptor Domains

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Neurotransmitter receptor molecules, concentrated in postsynaptic domains along with scaffold and a number of other molecules, are key regulators of signal transmission across synapses. Combining experiment and theory, we develop a quantitative description of synaptic receptor domains in terms of a reaction-diffusion model. We show that interactions between only receptors and scaffolds, together with the rapid diffusion of receptors on the cell membrane, are sufficient for the formation and stable characteristic size of synaptic receptor domains. Our work reconciles long-term stability of synaptic receptor domains with rapid turnover and diffusion of individual receptors, and suggests novel mechanisms for a form of short-term, postsynaptic plasticity.

PACS numbers: 87.16.-b, 82.40.-g, 87.19.lp, 87.19.lw

How the physiological stability necessary for memory storage can be achieved in the presence of rapid molecular turnover and diffusion is a central problem in neurobiology [1]. Synapses, in particular, are believed to be the physiological seat of memory, and rely on the stability of postsynaptic domains containing neurotransmitter receptor molecules, as well as scaffold and a number of other molecules, over days, months, or even longer periods of time [2, 3]. Yet, recent experiments have demonstrated that individual receptor [4–6] and scaffold [7–9] molecules leave and enter postsynaptic domains on typical time scales as short as seconds. How can these seemingly contradictory observations—long-term stability and a well-defined characteristic size of postsynaptic domains on the one hand, rapid molecular turnover and diffusion on the other hand—be integrated in a unified understanding of postsynaptic domain formation and stability?

Classically, it has been assumed that interactions between presynaptic and postsynaptic neurons play a paramount role in the stability and in setting the characteristic size of synaptic receptor domains [10]. Over recent years, though, a number of studies [4, 6, 11], carried out on a variety of chemical synapses, have indicated that molecular domains containing synaptic receptor molecules may form spontaneously even in the absence of presynaptic neurons. However, a detailed molecular understanding of the mechanism governing the formation and stability of synaptic receptor domains has remained elusive. In this Letter, we first discuss a minimal experimental system which enables us to determine the molecular components essential for the emergence of stable receptor domains of the characteristic size observed in neurons [9, 12, 13]. On this basis, we then formulate a mathematical model of the formation and stability of synaptic receptor domains which quantitatively explains our experimental observations, and also makes predictions pertaining to the stability and regulation of synap-

tic receptor domains.

In our experiments we used single fibroblast cells, which are devoid of the molecular machinery commonly associated with postsynaptic domain formation [10] but allow for the rapid turnover and diffusion of receptors observed in neurons [4, 6], as well as for interaction of receptors with scaffold molecules. Fibroblast cells were transfected [14] with glycine receptors, one of the main receptor types at inhibitory synapses, and their associated scaffolds, gephyrin molecules [9]. In our minimal system, the mere presence of both receptor and scaffold molecules led to the spontaneous emergence of stable receptor-scaffold domains (RSDs) [see Figs. 1(a,b)]. These domains corresponded to a joint enhancement of the receptor and scaffold molecule densities, over a characteristic area of 0.2 to 0.3  $\mu\text{m}^2$  [Fig. 1(c)]. Once the RSDs were formed, their mean area remained stable over a time scale of days, with little cell-to-cell variability in the mean area of RSDs but larger variability in the mean number of RSDs per cell [Fig. 1(c)]. If only receptors were transfected, in the absence of scaffold molecules, receptor domains did not emerge, apart from possible occurrences of transient microdomains [12, 15]. If only scaffold molecules were transfected, in the absence of receptors, then these formed large intracellular blobs but no association with the cell membrane was detected [12, 16].

