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Escape Through a Small Opening: Receptor Trafficking in a Synaptic Membrane

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We model the motion of a receptor on the membrane surface of a synapse as free Brownian motion in a planar domain with intermittent trappings in and escapes out of corrals with narrow openings. We compute the mean confinement time of the Brownian particle in the asymptotic limit of a narrow opening and calculate the probability to exit through a given small opening, when the boundary contains more than one. Using this approach, it is possible to describe the Brownian motion of a random particle in an environment containing domains with small openings by a coarse grained diffusion process. We use the results to estimate the confinement time as a function of the parameters and also the time it takes for a diffusing receptor to be anchored at its final destination on the postsynaptic membrane, after it is inserted in the membrane. This approach provides a framework for the theoretical study of receptor trafficking on membranes. This process underlies synaptic plasticity, which relates to learning and memory. In particular, it is believed that the memory state in the brain is stored primarily in the pattern of synaptic weight values, which are controlled by neuronal activity. At a molecular level, the synaptic weight is determined by the number and properties of protein channels (receptors) on the synapse. The synaptic receptors are trafficked in and out of synapses by a diffusion process. Following their synthesis in the endoplasmic reticulum, receptors are trafficked to their postsynaptic sites on dendrites and axons. In this model the receptors are first inserted into the extrasynaptic plasma membrane and then random walk in and out of corrals through narrow openings on their way to their final destination.

KEY WORDS: Diffusion, random walk, trafficking, receptor confined, synaptic plasticity.

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1. INTRODUCTION

The theoretical question we consider here is how receptors are directed toward their final destination on the membrane of a biological cell, if their movement is diffusion with neither a field of force nor a concentration gradient (see Fig. 1)? How long does it take for a receptor to diffuse from its point of insertion in the membrane to its final location? (by final location, we mean a specific place in the membrane that the receptor occupies for a period of time of between a few minutes to hours). What does this time depend on? In this paper, we attempt to answer some of these questions by analyzing a mathematical model of the motion of the receptors.

The mathematical description of the diffusive motion of a receptor on the cell membrane begins with the geometrical description of the membrane and of the obstacles the random walking receptor encounters. We describe the motion of the receptor on the membrane as free Brownian motion in the plane (thus neglecting the surface curvature), with occasional trappings in and escapes from confinement regions, called *corrals* (see Fig. 1). We describe the corrals as smooth two-dimensional domains, whose boundary is reflecting, except for a narrow opening. The

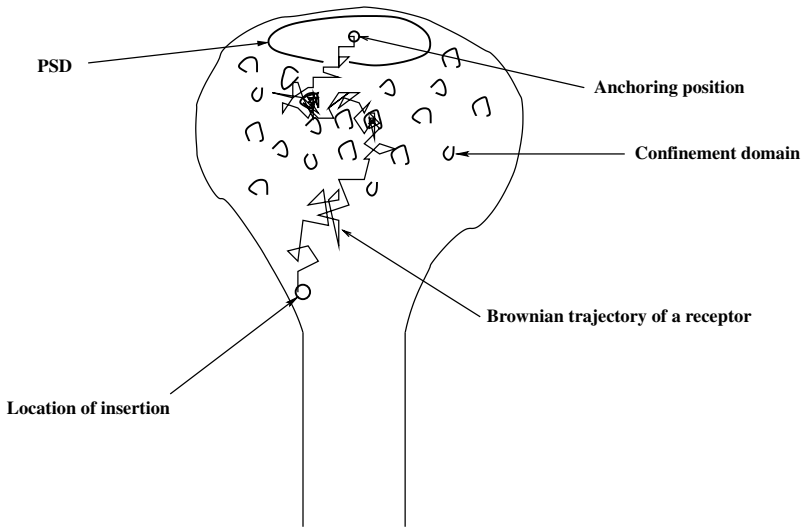

Fig. 1. Trajectory of a receptor on the surface of a dendritic spine. The receptor is inserted somewhere on the spine and moves by diffusion until it finds its final location inside a confinement domain. In part of its trajectory the receptor may be attached to a protein such as stargazin, which slows it down. Attached proteins may have a tail inside the cell, interacting with other plasmic proteins, located inside the cell.

mean time the receptor spends in a corral is called the *confinement time* of the receptor (see Fig. 2). The main result of this paper is the calculation of the confinement time as a function of the parameters of the problem, and the application of this result to the interpretation of experimental measurements. This mean first passage problem is different than activated escape problems and its analysis leads to a different singular perturbation problem than classical escape from an attractor. The escape of the receptor can be effected also by thermal activation over the fence.

In Sections 2 and 3, we describe the biological context by recalling some basic facts of receptor trafficking and its relation to synaptic plasticity. In Section 4, we calculate the confinement time of a free

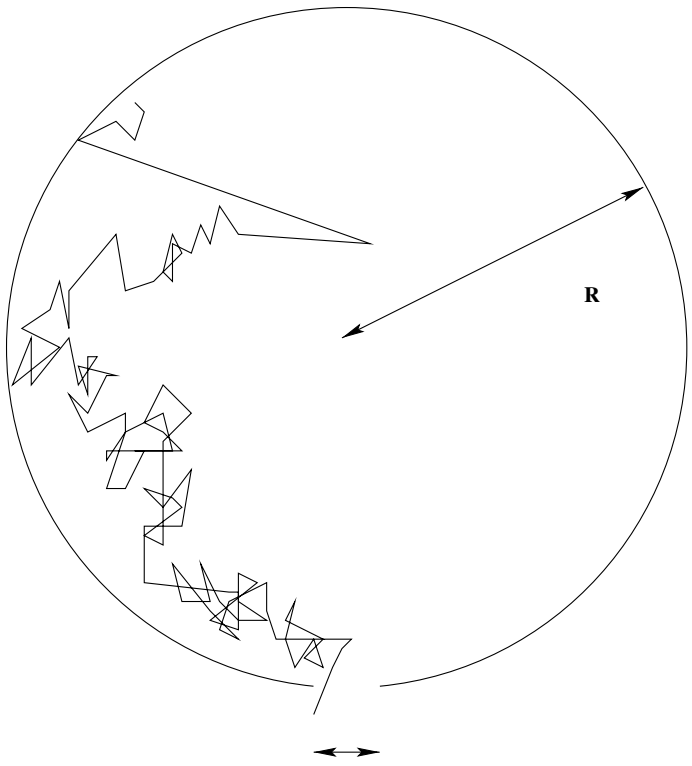

Fig. 2. A Brownian trajectory reflected at the boundary and exits through a narrow opening. Typically, the trajectory fills a larger part of the domain.

Brownian particle in a general domain with a small opening. We consider confinement domains that are either obstacles or termination domains. We apply the result to the estimation of the time it takes for a receptor to enter its final destination domain. Such estimation is relevant in the context of protein trafficking on a postsynaptic membrane. In Section 5, the confinement time is computed when the boundary of the confinement domain is made of charged proteins, creating a potential barrier with a small opening. In Section 6, we compute the probability that a Brownian particle exits a confinement domain when its trajectory can be terminated inside the domain. Termination of the trajectory corresponds to the anchoring of a receptor to a binding protein molecule. The notion of a final location, or termination of trajectories by anchoring may not reflect the fact that anchoring is very likely to be a reversible process. Anchoring is itself a reversible process, whose lifetime may be quite short, on the order of minutes, and it is known that even in the absence of synaptic activity receptors can enter and leave a synapse. The present computations can be used to estimate the confinement time as a function of biological parameters and also to estimate the time it takes for a diffusing receptor to find its functional destination, after insertion in the membrane. An acronym identification is presented at the end of the paper.

2. FROM NEURO-BIOLOGY TO STATISTICAL PHYSICS

A synapse[1] is functionally the place of physical storage of the "synaptic weight", by which a signal coming from a pre-synaptic neuron is modulated by the post-synaptic neuron. Brief repetitive electrical stimulations of hippocampal neurons[2] are known to lead to a long lasting enhancement in synaptic strength.[3,4] This phenomenon, referred to as *long term potentiation* (LTP), is the evidence that activity induces persistent changes in synapses and is believed to underlie learning and memory. Stimulation at low frequencies induces a long lasting decrease in synaptic strength, called *long term depression* (LTD). However, the various steps of LTP/LTD induction are not yet fully elucidated and it is a challenge of modern neurobiology to identify all the biochemical mechanisms involved in synapse regulation. In particular, modification of the synaptic weight (the measure of synaptic strength) during LTP can be caused by a change in the biophysical properties of channels, such as conductances, selectivity to ions, gating, and/or by an increase in the total number of protein channels (receptors).[5] Moreover, experimental evidence indicates that new AMPA receptors (see table of acronyms at the end of the paper) are inserted into synapses during LTP. AMPA receptors provide the

primary depolarization[6] in excitatory neurotransmission and the insertion or removal of the receptors affects the synaptic weight and therefore has to be very well controlled.[7,8] Not only AMPA receptors are trafficked, but also NMDA-receptors, which mediate Ca^{2+} influx into the synapse. Both are glutamate-activated transmitters.

The number of AMPA receptors changes during synaptic plasticity and, in addition, a specific form of the receptor cycles continuously on and off the synaptic membrane. After their synthesis in the endoplasmic reticulum AMPA receptors are trafficked to post-synaptic sites on either neuronal dendrites or axons, but the route they take from intracellular vesicles to synapses is not yet clear. From a biological point of view, a critical question is whether the receptors are directly inserted to the post-synaptic density (PSD), which is the area of the membrane where synaptic sites face the pre-synaptic terminal, or if they are first inserted into the extrasynaptic plasma membrane and later on move to the PSD.

There are various forms of AMPA receptors, identified by their GluR-subunits, which determine the biophysical properties of a channel, e.g., their diffusion coefficient on the membrane, and therefore their confinement times.[9] AMPA receptors containing GluR2-subunit are impermeable to calcium, whereas AMPA receptors with GluR1, three and four subunits are permeable. Moreover, each subunit has a different cytoplasmic tail (which dangle under the membrane), so that AMPA receptors can be classified into two classes: first, the AMPA receptors with long tails, such as GluR1, can only be inserted after synaptic activity, and second, the AMPA receptors containing a GluR2 subunit, have a short tail and are inserted constitutively.[8] Long and short tail AMPA receptors trafficked on the surface membrane are associated with different proteins. Recently,[9–11] single AMPA receptors attached to a Green Fluorescent Protein have been observed to diffuse in the extrasynaptic membrane, but to lose mobility when they enter a synaptic region. During their movement, AMPA receptors associate with accessory and scaffolding proteins, which are intracellular proteins that bind receptors and contribute to their stabilization at synapses and assist their trafficking in various subcellar domains.[8]

The turnover of AMPA receptors at synapses is regulated by a large family of interacting proteins that thereby influence synaptic strength. Receptor movement on the membrane of a neuron seems to be a diffusion process (see review[9]), that moves rapidly within a constrained space (corral) for short periods of time, and then periodically escapes from these areas. The escape of a protein from any of these domains can be accomplished either by hopping over the the corral fence and/or by passing through the gaps when the membrane skeleton is transiently

dissociated. Thus the membrane can be viewed as a patchwork of submicron domains, within which diffusion is as fast as expected from theory. Fences that restrict transitions from one compartment to another separate these domains, thereby decreasing overall diffusion. Thus receptor trafficking leads to the ubiquitous problem of escape of a random walker, as well as to many other related mathematical problems.

3. LATERAL MOVEMENT ON A POSTSYNAPTIC MEMBRANE

Postsynaptic membranes of neurons contain specialized sub-domains, referred to as PSD, where hundreds of different proteins and other molecules are clustered, all playing a specific role in the functioning of the synapse. In particular, a change in synaptic plasticity is correlated with a change of the biophysical properties of protein channels, due to covalent modifications of channels [7], or with a change in the total number of channels due, for example, to the insertion of new AMPA receptor channels. It has been demonstrated in refs. 9–12 that receptors can diffuse on the surface membrane of neurons and prior to their anchoring the diffusive motion of receptors in the membrane is nearly free diffusion. The random motion of receptors was observed in Ref. 9, and more specifically, it has been reported that the motion of a receptor can switch between two different stages. In one stage, the receptor diffuses freely on the surface, and in the second stage, it diffuses in a confined region, where the diffusion constant is much smaller than that in the free diffusion stage. The confined regions are described as specific subdomains of the synaptic membrane and are typically few hundreds nanometers across.

