Synaptic Bistability Due to Nucleation and Evaporation of Receptor Clusters

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We introduce a bistability mechanism for long-term synaptic plasticity based on switching between two metastable states that contain significantly different numbers of synaptic receptors. One state is characterized by a two-dimensional gas of mobile interacting receptors and is stabilized against clustering by a high nucleation barrier. The other state contains a receptor gas in equilibrium with a large cluster of immobile receptors, which is stabilized by the turnover rate of receptors into and out of the synapse. Transitions between the two states can be initiated by either an increase (potentiation) or a decrease (depotentiation) of the net receptor flux into the synapse. This changes the saturation level of the receptor gas and triggers nucleation or evaporation of receptor clusters.

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According to current understanding, the main biophysical mechanism for storing information in the central nervous system is activity-based changes in the strengths (weights, or efficacies) of synaptic connections between neurons (synaptic plasticity) [1–4]. In many cases, synaptic plasticity is expressed by changes in the number of neurotransmitter protein receptors (AMPARs) within the postsynaptic membrane of a stimulated neuron [5,6]. However any modification in the number of independent receptors cannot fully account for long-term memory because of the limited dwell time of individual receptors, which are recycled into and out of a synapse several times an hour [7]. One suggested mechanism for stabilizing higher concentrations of receptors at a synapse is through interactions with submembrane scaffolding proteins [8–10]. An alternative mechanism has recently been proposed by Shouval [11], in which receptor clusters, if formed, can survive much longer than individual receptors if the rate of receptor insertion into the membrane (exocytosis) is higher in the vicinity of other receptors due to receptor-receptor interactions and the rate of receptor removal from the membrane (endocytosis) is independent of such interactions. However, no particular mechanism for cluster formation has been proposed.

In this Letter we present a physical model of synaptic weight stabilization that exhibits two stable states with different efficacies (receptor numbers), i.e., bistability, and includes an explicit mechanism of transition between these states by receptor clustering. The latter is formulated along analogous lines to the well-known physical phenomenon of vapor-liquid phase transformations where supercritical liquid droplets are formed from supersaturated vapor [12]. The vapor in our model is represented by mobile receptors within the postsynaptic membrane, which together with a population of immobile localized receptors bound to scaffolding proteins, determines the strength of the synapse. Each immobile receptor can act as a preexisting nucleation site for supercritical cluster formation (heterogeneous nucleation, see for example [13] and references therein), which rapidly occurs if the surrounding vapor becomes dense enough, e.g., after an excitatory signal has stimulated the synapse. Once nucleated, the cluster grows until it reaches an equilibrium size determined by the balance between receptor influx and outflow from the synapse. The cluster can also be evaporated if the receptor concentration in the vapor becomes too low, e.g., after an inhibitory signal. We show that such a mechanism of cluster formation or evaporation allows a synapse to exhibit bistability; i.e., there exist two distinct stable states with different receptor numbers for the same value of receptor influx. Receptors in the state with low efficacy are either in the vapor phase or are immobile and isolated, while the efficacy of the other state is significantly increased by the formation of one or more receptor clusters. Transitions between the two stable states correspond to writing or erasing information. We also show that synaptic bistability is controlled by the total influx of receptors into the postsynaptic terminal and depends on the area of the postsynaptic membrane. Changing any of these parameters modifies the size and stability of the receptor cluster and modulates synaptic efficacy accordingly.

Model description.—At most excitatory synapses in the brain, AMPA receptors are highly clustered at the postsynaptic density (PSD), which is the protein-rich domain in the postsynaptic membrane of a dendritic spine (Fig. 1). The dendritic spine is a small (submicrometer) membranous extrusion that protrudes from a dendrite. Typically spines have a bulbous head that is connected to the parent dendrite through a thin spine neck. There are two main mechanisms for the transport of receptors between the PSD and extrasynaptic regions [9]. First, surface receptors can undergo lateral membrane diffusion, in which the PSD acts

