The effects of noradrenaline and corticosterone on dendritic signal processing in cortical pyramidal cells of the rat hippocampus

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Abstract

The ability of neurons to encode information and communicate with each other is vital for the normal functioning of our brain and body. The large pyramidal cells of the hippocampus have been of particular interest, due to the well-known role of the hippocampus in learning and memory, as well as epilepsy and stress. During a stressful event, noradrenaline and corticosterone are released. They act as neuromodulators, influencing signal processing and synaptic plasticity. The actions of these two neuromodulators have previously been studied separately, but not much is known about their interactions.

The overall goal of this study was to investigate how noradrenaline and corticosterone influence and modulate dendritic signal processing in CA1 pyramidal cells of the hippocampus. It has become clear that the dendrites of neurons actively participate in signal processing, instead of just being passive conductors. The main aim of the study was to examine if corticosterone modulates the effect of noradrenaline on signaling processing. Additionally, the influence of these modulators of different ion channels that regulate postsynaptic potentials and slow afterhyperpolarizations were investigated, as these have been shown to influence synaptic plasticity.

Using the whole-cell patch clamp technique, brain slices containing CA1 pyramidal cells from the hippocampus of rats were examined. Half the slices were incubated in 100 nM corticosterone 1 h prior to recording, the other half were used as controls. Recordings were made using specially constructed protocols. Corticosterone was not found to significantly modulate the effect of noradrenaline. The effects of noradrenaline on postsynaptic potentials, slow afterhyperpolarization, sag and input resistance all remained largely the same in the presence of corticosterone.

In conclusion, corticosterone did not significantly modulate the effect of noradrenaline on the electrophysiological parameters studied in CA1 pyramidal cells in the rat hippocampus.

Abbreviations

ACTH	adrenocorticotropic hormone
AHP	afterhyperpolarization
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP	action potential
APV	D-2-amino-5-phosphonopentanoic acid
AVP	vasopressin
CA	Cornu Ammonis
CaMKII	Ca^{2+} calmodulin kinase II
cAMP	cyclic adenosine monophosphate
CREB	cAMP response-element binding protein
CRH	corticotropin-releasing hormone
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
GR	glucocorticoid receptor
HCN-channel	hyperpolarization-activated cyclic nucleotide-gated channel
HPA axis	hypothalamic-pituitary-adrenocortical axis
IPSP	inhibitory postsynaptic potential
LTD	long-term depression
LTP	long-term potentiation
MR	mineralocorticoid receptor
NMDA	N-methyl-D-aspartate
PKA	protein kinase A
RMP	resting membrane potential
sADP	slow afterdepolarization
sAHP	slow afterhyperpolarization
Gabazine	SR 95531

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Yvette Dirven

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1 Introduction

1.1 Information encoding and processing in the brain

The ability of neurons to encode information and communicate with each other is vital for the normal functioning of our brain and body. Neurons communicate with each other through the generation of action potentials (APs). An AP is a rapid increase and subsequent decrease in the membrane potential of a neuron. An AP is triggered when chemical and electrical signals cause the membrane potential of a neuron to depolarize above a certain threshold. Several factors influence a neurons ability to generate an AP.

The process of generating an AP starts when a postsynaptic neuron receives neurotransmitters from a presynaptic neuron, through a process called synaptic transmission. Neurotransmitters, such as noradrenaline, dopamine and acetylcholine, bind to different receptors and ion channels on the postsynaptic neuron affecting their activity, resulting in the generation of a postsynaptic potential. The postsynaptic potential can either be an excitatory postsynaptic potential (EPSP) and depolarize the membrane, or an inhibitory postsynaptic potential (IPSP) that hyperpolarizes the membrane. It is the integration and summation of the postsynaptic potentials at the axon hillock that determines whether or not the threshold for AP generation is reached (Purves, 2012). In addition to neurotransmitters, there are neuromodulators such as corticosterone. Neuromodulators work like neurotransmitters, but they can diffuse through the extracellular fluid and affect the activity of a population of neurons rather than a single neuron (Carlson, 2014). Noradrenaline can act both as a neurotransmitter and neuromodulator.

A neurons ability to generate a single AP is not enough to explain how the brain encodes information and performs complex tasks and behaviors. Instead it is the firing frequency and firing pattern of several action potentials that encodes information. Additionally, it is not the activity of a single neuron, but rather the combined activity of a population of neurons that underlies a behavior (Purves, 2012).

When a population of neurons displays synchronous and rhythmic firing of APs, and the electric activity of the population is measured as a whole, it is referred to as an oscillation. Oscillations have been shown to increase the AP firing rate, as well as the speed at which

APs are transmitted (Rolls and Treves, 2011, Stanley, 2013, Gasparini et al., 2004). As a result, oscillations influence information encoding. Neural populations can consist of several different types of neurons with diverse functions that impact the way they process and transmit information.

1.1.1 Information processing in neurons and dendritic integration

Understanding how neurons process and integrate information is crucial for understanding brain function. Information processing in neurons begins when they receive input from other neurons. Neurons are connected in complex circuits and networks in order to effectively communicate with each other and transmit information. A single neuron can receive input from hundreds to thousands of other neurons. Usually, the input from a single synapse is not enough to change the membrane potential in such a way that it triggers an AP. The integration and processing of many synaptic inputs therefore plays a critical role, promoting or inhibiting a neurons ability to propagate information through AP generation (Gulledge et al., 2005).

Neurons receive input through their dendrites, which are extensions from the soma that can branch out into extensive tree-like structures. Although they were previously thought of as passive conductors that transmitt input to the soma, it has become clear that dendrites play a complex role in information processing (Stuart and Spruston, 2015, Magee, 1998).

