# PhD thesis

## Single-molecule imaging reveals rapid estradiol action on the surface movement of AMPA receptors in live neurons


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# 1. Introduction

## 1.1. 17β-estradiol

Estrogens are gonadal steroid hormons playing major role in the reproductive system but they are also crucial for osteogeneis, cardiovascular health, lipid metabolism and nervous system functions. (Ábrahám et al., 2009)

E2 is mainly produced by the ovaries in premenopausal woman. The synthesized E2 diffuses into the circulatory system where it binds to sex hormone binding globulins and transported to target tissues. E2 production is under the strict control of hypothalamic-pituitary-gonadal axis (HPG axis). E2 is also produced by extragonadal tissues. For instance, the brain can synthetize E2 locally as aromatase enzyme is also expressed by neurons in different parts of the brain such as hippocampus, medial preoptic area, medialis amygdala and cortex. Aromatase enzyme activity and neurosteroid E2 have been linked to several physiological functions such as neurogenesis, synaptic plasticity, neuroprotection and cognitive behaviour. It has also been shown that aromatase activity is disturbed in the pathophysiology of Alzheimer's disease or autism spectrum disorder. (Ubuka and Tsutsui, 2014)

## 1.2. Estradiol receptors

The first estrogen receptor (ER), the estrogen receptor α (ERα) was described in 1958 and was classified as a ligand-activated nuclear receptor. ERα consist of an N-terminal domain, a DNA-binding domain, a hinge region, a ligand-binding domain and a C-terminal domain. There are three more isoforms that have been described since then, two of them lack the N-terminal domain, which hinders the autoactivation of the receptor.

The second ER was cloned and named to estrogen receptor β (ERβ) in 1996. This receptor is also the member of the ligand-activated nuclear receptor superfamily and contains the same domains as ERα. Five shorter isoforms have also been identified. These isoforms are unable to bind ligands and has no transcriptional activity but are able to form dimers with ERα to reduce its effectivity. (Heldring et al., 2007)

In 1997, a G-protein-coupled receptor was identified in cell lines responsive to E2. (Carmeci et al., 1997; Thomas et al., 2005) GPER1 is a seven transmembrane receptor and localizes in the cell membrane and the endoplasmatic reticulum. E2 has a lower affinity to

GPER1 than to ERα or ERβ, but the ligand binding and release occur more rapidly. (Filardo and Thomas, 2012)

## 1.3. Classical estradiol pathway

According to the classic paradigm, the „free hormone hypothesis" E2 as a lipohpilic molecule enters cells by diffusing through the cell membrane and binds to the cytoplasmic classical genomic receptors. However, several paper suggested that the cellular uptake of E2 is mediated and controlled by specific carrier proteins. (Hammond and Bocchinfuso, 1995; Hammes et al., 2005) During the classical or genomic effect of E2, ERα and ERβ act as ligand activated transcriptional factors. (Marino et al., 2006) Upon E2 binding, ERs dimerize and translocate into the nucleus from the cytoplasm. (Le Dily and Beato, 2018) In the nucleus the ERα and ERβ dimers bind to the promoter region of their target sequences, the so called Estrogen Responsive Elements (ERE). ERE is found throughout the genom as part of a complex regulatory system for hundreds of proteins involved in reproduction, cardiovascular system, neuronal development or cognitive system. (Bourdeau et al., 2004)

## 1.4. Non-classical estradiol pathway

E2 affects several cellular processes in seconds or minutes that cannot be explained by the action of the slow classical pathway. This suggest the presence of a non-classical pathway for E2 effects where E2 modulates gene expression without directly interacting with DNA. (Vrtačnik et al., 2014)