The experiments carried out on our minimal system indicate that the reaction and diffusion properties of receptors and scaffolds at the membrane are *necessary and sufficient* for RSD formation and stability. In particular, the presence of a presynaptic terminal is not essential for the occurrence of stable RSDs. In agreement with previous studies [4, 6, 11], our results point to a picture in which postsynaptic domains form in the absence of presynaptic stimulation, which subsequently intervenes in their maturation and regulation. Both the characteristic size and the stability of the RSDs observed in our experiments are

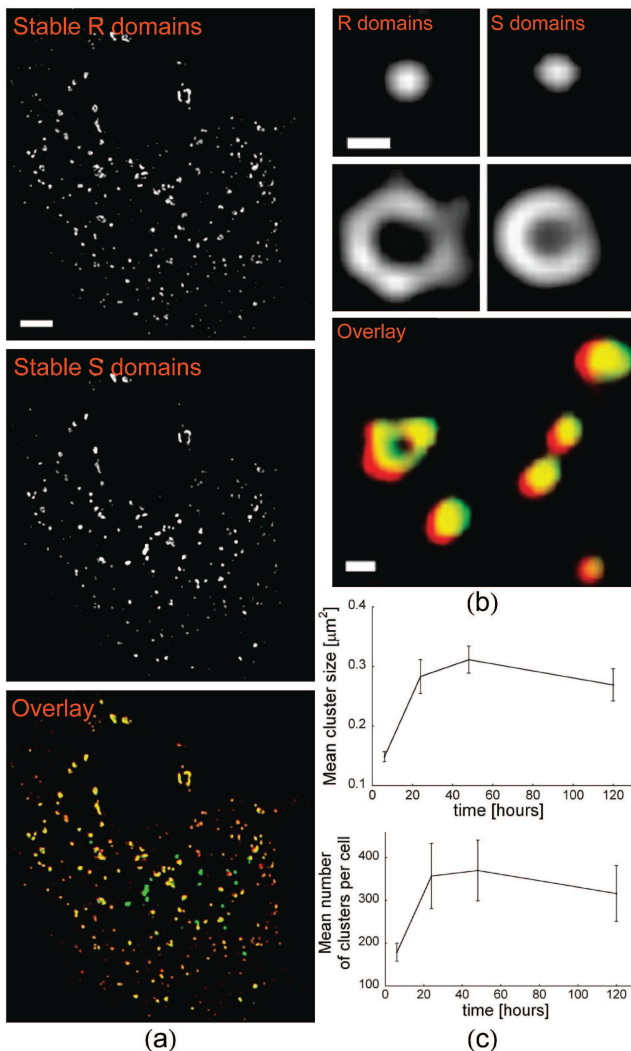


FIG. 1: (color online). Experimental results on the formation and stable characteristic size of RSDs composed of glycine receptors and gephyrin scaffolds [14]. (a) Example of a transfected COS-7 cell with domains on its membrane: Receptor (R, red) and scaffold (S, green) concentration and overlay (co-domains in yellow). A fraction of the apparent green-labeled scaffolds is endoplasmic. Scale bar,  $5 \mu\text{m}$ . (b) Examples of selected RSDs at higher resolution. For ease of visualization, the concentration maps of the two molecular species were slightly shifted with respect to one another in the color panel. Scale bars,  $0.5 \mu\text{m}$ . (c) Mean RSD (cluster) area and number of RSDs (clusters) per cell versus time. Error bars: standard errors;  $n > 10$  cells from two independent experiments for each point.

similar to those of synaptic receptor domains in neurons. Indeed, when scaffold molecules are transfected to young neurons devoid of synapses, domains of a comparable size arise [12]. When they are transfected to mature neurons with synapses, the domain size remains unchanged [13]. Finally, the diffusion properties of receptors are similar in cells with transfected [9] and endogenous [17] scaffold molecules. Thus, we expect that receptors and scaffolds

in neurons exhibit the necessary and sufficient properties for RSD formation and stability, as they do in our experiments.