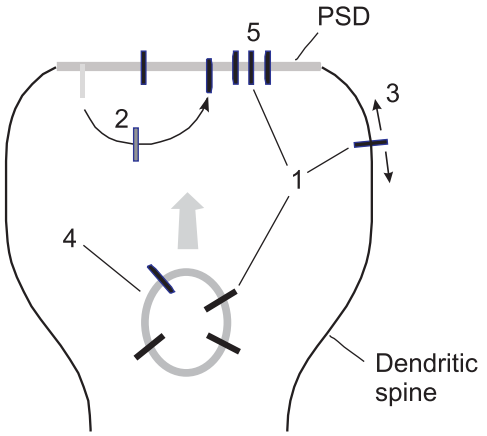

FIG. 1 (color online). Schematic representation of the PSD. The number of receptors (1) within the PSD is maintained by receptor recycling (2) and diffusion from extrasynaptic areas (3). Extra receptors can be delivered to the PSD from an intracellular pool (4) following an excitatory signal to form stable clusters (5), resulting in potentiation (strengthening) of the synapse. An inhibitory signal would cause an increase in the rate of receptor removal (endocytosis), resulting in receptor cluster evaporation and weakening of the synapse (depotentiation).

as a spatially localized trap through receptor interactions with scaffolding proteins and the cytoskeleton. This is consistent with single-particle tracking experiments, which show surface receptors undergoing periods of free diffusion interspersed with periods of restricted motion in confinement domains that coincide with synapses [14–17]. Second, surface receptors may be internalized via endocytosis and either stored within an intracellular pool or recycled to the surface via exocytosis [18].

In our model the mechanism of receptor delivery to the PSD combines both the diffusive flux from extrasynaptic areas and direct insertion into the PSD via exocytosis (Fig. 1). It is assumed that receptor outflow from the PSD occurs mainly via endocytosis; i.e., the diffusive flux of receptors out of the PSD is nonvanishing but small enough. The latter is required for maintaining the receptor pool in the PSD out of equilibrium with the extrasynaptic pool of receptors. At any time t the total number of receptors of the PSD will depend on the balance between fluxes into and out of the postsynaptic membrane. The total receptor influx has two components $y = y_1 + y_2$, where y_1 is the combined background influx due to local receptor recycling and diffusion from the extrasynaptic area, and y_2 is the contribution from an intracellular receptor pool triggered upon synaptic excitation (see Fig. 1). We assume that the background receptor influx y_1 is constant in the absence of any external excitations. This is important for maintaining dynamic equilibrium of receptor influx-outflow at synapses. The outflow of receptors depends on the number of clustered and mobile receptors, with endocytosis rates determined by their local environments. (For simplicity, we assume that immobile nonclustered receptors are stable within the PSD and so do not contribute to any fluxes; they only contribute to cluster formation by acting as potential nucleation sites). One candidate mechanism for receptor clustering is short-range (van der Waals) interactions between receptors with a characteristic energy of the order k_BT, where T is the system temperature [19]. This relatively low binding energy allows us to treat receptor clusters as two-dimensional liquid droplets, which are approximately circular as they minimize their surface energy. Hence, any receptor removal from the cluster does not change the cluster shape and integrity.

Energy analysis.—Although AMPA receptor diffusion within the PSD is 10–50 times slower than within the extrasynaptic membrane [14–16], it is fast enough to establish thermodynamic equilibrium of the receptor vapor (see Section 1 in the Supplemental Material [20]). This allows us to define the chemical potential of receptors in the cluster $\mu_{\rm cl}$ and in the vapor $\mu_{\rm vap}$ as $\mu_{\rm cl} = -\varepsilon_{\rm cl} - \varepsilon_{\rm adh} + \gamma\frac{a}{R}$ and $\mu_{\rm vap} = k_BT\ln(x_{\rm vap}a^2) - \varepsilon_{\rm adh}$, where $\varepsilon_{\rm cl}$ is the binding energy of receptors in the cluster, R is the cluster radius, $\varepsilon_{\rm adh}$ is the adhesive energy of receptors in the membrane, γ is the cluster surface energy per receptor, a is the spacing between receptors in the cluster, and $x_{\rm vap} \ll 1$ is the vapor (mobile receptor) concentration measured relative to receptor concentration in the cluster. The vapor concentration $x_{\rm vap}$ is obtained from the condition that at steady state the receptor influx y is equal to the receptor outflow. We assume that the area S_0 occupied by the mobile receptors (vapor) is approximately independent of the size of the cluster. In general the outflow of receptors contains three contributions, which are due to (1) endocytosis of the clustered receptors, (2) endocytosis of vapor receptors, and (3) diffusion of receptors out of the PSD. Here we consider the case when the latter contribution, whose role is analyzed in the Supplemental Material [20] (Section 5), can be neglected. The balance of receptor fluxes in and out of the PSD is $y = \pi R^2 P_{\rm cl} + S_0 x_{\rm vap} P_{\rm vap}$, where all lengths are in a units, $P_{\rm cl}$ and $P_{\rm vap}$ are endocytosis rates of receptors in the cluster and in the vapor, respectively. In contrast to receptors in the vapor the endocytosis of clustered receptors requires extra work $\delta A = \varepsilon_{\rm cl} + \gamma\frac{1}{R}$ to be performed against receptor-receptor interactions. Therefore this effect decreases the endocytosis rate $P_{\rm cl}$ compared to $P_{\rm vap}$ by a factor $e^{-(\varepsilon_{\rm cl}/k_BT)+(\gamma/Rk_BT)}$. Note that this is completely different to the approach used in Ref. [11], where receptor removal rates were assumed to be equal for mobile and bound receptors. Therefore, the vapor concentration as a function of cluster size is