The first evidence of non-classical E2 effect was described by Szego and Davis in 1967. The level of cAMP was increased double-fold after E2 treatment in rat uterus in less than a minute. (Szego and Davis, 1967) Since then several studies reported non-classical effects of different steroid hormones. (Fujimoto and Kitamura, 2004; Glidewell-Kenney et al., 2007; McDevitt et al., 2008; Rudolph et al., 2016) The non-classical effect of E2 is mostly initiated by GPER1 and membrane associated ERα and ERβ. E2 can change the function of ion channels (Kelly and Rønnekleiv, 2009), modulate membrane fluidity (Kumar et al., 2011) or induce activation of signaling pathways and second messengers such as phospholipase C (Marino et al., 1998), adenyl cyclase, protein kinase A (PKA), (Gu and Moss, 1996) protein kinase C (PKC) (Marino et al., 1998), the phophatidyl inosytol 3 kinase A cascade, the ERK pathway (Dos Santos et al., 2002), the intracellular $Ca^{2+}$ and cAMP levels. (Björnström and

Sjöberg, 2005) These mechanisms finally also lead to gene expression changes: gene silencing or enhancing.

## 1.5. Glutamaterg neurotransmission

Glutamate is a neurotransmitter that plays a pivotal role in most excitatory synapses in the CNS. (Izquierdo, 1994) Glutamaterg neurotransmission is essential for almost every sensory and motor function, neuronal development, memory formation and cognitive functions. (Niciu et al., 2012) In the CNS the extra- and intracellular levels of glutamate are tightly regulated by a vast number of molecular mechanisms. These mechanisms control the expression and release of glutamate at the synaptic site as well as their clearance and recycling.

Glutamate is not able to cross the blood-brain barrier, thus it is generated from glucose in the brain. The synthesized glutamate is transported via vesicular glutamate transporters into the presynaptic site of synapses and released into the synaptic cleft. When glutamate release is triggered by an action potential, the glutamate loaded membrane vesicles fuse with the presynaptic membrane of the synapse. (Pang and Südhof, 2010) Glutamate molecules then diffuse through the synaptic cleft and bind the receptors located in the postsynaptic membrane, where action potential is provoked if treshold value is reached.

## 1.6. Glutamate receptors

### 1.6.1. Metabotropic glutamate receptors

Metabotropic glutamate receptors (mGluRs) are the members of the seven transmembrane domain-spanning receptor family. Most metabotropic glutamate receptors are located outside the active site of synapses, but also presented in glia cells. Metabotropic glutamate receptors are slow acting receptors and activate membrane bound G-proteins which induces intracellular $Ca^{2+}$ release or protein kinaseA or C phosporylation. (Niciu et al., 2012) These events precisely control the sensitivity of cells to neurotransmitters, fine tune excitatory and inhibitory neurotransmission and enhance synapse development. (Lesage and Steckler, 2010)

### 1.6.2. Ionotropic glutamate receptors

Ionotropic glutamate receptors are ligand-gated ion channels that are activated by the neurotransmitter glutamate. They are the fast acting component of the synapse and responsible for most of the excitatory synaptic transmission. Ionotropic glutamate receptors are subdivided into three categories based on their selective agonists: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) and kainate.

AMPA receptors are heterotetrameric ion channels located in the post and perisynaptic areas and responsible for the rapid postsynaptic response to the presynaptic glutamate release. (Diering and Huganir, 2018) Four AMPA subunits (GluA1-4) assemble a functional AMPA ion channel. The most abundant AMPA receptor subunit in neurons is GluA2 paired with GluA1 or GluA3. (Wenthold et al., 1996) Subunit composition of AMPA receptors, but mostly the presence or absence of GluA2 subunit determines the main properties of the ion channel. GluA2 containing receptors are $Ca^{2+}$ impermeable and show a slow decay kinetics. Lack of GluA2 subunit turns AMPA receptors into $Ca^{2+}$ permeable channels with high conductance and rapid decay kinetics.