Dendrites have both active and passive electrical properties that influence how they process information. The passive properties of dendrites include their membrane resistance, capacitance and geometry. Together these influence the size, shape and dynamic interactions of input in the dendrites (Gulledge et al., 2005). The active properties of dendrites are caused by voltage-gated channels. Several voltage-gated channels such as sodium, calcium, potassium and hyperpolarization cyclic nucleotide-gated channels (HCN channels) have been found in dendrites. The active conductance through these channels impacts signal processing and AP output by affecting the speed and size of EPSPs and IPSPs (Stuart and Spruston, 2015, Gulledge et al., 2005).

The difference between passive and active properties of dendrites, and their effect on dendritic integration, can be illustrated by looking at the leak potassium channel and voltage-gated potassium channel. The leak potassium channel plays an important role in maintaining

the resting membrane potential (RMP) of a neuron, which is a passive property. When the membrane potential of a neuron is at rest, the K^+ ions are at equilibrium. When there is a change in the RMP, K^+ leakage through the potassium leak channel will oppose the change in RMP and bring it back to rest. As a result, the potassium leak channel passively influences dendritic integration by bringing the membrane potential back to rest after it changes. The voltage-gated potassium channel on the other hand, opens when a neuron depolarizes above the threshold for AP generation. When these channels open, they allow K^+ to leave the neuron along its electrochemical gradient. The efflux of potassium brings the membrane potential towards rest, terminating the AP. The voltage-gated potassium channel actively influences dendritic integration by affecting the shape and propagation of APs (Purves, 2012, Carlson, 2014).

Dendritic integration has been extensively studied in the hippocampus due to its very organized structure. Different types of neurons are organized in distinct layers, making the hippocampus a good model to study signal processing in specific cell types.

1.2 The hippocampus

The hippocampus is one of the most studied structures in the brain, and it is mostly known for its role in learning, memory, plasticity, spatial navigation and stress (Anand and Dhikav, 2012, de Kloet et al., 2005, Howland and Wang, 2008, Joels et al., 2006, Hartley et al., 2014, Moser et al., 2015). Understanding the information encoding and signal processing that underlies these complex processes will enable us to better understand their role in normal brain functioning. Studies of damage or abnormal functioning of the hippocampus have unraveled its role in several neurological and psychiatric disorders, including Alzheimer's, Schizophrenia, epilepsy, depression and posttraumatic stress disorder (de Kloet et al., 2005, Anand and Dhikav, 2012). The initial interest in studying the hippocampus arose from patient H. M. in 1953. He had a bilateral removal of the medial temporal lobe, where the hippocampus is situated, in order to treat his severe epilepsy. After the surgery he suffered from anterograde amnesia, which gave the first clue that the hippocampus plays a role in the formation of new memories (Carlson, 2014).

1.2.1 Anatomy of the hippocampus and dentate gyrus

The hippocampus is situated in the medial temporal lobes of the cerebral cortex, and can be divided into five subfields: three Cornu Ammonis (CA) fields (CA1, CA2 and CA3), the dentate gyrus and the subiculum (Boccara et al., 2015, Schultz and Engelhardt, 2014, Byrne, 1997). In terms of structure, the CA and the dentate gyrus resemble two interlocked C's, with the CA3 field covered by the dentate gyrus (Figure 1) (Boccara et al., 2015, Byrne, 1997).