The mean time a Brownian trajectory reaches a given subdomain (or any one of a number of subdomains) of a given bounded domain, to which it is confined, depends on the domain, on the number, and on the sizes of the subdomains. The size of the confinement subdomain on a surface of the post-synaptic membrane is not known exactly. However, when a receptor enters a subdomain, where it can be anchored, the mean time it stays there provides much information about the possible bonds the diffusing receptor can make with scaffolding proteins. As a consequence of such binding the speed of diffusion is reduced, thus increasing the mean exit time and increasing the probability that the complex channel-scaffolding protein meets a protein that will ultimately stop the complex at its final location.

Once a receptor is inserted into the membrane far from the PSD, it can remain in the extrasynaptic membrane instead of diffusing to the PSD. It can even diffuse in the direction of the dendrite, never to come back, and find another synapse, unless a potential barrier prevents the receptor

from escaping. Such a barrier has not been reported so far. If we assume that such a barrier exists, the mean time to reach a given confinement subdomain is finite. The purpose of this work is to describe the movement of a receptor from the time it is inserted in the membrane until it is anchored at the PSD.

When a receptor enters a confinement subdomain, it can either be anchored there immediately or leave. We compute the time it takes for a receptor to leave the confinement subdomain in two cases. First, when the confinement subdomain can be approximated by a disk, whose boundary is reflecting, except for one or more small openings that allow the receptor to escape. Second, when the confinement subdomain is bounded by a known potential barrier created by proteins. Explicit computation of the mean confinement time relates it to the geometry of the domain and to the diffusion coefficient of the complex receptor-scaffolding protein. Thus, we expect that combining those computational results with experimental studies, it will becomes possible to study the effect on the movement of potential candidates for scaffolding proteins that bind to the receptor, thereby decreasing its diffusion coefficient. The increase in the confinement time was reported in ref. 9 when a receptor diffuse inside a confinement domain: it can be due to the binding with a scaffolding protein. To take into account the effect of the confinement subdomains, observed in a synapse, we will define later on, an effective diffusion constant that describes the random walk of ideal receptors in synapse. The definition is based on the diffusion time from one confinement subdomain to another. The coarse grained diffusion constant is computed by using the mean confinement time.

The increase in confinement time was reported in ref. 9. Combining the probability that a receptor enters and leaves a confinement domain without being anchored (a synapse contains many confinement subdomains), we define an effective diffusion coefficient that describes the random walk of receptors from one confinement subdomain to another as a coarse grained diffusion process.

Finally, a synapse is considered to be the fundamental unit of the memory at a subcellular level and is a reliable storage compartment of information over years, while the life time of its basic constituent receptors, such as AMPA receptors, is of the order of few hours.[13] In order to maintain the synaptic weight and to insure the stability of the synapse in the absence of any input signal, a daily turnover of receptors has to be very well regulated. Defected receptors have to be replaced without increasing the total number of active receptors. It is not clear what are the fundamental mechanisms that regulate this turnover, neither is known the precise ways by which the number of receptors is detected at each moment

of time. Finally, the estimation of the confinement time gives a constraint of the time it takes for a receptor to travel on the membrane before being anchored.

4. RECEPTOR MOVEMENT ON A MEMBRANE

Receptors diffuse on the surface membrane of a nerve cell, which is composed of many sub-compartments of various sizes and contains assemblies of various proteins, such as the PSD. Each compartment can absorb a receptor or release one. The movement of receptors is not simply described as a free diffusion in a surface with obstacles, but rather the movement can be decomposed into two type of time-periods; one time period is defined when the receptor diffuses freely and the second when it is confined in a corral. There, the receptor is trapped, but eventually escapes. Back on the free side of the membrane, it can reach another confinement domain, until it is finally anchored for a certain time somewhere. We calculate below the mean time of each type.

4.1. Mean Escape Time from a Bounded Domain

We begin with a receptor inside a confinement subdomain Ω, where it can be bound to a protein. The mean time it stays in the confinement subdomain is called the *confinement time*. We assume that the boundary $\partial\Omega$, is reflecting for the diffusing receptor, except for a small opening. We represent the opening as an absorbing part of the boundary, $\partial\Omega_a$, and the remaining part of the boundary, $\partial\Omega_r = \partial\Omega - \partial\Omega_a$, is reflecting. The length of $\partial\Omega_a$ is assumed small. More specifically, if $\partial\Omega_1$ is the connected component of $\partial\Omega$ that contains $\partial\Omega_a$, assume that

$$\varepsilon = \frac{|\partial\Omega_a|}{|\partial\Omega_1|} \ll 1.$$

First, we review the general theory.[14,15] We assume that $\partial\Omega$ is an analytic surface and that $\partial\Omega_a$ is a $d-1$-dimensional subdomain of $\partial\Omega$, whose $d-2$-dimensional boundary is also analytic (for $d=2$ the latter boundary consists of isolated points). The transition probability density function of a Brownian trajectory $\boldsymbol{x}(t)$, with diffusion constant D, is defined as

$$p(\boldsymbol{x}, t \,|\, \boldsymbol{y})\, d\boldsymbol{x} = \Pr\{\boldsymbol{x}(t) \in \boldsymbol{x} + d\boldsymbol{x} \,|\, \boldsymbol{x}(0) = \boldsymbol{y}\}.$$

It satisfies the diffusion equation

$$\frac{\partial p(\boldsymbol{x}, t \mid \boldsymbol{y})}{\partial t} = D\Delta_{\boldsymbol{x}} p \quad \text{for } \boldsymbol{x}, \boldsymbol{y} \in \Omega$$

with the initial condition

$$p(\boldsymbol{x}, 0 \mid \boldsymbol{y}) = \delta(\boldsymbol{x} - \boldsymbol{y})$$

and the boundary conditions

$$\begin{aligned} \frac{\partial p(\boldsymbol{x}, t \mid \boldsymbol{y})}{\partial n(\boldsymbol{x})} &= 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_r, \ \ \boldsymbol{y} \in \Omega, \\ p(\boldsymbol{x}, t \mid \boldsymbol{y}) &= 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_a, \ \ \boldsymbol{y} \in \Omega. \end{aligned}$$

The first passage time to the absorbing boundary is defined as

$$\tau = \inf \{t > 0 : \boldsymbol{x}(t) \in \partial\Omega_a\}$$

and the the mean first passage time (MFPT) to $\partial\Omega_a$, given that $\boldsymbol{x}(0) = \boldsymbol{y}$, is defined as the conditional expectation

$$\bar{\tau}_{\boldsymbol{y}} = E[\tau \mid \boldsymbol{x}(0) = \boldsymbol{y}] = \int_0^\infty \int_\Omega p(\boldsymbol{x}, t \mid \boldsymbol{y})\, d\boldsymbol{x}\, dt.$$

The *confinement time* $\bar{\tau}$ is defined as

$$\bar{\tau} = E\tau = \int_\Omega E[\tau \mid \boldsymbol{x}(0) = \boldsymbol{y}]\, p_0(\boldsymbol{y})\, d\boldsymbol{y},$$

where $p_0(\boldsymbol{y})$ is the probability density function (pdf) of the initial point $\boldsymbol{y}$.

4.2. The Boundary Value Problem for $\bar{\tau}_{\boldsymbol{x}}$

To facilitate notation we use

$$u(\boldsymbol{x}) = \bar{\tau}_{\boldsymbol{x}}.$$

The function $u(\boldsymbol{x})$ satisfies the mixed Neumann–Dirichlet boundary value problem (see for example, ref. 14)

$$D\Delta u(\boldsymbol{x}) = -1 \quad \text{for } \boldsymbol{x} \in \Omega, \tag{4.1}$$

$$\frac{\partial u(\boldsymbol{x})}{\partial n} = 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega - \partial\Omega_a, \tag{4.2}$$

$$u(\boldsymbol{x}) = 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_a, \tag{4.3}$$

where D is the diffusion coefficient. Eqs. (4.1)–(4.3) are a classical mixed boundary value problem in potential theory that has been discussed at length in the literature. Explicit expressions for the solution are known for several domains, including a circular disk[16] (see Section 4.3.1). The singular perturbation problem for a general domain with a small opening has not been solved so far.

We assume, for convenience, that $D = 1$. To determine the solution of the mixed boundary value problem (4.1)–(4.3) in terms of Neumann's function $N(\boldsymbol{x}, \boldsymbol{\xi})$, we recall[17] that $N(\boldsymbol{x}, \boldsymbol{\xi})$ is the solution of the boundary value problem

$$\Delta_{\boldsymbol{x}} N(\boldsymbol{x}, \boldsymbol{\xi}) = -\delta(\boldsymbol{x} - \boldsymbol{\xi}) \quad \text{for } \boldsymbol{x}, \boldsymbol{\xi} \in \Omega, \tag{4.4}$$

$$\frac{\partial N(\boldsymbol{x}, \boldsymbol{\xi})}{\partial n(\boldsymbol{x})} = -\frac{1}{|\partial\Omega|} \quad \text{for } \boldsymbol{x} \in \partial\Omega, \ \boldsymbol{\xi} \in \Omega, \tag{4.5}$$

and is defined up to an additive constant. It has the form

$$N(\boldsymbol{x}, \boldsymbol{\xi}) = \begin{cases} \dfrac{1}{\sigma_{d-1}} |\boldsymbol{x} - \boldsymbol{\xi}|^{-d+2} + v_S(\boldsymbol{x}, \boldsymbol{\xi}) & \text{for } d > 2, \ \ \boldsymbol{x}, \boldsymbol{\xi} \in \Omega, \\ -\dfrac{1}{2\pi} \log |\boldsymbol{x} - \boldsymbol{\xi}| + v_S(\boldsymbol{x}, \boldsymbol{\xi}) & \text{for } d = 2, \ \ \boldsymbol{x}, \boldsymbol{\xi} \in \Omega, \end{cases} \tag{4.6}$$

where $v_S(\boldsymbol{x}, \boldsymbol{\xi})$ is a regular harmonic function, σ_{d-1} is the surface area of the unit sphere in $\mathbb{R}^d$.