Binding of glutamate to AMPA receptor quickly opens the ion channel, $Na^{+}$, $K^{+}$ and $Ca^{2+}$ flow in for a brief period of time (Collingridge et al., 2004; Traynelis et al., 2010) that results in membrane depolarization and subsequent excitatory postsynaptic potential if threshold is reached. Deactivation and desenzitization of the AMPA receptors terminate the ion influx.

The number, type and localization of glutamate receptors in and around the PSD are dynamic. Rapid redistribution of glutamate receptors contributes to synaptogenesis, synapse maturation, normal synaptic function and one of the key steps in the process of synaptic plasticity. (Diering and Huganir, 2018)

## 1.7. Synaptic plasticity

One of the most complex properties of the adult brain is the synaptic plasticity, its capacity to adapt to external factors and effects. It was first described by Eric Kandel, who demonstrated in sea slug that the learning is accompanied with the strengthening of preexisting synapses. (Castellucci et al., 1970) Synaptic plasticity cannot be described as a single event, but a series of actions at molecular and cellular levels. The most dominant change during synaptic plasticity is the stimulus-dependent strengthening or weakening of

the already existing synapses. This change can be transient (milliseconds) only or persistent (days or longer) and subsequently influence future behaviour, learning and other cognitive functions. Synaptic plasticity is also crucial for development of CNS and its disturbance could lead to serious neurological disorders.

### 1.7.1. Long term synaptic plasticity

Long-term synaptic plasticity refers to the long-lasting answer to external or internal stimuli at the level of neuronal circuits and is believed to be the very basis of learning and memory formation. (Bliss and Gardner-Medwin, 1973; Fusi et al., 2005) These mechanism involve the two most abundant glutamate receptors: AMPA and NMDA receptors.

LTD is initiated when the synapse is triggered by minutes-long low-frequency stimulation. Formation of LTD involves NMDA-dependent postsynaptical $Ca^{2+}$ level increase (Mulkey and Malenka, 1992) following the activation of a series of phosphatases such as calcium-dependent protein phosphatase or calcium/calmodulin dependent phosphatase. (Lisman, 1989) The activation of these signal transduction mechanisms cause the removal of AMPA receptors from the active site of the synapse. (Bredt and Nicoll, 2003; Malenka and Bear, 2004) AMPA receptors move to perisynaptical areas where they are endocytosed in a process mediated by clathrin and dynamin. (Ashby et al., 2004; Groc et al., 2004)

LTP is one of the most studied neuronal phenomenon, which can be developed in every synapse counterbalancing LTD. Similar to LTD the receptors responsible for LTP are the NMDA and AMPA receptors. LTP can be induced by high-frequency tetanic stimulation, which depolarizes the postsynaptic membrane and activates NMDA receptors. (Malenka, 1991) The major mechanism of LTP is the enrichment of AMPA receptors in the PSD. This is achieved by incorporation of AMPA receptors from endosomes and the persisynaptic area into the PSD in a process mediated by the submembrane actin structure. It has been demonstrated that actin network and its dynamic remodeling is essential for the rearrangement of AMPA receptors. (Hanley, 2014; Baglietto-Vargas et al., 2018) The increased number of AMPA receptors enhance the probability of depolarization of the postsynaptic membrane. The synaptic strength is maintained for hours to days or even longer by upregulation of the synthesis of synaptic proteins. (Citri and Malenka, 2008)

## 1.8. Surface movement of transmembrane proteins

Since the trafficking of NMDA and AMPA receptors is crucial for LTP, many studies focus on the membrane movement of these proteins. The development of single molecule techniques enabled researchers to study the movement of single membrane receptors in live cells. (Kusumi et al., 1993) Receptors tend to move freely in small compartments and frequently shift to another compartment. (Sako and Kusumi, 1994) One possible theory of the non-free movement of proteins is that their intracellular domains interact with the submembrane actin network directly or through anchoring proteins that restricts receptor movement. This hypothesis is termed as „fence model", in which the actin filaments act as fence creating compartments and the transition of receptors between these regions is called hop diffusion. (Kusumi et al., 1993) Actin fenced domains can range from 40 to 300 nm.