$$x_{\rm vap}(R) = y/(S_0P_{\rm vap}) - [\pi R^2/S_0]e^{-(\varepsilon_{\rm cl}/k_BT)+(\gamma/Rk_BT)}. \quad (1)$$

In the absence of a cluster the second term on the right-hand side of Eq. (1) can be neglected so that $x_{\rm vap}(0) = y/(S_0P_{\rm vap})$. Using this expression and writing the cluster

radius $R = R(n_{cl}) \equiv \sqrt{n_{cl}/\pi}$ in terms of receptor number in the cluster n_{cl}, the energy required for the formation of the receptor cluster from vapor is

$$\Delta E = \int_0^{n_{cl}} (\mu_{cl} - \mu_{vap}) dn + S_0[F(n_{cl}) - F(0)]$$

where $F(n) = k_B T x_{vap}(R(n)) \ln[x_{vap}(R(n))]$. Evaluating the integrals then gives

$$\Delta E = -n_{cl}\varepsilon_{cl} - \int_0^{n_{cl}} \ln\left[x_{vap}(0) - \frac{n}{S_0} e^{-(\varepsilon_{cl} - \gamma\sqrt{\pi/n}/k_B T)}\right] dn + 2\gamma\sqrt{\pi n_{cl}} + S_0[F(n_{cl}) - F(0)]. \quad (2)$$

Figure 2 shows how the change in the system energy depends on the cluster size for different values of the receptor influx y, endocytosis rate P_{vap}, and synaptic area S_0. γ is prescribed from the surface energy of a relatively small discrete cluster of receptors on a hexagonal grid (see Section 2 in [20]). Also, the value $P_{vap} = 0.1$ min^{-1} corresponds to the reported endocytosis time of mobile receptors given by $t_d = 10$ min [21]. For $y = 0.6$ min^{-1} (curve 1) the energy has two local minima. The one at $n_{cl} = 0$ corresponds to a state with no cluster (but still containing a mixture of mobile receptors and immobile isolated receptors), while the other around $n_{cl} = 200$ suggests the existence of a stable cluster [22]. To nucleate this cluster, the system has to overcome the energy barrier of about $\delta E \approx 17 k_B T$ in height and about $\delta n \approx 50$ receptors in width, which makes spontaneous cluster formation rather unlikely. If the overall receptor influx is increased up to $y = 0.9$ min^{-1} (curve 2), the energy of the clustered state rapidly decreases, making cluster formation more likely, as both the nucleation barrier height and the width are significantly decreased down to $\delta E \approx 10 k_B T$ and $\delta n \approx 15$, respectively. This suggests that an increase in y for a relatively short time period may be enough to initiate cluster formation by appropriate fluctuations. The cluster may then grow further and reach its stable size even after the influx y has already returned to its background value $y = y_1$.

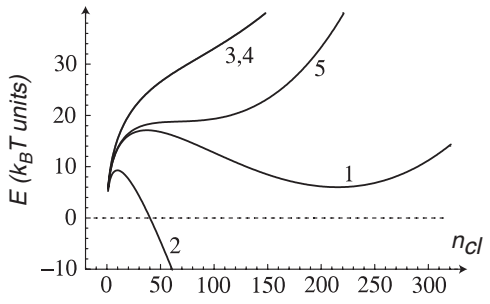

FIG. 2. The energy change associated with cluster formation as a function of receptors number n_{cl}. The energy was obtained by evaluating Eq. (2) for $\varepsilon_{cl} = 5 k_B T$, $\gamma = 1.646 k_B T$ and the triple (P_{vap}, S_0, y) equal to: (1) (0.1, 500, 0.6); (2) (0.1, 500, 0.9); (3) (0.1, 500, 0.5); (4) (0.12, 500, 0.6); (5) (0.1, 300, 0.36). The last value of y is chosen to keep $x_{vap}(0)$ the same as in 1. Units of y and P_{vap} are min^{-1} and S_0 is in a^2 units.