To derive an integral representation of the solution, we multiply Eq. (4.1) by $N(\boldsymbol{x}, \boldsymbol{\xi})$, Eq. (4.4) by $u(\boldsymbol{x})$, integrate with respect to $\boldsymbol{x}$ over Ω, and use Green's formula to obtain the identity

$$\begin{aligned} &\oint_{\partial\Omega} N(\boldsymbol{x}(\boldsymbol{S}), \boldsymbol{\xi}) \frac{\partial u(\boldsymbol{x}(\boldsymbol{S}))}{\partial n} \, dS + \frac{1}{|\partial\Omega|} \oint_{\partial\Omega} u(\boldsymbol{x}(\boldsymbol{S})) \, dS \\ &\quad = u(\boldsymbol{\xi}) - \int_{\Omega} N(\boldsymbol{x}, \boldsymbol{\xi}) \, d\boldsymbol{x}. \end{aligned} \tag{4.7}$$

The second integral on the left-hand side of Eq. (4.7) is an additive constant, so we obtain the representation

$$u(\boldsymbol{\xi}) = \int_{\Omega} N(\boldsymbol{x}, \boldsymbol{\xi})\, d\boldsymbol{x} + \int_{\partial\Omega_a} N(\boldsymbol{x}(\boldsymbol{S}), \boldsymbol{\xi}) \frac{\partial u(\boldsymbol{x}(\boldsymbol{S}))}{\partial n}\, dS + C', \tag{4.8}$$

where C' is a constant to be determined from the boundary condition (4.3), $\boldsymbol{S}$ is the $d-1$-dimensional coordinate of a point on $\partial\Omega_a$, and dS is a surface area element on $\partial\Omega_a$. We set

$$g(\boldsymbol{S}) = \frac{\partial u(\boldsymbol{x}(\boldsymbol{S}))}{\partial n},$$

choose $\boldsymbol{\xi} = \boldsymbol{\xi}(\boldsymbol{S}) \in \partial\Omega_a$, and use the boundary condition (4.3), to obtain the equation

$$0 = \int_{\Omega} N(\boldsymbol{x}, \boldsymbol{\xi}(\boldsymbol{S}))\, d\boldsymbol{x} + \int_{\partial\Omega_a} N(\boldsymbol{x}(\boldsymbol{S}'), \boldsymbol{\xi}(\boldsymbol{S})) g(\boldsymbol{S}')\, dS' + C' \tag{4.9}$$

for all $\boldsymbol{\xi}(\boldsymbol{S}) \in \partial\Omega_a$. The first integral in Eq. (4.9) is a regular function of $\boldsymbol{\xi}$ on the boundary. Indeed, due to the symmetry of the Neumann function we have from Eq. (4.4)

$$\Delta_{\boldsymbol{\xi}} \int_{\Omega} N(\boldsymbol{x}, \boldsymbol{\xi})\, d\boldsymbol{x} = -1 \quad \text{for } \boldsymbol{\xi} \in \Omega \tag{4.10}$$

and

$$\frac{\partial}{\partial n(\boldsymbol{\xi})} \int_{\Omega} N(\boldsymbol{x}, \boldsymbol{\xi})\, d\boldsymbol{x} = -\frac{|\Omega|}{|\partial\Omega|} \quad \text{for } \boldsymbol{\xi} \in \partial\Omega. \tag{4.11}$$

Equation (4.10) and the boundary condition (4.11) define the integral $\int_{\Omega} N(\boldsymbol{x}, \boldsymbol{\xi})\, d\boldsymbol{x}$ as a regular function, up to an additive constant. Thus Eq. (4.8) can be written as

$$u(\boldsymbol{\xi}) = \int_{\Omega} N(\boldsymbol{x}, \boldsymbol{\xi})\, d\boldsymbol{x} + \int_{\partial\Omega_a} N(\boldsymbol{x}(\boldsymbol{S}), \boldsymbol{\xi}) g(\boldsymbol{S})\, dS + C, \tag{4.12}$$

and both $g(\boldsymbol{S})$ and C are determined by the absorbing condition (4.3)

$$\begin{aligned} 0 = &\int_{\Omega} N(\boldsymbol{x}, \boldsymbol{\xi}(\boldsymbol{S}))\, d\boldsymbol{x} + \int_{\partial\Omega_a} N(\boldsymbol{x}(\boldsymbol{S}'), \boldsymbol{\xi}(\boldsymbol{S})) g(\boldsymbol{S}')\, dS' + C \\ &\text{for } \boldsymbol{\xi}(\boldsymbol{S}) \in \partial\Omega_a. \end{aligned} \tag{4.13}$$

Eq. (4.12) can be considered an integral equation for $g(\boldsymbol{S})$ and C. The normal derivative $g(\boldsymbol{S})$ is a regular function of the $d-1$ variables $\boldsymbol{S}=(s_1,\ldots,s_{d-1})$ for $\boldsymbol{\xi}(\boldsymbol{S})$ in the $d-1$ dimensional subdomain $\partial\Omega_a$, but develops a singularity as $\boldsymbol{\xi}(\boldsymbol{S})$ approaches the $d-2$-dimensional boundary of $\partial\Omega_a$ in $\partial\Omega$.[18] Both can be determined from the representation (4.12) if all functions in Eq. (4.13) and the boundary are analytic. In that case the solution has a series expansion in powers of arclength on Ω_a.

4.3. MFPT Through a Small Opening in a Planar Domain

When the size of the absorbing boundary is small an asymptotic approximation to the constant C can be found from Eq. (4.13). We can assume that the constant term in the expansion of the first integral in equation Eq. (4.13) vanishes, because otherwise, it can be incorporated into the constant C. With this assumption in mind, we rename the constant C_ε.

Consider now a bounded domain $\Omega\subset\mathbb{R}^2$, whose boundary $\partial\Omega$ has the representation $(x(s), y(s))$, the functions $x(s)$ and $y(s)$ are real analytic in the interval $2|s|\leqslant|\partial\Omega|=1$, and

$$\left(x\left(-\frac{1}{2}\right), y\left(-\frac{1}{2}\right)\right)=\left(x\left(\frac{1}{2}\right), y\left(\frac{1}{2}\right)\right).$$

We assume the absorbing part of the boundary $\partial\Omega_a$ is the arc

$$\partial\Omega_\varepsilon=\{|s|<\varepsilon\}$$

and $\partial\Omega-\partial\Omega_\varepsilon$ is reflecting to Brownian trajectories in Ω. All variables are assumed dimensionless. We assume here that Neumann's function,

$$N(x,y;\xi,\eta)=-\frac{1}{2\pi}\log\sqrt{(x-\xi)^2+(y-\eta)^2}+v_S(x,y;\xi,\eta), \qquad (4.14)$$

is known (that is, the harmonic function $v_S(x,y;\xi,\eta)$ is known). We note, however, that $v_S(x,y;\xi,\eta)$ is regular as long as either $(x,y)\in\Omega$ or $(\xi,\eta)\in\Omega$, or both. If $(x,y)\in\partial\Omega$ and $(\xi,\eta)\in\partial\Omega$, then the regular part contains the same singularity as $-(1/2\pi)\log\sqrt{(x-\xi)^2+(y-\eta)^2}$, so that the singular part acquires a factor of 2 on the boundary.

In this setup Eq. (4.13) can be written as

$$\int_\Omega \int \left\{ v_S(x(s'), y(s'); \xi(s), \eta(s)) \right\} dx\, dy - \frac{1}{2\pi} \log \sqrt{(x-\xi)^2 + (y-\eta)^2} + \int_{|s'|<\varepsilon} \left\{ \tilde{v}_S(x(s'), y(s'); \xi(s), \eta(s)) - \frac{1}{\pi} \log \sqrt{(x(s')-\xi(s))^2 + (y(s')-\eta(s))^2} \right\} \times g(s')\, ds' = -C_\varepsilon, \tag{4.15}$$

where

$$\tilde{v}_S(x(s'), y(s'); \xi(s), \eta(s)) = v_S(x(s'), y(s'); \xi(s), \eta(s)) + (1/2\pi) \log \sqrt{(x(s')-\xi(s))^2 + (y(s')-\eta(s))^2}$$

is a regular function of its variables. The double integral in the first line of Eq. (4.15) is the regular function $\int_\Omega \int N(x, y; \xi(s), \eta(s))\, dx\, dy$ and can be expanded into a power series in the interval $|s| < \varepsilon$,

$$\int_\Omega \int N(x, y; \xi(s), \eta(s))\, dx\, dy = \sum_{j=1}^{\infty} N_j s^j, \tag{4.16}$$

where N_j are known coefficients. As mentioned above, the sum is assumed to begin with $j = 1$. Now, we expand

$$g(s) = \sum_{j=0}^{\infty} g_j s^j, \qquad \tilde{v}_S(x(s'), y(s'); \xi(s), \eta(s)) = \sum_{j=0}^{\infty} v_j(s') s^j \tag{4.17}$$

for $|s| < \varepsilon$, where $v_j(s')$ are known coefficients and g_j are unknown coefficients, to be determined from Eq. (4.15).

To expand the logarithmic term in the last integral in Eq. (4.15), we recall that $x(s')$, $y(s')$, $\xi(s)$, and $\eta(s)$ are analytic functions of their arguments in the intervals $|s| < \varepsilon$ and $|s'| < \varepsilon$, respectively. In view of the obvious identities $(x(s), y(s)) = (\xi(s), \eta(s))$, and $[x'(s)]^2 + [y'(s)]^2 = 1$, we can write for all $n \geqslant 0$

$$\int_{-\varepsilon}^{\varepsilon} (s')^n \log \sqrt{(x(s')-\xi(s))^2 + (y(s')-\eta(s))^2}\, ds' = \int_{-\varepsilon}^{\varepsilon} (s')^n \log \left\{ |s'-s| \left(1 + O\left((s'-s)^2\right)\right) \right\} ds'. \tag{4.18}$$

We keep in Taylor's expansion of $\log\left\{|s'-s|\left(1+O\left((s'-s)^2\right)\right)\right\}$ only the leading term, because higher-order terms contribute positive powers of ε to the series

$$\int_{-\varepsilon}^{\varepsilon}\log(s-s')^2\,ds'=4\varepsilon\,(\ln|\varepsilon|-1)+2\sum_{j=1}^{\infty}\frac{1}{(2j-1)\,j}\frac{s^{2j}}{\varepsilon^{2j-1}}. \tag{4.19}$$

For even $n\geqslant 0$, we have

$$\begin{aligned}\int_{-\varepsilon}^{\varepsilon}(s')^n\log(s-s')^2\,ds' &= 4\left(\frac{\varepsilon^{n+1}}{n+1}\log\varepsilon-\frac{\varepsilon^{n+1}}{(n+1)^2}\right)\\ &\quad -2\sum_{j=1}^{\infty}s^{2j}\frac{\varepsilon^{n-2j+1}}{j\,(n-2j+1)},\end{aligned} \tag{4.20}$$

whereas for odd n, we have

$$\int_{-\varepsilon}^{\varepsilon}(s')^n\log(s-s')^2\,ds'=-4\sum_{j=1}^{\infty}\frac{s^{2j+1}}{2j+1}\frac{\varepsilon^{n-2j}}{n-2j}. \tag{4.21}$$

Using the above expansions in Eq. (4.15), we obtain a linear system of equations for the coefficients g_j, that define them as linear functions of the constant C_ε. In particular, g_0 is proportional to C_ε.

The system of equations is obtained by comparing the coefficients of like powers of s in the expansion of (4.15), using the expansions (4.16)–(4.21),

$$\begin{aligned}0=&-\sum_{j=1}^{\infty}N_j s^j+\int_{-\varepsilon}^{\varepsilon}\left\{\frac{-1}{2\pi}\log\left[|s'-s|^2\left(1+O\left((s'-s)^2\right)\right)\right]\right.\\ &\left.+\sum_{j=0}^{\infty}v_j(s')s^j\right\}\sum_{j=0}^{\infty}g_j s'^j\,ds'+C_\varepsilon,\end{aligned}$$

which gives the term of degree 0 as

$$\begin{aligned}&\varepsilon\,(\ln|\varepsilon|-1)\,g_0+\sum_p\left(\frac{\varepsilon^{2p+1}}{2p+1}\log\varepsilon-\frac{\varepsilon^{2p+1}}{(2p+1)^2}\right)g_{2p}\\ &\quad=\frac{\pi}{2}\int_{-\varepsilon}^{\varepsilon}v_0(s')\,ds'+C_\varepsilon.\end{aligned} \tag{4.22}$$

The general term for $j > 0$ is given by

$$0 = -N_{2j} + \frac{1}{\pi}\sum_{p=0}^{\infty} g_{2p}\frac{\varepsilon^{2p-2j+1}}{(2p-2j+1)j} + \int_{-\varepsilon}^{\varepsilon} v_{2j}(s')g(s')\,ds',$$

$$0 = -N_{2j+1} + \frac{2}{\pi}\sum_{p=0}^{\infty} g_{2p+1}\frac{\varepsilon^{2p-2j+1}}{(2p-2j+1)(2j+1)} + \int_{-\varepsilon}^{\varepsilon} v_{2j+1}(s')g(s')\,ds'.$$

Equation (4.22) and

$$\frac{1}{2}\int_{-\varepsilon}^{\varepsilon} g(s)ds = \sum_{p}\frac{\varepsilon^{2p+1}}{(2p+1)}g_{2p}$$

determine C_ε. Indeed, integrating Eq. (4.1) over the domain, we see that

$$\int_{-\varepsilon}^{\varepsilon} g(s)\,ds = -|\Omega|, \tag{4.23}$$

and using the fact that $\displaystyle\int_{-\varepsilon}^{\varepsilon} v_0(s')\,ds' = O(\varepsilon)$, we find that the leading term in the expansion of C_ε in Eq. (4.22) is

$$C_\varepsilon = \frac{|\Omega|}{\pi}\left[\log\frac{1}{\varepsilon} + O(1)\right] \quad \text{for } \varepsilon \ll 1. \tag{4.24}$$

If the diffusion coefficient is D, Eq. (4.12) gives the MFPT from a point $(\xi, \eta) \in \Omega$ as

$$\bar{\tau}_{(\xi,\eta)} = u(\xi,\eta) = \frac{1}{D}\int_{\Omega} N(\boldsymbol{x},\boldsymbol{\xi})\,d\boldsymbol{x} + \frac{|\Omega|}{\pi D}\left[\log\frac{1}{\varepsilon} + O(1)\right] \quad \text{for } \varepsilon \ll 1. \tag{4.25}$$

The leading term in the expansion (4.25) is insufficient in general, because $\log\varepsilon$ may be comparable to 1, even if epsilon is quite small. It is important to obtain the $O(1)$ term in the expansion. This is done below for a circular domain.