### 1.8.1. Glutamate receptor movement in synapse

The movement of glutamate receptors in the synapse under physiological conditions as well as during LTP was described in the last decades with the use of live-cell single molecule imaging and tracking. The turnover of AMPA receptors between the synaptic and perisynaptic areas is more dominant and faster during LTP than the lateral diffusion of NMDA receptors. Inside the active site of the synapse AMPA receptors are either immobile and possibly bound to PSD95 or exhibit Brownian diffusion (Lee et al., 2017), while extrasynaptic AMPA receptors are moving mostly with Brownian diffusion and rarely enter immobile state. (Borgdorff and Choquet, 2002) The high mobility of extrasynaptic AMPA receptors enables them to serve as a reservoir during LTP. Extrasynaptic AMPA receptors laterally diffuse into the PSD where they are trapped by anchoring proteins. (Triller and Choquet, 2005) The reservoirs are refilled with AMPA receptors stored in endosomes inside the postsynaptic button.

## 1.9. Effect of E2 on synaptic plasticity

In the last decades several studies described that E2 treatment potentiates glutamaterg neurotransmission in the hippocampus. (Teyler et al., 1980; Maggi et al., 1989; Gould et al., 1990; Wong and Moss, 1992) The physiological relevance of this effect was unknown until

the discovery of neurosteroids which are synthesized in the brain in both sexes. (Roselli et al., 1985)

The effect of E2 on synaptic potentiation is a complex mechanism, which is probably due to the neuron-type specific expression pattern of ERs. (Kramár et al., 2009; Kumar et al., 2015) E2 acts at the level of pre- and postsynaptic membrane as well. It was demonstrated that E2 treatment increases the probability of glutamate release in synapses, generating a higher excitatory postsynaptic current (EPSC). (Smejkalova and Woolley, 2010) Postsynaptically E2 was found to modulate dendritic spine formation, synapse size and the number of glutamate receptors in the PSD. This synaptic strengthening is acquired through the reinsertion of AMPA receptors into the active site. In summary, E2 can rewire the neuronal connectivity via potentiating the more active and weakening or abolishing the less used synapses. (Xie et al., 2007; Srivastava et al., 2008)

The continuous movement of glutamate receptors inside and outside of the synapse is a fundamental part of the effect of E2 on synaptic plasticity, however the precise mechanism in live cells has not been described. There are studies examining the effect of E2 on NMDA receptors or the effect of glucocorticoid hormones on the movement AMPA receptor (Groc et al., 2008; Potier et al., 2016), but the ability of E2 to modulate the membrane movement of AMPA receptors is unknown.

## 2. Aims of the study

The major scope of this study was to determine the non-classical effects of E2 on the membrane movement of AMPA and mGluR1 glutamate receptors in order to better understand the molecular mechanism of E2 improved synaptic plasticity.

Our aims were:

1. to determine the E2 effect on diffusion coefficient (D) of AMPA and mGluR1 receptors in differentiated PC12 cells
2. to explore which ERs are responsible for the E2 effect
3. to test the role of cortical actin network in the E2 effect
4. to measure the effect of E2 on the D and synaptic dwell time of AMPA receptors in cultured hippocampal neurons

# 3. Results

## 3.1. E2 rapidly decreases the surface movement of AMPAR in dPC12

The surface movement of glutamate receptors was detected in the plasma membrane of live dPC12. The diffusion coefficients of both receptors are significantly higher on the neurite than on soma, indicating that the surface movement of glutamate receptors is faster on neurites.

Administration of 100pM, 1 nM and 100 nM doses of E2 evoked a clear dose-dependent decrease in $D_{AMPAR}$ in neurites as measured in the first 20 minutes after treatment. In soma, 100 pM of E2 significantly decreased $D_{AMPAR}$, while 1 nM and 100 nM of E2 were ineffective. In contrast, E2 (100 nM, 1 nM or 100 pM) did not change $D_{mGluR1}$ either in soma or in neurites.