A reverse phenomenon of cluster evaporation takes place if the y value is decreased from 0.6 min^{-1} down to 0.5 min^{-1} or the endocytosis rate P_{vap} is increased from 0.1 min^{-1} up to 0.12 min^{-1}, as is illustrated by the coincident curves 3 and 4, respectively. Both these changes make the cluster highly unstable and result in its decay. This sequence of events is illustrated using the kinetic Monte Carlo technique in the next section. An interesting feature of the proposed mechanism is illustrated by curve 5 in Fig. 2. This curve is calculated for $S_0 = 300$ with the receptor influx $y = 0.36$ min^{-1}, which corresponds to the same influx density y/S_0 as for curve 1. This feature indicates that increasing S_0 (dendritic spine size) results in an increased and more stable synaptic efficacy, which is consistent with the experimental data [23,24]. In our model this effect is explained in terms of cluster surface energy. The latter is always positive and its fraction per one receptor decreases with increasing the cluster size. A higher receptor influx into a larger area at the same influx density y/S_0, as shown by comparing curve 1 and curve 5, generates a larger cluster, which is more stable due to a lower surface energy contribution per one receptor.

Model kinetics.—The transitions between the two metastable states are governed by the evolution of the populations of clustered n_{cl} and mobile N_{vap} receptors

$$\frac{dN_{vap}}{dt} = y - N_{vap}P_{vap} - 2\sqrt{\pi n_{cl}}(J_{con} - J_{evap})$$
$$\frac{dn_{cl}}{dt} = 2\sqrt{\pi n_{cl}}(J_{con} - J_{evap}) - n_{cl}P_{cl} \quad (3)$$

where J_{con} and J_{evap} are, respectively, the fluxes of condensing and evaporating receptors from the cluster. We take $J_{con} = k_{int} x_{vap}$, where k_{int} is the rate at which receptors join the cluster [25]. We have assumed that the receptor vapor is in thermodynamic equilibrium, i.e., homogeneous, which poses a restriction on the value of k_{int} (see Section 1 in [20]). The flux $J_{evap} = k_{int} x_{GT}$ can be obtained by assuming that the cluster evaporates with the same rate as if it was in equilibrium with the surrounding vapor, and its stability is due to the compensating condensation flux from the vapor. The so-called Gibbs-Thomson concentration x_{GT} of the vapor can be found from the equilibrium condition $\mu_{cl} = \mu_{vap}$ with the chemical potentials defined above. Hence, $x_{GT} = e^{-(\varepsilon_{cl} - \gamma\sqrt{\pi/n}/k_B T)}$. Equations (3) are solved by kinetic Monte Carlo simulations (see the Supplemental Material [20] and Ref. [26]). Typical simulation results illustrating responses of the receptor system to an increased receptor influx (potentiation) or receptor removal rate (depotentiation) are presented in Fig. 3. In the simulations we used $a = 5$ nm, $k_{int} = 1.3 \times 10^{-3}$ μ/s and a characteristic diffusion coefficient D for mobile receptors in the PSD [14–17]. We assumed that the PSD