4.3.1. MFPT Through a Small Opening in a Circular Domain

The explicit solution u_ε of the boundary value problem

$$\begin{aligned} D\Delta u_\varepsilon(r,\theta) &= -1 \quad \text{for } r<R, \\ \frac{\partial u_\varepsilon(R,\theta)}{\partial r} &= 0 \quad \text{for } \varepsilon<\theta<\pi,\ -\pi<\theta<-\varepsilon, \\ u_\varepsilon(R,\theta) &= 0 \quad \text{for } -\varepsilon<\theta<\varepsilon, \end{aligned} \tag{4.26}$$

is given in ref. 16. The application of the power series expansion method of the previous section begins with the solution of the Neumann problem in polar coordinates (see Appendix I)

$$\begin{aligned} D\Delta v_\varepsilon(R,\theta) &= 0 \quad \text{for } r<R, \\ \frac{\partial v_\varepsilon(R,\theta)}{\partial r} &= h(\theta) \quad \text{for } r=R. \end{aligned}$$

It has the representation

$$v_\varepsilon(r,\theta) = -\frac{R}{2\pi D}\int_0^{2\pi} \log\left(R^2 - 2rR\cos(\theta-\phi)+r^2\right) h(\phi)\,d\phi + C_\varepsilon, \tag{4.27}$$

where C_ε is a constant to be determined. To solve Eq. (4.26), we set

$$u_\varepsilon(r,\theta) = \frac{R^2-r^2}{4D} + \frac{v_\varepsilon(r,\theta)}{D}, \tag{4.28}$$

where

$$\Delta v_\varepsilon(R,\theta) = 0 \quad \text{for } r<R, \tag{4.29}$$

$$\frac{\partial v_\varepsilon(R,\theta)}{\partial r} = \frac{R}{2} = Rf(\theta) \quad \text{for } |\theta|>\varepsilon, \tag{4.30}$$

$$v_\varepsilon(R,\theta) = 0 \quad \text{for } |\theta|<\varepsilon. \tag{4.31}$$

We set

$$\frac{\partial v_\varepsilon(R,\theta)}{\partial r} = Rg(\theta) \quad \text{for } |\theta|<\varepsilon \tag{4.32}$$

and use the Green function of the Neumann problem for a disk to write the solution of the boundary value problem (4.29) as

$$v_\varepsilon(r,\theta) = -\frac{R^2}{4\pi}\int_{|\phi|>\varepsilon} \log\left(\frac{R^2 - 2rR\cos(\theta-\phi)+r^2}{R^2}\right) d\phi$$
$$-\frac{R^2}{2\pi}\int_{|\phi|<\varepsilon} \log\left(\frac{R^2 - 2rR\cos(\theta-\phi)+r^2}{R^2}\right) g(\phi)\, d\phi + C_\varepsilon. \tag{4.33}$$

This gives

$$u_\varepsilon(r,\theta)$$
$$= \frac{R^2-r^2}{4D} - \frac{R^2}{2\pi D}\int_{|\phi|<\varepsilon} \log\left(\frac{R^2 - 2rR\cos(\theta-\phi)+r^2}{R^2}\right)$$
$$\times\left(g(\phi) - \frac{1}{2}\right) d\phi + C_\varepsilon.$$

To estimate the unknown function g, we use the absorbing boundary condition of v_ε at $r = R$ and $\theta = 0$. The function g and the constant C_ε can be determined from

$$0 = v_\varepsilon(R,\theta) = -\frac{R^2}{2\pi}\int_{|\phi|<\varepsilon} \log(\cos 2[1-\cos(\theta-\phi)])$$
$$\times\left\{g(\phi) - \frac{1}{2}\right\} d\phi + C_\varepsilon, \tag{4.34}$$

because

$$\int_{|\phi|<\pi} \log\{2[1-\cos(\theta-\phi)]\}\, d\phi = 0.$$

Using the expansion procedure described above (see also Appendix II), we obtain that

$$C_\varepsilon = R^2\left(0.73 + (1+O(\varepsilon))\ln\frac{1}{\varepsilon}\right), \tag{4.35}$$

when all series are truncated at $O\left(\theta^{12}\right)$. The expansion of the exact solution of ref. 16 gives the value $\log 2 = 0.6931471806$. Now, in the limit of small opening Eq. (4.33) gives

$$v_\varepsilon(0,0) = C_\varepsilon \sim R^2\left(0.73 + \ln\frac{1}{\varepsilon}\right).$$

It follows from (4.28) that the MFPT from the center of the disk to the absorbing boundary is given by

$$\bar{\tau}_0 = u_\varepsilon(0,0) \sim \frac{R^2}{D}\left(0.98 + \ln\frac{1}{\varepsilon}\right). \tag{4.36}$$

The exact value of the constant term is $\log 2 + 1/4 = 0.9431471806$,[16] which indicates an error of about 4% of the power series approximation.

Remark 1. In three-dimensional diffusion, if a particle (a receptor inside the confinement domain) is bound to a scaffolding protein of mass M_s, the diffusion constant of the system of the two proteins has to be recompute according to Einstein's law[14]

$$D_s = \frac{k_B T}{(M + M_s)\gamma_{rs}},$$

where k_B is Boltzmann's constant, T is the absolute temperature, $M + M_s$ is the mass of the complex receptor–protein, and γ_{rs} is the viscosity coefficient of the complex. Assuming the volume of the complex is the sum of the volumes of its components, Stokes' law, as used in Einstein's formula,[14] gives

$$\gamma_{rs} = \gamma_r + \gamma_s,$$

where γ_r, γ_s are the friction coefficients of the receptor and the scaffolding protein, respectively. The new diffusion constant of the system is now,

$$\bar{D}_c = \frac{R^2}{k_B T}(M + M_s)(\gamma_r + \gamma_s).$$

Remark 2. For a cylindrical model of a protein moving on membrane surface, the diffusion constant has been derived in ref. 19 and is given by

$$D = \frac{kT}{4\pi\mu h}\left(\log\left(\frac{\mu h}{\mu' R}\right) - \gamma_E\right), \tag{4.37}$$

where R and h are, respectively, the radius and the height of the cylinder, μ is the viscosity, μ' is the viscosity coefficient of the aqueous phase and γ_E is Euler's constant. When a receptor of radius R_1 is bound to

a scaffolding protein such as stargazin of radius R_2, we approximate the shape of the two link proteins as a cylinder of radius $R_1 + R_2$. The diffusion constant for the two proteins becomes

$$D = \frac{kT}{4\pi\mu h}\left[\log\left(\frac{\mu h}{\mu'(R_1+R_2)}\right) - \gamma_{\mathrm{E}}\right].$$

Sometimes, the scaffolding protein is bound to a receptor and increases only the total length h and not the total radius. This is the case for PICK or GRIP proteins binding to an AMPA receptor, as describe in the review.[9] When the total length equals $h_1 + h_2$, the diffusion constant becomes:

$$D = \frac{kT}{4\pi\mu(h_1+h_2)}\left[\log\left(\frac{\mu(h_1+h_2)}{\mu' R}\right) - \gamma_{\mathrm{E}}\right].$$

In general, a receptor is made of several sub-units which are integral membrane proteins[20]. Accessory or scaffolding proteins may be bound to the receptors and it is not clear if these proteins are always bound to the receptors, or only under specific conditions. Some of the receptor's subunits may be stored in intracellular compartments and may be inserted in the plasma membrane only under specific circumstances.

Remark 3. If the surface of the membrane contains many confinement domains, the diffusion of a receptor can be described on a coarse time scale as a random walk between confinement domains (or slower Brownian motion). When the receptor is not in a confinement domain and is free of the scaffolding protein, its Brownian motion is much faster than that while it is inside a confinement domain and attached to a scaffolding protein, because its diffusion coefficient is larger in the former than in the latter case. Thus, we can describe the motion of the receptor as a random walk between the confinement domains.[21,22] Assuming that the characteristic distance between (circular) confinement domains is d, the coarser random walk can be described as diffusion with a diffusion constant

$$D_a = \frac{d^2}{\bar{\tau}_0} = \frac{d^2 D}{R^2\left(\log 2 + 1/4 + \ln\frac{1}{\varepsilon}\right)}, \tag{4.38}$$

assuming the diffusion is isotropic. This assumption is justified if the narrow openings are distributed uniformly on the circles. If there is a preferred direction, the two-dimensional diffusion tensor becomes anisotropic with a larger diffusion coefficient in the preferred direction.[14]

When the synapse contains circular confinement domains of typical area $350\,\mathrm{nm}^2$, (radius $R \sim 10.5\,\mathrm{nm}$), the mean distance between the domains is around $0.13\,\mu\mathrm{m}$, and the free diffusion constant is $0.1\mu\mathrm{m}^2/\mathrm{s}$, the effective coarse grained diffusion constant is about $0.02\,\mu\mathrm{m}^2/\mathrm{s}$, according to Eq. (4.38).

4.3.2. The Mean Confinement Time

Averaging the MFPT over a uniform distribution of initial positions inside the disk gives

$$\bar{\tau}_{\mathrm{m}} = \frac{1}{\pi R^2}\int_0^{2\pi}\int_0^R u_\varepsilon(r,\theta) r\,dr\,d\theta, \tag{4.39}$$

where $u_\varepsilon(r,\theta)$ is given by (4.28), and $v_\varepsilon(r,\theta)$ is the solution of Eq. (4.34). This gives

$$\frac{1}{\pi R^2}\int_0^{2\pi}\int_0^R \frac{R^2-r^2}{4D}\, r\,dr\,d\theta = \frac{R^2}{8D}$$

and

$$\frac{1}{\pi R^2}\int_0^{2\pi}\int_0^R v_\varepsilon(r,\theta)\, r\,dr\,d\theta = C_\varepsilon. \tag{4.40}$$

We have used the fact that for all $r < R$

$$\int_0^{2\pi} \log\left(\frac{R^2 - 2rR\cos(\theta-\phi)+r^2}{R^2}\right) d\theta = 0.$$

It follows that the mean confinement time $\bar{\tau}_{\mathrm{m}}$ is given by

$$\bar{\tau}_{\mathrm{m}} = C_\varepsilon + \frac{R^2}{8D} = R^2\left(\log 2 + \frac{1}{8} + (1+O(\varepsilon))\ln\frac{1}{\varepsilon}\right) \sim \frac{R^2}{D}\left(0.818 + \ln\frac{1}{\varepsilon}\right). \tag{4.41}$$

The difference between the mean time $\bar{\tau}_{\mathrm{m}}$ and the confinement time, computed at the origin, is not significant for the scale we are interested in. As is typical for the exit problem,[14] the MFPT is independent of the initial point, except for a layer near the absorbing boundary.