To examine the time dependence of the effect evoked by E2 on $D_{AMPAR}$ or $D_{mGluR1}$, we applied the most effective E2 doses on soma and neurites and measured D at different time points. The application of 100 pM of E2 resulted in a significant decrease in $D_{AMPAR}$ on soma within 5 min. This remained reduced at 10 min, 15 min, and 20 min on soma. In contrast, 100 nM of E2 only reduced $D_{AMPAR}$ on neurites at 10 min, 15 min, and 20 min. In contrast, 100 pM or 100 nM of E2 did not affect $D_{mGluR1}$ on neurites or soma, respectively, at any time point.

## 3.2. E2 effect is mediated by GPER1 and ERβ in dPC12

Our PCR results revealed that dPC12 expresses ERβ and GPER1, but not ERα. Although the addition of ERβ agonist DPN (10 pM) or specific GPER1 agonist G1 (100 nM) alone did not affect the surface movement of somatic GluR2-AMPAR molecules, co-administration of DPN and G1 decreased $D_{AMPAR}$ similar to 100 pM of E2. G1 (100 nM) mimicked the effect of 100 nM of E2 without and with 10 pM of DPN in neurites. However, 10 pM of DPN alone did not alter the $D_{AMPAR}$ in neurites. In addition, prior application of 1 μM of G15 blocked the effect of 100 pM of E2 on soma and 100 nM of E2 on neurites. G15 application alone did not alter the surface movement of GluR2-AMPAR in either neurites or soma.

Our results show that GPER1 mediates the effect of E2 on GluR2-AMPAR on both soma and neurites. To further analyze the relationship between GluR2-AMPAR and GPER1, we used STORM super-resolution imaging to examine the expression GPER1 and GluR2-AMPAR. In order to examine the number of GPER1 in relation to GluR2-AMPAR we normalized the number of GPER1 to GluR2-AMPAR using GPER1/GluR2-AMPAR ratio. Our analysis demonstrated that the GPER1/GluR2-AMPAR ratio was significantly higher in soma than in neurites of dPC12.

E2 can induce rapid internalization and consequent desensitization of GPER1 (Filardo and Thomas, 2012). The internalization of GPER1 may explain the different effects of E2 on the soma and neurites. To visualize whether GPER1 is internalized after E2 administration in soma, stimulated emission depletion (STED) microscopy was used. Super-resolution STED imaging revealed that the intensity of immunostaining of GPER1 was approximately 2 times higher in the membrane region than in the cytoplasm of vehicle-treated dPC12. After 10 min of 100 nM of E2 treatment, the intensity profile of GPER1 showed a significant decrease in the membrane region. In contrast, the majority of GPER1 immunoreactivity was located in the cytoplasm after treatment with 100 nM of E2, suggesting rapid internalization of GPER1 in response to high E2 exposure. There was no internalization of GPER1 observed in neurites after 100 nM of E2 treatment.

## 3.3. Function of cortical actin network in the effect of E2 on the membrane diffusion of AMPAR in dPC12

Cortical actin is a thin actin network that lies directly underneath the plasma membrane. The cortical actin network is essential in the organization of neuronal compartments and plays a crucial role in membrane receptor movement (Schevzov et al., 2012), thus we speculated that the cortical actin network may play a role in the effect of E2 on the receptor dynamics. Previous studies show that E2 induces cytoskeleton assembly mediated by GPER1 receptors via different intracellular signaling pathways, including the ROCK-cofilin (Gowrishankar et al., 2012; Wang et al., 2019) and JNK-cofilin (Kim et al., 2019) pathways. To determine the possible role of cortical actin in the effects of E2 on glutamate receptors, we treated cells with the actin polymerization inhibitor, latrunculinA (latA; l μM). To examine the role of the ROCK-cofilin and JNK-cofilin pathways in E2 action, we applied the ROCK inhibitor, GSK429286 (l μM) (Wang et al., 2019), and JNK inhibitor, SP600125 (l μM) (Kim et al., 2019), respectively.