We now turn to the mathematical description of our minimal experimental system. The concentrations of synaptic receptors and scaffolds are represented by the functions  $r(x, y, t)$  and  $s(x, y, t)$ , where the variables  $x$  and  $y$  denote coordinates along the cell membrane, and the variable  $t$  denotes time. The spatiotemporal evolution of these fields is governed by the reaction-diffusion equations

$$\frac{\partial r}{\partial t} = F(r, s) + \nu_r \nabla \cdot [(1-s)\nabla r + r\nabla s], \quad (1)$$

$$\frac{\partial s}{\partial t} = G(r, s) + \nu_s \nabla \cdot [(1-r)\nabla s + s\nabla r], \quad (2)$$

where  $F$  and  $G$  are cubic polynomials in  $r$  and  $s$  that describe the reactions in our system [14], and  $\nu_r$  and  $\nu_s$  are the receptor and scaffold diffusion coefficients. The non-linear corrections to the standard diffusion terms  $\nu_r \nabla^2 r$  and  $\nu_s \nabla^2 s$  in Eqs. (1) and (2) arise from the constraint  $0 \leq r + s \leq 1$ , which accounts for steric repulsion [4, 6] of receptors and scaffolds in the confined membrane environment of a living cell; we have normalized  $r$  and  $s$  so that the maximum concentration of receptors and scaffolds is equal to 1. The same constraint is imposed [14] on the reaction terms in Eqs. (1) and (2). Experimental studies [4–9, 15] of the diffusion properties of glycine receptors and gephyrin scaffolds, as well as of other types of receptors and scaffolds, yield  $\nu_r \gg \nu_s$ .

The reaction and diffusion properties of synaptic receptors and scaffolds [4–9, 15] suggest that Eqs. (1) and (2) exhibit pattern formation via a Turing instability [18, 19]: Receptors diffuse quickly and, due to steric constraints, passively inhibit increased molecular concentrations of receptors and scaffolds, whereas scaffolds diffuse more slowly and transiently bind receptors as well as scaffolds. In agreement with experiments, domain formation via a Turing mechanism necessarily relies on the presence of both receptors and scaffolds. Expressions of the reaction terms  $F$  and  $G$  in Eqs. (1) and (2) are obtained [14] from the relevant chemical interactions, reported previously [4–9], together with the general mathematical constraints associated with Turing instabilities [19], a point to which we return below. Reaction-diffusion models akin to the one described here have, in recent years, been used to describe molecular localization during cell division [20–22], and are to be contrasted with models of domain formation which rely on phase separation and coarsening [23].

We simulated Eqs. (1) and (2), starting from random initial conditions, with units of space and time set by the receptor diffusion coefficient and the rate of receptor endocytosis. Using typical values of these parameters taken from experiments [4–9, 15], we found that irregular patterns of stable RSDs similar to experimental ones emerged over a time scale of hours [see Figs. 2(a)