4.3.3. Numerical Evaluations

To estimate the mean confinement time $\bar{\tau}$ for a receptor, we use the values of the different parameters reported in refs. 10 and 23. For a receptor inside a confinement domain (see Fig. 2), we take $D = 0.004\,\mu\text{m}^2/\text{s}$, for $R = 0.25\,\mu\text{m}$, $\varepsilon = 10^{-3}\,\text{nm}/(2\pi \times 0.25)$ to find that $\bar{\tau} = 125\,\text{s}$. For a diffusion constant of $D = 0.02\,\mu\text{m}^2/\text{s}$, which is the free diffusion constant in a membrane, $\bar{\tau} = 25$. For a domain of area $350\,\text{nm}^2$, which we assume is well approximated by a disk, using a diffusion coefficient of $0.025\,\mu\text{m}^2/\text{s}$, we find that the mean confinement time is around $\bar{\tau} = 35\,\text{s}$.

4.3.4. Confinement by a Potential Barrier

If a receptor is confined to the corral by a high potential barrier $\Phi(x, y)$ (relative to the thermal energy per unit mass), with a single saddle point on its crest, the confinement domain Ω is bounded by the crest of the potential barrier (characterized by $\partial\Phi/\partial n = 0$ on the crest). We assume that the potential barrier is narrow relative to the size of the domain and that $\Phi(x, y) = 0$ away from the barrier. If there is a single minimum of the energy of the barrier (at a saddle point), the calculations of ref. 14, [Ch. 8.5, Eqs. (8.5.7)–(8.5.13)] give the confinement time for a three-dimensional diffusion as

$$\bar{\tau} = \frac{|\Omega|\omega_{\parallel}}{D\omega_{\perp}} \exp\left\{\frac{E}{\gamma D}\right\}, \tag{4.42}$$

where

$$\omega_{\parallel}^2 = \frac{\partial^2\Phi}{\partial s^2} \quad \text{at the saddle point,}$$

$$\omega_{\perp}^2 = -\frac{\partial^2\Phi}{\partial n^2} \quad \text{at the saddle point,}$$

s is arclength along $\partial\Omega$, D is the diffusion coefficient, E is the energy of the saddle point per unit mass on the barrier (the lowest energy of the barrier), and T is absolute temperature. The factor $\omega_{\parallel}$ is the frequency of oscillation in the stable direction of the saddle point (parallel to the boundary), and $\omega_{\perp}$ is the imaginary frequency in the unstable direction of the saddle point (e.g., perpendicular to the boundary). Note that in the case at hand $\Phi = 0$ throughout Ω, except for a boundary layer, whose contribution to the integral is negligible. Thus

$$\int_{\Omega}\int e^{-\Phi/\gamma D}\,dx\,dy = |\Omega|,$$

which simplifies Eq. (8.5.13) in ref. 14 to the result (4.42). The case of multiple saddle points is discussed in.[14]

If the energy of the boundary is constant, E, the MFPT is given by

$$\bar{\tau} = \sqrt{\frac{2\pi}{D}} \frac{\sqrt{\gamma}|\Omega|}{\omega_\perp |\partial\Omega|} \exp\left\{\frac{E}{\gamma D}\right\}, \tag{4.43}$$

where γ is the friction coefficient (this is case (i) in [ref. 14, Eq. (8.5.15)]). It has not been established experimentally that there is hopping of AMPA receptors over a potential barrier. Rather, it is believed that the barrier of the corral is not stable and breaks down intermittently.

4.3.5. Mean Time to Enter the PSD

The mean time for a receptor to enter the PSD after insertion in the membrane depends on the diffusion coefficient, the organization of the synapse, the layout of confinement domains, and the distribution of scaffolding proteins. The latter can decrease the diffusion constant when attached to the receptor (see Fig. 1). When the diffusion of the receptor is confined by a reflecting barrier to a domain Ω that contains a corral ω, and the receptor is inserted somewhere is $\Omega - \omega$, the entrance problem to ω is the exit problem from $\Omega - \omega$. Thus, if the opening $\partial\omega_a$ in $\partial\omega$ is small, that is, if $\varepsilon = |\partial\omega_a|/|\partial\omega| \ll 1$, the result (4.25) is still valid. In particular, for an annulus $D(R_1, R_2)$, of inner radius R_1 and outer radius R_2, where the inner circle $r = R_1$ represents the boundary of a PSD and contains a small opening of length εR_1, and the outer circle models a barrier that prevents the escape of the receptor, Eq. (4.25) gives

$$\bar{\tau} \sim \frac{R_2^2 - R_1^2}{D} \ln \frac{1}{\varepsilon}. \tag{4.44}$$

The mean entrance time for the annulus $D(R_1, R_2)$ can be found explicitly if the inner circle is absorbing while the outer circle is reflecting. The boundary value problem (4.1)–(4.3) becomes

$$\begin{aligned} D\Delta u &= -1 \qquad \text{for } R_1 < r < R_2 \\ \frac{\partial u(R_2, \theta)}{\partial r} &= 0, \qquad u(R_1, \theta) = 0. \end{aligned} \tag{4.45}$$

The solution (in radial symmetry) is given by

$$u(r, \theta) = \frac{R_1^2 - r^2}{4D} + \frac{R_2^2}{2D} \log \frac{r}{R_1}.$$

In particular, if $R_1 \ll R_2$, we can write $R_2 = R$, $R_1 = \varepsilon R$, with $\varepsilon \ll 1$. Asymptotically, the MFPT from the outer circle to the inner circle is

$$\bar{\tau} \sim \frac{R^2}{2D} \ln \frac{1}{\varepsilon}. \tag{4.46}$$

In the same limit Eq. (4.44) becomes

$$\bar{\tau} \sim \frac{R^2}{D} \ln \frac{1}{\varepsilon}. \tag{4.47}$$

Comparing (4.46) with (4.47), we find that one is twice the other. This result indicates that the aspect angle of the absorbing boundary from it's center determines the pre-logarithmic factor. While 2π for a full circle, it is π for an arc of length 2ε on an arc of length $O(1)$.

4.3.6. Numerical Computation of the Time to Enter into a Confinement Domain

The range of exit times from a confinement domain is between 35 and 125 s, depending on the diffusion constant and on the size of the domain.

Using a free diffusion constant $D = 0.1\,\mu\text{m}^2/\text{s}$, for a domain of area $350\,\text{nm}^2$, when the receptor is inserted at a distance of $1\,\mu$m (we assume that the radius R of the unfolded synapse is $1\,\mu$m), a lower bound on the expected insertion time is $\bar{\tau} = 25\,\text{s}$. This is an underestimate, because we have used only one the leading term in the expansion of the MFPT in Eq. (4.25).

For a diffusion constant $D = 0.02\,\mu\text{m}^2/\text{s}$, which is calculated by averaging over many confinement periods, a PSD of diameter 350 nm, (that is, for $R = 4\,\mu$m), we find that a receptor enters in about 78 s. These numbers are within the range of values communicated in ref. 9.

Remarks

(i) The diffusion process does not require any other energy than the temperature of the cell, and for that reason receptor movement does not cost any chemical energy, but it requires some time, of the order of a few minutes. (ii) The time to anchoring is the sum of the time to enter and time the to reach the final position, which is of the order of the confinement time. The time to anchoring, after insertion of the receptor in a membrane containing several confinement domains, is of the order of a few minutes. The more often a receptor's trajectory enters confinement

domains, the longer is the time to to its anchoring, up to several minutes. Binding to scaffolding proteins that change the diffusion constant increases the mean time to anchoring. (iii) The time to enter a PSD is more sensitive to the location of the point of insertion rather than to the size of the small opening in the barrier. In the regime, where the diffusion outside is faster than inside the confinement domain, the time spent inside is the main contributor to the anchoring time.

5. THE EXIT DISTRIBUTION

When the barrier contains several narrow openings of various sizes the probabilities of exit through given openings are not necessarily the same. Specifically, we consider the problem of escape from a planar domain Ω, whose boundary, $\partial\Omega$ ($|\partial\Omega| = 1$), is reflecting, except for the n absorbing arcs $|s - s_k| < \varepsilon_k$, with $\sum_{k=1}^{n} \varepsilon_k = \varepsilon \ll 1$. The probability that a trajectory that starts at the point $(x, y) \in \Omega$ escapes through arc i is the solution of the boundary value problem

$$\begin{aligned} \Delta u(x, y) &= 0 \quad \text{for } (x, y) \in \Omega \\ \frac{\partial u(x(s), y(s))}{\partial n} &= 0 \quad \text{for } |s - s_k| > \varepsilon_k, \ \ \forall k \\ u(x(s), y(s)) &= \delta_{i,k} \quad \text{for } |s - s_k| < \varepsilon_k, \quad \text{for each } k = 1, 2, \ldots, n, \end{aligned} \tag{5.48}$$

$\delta_{i,k} = 1$ if $i = k$ and zero otherwise. As above, we define the flux density on the absorbing boundary as an unknown function

$$g(s) = \frac{\partial u(x(s), y(s))}{\partial n}.$$

The representation formula for the solution is given by

$$u(\xi, \eta) = \sum_{k=1}^{n} \int_{s_k - \varepsilon_k}^{s_k + \varepsilon_k} N(x(s), y(s); \xi, \eta) g(s)\, ds + C, \tag{5.49}$$

where $N(x, y; \xi, \eta)$ is given in (4.14) and C is a constant. The function $g(s)$ is defined in each one of the intervals $|s - s_k| < \varepsilon_k$ and has to satisfy

the boundary condition

$$\int_{s_k-\varepsilon_k}^{s_k+\varepsilon_k} \Big\{ v_S(x(s'), y(s'); \xi(s), \eta(s)) - \frac{1}{2\pi} \log \sqrt{(x(s')-\xi(s))^2 + (y(s')-\eta(s))^2} \Big\} \times g(s')\, ds' = -C + \delta_{i,k} \quad \text{for all } |s - s_j| < \varepsilon_j, \quad j,k = 1,2,\ldots,n. \tag{5.50}$$

Next, we expand $g(s)$ in Taylor's series in each interval $|s - s_k| < \varepsilon_k$,

$$g(s) = \sum_{j=0}^{\infty} \frac{g^{(j)}(s_k)}{j!} (s - s_k)^j \tag{5.51}$$

and determine the coefficients. The solvability condition for the problem (5.48) is

$$\sum_{k=1}^{n} \int_{s_k-\varepsilon_k}^{s_k+\varepsilon_k} \Big\{ v_S(x(s'), y(s'); \xi(s), \eta(s)) - \frac{1}{2\pi} \log \sqrt{(x(s')-\xi(s))^2 + (y(s')-\eta(s))^2} \Big\} \times g(s')\, ds' = 0, \tag{5.52}$$

Using the expansions (4.19)–(4.21) and (5.51) in the solvability condition (5.52), we obtain

$$\sum_{k=1}^{n} \int_{-\varepsilon_k}^{\varepsilon_k} \sum_{j=0}^{\infty} (1 + O(\varepsilon_k)) \frac{g^{(j)}(s_k)}{j!} s^j\, ds = 0,$$

which is

$$\sum_{k=1}^{n} \sum_{j=0}^{\infty} \frac{g^{(2j)}(s_k)\,(1 + O(\varepsilon_k))}{(2j)!} \frac{\varepsilon_k^{2j+1}}{2j+1} = 0. \tag{5.53}$$

Using the expansions (4.19)–(4.21) in Eqs. (5.50) and (5.52) and equating the coefficients of like powers of $s - s_k$ on both sides of Eq. (5.50), we obtain at the leading order

$$\sum_{j=0}^{\infty} \frac{\varepsilon_k^{2j+1} g^{(2j)}(s_k)}{(2j)!(2j+1)} \left(\log \varepsilon_k - \frac{1}{2j+1} \right) = \frac{\delta_{i,k} - C}{4}$$

and for higher orders

$$\sum_{j=0}^{\infty} \frac{\varepsilon_k^{j+1} g^{(j)}(s_k)}{j!\,(j-2m+1)} = 0 \quad \text{for } k = 1, 2, ..., n, \quad m = 1, 2, ...$$