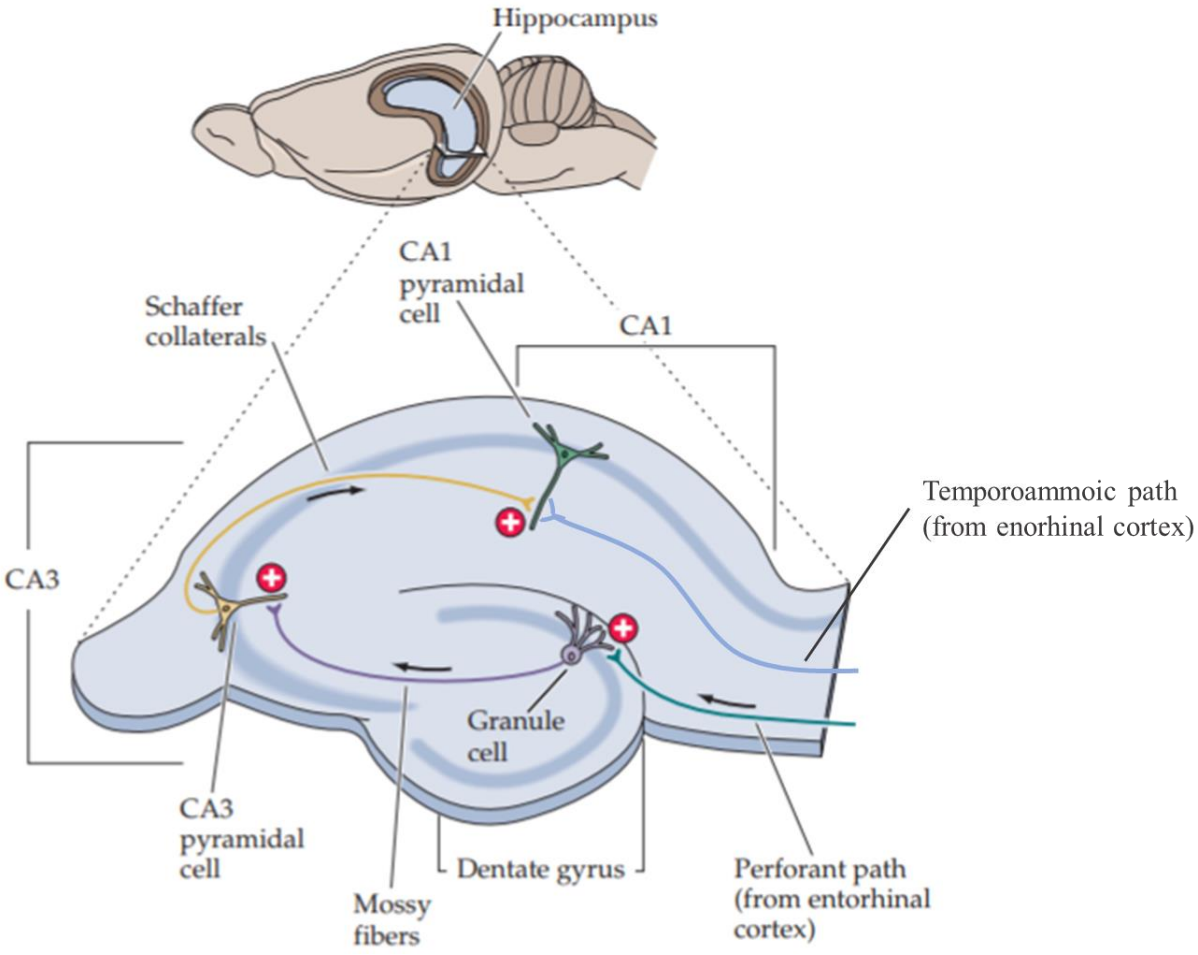

Figure 1: Connectivity and anatomy of the hippocampus in rats. Information enters the dentate gyrus from the entorhinal cortex via the perforant path. From the dentate gyrus the input travels to the CA3 via Mossy fibers and further to the CA1 via Schaffer collaterals. Information can also enter directly to the CA1 from the entorhinal cortex via the temporoammonic path. The + in the red circle indicates an excitatory synaptic pathway. Figure adapted from (Purves, 2012).

The entorhinal cortex is the main connection between the hippocampus and the neocortex, and it is also the main input and output structure of the hippocampus. (Witter et al., 2017). There are two major synaptic pathways between the entorhinal cortex and the CA1 area of

the hippocampus: the perforant path and the temporoammonic path (Figure 1). The perforant path, also known as the indirect path, goes from the entorhinal cortex to the dentate gyrus and CA3. From the dentate gyrus the input is passed to the CA3 area via mossy fibers, and from the CA3 the input goes to the CA1 area via the Schaffer collaterals. The temporoammonic path goes directly from the entorhinal cortex to the CA1 area (Li et al., 2017, Purves, 2012).

The CA region of the hippocampus can be further divided into different layers called strata (Figure 2). The soma of pyramidal cells make up the stratum pyramidale. The pyramidal cells are named after their pyramid-shaped cell bodies. They are organized in a very structured manner, forming a visible layer of cell bodies with dendrites laying parallel to each other. Above the stratum pyramidale is the stratum oriens. Below the stratum pyramidale is the stratum lucidum where mossy fibers projecting to the dentate gyrus lie (this layer is only present in CA3). Underneath the stratum lucidum lies the stratum radiatum, in which Schaffer collaterals, axons that connect CA3 to CA1, are located. Below the stratum radiatum is the stratum lacunosum-moleculare, where input from the entorhinal cortex is received (Fröhlich, 2016).

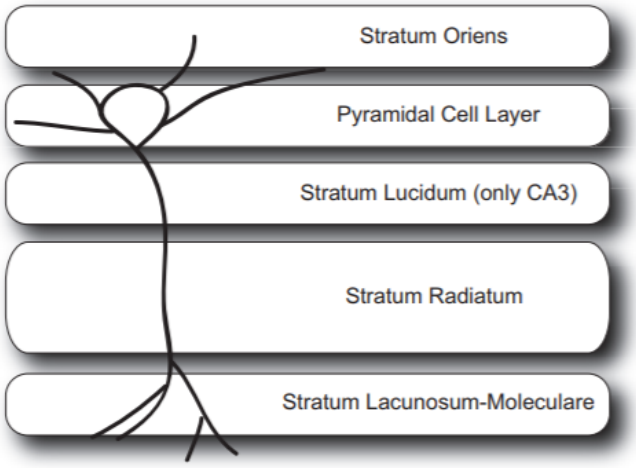

Figure 2: Strata of the hippocampus relative to a pyramidal cell. Source: (Fröhlich, 2016).

It is often quite difficult to distinguish the different strata from one another, which is why they are more commonly grouped into three layers. The hippocampus is therefore said to be a three-layered cortex, rather than a six layered cortex as found in the neocortex. The three layers are the polymorphic layer, pyramidal layer, and the molecular layer. The stratum oriens makes up the polymorphic layer, the stratum pyramidale makes up the pyramidal layer, and the remaining strata are grouped into the molecular layer (Byrne, 1997).

1.3 Stress and its effect on signal processing

One of the many functions of the brain is to ensure homeostasis and respond appropriately to situations and conditions in which homeostasis is interrupted, such as during stress. Many studies have shown that the experience of stress releases endogenous chemicals in the brain that affect signal processing (Arnsten, 2009, de Kloet et al., 2005, Krugers et al., 2012). In addition, stress has an impact on synaptic plasticity, learning and memory (Arnsten, 2009, de Kloet et al., 2005, Howland and Wang, 2008).

When we experience stress, hormones and neurotransmitters such as corticosterone and noradrenaline are released. Corticosterone (cortisol in humans) is a steroid hormone and glucocorticoid in rats (PubChem, Corticosterone). Noradrenaline, also called norepinephrine, is a monoamine transmitter in the catecholamine family (PubChem, Noradrenaline).

Stress activates two different systems that each aim at helping to adapt to the stressful situation: activation of the hypothalamus-pituitary-adrenal axis, and activation of the sympathetic part of the autonomic nervous system with the release of noradrenaline (de Kloet et al., 2005, Krugers et al., 2012, McEwen and Gianaros, 2011).