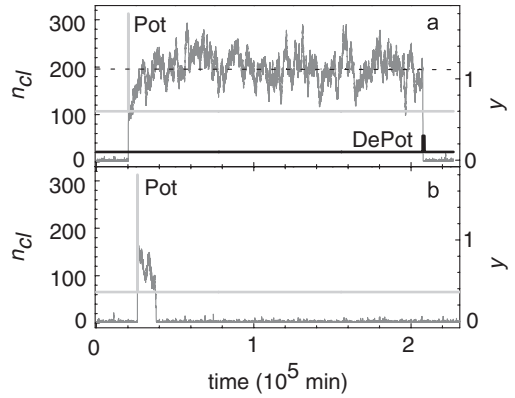

FIG. 3. Evolution of the receptor numbers in the cluster n_{cl} (dark grey) obtained by kinetic Monte Carlo simulation using Eq. (3) with $P_{vap} = 0.1$, $\varepsilon_{cl} = 5$, $\gamma = 1.646$, $D = 10^{-2}$ μ^2/s, $k_{int} = 1.3 \times 10^{-3}$ μ/s.(a)$S_0 = 500$, $y_1 = 0.6$; (b) $S_0 = 300$, $y_1 = 0.36$. The values of y and P_{vap} are given in min^{-1}, and S_0 is given in a^2 units. Straight grey lines show $y = y_1 + y_2$, where y_1 represents the background exocytosis rate due to receptor recycling and y_2 represents the spike in receptor influx from an intracellular pool (potentiation). Dashed line indicates the steady state cluster size. Black solid line indicates the spike in the endocytosis rate corresponding to a depotentiation signal.

contains a single nucleation site with at least two receptors. The impact of the parameter k_{int} on the cluster size and its fluctuations is described in the Supplemental Material [20]. We find that our simulation results are in excellent agreement with the energy analysis if $k_{int} > 10^{-3}$ μ/s. The values of other parameters used for the simulations shown in Fig. 3(a) are the same as those used to calculate curve 1 in Fig. 2. Cluster formation was triggered by a short increase in y_2 from 0 to 1.8 min^{-1}, after which the cluster grows to its stable size of around 200 receptors as shown by the dashed line, and fluctuates about this stable value. To initiate cluster evaporation we increased P_{vap} by a factor of 3. Note that by tuning the model parameters, it is possible to reduce the quasiequilibrium cluster size down to 100 receptors or less. However, this also significantly reduces the cluster nucleation barrier so that the system can now spontaneously transition between the two quasistable states much more rapidly. The results shown in Fig. 3(b) were obtained using parameter values for curve 5 in Fig. 2 and cluster formation was initiated by an increase in y_2 from 0 up to 1.8 min^{-1}. According to curve 5 in Fig. 2, the resulting cluster is unstable and, as can be seen from Fig. 3(b), it decays spontaneously after about 150 hours.

So far we have assumed that the cluster has nucleated around a single isolated nucleation site. In reality the PSD contains a significant number of receptors that are immobilized due to their interaction with submembrane scaffold proteins. Each of these immobile receptors can act as a nucleation site initiating cluster formation. Moreover, individual clusters could coalesce making the formation of the final stable cluster rather complicated. It is clear that the stable cluster should be of the same size as in the case of a single nucleation site, as it corresponds to the minimum energy of the system. Any other cluster, on the other hand, is not that well defined because of the uncertainty in its shape and, hence, surface energy. However, as illustrated in Section 6 of [20], the bistability of the synapse (energy barrier between the stable states) is at least as strong as in case of a single nucleation site. As we have highlighted above, the presence of multiple nucleation sites (immobile receptors) means that the state without any receptor clusters still has a significant number of receptors, and thus a nonzero synaptic efficacy.

Discussion.—In this Letter we presented a model of synaptic bistability based on the nucleation and evaporation of receptor clusters in the postsynaptic membrane. Bistability is controlled by the area of the synapse and the background receptor vapor concentration. Both metastable states of the synapse can be maintained with the same receptor influx hence at the same metabolic cost to the cell. We found that the long-term stability (lifetime) of synaptic efficacy is achieved with the formation of large (200 receptors) clusters while smaller clusters may account for the shorter term potentiation of synaptic efficacy. A major simplification of the model was to consider only a single cluster. Further simulations show that the nucleation of more than one cluster at different isolated nucleation sites does not change the main result related to synaptic efficacy, as only one stable cluster is eventually formed and all others vanish. This result also suggest that nonclustered immobile receptors do not contribute to the vapor-cluster dynamics and need only to be treated as additional nucleation sites. A more detailed study, however, is required to investigate the impact of multiple nucleation sites on fluctuations in the number of clustered receptors. Finally, note that there is still some disagreement on whether or not changes at individual synapses occur in an analog or digital manner. Most experiments on synaptic plasticity involve stimulation of and recordings from an ensemble of synapses, making it difficult to address this issue. However, several groups have used minimal stimulation protocols in order to determine changes in the electrophysiological response properties of single synapses, and these suggest that such changes are all-or-none switchlike events consistent with bistability [27,28].

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