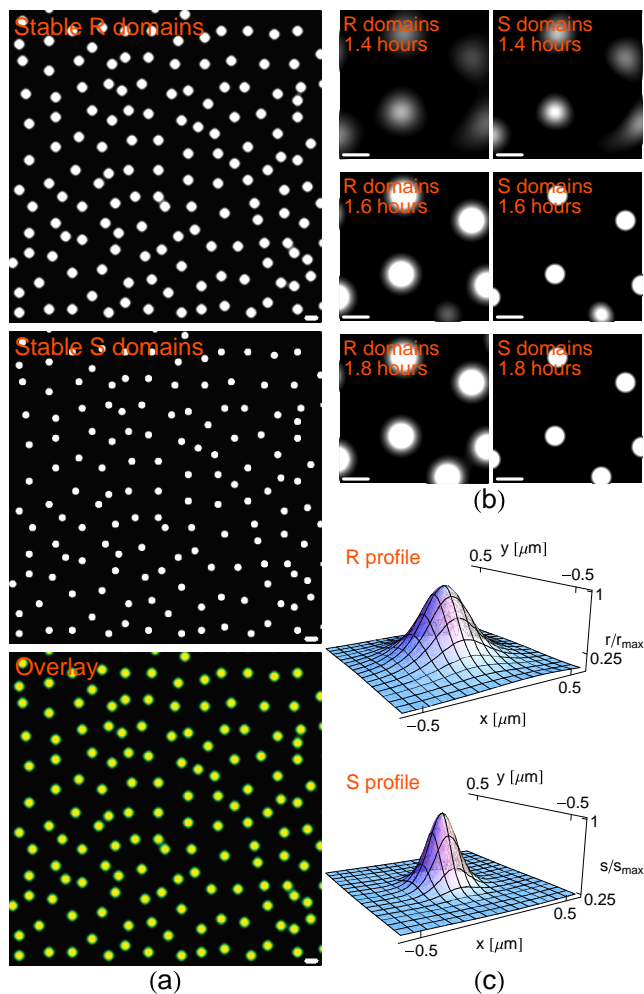


FIG. 2: (color online). Model results on the formation and stable characteristic size of RSDs [14]. (a) Irregular patterns of stable RSDs, with an area of approximately  $0.2$  to  $0.3 \mu\text{m}^2$  each, emerge on a time scale of hours. The distributions of receptors (upper panel) and scaffolds (middle panel) are overlaid (lower panel), and the domain shapes and patterns are stable. (b) Formation and (c) shape of RSDs at higher resolution. The fields  $r$  and  $s$  in panel (c) are scaled with respect to their maximum values inside RSDs. Scale bars,  $0.5 \mu\text{m}$ .

and 1(a)]. Individual domains resulted from a coordinated increase of receptor and scaffold densities. Occasionally, we observed sets of closely-spaced RSDs in the outcomes of simulations [see Figs. 2(a,b)], which resulted from initial random fluctuations and are expected to be further distorted in the presence of molecular noise [24]. At lower resolution, these appeared as larger than average, ring-shaped domains reminiscent of similar instances observed experimentally at a comparable resolution [see Fig. 1(a) and the lower panels in Fig. 1(b)]. Moreover, we found in our simulations that receptor aggregation trails behind scaffold aggregation in time [see Fig. 2(b)], which is in fact a general prediction of our model and is also in agreement with experiments [25, 26].

While, in accordance with experimental observations [4–9, 15], we allowed [14] for a variety of interactions between receptors and scaffolds when simulating Eqs. (1) and (2), only a handful of chemical reactions were actually crucial for the emergence of a Turing instability. To lowest order, these reactions correspond to  $R \rightarrow R_b$  and  $R_b + S \rightarrow R + S$  for the receptors, and to  $S \rightarrow S_b$  and  $S_b + 2S \rightarrow 3S$  for the scaffolds, respectively. In these expressions, the symbols  $R$  and  $S$  stand for receptors and scaffolds at the membrane, and  $R_b$  and  $S_b$  denote molecules in the bulk of the cell. In particular, the reaction  $S_b + 2S \rightarrow 3S$ , in which a scaffold molecule from the bulk is adsorbed onto the membrane by two other scaffold molecules into a trimer, is key to domain formation, whereas the simpler reaction  $S_b + S \rightarrow 2S$  alone is not sufficient. Indeed, gephyrin scaffold molecules are thought to form both dimers and trimers under the usual conditions in which neural domains are observed [4]. However, if trimerization is prevented, no domains (or only very small ones) appear [9]. Conversely, our model suggests that experimentally inducing attractive receptor-receptor interactions could prevent the formation of stable RSDs.

The Turing mechanism implies that individual RSDs stabilize once scaffold-induced activation and receptor-induced inhibition of increased molecular concentrations are balanced by rapid receptor diffusion. Our simulations showed, in line with the linear stability analysis of our model [14], that Eqs. (1) and (2) can quantitatively account for the characteristic size of RSDs and the time scale of their formation observed in experiments [see Figs. 2(b,c) and 1(c)]. These results relied on the aforementioned reactions crucial to the Turing instability but, apart from that, did not depend on the particular reaction scheme considered. Similarly to other Turing instabilities [19], our model predicts that changes in diffusion rates can affect the size, stability, and large-scale pattern of RSDs. The Turing mechanism for RSD formation and stability also indicates that the receptor profile is broader than the scaffold profile across any given domain [see Fig. 2(c)], although the numerical values of  $r$  and  $s$  inside and outside RSDs depend on the specific reaction kinetics considered. Such details of RSDs may soon be [6, 8] within reach of experimental observation.