1.3.1 Stress activates the hypothalamic-pituitary-adrenocortical axis

When animals experience a stressful event, their brain activates specific neural circuits in response. Firstly, the neuropeptides corticotropin-releasing hormone (CRH) and vasopressin (AVP) are released from the hypothalamus (Figure 3). The hypothalamus is a brain structure that links the nervous system to the endocrine system. Additionally, the hypothalamus plays an important role in maintaining homeostasis in the body. CRH and AVP are essential for initiating the stress response, as they activate the hypothalamic-pituitary-adrenocortical (HPA) axis. The HPA axis consists of the hypothalamus, the anterior and posterior pituitary glands and the adrenal cortex (Smith and Vale, 2006). In response to stress, CRH is released into the blood and binds to its receptor in the anterior pituitary gland, while AVP is transported via axonal transport to the posterior pituitary gland. Binding of CRH and AVP causes the pituitary gland to release adrenocorticotropic hormone (ACTH) into the blood. ACTH travels to the adrenal cortex, where it stimulates the synthesis and release of glucocorticoid hormones, such as corticosterone. The glucocorticoids travel via the blood to every organ in the body, including the brain (de Kloet et al., 2005, Smith and Vale, 2006,

Krugers et al., 2012). This allows the brain to initiate and coordinate the body's response to stress.

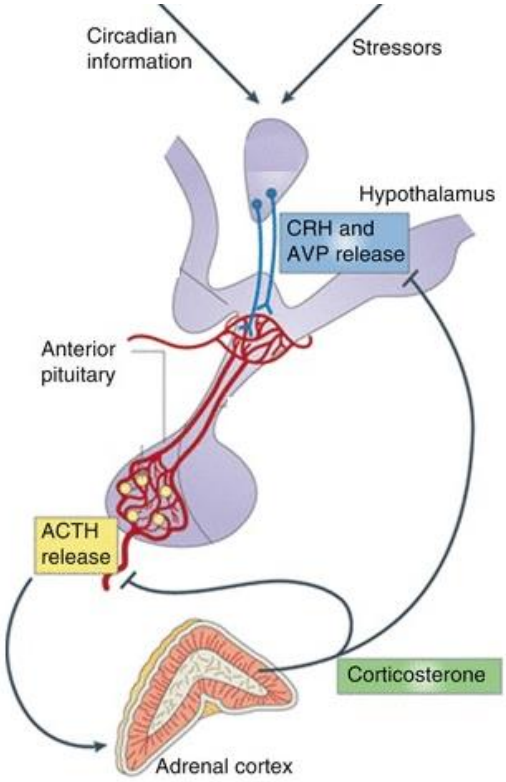

Figure 3: The hypothalamic-pituitary adrenal (HPA) axis. Circadian information or stressors cause the hypothalamus to release CHR and AVP which travel to the pituitary. As a result, the pituitary releases ACTH which stimulates the release of corticosterone from the adrenal cortex. Corticosterone has a negative feedback loop that prevents the further release of CRH, AVP, ACTH, and corticosterone. Adapted from (Lightman, 2016).

Glucocorticoids like corticosterone do not only play a role in stress, but have been shown to play a role in the regulation of the circadian rhythm (sleep-wake cycle) as well. The production of corticosterone is highest at the beginning of the cycle (Arriza et al., 1988, Gomez-Sanchez and Gomez-Sanchez, 2014). The circadian rhythm is an internal process where the release of different endogenous chemicals (including corticosterone and noradrenaline) as well as external ques such as light, regulate brain activity in a rhythmic cycle lasting about 24 h (Carlson, 2014).

1.3.2 The role of corticosterone in stress

Corticosterone is a steroid hormone, passing through the plasma membrane and binding to intracellular mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). MRs and GRs are ligand activated nuclear transcription factors, but they can also act in a non-nuclear fashion (Gomez-Sanchez and Gomez-Sanchez, 2014, Finsterwald and Alberini, 2014).

When MR and GR function as nuclear transcription factors, their activation by corticosterone initiates a nuclear localization signal, translocating the receptor-ligand complex to the nucleus. In the nucleus, the receptor-ligand complex binds to glucocorticoid response elements in the DNA, which affect the transcription of glucocorticoid responsive genes (Gomez-Sanchez and Gomez-Sanchez, 2014, Smith and Vale, 2006, Sarabdjitsingh et al., 2009).

Both MRs and GRs are expressed in the hippocampus, where they have been implicated to play a role in the negative feedback regulation of the HPA axis, reducing the release of glucocorticoids from the adrenal cortex during acute stress (Smith and Vale, 2006). The expression pattern of MR and GR in the hippocampus is, however, different. Sarabdjitsingh et al. found that both GR and MR were expressed in the CA1, CA2 and dentate gyrus, whereas only MR was expressed in CA3. The affinity of MR and GR to corticosterone is different as well (Sarabdjitsingh et al., 2009).

As mentioned in 1.3.1, corticosterone plays a role in regulating the circadian cycle and is released just before awakening. The MR has a higher affinity to corticosterone than the GR, and as result the MR is occupied by the corticosterone that is normally released during the day. When additional corticosterone is released by the adrenal cortex during stress, or at the peak of the circadian rhythm, the MR receptors become saturated, and the corticosterone binds to the GR as well (Arriza et al., 1988, Sarabdjitsingh et al., 2009, Finsterwald and Alberini, 2014). Both MR and GR act as transcription factors and translocate to the nucleus when corticosterone binds, regulating the transcription of genes. Activation of GR receptors in response to elevated corticosterone levels during stress results in the altered expression of 105 genes (Datson et al., 2001).

1.3.3 Stress activates the sympathetic nervous system and the release of noradrenaline.

Besides activating the HPA axis, exposure to stress releases noradrenaline through the locus coeruleus noradrenergic system. The locus coeruleus is located in the pons and has noradrenergic neurons that project to the cortex and hippocampus. During stress, cells in the locus coeruleus produce and release noradrenaline. These cells have been shown to respond to several stressors, amongst them CRH (Hagena et al., 2016, Valentino and Van Bockstaele, 2008).

1.3.4 Actions of noradrenaline in the hippocampus

Noradrenaline acts as a neurotransmitter binding to α- and β-adrenergic receptors, both of which have several subtypes. Adrenergic receptors are G-protein coupled receptors that act via second messengers to open or close ion channels, ultimately altering the electrical properties of neurons (Krugers et al., 2012, Hagena et al., 2016, Morilak et al., 2005, Madison and Nicoll, 1986a).