First, we observe that

$$\frac{g^{(2j+1)}(s_k)}{(2j+1)!} = 0 \quad \text{for } k = 1, 2, ..., n, \quad j = 1, 2, ...$$

To determine the even order derivatives and the constant C, we set

$$x_{j,k} = \frac{\varepsilon_k^{2j+1} g^{(2j)}(s_k)}{(2j)!},$$

and find that $x_{j,k}$ and C are the solutions of the system

$$\sum_{j=0}^{\infty} \frac{x_{j,k}}{2j+1} \left(\log \varepsilon_k - \frac{1}{2j+1} \right) = \frac{\delta_{i,k} - C}{4}, \quad \text{for } k = 1, 2, ..., n, \tag{5.54}$$

$$\sum_{j=0}^{\infty} \frac{x_{j,k}}{2j-2m+1} = 0, \quad \text{for } k = 1, 2, ..., n, \quad m = 1, 2, ... \tag{5.55}$$

$$\sum_{k=1}^{n} \sum_{j=0}^{\infty} \frac{x_{j,k} \varepsilon_k^{2j+1}}{2j+1} = 0. \tag{5.56}$$

If $y_{j,k}$ is the solution of the system

$$\sum_{j=0}^{\infty} \frac{y_{j,k}}{2j+1} = 1,$$

$$\sum_{j=0}^{\infty} \frac{y_{j,k}}{2j-2m+1} = 0 \quad \text{for } k = 1, 2, ..., n, \quad m = 1, 2, ...$$

then

$$x_{j,k} = \frac{\delta_{i,k} - C}{4 \log \varepsilon_k} y_{j,k} \left(1 + O\left(\frac{1}{\log \varepsilon_k} \right) \right)$$

and Eq. (5.56) gives

$$C \sim \frac{\dfrac{1}{\log \varepsilon_i} \displaystyle\sum_{j=0}^{\infty} \frac{y_{j,i} \varepsilon_i^{2j+1}}{2j+1}}{\displaystyle\sum_{k=1}^{n} \frac{1}{\log \varepsilon_k} \sum_{j=0}^{\infty} \frac{y_{j,k} \varepsilon_k^{2j+1}}{2j+1}}.$$

Note that

$$\sum_{k=1}^{n} \int_{s_k-\varepsilon_k}^{s_k+\varepsilon_k} v_S(x(s), y(s); \xi, \eta) g(s)\, ds = O(\varepsilon)$$

in the representation formula (5.49). It follows that the exit probability through arc i is

$$u(\xi, \eta) \sim \frac{\dfrac{1}{\log \varepsilon_i} \displaystyle\sum_{j=0}^{\infty} \frac{y_{j,i} \varepsilon_i^{2j+1}}{2j+1}}{\displaystyle\sum_{k=1}^{n} \frac{1}{\log \varepsilon_k} \sum_{j=0}^{\infty} \frac{y_{j,k} \varepsilon_k^{2j+1}}{2j+1}}. \tag{5.57}$$

If all ε_k are equal, Eq. (5.57) reduces to the obvious result

$$u(\xi, \eta) = \frac{1}{n}.$$

The above equations can be solved explicitly for a disk. When the series are truncated at 10 terms, we obtain the probability of escape at arc i as

$$C_i \sim \frac{\dfrac{\varepsilon_i y_{0,i}}{\ln \varepsilon_i}}{\displaystyle\sum_{k=1}^{n} \frac{\varepsilon_k y_{0,k}}{\ln \varepsilon_k}}. \tag{5.58}$$

As mentioned in Section 5, if the openings on the circles are not distributed uniformly, the diffusion tensor of the coarse grained Brownian motion becomes anisotropic and the diffusion in one direction will be faster than in the orthogonal direction, depending on the distribution of exit points.

6. ESCAPE BEFORE ANCHORING

When a receptor enters a PSD Ω, it can either be anchored for a certain time there by a specific protein or it can leave the PSD without binding. In this section, we calculate the probability of such an event. We formulate the problem for a general domain and give an explicit computation for a planar disk.

We model the anchoring of the receptor as the termination of its trajectory. Termination of diffusing trajectories introduces a killing measure.[14] In the presence of a killing measure $k(\boldsymbol{x})$ the transition probability density of a trajectory, $p(\boldsymbol{x}, t \mid \boldsymbol{y})$ is in fact the probability density to reach the point $\boldsymbol{x}$ at time t without being killed or absorbed. It satisfies the initial-boundary value problem.[14]

$$\frac{\partial p(\boldsymbol{x}, t \mid \boldsymbol{y})}{\partial t} = -\nabla_{\boldsymbol{x}} \cdot \boldsymbol{J}(\boldsymbol{x}, t \mid \boldsymbol{y}) - k(\boldsymbol{x}) p(\boldsymbol{x}, t \mid \boldsymbol{y}) \quad \text{for } \boldsymbol{x}, \boldsymbol{y} \in \Omega, \tag{6.59}$$

$$p(\boldsymbol{x}, t \mid \boldsymbol{y}) = 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_a \quad \boldsymbol{y} \in \Omega,$$

$$\frac{\partial p(\boldsymbol{x}, t \mid \boldsymbol{y})}{\partial n(\boldsymbol{x})} = 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_r, \quad \boldsymbol{y} \in \Omega, \tag{6.60}$$

$$p(\boldsymbol{x}, 0 \mid \boldsymbol{y}) = \delta(\boldsymbol{x} - \boldsymbol{y}) \quad \text{for } \boldsymbol{x}, \boldsymbol{y} \in \Omega, \tag{6.61}$$

where the probability flux density vector is given by

$$\boldsymbol{J}(\boldsymbol{x}, t \mid \boldsymbol{y}) = -D \nabla_{\boldsymbol{x}} p(\boldsymbol{x}, t \mid \boldsymbol{y}),$$

and $\partial\Omega_r$ is the reflecting part of the boundary and $\partial\Omega_a$ the absorbing part. For a general domain binding proteins are spread over a subdomain $\Omega_p \subset \Omega$. We denote by T the time to killing and by τ the time to leave through $\partial\Omega_a$. The probability of a trajectory that starts at $\boldsymbol{y}$ to leave before being killed is the total flux through the absorbing boundary,

$$\Pr\{\tau < T \mid \boldsymbol{y}\} = \int_0^\infty \int_{\partial\Omega_a} \boldsymbol{J}(\boldsymbol{x}, t \mid \boldsymbol{y}) \cdot \boldsymbol{n}(\boldsymbol{x}) \, dS_{\boldsymbol{x}} \, dt. \tag{6.62}$$

Integrating Eq. (6.59) with respect to $\boldsymbol{x}$ and t and using the boundary and initial conditions (6.60) and (6.61), we obtain from (6.62) the representation

$$\Pr\{\tau < T \mid \boldsymbol{y}\} = 1 - \int_\Omega k(\boldsymbol{x}) G(\boldsymbol{x} \mid \boldsymbol{y}) \, d\boldsymbol{x}, \tag{6.63}$$

where

$$G(\boldsymbol{x} \mid \boldsymbol{y}) = \int_0^\infty p(\boldsymbol{x}, t \mid \boldsymbol{y})\, dt.$$

Integrating Eq. (6.59) only with respect to t, we see that the function $G(\boldsymbol{x} \mid \boldsymbol{y})$ is the solution of the boundary value problem

$$\begin{aligned} D\Delta_{\boldsymbol{x}} G(\boldsymbol{x} \mid \boldsymbol{y}) - k(\boldsymbol{x}) G(\boldsymbol{x} \mid \boldsymbol{y}) &= -\delta(\boldsymbol{x} - \boldsymbol{y}), \\ \frac{\partial G(\boldsymbol{x} \mid \boldsymbol{y})}{\partial n(\boldsymbol{x})} &= 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_r, \ \ \boldsymbol{y} \in \Omega, \\ G(\boldsymbol{x} \mid \boldsymbol{y}) &= 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_a, \ \ \boldsymbol{y} \in \Omega. \end{aligned} \tag{6.64}$$

That is, $G(\boldsymbol{x} \mid \boldsymbol{y})$ is Green's function for the inhomogeneous problem

$$\begin{aligned} D\Delta_{\boldsymbol{x}} u(\boldsymbol{x}) - k(\boldsymbol{x}) u(\boldsymbol{x}) &= -f(\boldsymbol{x}), \\ \frac{\partial u(\boldsymbol{x})}{\partial n(\boldsymbol{x})} &= 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_r, \\ u(\boldsymbol{x}) &= 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_a, \end{aligned}$$

where $f(\boldsymbol{x})$ is any square integrable function. It follows that Eq. (6.63) can be rewritten in terms of Green's function as

$$\Pr\{T < \tau \mid \boldsymbol{y}\} = \int_\Omega k(\boldsymbol{x}) G(\boldsymbol{x} \mid \boldsymbol{y})\, d\boldsymbol{x}.$$

The chance to leave before being anchored is found by integrating the conditional probability with respect to the initial uniform distribution of $\boldsymbol{y} \in \Omega$. By definition,

$$\Pr\{T < \tau\} = \frac{1}{|\Omega|} \int_\Omega \Pr\{T < \tau \mid \boldsymbol{y}\}\, d\boldsymbol{y} = \frac{1}{|\Omega|} \int_\Omega k(\boldsymbol{x}) \int_\Omega G(\boldsymbol{x} \mid \boldsymbol{y})\, d\boldsymbol{y}\, d\boldsymbol{x}. \tag{6.65}$$

The function

$$u(\boldsymbol{x}) = \int_\Omega G(\boldsymbol{x} \mid \boldsymbol{y})\, d\boldsymbol{y},$$

is the solution of the boundary value problem

$$D\Delta u(\boldsymbol{x}) - k(\boldsymbol{x}) u(\boldsymbol{x}) = -1 \quad \text{for } \boldsymbol{x} \in \Omega, \tag{6.66}$$

$$u(\boldsymbol{x}) = 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_a,$$

$$\frac{\partial u(\boldsymbol{x})}{\partial n} = 0 \quad \text{for } \boldsymbol{x} \in \partial\Omega_r \tag{6.67}$$

and

$$\Pr\{T<\tau\}=\frac{1}{|\Omega|}\int_{\Omega}k(\boldsymbol{x})u(\boldsymbol{x})\,d\boldsymbol{x}. \tag{6.68}$$

To find the asymptotic expansion of $\Pr\{T<\tau\}$ for a small opening, we proceed as above. We compute $u(\boldsymbol{x})$ from the Neumann function, which is the solution of

$$\begin{aligned} D\Delta N(\boldsymbol{x}\mid\boldsymbol{y})-k(\boldsymbol{x})N(\boldsymbol{x}\mid\boldsymbol{y}) &= -\delta(\boldsymbol{x}-\boldsymbol{y}) \quad \text{for } \boldsymbol{x}\neq\boldsymbol{y}\in\Omega,\\ \frac{\partial N(\boldsymbol{x}\mid\boldsymbol{y})}{\partial n(\boldsymbol{x})} &= 0 \quad \text{for } \boldsymbol{x}\in\partial\Omega,\quad \boldsymbol{y}\in\Omega. \end{aligned} \tag{6.69}$$

From Green's formula, we obtain

$$u(\boldsymbol{y})=\int_{\partial\Omega_a}N(\boldsymbol{x}\mid\boldsymbol{y})\frac{\partial u(\boldsymbol{x})}{\partial n(\boldsymbol{x})}\,dS_{\boldsymbol{x}}+\int_{\Omega}N(\boldsymbol{x}\mid\boldsymbol{y})\,d\boldsymbol{x}. \tag{6.70}$$