First, we validated whether latA, or ROCK and JNK inhibitors altered the morphology of cortical actin. Phalloidin immunostaining demonstrated cortical F-actin in dPC12. The density of the cortical actin network in dPC12 was decreased by latA, GSK429286, or SP600125 administration. In single-molecule tracking experiments, 10 min of latA, or pretreatment with GSK429286 or SP600125 for 60 min significantly increased $D_{AMPAR}$ on soma without affecting $D_{AMPAR}$ on neurites in dPC12. Pretreatment with latA, GSK429286, or SP600125 decreased the effect of 100 pM of E2 on soma and 100 nM of E2 on neurites on the surface movement of GluR2-AMPAR molecules.

### 3.4. E2 rapidly decreases the surface movement and increases the synaptic dwell time of AMPAR in hippocampal neurons

To validate the effect of E2 on the surface movement of GluR2-AMPAR in another *in vitro* neuron system and examine the effect of E2 on synaptic GluR2-AMPAR, we performed single-molecule tracking experiments on primary hippocampal neuron culture.

Our single-molecule imaging experiment revealed the surface movement of ATTO 488-labeled GluR2-AMPAR on neurites in extrasynaptic and synaptic regions. D values of GluR2-AMPAR molecules were significantly lower in synapse compared to extrasynaptic regions.

Both 100 pM and 100 nM of E2 decreased extrasynaptic and synaptic $D_{AMPAR}$ in neurites. Similar to E2, chemical strengthening of synapses (chemical long term potentiation (cLTP)) elicited a decrease in synaptic $D_{AMPAR}$. Furthermore, 100 nM, but not 100 pM of E2, increased the synaptic dwell time of GluR2-AMPAR to a similar extent as cLTP. Treatment with 100 nM of E2 did not change the cLTP-induced increase in the synaptic dwell time of GluR2-AMPAR. E2 (100 nM, 100 pM) did not affect synaptic AMPAR content, and it did not alter cLTP-induced increase in synaptic AMPAR content.

## 4. Discussion

We found that E2 rapidly decreased the $D_{AMPAR}$ in live dPC12 via rapid membrane-initiated GPER1 signaling in neurites but both GPER1 and ERβ was required for the effect of E2 in soma. Nevertheless, different dose was effective on soma compared to neurites. On soma 100 pM E2, while on neurites 1 nM or 100 nM E2 decreased the $D_{AMPAR}$. This difference may be the consequence of GPER1 internalization in soma induced by 100 nM

E2. We showed that $D_{AMPAR}$ was affected by the cortical actin network in dPC12 cells. Furthermore, the effects of E2 on $D_{AMPAR}$ in soma were mediated by actin via the ROCK-cofilin and JNK-cofilin pathways. Importantly, we confirmed our results on live hippocampal neurons: we showed that E2 also decreases $D_{AMPAR.}$ Similarly to cLTP induction, E2 decreases $D_{AMPAR}$ and increases the synaptic dwell time of GluR2-AMPAR.

## 4.1. Compartment specific effect of E2

Here, we show that E2 decreases $D_{AMPAR}$ in a concentration-dependent manner, with distinct effects on soma and neurites in dPC12. However, E2 alters only $D_{AMPAR}$ but not $D_{mGluR1}$, suggesting that the rapid modulation of glutamatergic receptor surface diffusion by E2 is type-dependent. It is worth noting that the rapidity of E2 action on $D_{AMPAR}$ ($\leq$ 5 min) indicates a non-classical mechanism.