In the hippocampus, noradrenaline mainly exerts its effect through the β-adrenergic receptor (Hagena et al., 2016). The main pathway that is activated when noradrenaline binds the β-adrenergic receptor is cyclic adenosine monophosphate (cAMP) dependent (Figure 4). Noradrenaline binds the β-adrenergic receptor which activates adenylyl cyclase. Adenylyl cyclase produces cAMP from adenosine triphosphate, and cAMP acts as a second messenger activating protein kinase A (PKA) (Purves, 2012). cAMP can also act on ion channels independently of PKA (Wainger et al., 2001, Schroeder et al., 1998, Pedarzani and Storm, 1995). When cAMP activates PKA, PKA increases protein phosphorylation, including phosphorylation of the transcription factor cAMP response element-binding protein (CREB), which activates the transcription of several cAMP responsive genes (Lorton and Bellinger, 2015, Marzo et al., 2009). Madison and Nicoll found that cAMP mediates the actions of noradrenaline on the β-adrenergic receptor in hippocampal pyramidal cells (Madison and Nicoll, 1986b). Additionally, the release of noradrenaline in the hippocampus causes changes in neural excitability through changing the activity of ion channels (Madison and Nicoll, 1986a, Krugers et al., 2012).

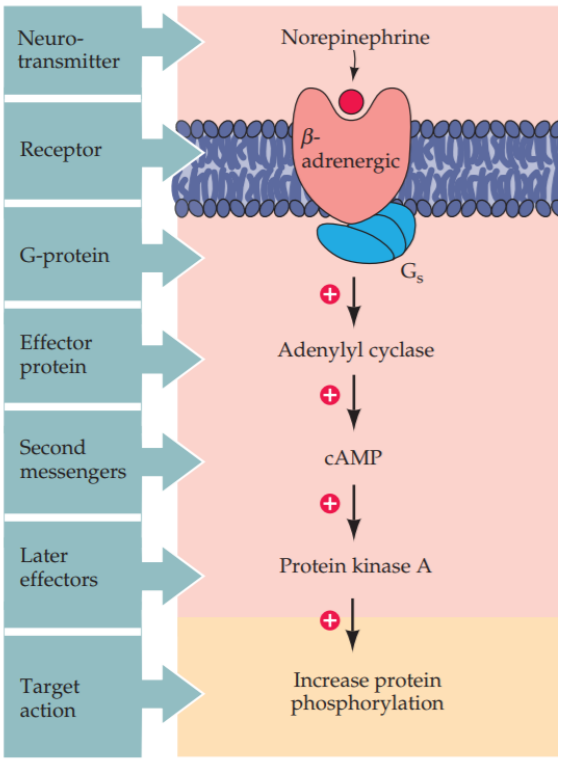

Figure 4: Signaling pathway of noradrenaline (norepinephrine). Noradrenaline binds to the β-adrenergic receptor which leads to the activation of the G-protein and the recruitments of the adenylyl cyclase, cAMP, PKA second messenger pathway. Source: (Purves, 2012).

1.3.5 Timing of noradrenaline and corticosterone release after stress

After exposure to stress noradrenaline is released instantly from the locus coeruleus, exerting its effect on the β-adrenergic receptor. Via a second messenger cascade, this alters the activity of ion channels, leading to changes in neural excitability. In addition, noradrenaline exerts a slower effect through the activation of gene expression via CREB. Corticosterone, on the other hand, is released with a 20 min delay because the synthesis of corticosterone only starts after exposure to stress. Furthermore, corticosterone acts by binding to GRs and MRs, which translocate to the nucleus and regulate the transcription of genes (Krugers et al., 2012, Groeneweg et al., 2011). Thus, the genomic corticosterone related response during stress is delayed in comparison to the noradrenergic response.

For a long time, corticosterone was believed to only be responsible for the delayed genomic response to stress, while monoamines such as noradrenaline were responsible for the rapid effect. Over the last years, research has shown that corticosterone also exhibits rapid, non-genomic functions in response to stress, changing the excitability and activity of neurons in

brain areas such as the hippocampus. The non-genomic effects of corticosterone are mediated through non-genomic membrane-located mineralocorticoid receptors. The non-genomic MR has a lower affinity for corticosterone than the genomic MR (Karst et al., 2005). Binding of low levels of corticosterone to non-genomic MR has been shown to promote synaptic plasticity and memory, whereas high levels of corticosterone during stress impairs plasticity and memory (Groeneweg et al., 2011, ter Heegde et al., 2015, Kim et al., 2015). The rapid non-genomic effect of corticosterone coincides with noradrenaline release during stress (Krugers et al., 2012).

1.4 Synaptic plasticity, learning and memory

The hippocampus plays an important role in synaptic plasticity, which is the mechanism underlying learning and memory. The two most recognized models for the molecular mechanism for synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD) (Lomo, 2003, Howland and Wang, 2008, Purves, 2012, Carlson, 2014). Other forms of activity dependent plasticity have also been found, including EPSP-spike potentiation and spike-timing-dependent-plasticity (Neves et al., 2008).

1.4.1 Long-term potentiation

LTP was discovered in the hippocampus of rabbits in 1966 by Terje Lømo and Timothy Bliss. LTP has since been regarded as the molecular mechanism behind learning and memory. LTP was discovered when Lømo and Bliss observed that high-frequency stimulation of neurons in the hippocampus caused prolonged and enhanced synaptic transmission (Lomo, 2003).