The above results demonstrate how stable synaptic receptor domains can emerge in the absence of presynaptic stimulation. In a synapse, however, presynaptic activity is thought to regulate [27] the concentration of receptors in the postsynaptic domain. Our reaction-diffusion model suggests novel postsynaptic mechanisms for such regulation. The diffusion of receptors on the postsynaptic membrane can be modified through binding of presynaptic neurotransmitters [8, 28]. Similarly, scaffold diffusion may be modulated by synaptic activity [29, 30]. This suggests that local modification of the diffusion properties of receptors or scaffolds may contribute to the regulation of postsynaptic receptor concentration.

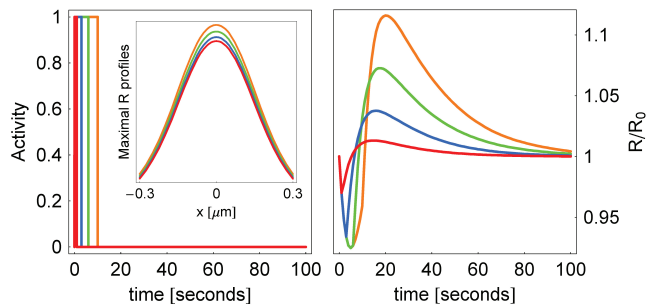


FIG. 3: (color online). Model results on the regulation of mature RSDs [14]. Left panel: temporal profiles of step stimulations. Inset: receptor concentration profiles at times of maximum domain size. Right panel: time course of the in-domain receptor population size,  $R$ , following stimulation, normalized by the in-domain receptor population size in the absence of any stimulation,  $R_0$ .

As a simple phenomenological perturbation to our model, we therefore implemented pre- and postsynaptic interactions through a local increase of the receptor diffusion rate which, within the framework of our model, has a similar effect as a local decrease of the scaffold diffusion rate.

As shown in Fig. 3, our model predicts that modulation of receptor or scaffold diffusion following presynaptic activity transiently changes the in-domain receptor population. Clearly, this speculative, purely biophysical mechanism may coexist with biochemical mechanisms of postsynaptic plasticity. Applying a few seconds of stimulation at a time (Fig. 3, left panel), we found a correlation between the increase in in-domain receptor population and the duration of stimulation (Fig. 3, right panel). After an initial, short-lived suppression, the population increase lasted for a few tens of seconds—the time scale typically associated with short-term plasticity. In this window of time, RSDs were richer in receptors (Fig. 3, inset) and, hence, yielded a larger synaptic efficacy. This phenomenon has a simple explanation in terms of the Turing instability exhibited by our model: Enhanced (diminished) receptor (scaffold) diffusion depletes the receptor (increases the scaffold) population in a transient manner which, because receptors are inhibitors and scaffolds are activators, in turn attracts even more receptors and scaffolds into RSDs.

In summary, we have used a minimal experimental system devoid of synaptic machinery to show that neurotransmitter receptor domains of the stable characteristic size observed in neurons can emerge from nothing more than interactions between receptors and scaffolds, together with the rapid diffusion of receptors on the cell membrane. A reaction-diffusion model quantitatively accounts for our experimental results, yielding spontaneous formation of stable receptor domains and their observed characteristic size, as well as new putative mechanisms

for the regulation of synaptic strength. Our results show how stable synaptic receptor domains may form even in the absence of presynaptic stimulation [4, 6, 11], and how rapid turnover and diffusion of receptors [4, 6], far from being a hindrance, may in fact be crucial [1] for ensuring overall stability and delicate control of synaptic receptor domains.

This work was supported by the Austrian Science Fund (C.A.H.), the Pierre-Gilles de Gennes Foundation and a FEBS grant (M.C.), the NSF through grant No. DMR-08-03315 and a Visiting Professorship at the Ecole Normale Supérieure (M.K.), the Inserm UR 789 (A.T.), and the CNRS through UMR 8550 (R.A.d.S.).

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