Now

$$\begin{aligned} \Pr\{T<\tau\} &= \frac{1}{|\Omega|}\int_{\Omega}k(\boldsymbol{y})u(\boldsymbol{y})\,d\boldsymbol{y}\\ &= \frac{1}{|\Omega|}\int_{\Omega}k(\boldsymbol{y})\left\{\int_{\partial\Omega_a}N(\boldsymbol{x}\mid\boldsymbol{y})\frac{\partial u(\boldsymbol{x})}{\partial n(\boldsymbol{x})}\,dS_{\boldsymbol{x}}+\int_{\Omega}N(\boldsymbol{x}\mid\boldsymbol{y})\,d\boldsymbol{x}\right\}d\boldsymbol{y}\\ &= \frac{1}{|\Omega|}\int_{\Omega}k(\boldsymbol{y})\int_{\partial\Omega_a}N(\boldsymbol{x}\mid\boldsymbol{y})\frac{\partial u(\boldsymbol{x})}{\partial n(\boldsymbol{x})}\,dS_{\boldsymbol{x}}\,d\boldsymbol{y}+1, \end{aligned} \tag{6.71}$$

so that

$$\Pr\{\tau<T\}=-\frac{1}{|\Omega|}\int_{\Omega}k(\boldsymbol{y})\int_{\partial\Omega_a}N(\boldsymbol{x}\mid\boldsymbol{y})g(\boldsymbol{x})\,dS_{\boldsymbol{x}}\,d\boldsymbol{y}, \tag{6.72}$$

where only the function $g(\boldsymbol{x})=\partial u(\boldsymbol{x})/\partial n(\boldsymbol{x})$ is not known explicitly. It can be, however, recovered by using the absorbing boundary condition

$$u(\boldsymbol{y})=0 \quad \text{for } \boldsymbol{y}\in\partial\Omega_a.$$

We obtain

$$\int_{\partial\Omega_a}N(\boldsymbol{x}\mid\boldsymbol{y})g(\boldsymbol{x})\,dS_{\boldsymbol{x}}+\int_{\Omega}N(\boldsymbol{x}\mid\boldsymbol{y})\,d\boldsymbol{x}=0 \quad \text{for } \boldsymbol{y}\in\partial\Omega_a. \tag{6.73}$$

The singularity of Neumann's function for a planar domain is logarithmic, that is,

$$N(\boldsymbol{x} \mid \boldsymbol{y}) = -\frac{1}{2\pi} \log |\boldsymbol{x} - \boldsymbol{y}| + v_S(\boldsymbol{x}, \boldsymbol{y}) \quad \text{for } \boldsymbol{x}, \boldsymbol{y} \in \Omega, \tag{6.74}$$

where $v_S(\boldsymbol{x}, \boldsymbol{y})$ is the regular function.

For a planar domain Ω we use the parametrization of the boundary by arclength $(x(s), y(s))$. We assume, as above, that $|\partial\Omega_a|/|\partial\Omega_r| = \varepsilon \ll 1$. In the case of a unique opening located symmetrically around a point $\boldsymbol{x}_0 \in \partial\Omega_a$, the function g can be approximated using condition (6.73) and a Taylor expansion. We write (6.73) at the boundary point $\boldsymbol{y} = (x(s'), y(s'))$ as

$$\begin{aligned} &-\frac{1}{2\pi} \int_{-\varepsilon}^{\varepsilon} \log(s - s')^2 \left(g(0) + \frac{g''(0)}{2} s^2 + \cdots \right) ds \\ &\quad = -\int_{\Omega} N(\boldsymbol{x} \mid (x(s'), y(s'))\, d\boldsymbol{x}. \end{aligned} \tag{6.75}$$

The first-order term is

$$g(0) = \frac{\pi \displaystyle\int_{\Omega} N(\boldsymbol{x} \mid x(0), y(0))\, d\boldsymbol{x}}{2\varepsilon \log \varepsilon}. \tag{6.76}$$

In general, all derivatives $g^{(k)}(0)$ in identity (6.75) can be computed. An infinite system of equations has to be solved, in a similar way as it is done in Appendix II. Here, using (6.76) in Eq. (6.72) and writing

$$\varepsilon \log \varepsilon \Pr\{\tau < T\} = F(\varepsilon), \tag{6.77}$$

we find that $F(0) = F'(0) = 0$, $F''(0) \neq 0$. It follows that for $\varepsilon \ll 1$

$$\Pr\{\tau < T\} = O\left(\frac{\varepsilon}{\log \varepsilon} \right). \tag{6.78}$$

More precisely, using only the leading order term in the expansion of $\Pr\{\tau < T\}$ for small ε,

$$\Pr\{\tau < T\} = -\int_{-\varepsilon}^{\varepsilon} \frac{1}{|\Omega|} \int_{\Omega} k(\boldsymbol{x}) N(\boldsymbol{x} \mid \boldsymbol{y}(s)) g(s)\, ds\, d\boldsymbol{x},$$

and using $g'(0)=0$, we obtain

$$\frac{\partial^2 \Pr\{\tau < T\}}{\partial \varepsilon^2}|_{\varepsilon=0} = -\frac{1}{|\Omega|}\int_\Omega k(\boldsymbol{x})\left.\frac{\partial N(\boldsymbol{x}\mid \boldsymbol{y}(s))}{\partial s}\right|_{s=0} g(0)\,d\boldsymbol{x}. \tag{6.79}$$

Thus the leading order term is

$$\Pr\{\tau < T\} = -\frac{\pi}{2|\Omega|}\left(\int_\Omega k(\boldsymbol{x})\left.\frac{\partial N(\boldsymbol{x}\mid \boldsymbol{y}(s))}{\partial s}\right|_{s=0} d\boldsymbol{x}\right) \times \left(\int_\Omega N(\boldsymbol{x}\mid \boldsymbol{y}(0))\,d\boldsymbol{x}\right)\frac{\varepsilon}{\log\varepsilon} + o\left(\frac{\varepsilon}{\log\varepsilon}\right).$$

7. CONCLUSION AND BIOLOGICAL IMPLICATIONS

The mathematical problem considered here is that of the exit of a Brownian motion from a bounded planar domain Ω, whose boundary is reflecting, except for a small absorbing arc $\partial\Omega_a$. Setting $\varepsilon = |\partial\Omega_a|/|\partial\Omega|$, we found that the confinement time of the Brownian particle in the domain is

$$O\left(\log\frac{1}{\varepsilon}\right)$$

for $\varepsilon \ll 1$. If there is an anchor in Ω, that can terminate the trajectory of the Brownian motion with a given killing rate, we found that the probability of reaching $\partial\Omega_a$ is

$$O\left(\frac{\varepsilon}{\log\varepsilon}\right)$$

for $\varepsilon \ll 1$.

The biological consequence of these results is to derive a coarse grained diffusion constant and to estimate the mean time for a receptor, such as AMPA, to be fixed in the PSD, after it's lateral insertion in the post-synaptic membrane. Under the assumption that the motion of the receptor in the complex environment of the synapse surface is Brownian, our computation shows that the mean time to anchoring is of the order of several minutes, not seconds. This estimate is relevant in the context of receptor trafficking, induced by LTP: the number of activated AMPA receptors increases during LTP (see the recent review ref. 8). The increase in the number of activated receptors can occur in about a minute. We may

surmise that if the bigger current response after LTP is due to the insertion of new receptors, not to the activation of already anchored receptors, then some AMPA receptors must already be present extra-synaptically on the synapse's membrane, so they won't have to diffuse all the way from the point of insertion to their final destination. Thus extrasynaptic receptors may serve the role of a reserve pool.

Under standard conditions, when no LTP is induced, the floating receptors should not be able to enter the PSD, to avoid significant fluctuations in the synaptic weight. In reality, however, there is evidence that receptors traffick in and out of synapses even in the absence of synaptic activity. The concentration of synaptic receptors is maintained constant by a hitherto unknown mechanism that has to be elucidated. A possible explanation may be that LTP induction induces disruptions, of size ε, say, in the boundaries of corrals of. This would allow receptors to enter. Such a prediction is based on the fact that AMPA receptors cannot both be inserted and reach the PSD in a minute. They should be already there and ready to move inside the PSD domain.

The lifetime of an AMPA receptor is of the order of 24 h, while the lifetime of a synapse is of the order of years, so a regulation mechanism, called the turnover of receptors, is necessary to maintain the number of receptors, and thus to maintain the synaptic weight.[7,8] Corrals can allow receptors to move inside the PSD domain, and thus allow the turnover by, intermittent disruptions of their barriers. It is also not clear how the membrane disruption occurs in the absence of any LTP induction. In particular, it is not known if new receptors, induced by LTP, follow the same pathway as the turnover receptors. It is well known that the forming of AMPA receptors is aided by different transmembrane subunits, GluR1 to GluR4, that could also play a key role in routing the receptors. If this is so, one would expect that specific proteins allow turnover receptors to penetrate the corral barrier, so they don't have to wait for any disruptions, induced under specific conditions only.

Another possible scenario in trafficking is that AMPA receptors are waiting extra-synaptically for the disruption of a corral barrier to facilitate their diffusion across sub-domains. It is unclear, however, what produces these disruptions. In vivo, the mean electrical activity of neurons can control trafficking for the following reason. It has been demonstrated recently[24] that at every synapse, the total number of AMPA receptors can be scaled with the activity: the total number of receptors *increases* at all synapses when the mean spontaneous activity decreases, but the number of receptors *decreases* at synapses when the mean spontaneous activity increases.

In molecular terms this means that when calcium enters a synapse, extrasynaptic AMPA receptors are slowed down, or altogether stopped.[9] It is then conceivable that spontaneous activity regulates AMPA receptor trafficking to the PSD by regulating calcium dynamics, and trafficking regulation is responsible for the scaling property reported in ref. 24. If so, the role of the spontaneous activity would be to allow the turnover of receptors and thus cause also the scaling of the synaptic weight by the mean electrical activity. The precise molecular pathways for such regulation have yet to be determined. In any case, when the mean activity decreases, less calcium enters the synapse, and if calcium can for example depolymerize actin molecules and create corral disruption, then by decreasing the mean activity, less polymerization occurs and less corral zones are open, on the average. This would educe the probability that receptors move to the PSD. Under this scenario, spontaneous activity is necessary for receptors to diffuse to the PSD. New models are necessary to describe the regulation between trafficking and spontaneous activity. Finally, further experiments should reveal if after LTP, AMPA receptors indeed move away from their extra-synaptic positions to the PSD. They should also clarify the role of extra-synaptic receptors in synaptic plasticity.

Acronyms identification

- GABA ([γ] -aminobutyric acid),
- GABAr=GABA receptor,
- AMPA([α] -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid),
- AMPAr=AMPA receptor,
- NMDA (N-methyl-D-aspartate),
- NMDAr=NMDA receptors,
- GRIP, glutamate-receptor-interacting protein (scaffolding proteins),
- PICK, protein that interacts with C kinase (scaffolding proteins),
- mGluRs metabotropic glutamate receptors (mGluRs),
- PSD Postsynaptic densities.

APPENDIX I. FROM A MIXED BOUNDARY VALUE PROBLEM TO THE NEUMANN PROBLEM

The asymptotic analysis of the confinement time depends on the representation of the solution of a mixed boundary value problem in terms of

the Neumann function. The representation is defined as follow. Consider the unique solution $u_{f,g}$ of the mixed Neumann–Dirichlet boundary value problem

$$\begin{aligned} \Delta u(\boldsymbol{x}) &= 0 \quad \text{for } \boldsymbol{x} \in \Omega, \\ \frac{\partial u(\boldsymbol{x})}{\partial n} &= f(\boldsymbol{x}) \quad \text{for } \boldsymbol{x} \in \partial\Omega_r, \\ u(\boldsymbol{x}) &= g(\boldsymbol{x}) \quad \text{for } \boldsymbol{x} \in \partial\Omega_a, \end{aligned} \tag{8.80}$$

where f, g are two given regular functions, and consider a function $v_{\tilde{g},g}$, the solution of

$$\begin{aligned} \Delta u(\boldsymbol{x}) &= 0 \quad \text{for } \boldsymbol{x} \in \Omega, \\ \frac{\partial u(\boldsymbol{x})}{\partial n} &= \tilde{g} \quad \text{for } \boldsymbol{x} \in \partial\Omega_r, \\ \frac{\partial u(\boldsymbol{x})}{\partial n} &= g \quad \text{for } \boldsymbol{x} \in \partial\Omega_a. \end{aligned} \tag{8.81}$$

Given $u_{f,g}$, there exists a unique function $\tilde{g}$, which is a function of (f, g), and a constant $C(\tilde{g}, g)$, such that

$$u_{f,g} = v_{\tilde{g},g} + C(\tilde{g}, g). \tag{8.82}$$

Moreover $\tilde{g}$ has to satisfy the compatibility condition

$$\int_{\partial\Omega_r} g(\boldsymbol{x})\, dS_{\boldsymbol{x}} + \int_{\partial\Omega_a} \tilde{g}(\boldsymbol{x})\, dS_{\boldsymbol{x}} = 0. \tag{8.83}$$

This representation is used in Section 1 of this paper, where the Neumann function is known explicitly for some simple geometric cases.