During synaptic transmission the neurotransmitter glutamate is released from the presynaptic terminal (Figure 5). Glutamate binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on the dendritic spine of the postsynaptic neuron. This causes an influx of Na^+, which causes the postsynaptic cell to depolarize. The influx of Na^+ opens N-methyl-D-aspartate (NMDA) receptors in the postsynaptic dendrite. NMDA receptors are normally blocked by the physiological concentration of Mg^{2+} or Zn^{2+} ions, and the block is voltage-dependent. When the postsynaptic dendrite depolarizes after the influx of Na^+ caused by the AMPA receptor, the ion block is lifted. As a result the NMDA channel opens and allows Ca^{2+} to enter the neuron. The increase in Ca^{2+} concentration leads to the activation of

Ca^{2+} calmodulin kinase II (CaMKII) and protein kinase C. The downstream effect of these kinases is the insertion of more AMPA receptors into the postsynaptic membrane, which makes it more sensitive to glutamate release from the presynaptic neuron (Purves, 2012). In addition, the kinases can phosphorylate already present AMPA receptors, increasing their conductance (Derkach et al., 1999). The strengthening of the synapse in response to recent activity is referred to as LTP.

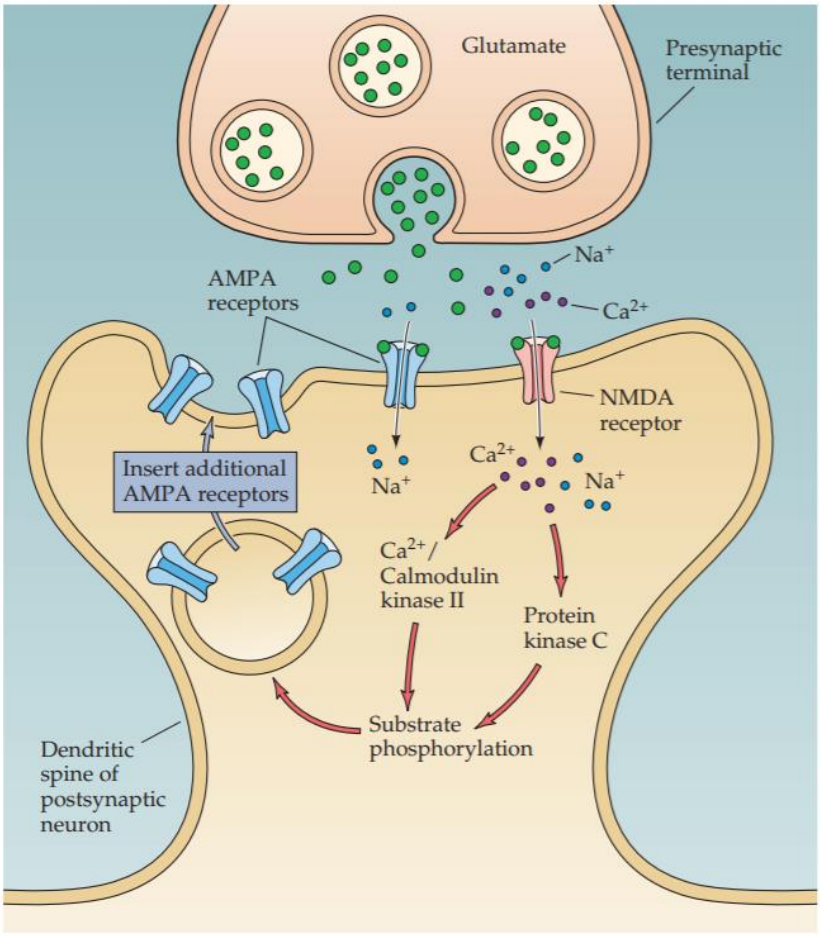

Figure 5: Molecular mechanism behind long term potentiation. During synaptic transmission glutamate is released from the presynaptic terminal. Glutamate binds to AMPA receptors and causes the cell to depolarize. The depolarization unblocks the NMDA receptor and allows Ca^{2+} to enter the neuron. The increase in Ca^{2+} concentration leads to the activation of Ca^{2+} calmodulin kinase II (CaMKII) and protein kinase C. The downstream effect of these kinases is the insertion of more AMPA receptors into the postsynaptic membrane, which increases the neurons sensitivity to glutamate and results in LTP. Source: (Purves, 2012).

1.4.2 The role of stress in synaptic plasticity, learning and memory

In addition to playing an important role in synaptic plasticity, learning and memory, the hippocampus is very susceptible to stress. The MR, GR and β-adrenergic receptor are all enriched in the hippocampus (Sarabdjitsingh et al., 2009, Hagena et al., 2016). Furthermore, the hippocampus provides a negative feedback loop, inhibiting corticosterone release from the adrenal cortex (Smith and Vale, 2006). Studies have also shown that both noradrenaline and corticosterone can regulate AMPA receptors in the neurons membrane, thereby influencing LTP and synaptic plasticity (Krugers et al., 2012). Noradrenaline does this by binding to β-adrenergic receptors which activate CaMKII. CaMKII is important for the insertion of new AMPA receptors in the membrane (Hu et al., 2007). Corticosterone has been shown to increase AMPA receptor trafficking to the membrane. Groc et al. showed that application of 100 nM corticosterone in the hippocampus, acting via the MR, increased the trafficking of AMPA receptors to the neurons membrane (Groc et al., 2008).

Several studies have looked at the relationship between stress and learning and memory, and there have been some contradictory results. Some studies suggested that stress impairs learning and memory, while other studies suggested that stress enhances learning and memory. It is now generally accepted that there is an inverted U-shaped relationship between stress, learning and memory. Medium levels of stress improves memory by enhancing LTP, whereas both low and high levels of stress impair learning and memory by enhancing LTD (Diamond et al., 1992, Salehi et al., 2010, Howland and Wang, 2008).

Experiences of acute stress can enhance cognitive performance and lead to the formation and consolidation of new long-term memories that help us respond more quickly next time a similarly stressful situation is experienced (Finsterwald and Alberini, 2014). Additionally, acute stress causes changes in the brain and body that help overcome the changes in homeostasis that caused stress (Howland and Wang, 2008). On the other hand, experiences of chronic stress impair cognitive performance and inhibits learning and memory. Additionally, chronic stress can also lead to the development of anxiety disorders, depression and post-traumatic stress disorder (de Kloet et al., 2005, Finsterwald and Alberini, 2014, Howland and Wang, 2008).