In our experiments, ER agonists and antagonists demonstrated a compartment-specific effect on dPC12, as they had different effects on soma and neurites. Both ERβ and GPER1 are required for E2 effect on soma, but on neurite E2 effect occurs through GPER1 only. Studies have revealed that cortical actin network differs in soma and neurite and its dynamics is regulated by ERβ. As discussed later, we found in dPC12 that the actin structure influences the membrane movement of receptors differently on soma and neurite. We assume that on soma ERβ and GPER1 regulates receptor dynamics through cortical actin rearrangement, while on neurite GPER1 alone affects receptor movements via an unknown mechanism unrelated to cortical actin network.

The concentration dependence of E2 action differs between soma and neurites in dPC12. While 100 pM of E2 reduced $D_{AMPAR}$ in soma, higher concentrations (1 nM or 100 nM) were required to decrease the $D_{AMPAR}$ in neurites. One possible reason for the compartement-specific E2 action may be the difference in the distribution of GPER1 molecules on the membrane of soma and neurites. Indeed, our STORM experiments showed that the GPER1/GluR2-AMPAR ratio was higher in soma than in neurites, indicating that neurites express less GPER1 than soma.

Interestingly, high doses of E2 (1 nM, 100 nM) did not alter $D_{AMPAR}$ in soma. Previous studies have indicated that GPER1 undergoes desensitization after the administration of the ligand at high concentrations. Thus, it is likely that a high concentration of E2 induces GPER1 desensitization in the soma. Previous experiments demonstrated that E2 administration could induce translocation of GPER1 from the cell membrane to the

cytoplasm, resulting in the desensitization of the receptor. Our STED experiments corroborated these findings because 10 min after administration of 100 nM of E2, GPER1 immunolabeling relocated from the membrane region to the cytoplasm, indicating a rapid internalization of GPER1 on soma. Rapid internalization indicates the desensitization of GPER1, which may explain why high doses of E2 were ineffective on the soma. We hypothesize that an even higher concentration of E2 would be sufficient to induce internalization due to the low level of GPER1.

## 4.2. Role of cortical actin network in the effect of E2

It has been shown earlier that the actin cytoskeleton can interact with the intracellular domains of membrane receptors, thus regulating their movement. Our present findings confirm these previous observations, as the disruption of cortical actin by latA increased $D_{AMPAR}$ in soma. Interestingly, latA has a compartment-specific effect because it is not effective in neurites. Super-resolution imaging studies revealed that soma and neurites have different cortical actin structures. Actin has a polygonal lattice structure in soma, and its associated proteins such as adducin and spectrin form 190-nm-spaced ring-like structures around the circumference of neurites. We hypothesize that the higher D values measured on neurites arise from the difference between the structural arrangement of actin in soma and neurites. This may also provide an effective basis for the compartment-specific effect of latA and surface dynamics of GluR2-AMPARs.

Recent evidence implicates that cortical actin is important in receptor crosstalk through modulation of protein dynamics. Cofilin is a highly abundant constitutively active actin-binding protein that alters the properties of F-actin and is regulated by the ROCK-cofilin and JNK-cofilin pathways. Phosphorylation inactivates cofilin and facilitates actin filament assembly. E2 increases the activity of cofilin and stabilizes the F-actin cytoskeleton via GPER1. Cofilin has been reported to mediate cortical actin dynamics that regulate AMPAR trafficking in synaptic plasticity. Therefore, we investigated the role of actin in the effect of E2 on $D_{AMPAR}$. Our results demonstrated that latA diminished the effect of E2, indicating that cortical actin plays a pivotal role in E2 action on $D_{AMPAR}$. Our results also demonstrated that the E2-induced decrease in $D_{AMPAR}$ is completely blocked by the inhibition of the ROCK-cofilin or JNK-cofilin pathways in soma and neurites. We suggest that E2 binding to

GPER1 activates both the ROCK-cofilin and JNK-cofilin pathways, which then change the cortical actin dynamics and decrease the surface movement of GluR2-AMPAR.