The Neumann function for the problem (8.80) gives the representation

$$f(\boldsymbol{y}) = \int_{\partial\Omega_a} N(\boldsymbol{x} \mid \boldsymbol{y})\tilde{g}(\boldsymbol{x})\, dS_{\boldsymbol{x}} + \int_{\partial\Omega_r} N(\boldsymbol{x} \mid \boldsymbol{y}) g(\boldsymbol{x})\, dS_{\boldsymbol{x}} \quad \text{for } \boldsymbol{y} \in \partial\Omega_a. \tag{8.84}$$

Eq. (8.84) is and integral equation for $\tilde{g}(\boldsymbol{x})$, given $f(\boldsymbol{x})$ and $g(\boldsymbol{x})$.

APPENDIX II. EXPLICIT COMPUTATION OF THE CONFINEMENT TIME IN A DISK

In this Appendix, we provide explicit computations to determine the leading term C_ε and the zero order term of the confinement time given by Eq. (4.35). To determine the function $g(\theta)$, as discussed in Section 1, we expand it in Taylor's series in the interval $|\theta| < \varepsilon$ and expand the integral in (4.34) in powers of θ. The boundary condition (4.31) implies that the power series has to vanish identically. Truncating the series expansion at n terms leads to a system of n linear equations for $g(0)$, for the derivatives $g^{(i)}(0)$, $(i = 1, 2, \ldots, n-1)$, and for the unknown constant C_ε. An additional equation is obtained by integrating Eq. (4.29) over the disk,

$$0 = \int_{-\pi}^{\pi} \frac{\partial v_\varepsilon(R,\theta)}{\partial r}\, d\theta = \pi - \varepsilon + \int_{|\theta|<\varepsilon} g(\theta)\, d\theta. \tag{8.85}$$

The absorbing boundary condition $v_\varepsilon(R,\theta) = 0$ implies that

$$\begin{aligned} &\int_{-\varepsilon}^{\varepsilon} \log\{2[1-\cos(\theta-\phi)]\} \\ &\quad \times \left[g(0) + \frac{g''(0)}{2}\phi^2 + \frac{g^{(iv)}(0)}{24}\phi^4 + \cdots + O\left(\phi^{10}\right) - \frac{1}{2} \right] d\phi \\ &\quad - \frac{2\pi C_\varepsilon}{R^2} = 0, \end{aligned} \tag{8.86}$$

where g is and even function. The integrals are estimated up to the order 10 as follows,

$$\begin{aligned} &\int_{-\varepsilon}^{\varepsilon} \log\{2[1-\cos(\theta-\phi)]\}\, d\phi \\ &\quad = -4\varepsilon + 4\varepsilon \ln|\varepsilon| + \left(\frac{2}{\varepsilon}\right)\theta^2 + \frac{1}{3\varepsilon^3}\theta^4 + \frac{2}{15\varepsilon^5}\theta^6 + \frac{1}{14\varepsilon^7}\theta^8 + \frac{2}{45\varepsilon^9}\theta^{10} + o(\theta^{10}), \\ &\int_{-\varepsilon}^{\varepsilon} \phi^2 \log|\theta-\phi|^2\, d\phi = \left(\frac{4}{3}\varepsilon^3 \ln\varepsilon - \frac{4}{9}\varepsilon^3\right) + (-2\varepsilon)\theta^2 + \frac{1}{\varepsilon}\theta^4 + \frac{2}{9\varepsilon^3}\theta^6 \\ &\qquad\qquad + \frac{1}{10\varepsilon^5}\theta^8 + \frac{2}{35\varepsilon^7}\theta^{10} + o(\theta^{10}), \\ &\int_{-\varepsilon}^{\varepsilon} \phi^4 \log|\theta-\phi|^2\, d\phi = \left(-\frac{4}{25}\varepsilon^5 + \frac{4}{5}\varepsilon^5 \ln\varepsilon\right) + \left(-\frac{2}{3}\varepsilon^3\right)\theta^2 + (-\varepsilon)\theta^4 \\ &\qquad\qquad + \frac{2}{3\varepsilon}\theta^6 + \frac{1}{6\varepsilon^3}\theta^8 + \frac{2}{25\varepsilon^5}\theta^{10} + o(\theta^{10}), \end{aligned}$$

$$\int_{-\varepsilon}^{\varepsilon} \phi^6 \log|\theta-\phi|^2\, d\phi = \left(\frac{4\varepsilon^7}{7}\ln\varepsilon - \frac{4}{49}\varepsilon^7\right) + \left(-\frac{2}{5}\varepsilon^5\right)\theta^2 + \left(-\frac{1}{3}\varepsilon^3\right)\theta^4 + \left(-\frac{2}{3}\varepsilon\right)\theta^6 + \frac{1}{2\varepsilon}\theta^8 + \frac{2}{15\varepsilon^3}\theta^{10} + o(\theta^{10}),$$

$$\int_{-\varepsilon}^{\varepsilon} \phi^8 \log|\theta-\phi|^2\, d\phi = \left(\frac{4\varepsilon^9}{9}\ln\varepsilon - \frac{4}{81}\varepsilon^9\right) + \left(-\frac{2}{7}\varepsilon^7\right)\theta^2 + \left(-\frac{1}{5}\varepsilon^5\right)\theta^4 + \left(-\frac{2}{9}\varepsilon^3\right)\theta^6 + \left(-\frac{1}{2}\varepsilon\right)\theta^8 + \frac{2}{5\varepsilon}\theta^{10} + o(\theta^{10}),$$

$$\int_{-\varepsilon}^{\varepsilon} \phi^{10} \log|\theta-\phi|^2\, d\phi = \left(\frac{4\varepsilon^{11}}{11}\ln\varepsilon - \frac{4}{121}\varepsilon^{11}\right) + \left(-\frac{2}{9}\varepsilon^9\right)\theta^2 + \left(-\frac{1}{7}\varepsilon^7\right)\theta^4 + \left(-\frac{2}{15}\varepsilon^5\right)\theta^6 + \left(-\frac{1}{6}\varepsilon^3\right)\theta^8 + \left(-\frac{2}{5}\varepsilon\right)\theta^{10} + o(\theta^{10}).$$

We denote the unknowns of the system by

$$a = g(0) - \frac{1}{2}, \quad b = \frac{g''(0)}{2}, \quad c = \frac{g^{(iv)}(0)}{24},$$
$$d = \frac{g^{(6)}(0)}{6!}, \quad e = \frac{g^{(8)}(0)}{8!}, \quad f = \frac{g^{(10)}(0)}{10!}.$$

Substituting the Taylor expansions into the expression (8.86), we obtain that

$$\begin{aligned}
&(-4\varepsilon + 4\varepsilon\ln\varepsilon)\,a + \left(\frac{4}{3}\varepsilon^3\ln\varepsilon - \frac{4}{9}\varepsilon^3\right)b + \left(-\frac{4}{25}\varepsilon^5 + \frac{4}{5}\varepsilon^5\ln\varepsilon\right)c \\
&\quad + \left(\frac{4\varepsilon^7}{7}\ln\varepsilon - \frac{4}{49}\varepsilon^7\right)d + \left(\frac{4\varepsilon^9}{9}\ln\varepsilon - \frac{4}{81}\varepsilon^9\right)e \\
&\quad + \left(\frac{4\varepsilon^{11}}{11}\ln\varepsilon - \frac{4}{121}\varepsilon^{11}\right)f = \frac{2\pi C_\varepsilon}{R^2}, \\
&\left(\frac{2}{\varepsilon}\right)a + (-2\varepsilon)\,b + \left(-\frac{2}{3}\varepsilon^3\right)c + \left(-\frac{2}{5}\varepsilon^5\right)d + \left(-\frac{2}{7}\varepsilon^7\right)e + \left(-\frac{2}{9}\varepsilon^9\right)f = 0, \\
&\frac{1}{3\varepsilon^3}a + \frac{1}{\varepsilon}b + (-\varepsilon)\,c + \left(-\frac{1}{3}\varepsilon^3\right)d + \left(-\frac{1}{5}\varepsilon^5\right)e + \left(-\frac{1}{7}\varepsilon^7\right)f = 0, \\
&\frac{2}{15\varepsilon^5}a + \frac{2}{9\varepsilon^3}b + \frac{2}{3\varepsilon}c + \left(-\frac{2}{3}\varepsilon\right)d + \left(-\frac{2}{9}\varepsilon^3\right)e + \left(-\frac{2}{15}\varepsilon^5\right)f = 0,
\end{aligned}$$

$$\frac{1}{14\varepsilon^7}a+\frac{1}{10\varepsilon^5}b+\frac{1}{6\varepsilon^3}c+\frac{1}{2\varepsilon}d+\left(-\frac{1}{2}\varepsilon\right)e+\left(-\frac{1}{6}\varepsilon^3\right)f=0,$$
$$\frac{2}{45\varepsilon^9}a+\frac{2}{35\varepsilon^7}b+\frac{2}{25\varepsilon^5}c+\frac{2}{15\varepsilon^3}d+\frac{2}{5\varepsilon}e+\left(-\frac{2}{5}\varepsilon\right)f=0.$$

The solutions are

$$g(0)=a+\frac{1}{2}=\frac{1}{2}+\frac{\pi C_\varepsilon}{\varepsilon R^2(-2.2112+3.0022\ln\varepsilon)},$$
$$b=\frac{\pi C_\varepsilon}{\varepsilon^3 R^2(-3.9802+5.4039\ln\varepsilon)},$$
$$c=\frac{\pi C_\varepsilon}{\varepsilon^5 R^2(-4.6436+6.3046\ln\varepsilon)},$$
$$d=\frac{\pi C_\varepsilon}{\varepsilon^7 R^2(-4.6436+6.3046\ln\varepsilon)},$$
$$e=\frac{\pi C_\varepsilon}{\varepsilon^9 R^2(-3.9802+5.4039\ln\varepsilon)},$$
$$f=\frac{\pi C_\varepsilon}{\varepsilon^{11} R^2(-2.2112+3.0022\ln\varepsilon)}.$$

Integrating Eq. (8.85), we obtain

$$0=\pi-\varepsilon+2\varepsilon g(0)+\frac{2\varepsilon^3}{3!}g''(0)+\cdots+\frac{2\varepsilon^{11}}{11!}g^{(10)}(0).$$

By replacing in this expression the value of $g^{(k)}(0)$, we obtain that

$$C_\varepsilon=0.73654+(1+O(\varepsilon))\ln\frac{1}{\varepsilon}.$$

Hence Eq. (4.36).

In the expansion

$$\bar{\tau}_\varepsilon=C_1(\Omega)\ln\frac{1}{\varepsilon}+C_2(\Omega)+O\left(\varepsilon\ln\frac{1}{\varepsilon}\right),$$

Eq. (4.25) gives an explicit expression for $C_2(\Omega)$ in terms of the area of Ω. A similar evaluation of $C_2(\Omega)$ in terms of geometric properties of Ω is still an open problem.

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