1.5 Patch clamp technique for measurement of electrical properties in cells

The patch clamp technique was invented by Neher and Sakmann. In 1991 they received the Nobel Prize in Physiology or Medicine for their achievements (Nobel Prize). The technique allows researchers to investigate the precise electrical potential of individual neurons.

There are several patch clamp configurations that can be used when recording from a neuron (Figure 6). In this study, whole-cell recordings were obtained from CA1 pyramidal neurons. During a whole-cell recording, the pipette forms a tight seal with the cell membrane before breaking into the cell. This means that the interior of the pipette becomes continuous with the cell cytoplasm. The whole-cell configuration allows for the study of how different drugs and neuromodulators influence the electrical potential, or membrane potential of a neuron.

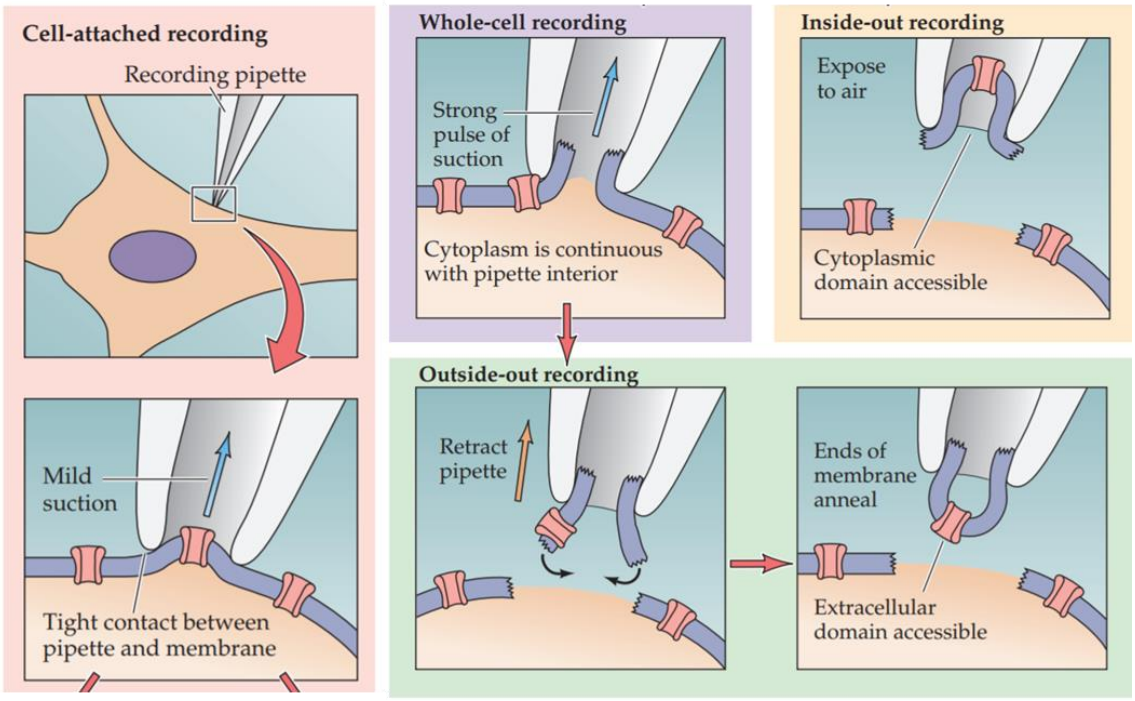

Figure 6: Four different patch clamp configurations. For all four configurations the recording pipette is first brought close to the cell membrane, and a tight seal is formed between the pipette and the membrane. During a cell-attached recording no more is done. For a whole-cell recording strong suction is used to make the cytoplasm of the cell continuous with the interior of the pipette. During an inside-out recording, a small section of the membrane is pulled away by the pipette in the cell-attached configuration so that the intracellular side of the membrane is exposed. For an outside-out recording, the pipette is pulled away in the whole-cell configuration so the membrane patch reseals with the extracellular side exposed.

In addition to having several different configurations, there are also two different modes in which patch clamp recordings can be made: current-clamp and voltage-clamp. In current-clamp mode the researcher controls, or clamps, the amount of current that is injected into the cell, which allows for the investigation of the changes in membrane potential that occur as a result of the applied current. In voltage-clamp the researcher clamps the membrane potential and measures the current across the membrane.

During the course of this study only the current-clamp mode was used for the recordings. Several electrophysiological properties and their response to applications of corticosterone and noradrenaline were investigated; excitatory postsynaptic potential summation, sag, and slow-afterhyperpolarizations.

1.5.1 Excitatory postsynaptic potentials

An excitatory postsynaptic potential (EPSP) is caused by the influx of positively charged ions into the neurons, via ligand gated ion channels, following transmitter binding. This causes a temporary depolarization in the membrane potential. The opposite phenomenon leads to a hyperpolarization of the membrane potential and is called an inhibitory postsynaptic potential (IPSP) (Purves, 2012, Carlson, 2014). In the CA1 of the hippocampus, EPSPs can be generated from the stimulation of several pathways, including the temporoammonic path, perforant path and Schaffer collaterals.

One postsynaptic neuron can receive several EPSPs and IPSPs at the same time. Summation of the EPSPs and IPSPs in the postsynaptic neuron, a form of information processing, determines the total outcome of the potentials. If there is more excitatory input, the EPSPs can summate and increase in amplitude, resulting in a depolarization of the membrane. If the amplitude of the depolarization is large enough, it will reach threshold and elicit and AP. Following LTP, AMPA receptors are inserted into the neuron membrane the strength of the synapse in enhanced, hence there is an increase in EPSP amplitude (Purves, 2012).