## 4.3. E2 effect on AMPAR in hippocampal neurons

To confirm the effect of E2 on $D_{AMPAR}$ in another *in vitro* neuron system and examine the effect of E2 on synaptic GluR2-AMPAR, we performed single-molecule tracking experiments on a primary hippocampal neuron culture. Our results showed that E2 administration (100 pM and 100 nM) rapidly decreased the synaptic and extrasynaptic $D_{AMPAR}$ in hippocampal neurons similar to dPC12.

LTP of excitatory synaptic transmission is a well-known form of synaptic plasticity and is considered a cellular model for learning and memory. Although several studies have demonstrated that E2 plays an essential role in LTP and alters memory formation, the precise molecular mechanism is not clear. AMPAR plays a pivotal role in synaptic alterations involved in synaptic transmission, synaptic plasticity, LTP, learning, and memory. Using single-molecule tracking experiments and AMPAR immobilization techniques studies have shown that the surface movement of AMPARs is a key factor in the modulation of synaptic potentiation and learning. At the molecular level, the recruitment and slow diffusion of glutamate receptors at the postsynaptic site have been shown after LTP. Indeed, our single-molecule tracking of hippocampal neurons demonstrated that cLTP decreased $D_{AMPAR}$ in synapses and increased the synaptic dwell time and content of GluR2-AMPARs. Similar to cLTP, 100 nM of E2 decreased $D_{AMPAR}$ and increased the dwell time of GluR2-AMPA in the synapse. We suggest that E2 can rapidly enhance the synaptic efficacy of glutamatergic synapses by decreasing $D_{AMPAR}$. Interestingly, E2 did not change the effect of cLTP on $D_{AMPAR}$, dwell time, and synaptic content of GluR2-AMPAR. However, E2 can likely increase the efficacy of cLTP by retaining the AMPARs in the synapses.

In conclusion, our study demonstrates that E2 rapidly and dose-dependently decreases the surface movement of GluR2-AMPARs via compartment-specific ER-mediated mechanisms in live neurons. Our results also suggest that cortical actin mediates liganded GPER1 action on the surface movement of GluR2-AMPARs via the ROCK-cofilin and JNK-cofilin pathways. This study provides the first evidence that E2 decreases the surface movement and increases the dwell time of GluR2-AMPARs in the synapses. These results provide a

strong foundation for understanding the molecular mechanism by which E2 affects neuronal plasticity and glutamatergic neurotransmission.

## 5. List of publications

This dissertation is based on the following articles:

Godó, S., Barabás, K., Lengyel, F., Ernszt, D., Kovács, T., Kecskés, M., et al. (2021). Single-Molecule Imaging Reveals Rapid Estradiol Action on the Surface Movement of AMPA Receptors in Live Neurons. *Front. Cell Dev. Biol.* 9, 2698.

impact factor: 6.684

Barabás, K., Godó, S., Lengyel, F., Ernszt, D., Pál, J., and Ábrahám, I. M. (2018). Rapid non-classical effects of steroids on the membrane receptor dynamics and downstream signaling in neurons. Horm. Behav., 0–1

impact factor: 3.949

Other publication:

Payrits, M., Borbely, E., Godo, S., Ernszt, D., Kemeny, A., Kardos, J., et al. (2020). Genetic deletion of TRPA1 receptor attenuates amyloid beta- 1-42 (Aβ(1-42))-induced neurotoxicity in the mouse basal forebrain in vivo. Mech. Ageing Dev. 189, 111268.

impact factor: 4.304

Barabás, K., Kobolák, J., Godó, S., Kovács, T., Ernszt, D., Ábrahám, IM., et al (2021). Live-Cell Imaging of Single Neurotrophin Receptor Molecules on Human Neurons in Alzheimer's Disease. Int J Mol Sci. 2021 Dec 9;22(24):13260.

impact factor: 5.923

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