Neuromodulators such as noradrenaline can also influence the summation of EPSPs. Noradrenaline is an excitatory neurotransmitter, and a study by Madison and Nicoll found that noradrenaline had a disinhibitory effect on CA1 pyramidal cells in the hippocampus. They found that the application of noradrenaline decreased the size of the IPSP. An increase in EPSP amplitude was also observed, but this was not believed to be caused by

noradrenaline, as the current underlying the EPSP did not change. (Madison and Nicoll, 1988).

1.5.2 Hyperpolarization-activated cyclic nucleotide-gated channels and sag

The hyperpolarization-activated cyclic nucleotide-gated (HCN) channel is one of the ion channels that influence the electrophysical properties of neurons. The HCN channel is a voltage-gated channel. The channel activates when the membrane potential of the neuron hyperpolarizes below -50 mV. Additionally, the channel is gated by cAMP. When cAMP is bound to the cytoplasmic site it facilitates opening of the HCN channel (Wainger et al., 2001, He et al., 2014, Wahl-Schott and Biel, 2009). When the HCN channel is open it allows K^+ and Na^+ to enter the cell, generating an excitatory inward current named I_h. Opening of the HCN channel during a hyperpolarization causes a "sag", where the neuron depolarizes towards the RMP (Pape, 1996) (For an illustration of the sag, see Figure 13 in Materials and Methods).

The difference between the peak hyperpolarization and the steady state decrease in membrane potential is referred to as "sag". Sag is a measure of the depolarization that is triggered in response to an initial hyperpolarization of the membrane potential. The sag is voltage dependent, in addition to being influenced by the presence of HCN channels. During a current clamp recording the membrane voltage measured is dependent on the current applied to the cell and its input resistance (Ohm's law: V = I * R). The sag is therefore indirectly dependent on input resistance as well.

In general, I_h decreases or inhibits the excitability of dendrites (Magee, 1998). I_h has been shown to decrease the input resistance of a cell, thus also decreasing the change in voltage potential in response to a current injection (He et al., 2014). Additionally, blocking HCN channels increases EPSP amplitude and summation (Magee, 1998).

1.5.3 Slow-afterhyperpolarizations

In several excitable cell types, such as the pyramidal cell in CA1 of the hippocampus, the firing of action potentials is followed by a hyperpolarization of the membrane below the resting membrane potential (RMP). This afterhyperpolarization can last from a few milliseconds to several seconds. The afterhyperpolarization (AHP) can be divided into three

different after-potentials; the fast AHP lasting 2-5 ms, medium AHP lasting 50-100 ms, and a slow AHP lasting 1-2 s (Storm, 1987).

The slow afterhyperpolarization (sAHP) is caused by the opening of Ca^{2+} activated K^+ channels following a train of action potentials. The subsequent K^+ efflux hyperpolarizes the cell (Gu et al., 2005, Storm, 1987). sAHPs can thus be said to be a Ca^{2+}-activated K^+-current (I_{sAHP}). The K^+ channel underlying the I_{sAHP} has yet to be found, even though a lot of research has been done on sAHP (Wang et al., 2016). sAHP is modulated by neurotransmitters like noradrenaline. Noradrenaline has been shown to block the current underlying the sAHP (Storm, 1987, Lancaster and Nicoll, 1987). It does this by blocking the Ca^{2+}-activated K^+-current (Madison and Nicoll, 1988).

AHPs play an essential role in regulating the excitability and repolarization of neurons. sAHPs have been shown to affect the shape and firing pattern of APs (Andrade et al., 2012). During LTP, the amplitude of AHP has been reported to be reduced. As a result, an increased sAHP has been suggested to play a role in learning and memory impairment (Kaczorowski et al., 2007). When the AHP is reduced, the neuron is more excitable and can repolarize faster after firing APs. This is important for neural processing, as it increases the speed and efficiency of AP generation (Storm, 1987).

sAHP have also been shown to influence EPSPs. In a study by Lancaster et al., the hyperpolarization that usually follows an EPSP was reduced when I_{sAHP} was blocked by noradrenaline. Additionally, when I_{sAHP} was increased, following a longer burst of action potentials, the summation of EPSPs was decreased. Lancaster et al. concluded that EPSPs could activate the Ca^{2+}-activated K^+-current responsible for sAHP, and that the activation of I_{sAHP} decreased subsequent EPSP summation (Lancaster et al., 2001).

1.5.4 Input resistance

Input resistance is another electrophysiological property of neurons. It is a measure of how much current needs to be injected into the cell to change the membrane potential of a cell by a given voltage. When the input resistance is high it means that less current needs to be applied to the neuron to obtain a given voltage change than if the input resistance is lower. Input resistance is a passive property and can be influenced by several factors including ion channel composition, membrane capacitance, and size of the neuron (Purves, 2012).

1.6 Interaction between noradrenaline, corticosterone and I_h

When noradrenaline is released during stress, it binds to β-adrenergic receptors in the brain, activating a second messenger cascade that ends with the production of cAMP and PKA (see point 1.3.4). cAMP also binds to HCN-channels, shifting their voltage activation curve and facilitating the voltage dependent opening of these channels, resulting in an increased I_h current at relatively depolarized potentials (Marzo et al., 2009) (Pedarzani and Storm, 1995).

The non-genomic, short term action of corticosterone has been shown to enhance the actions of noradrenaline and facilitate synaptic transmission when they both are present at the same time, as during stress (Joels et al., 2011).

As mentioned in section 1.4.2, both noradrenaline and corticosterone influence synaptic plasticity during stress, by increasing AMPA receptor trafficking and insertion into the membrane. Zhou et al. found that a combination of noradrenaline and corticosterone had a stronger effect on the mediation of the AMPA receptor, than each of the compounds separately. Corticosterone mediates the effect of the β-adrenergic receptor, and also influences the cAMP, PKA pathway. (Zhou et al., 2012).

1.7 Aims of the study

The overall goal of this study was to investigate how noradrenaline and corticosterone, both of which are released during stress, influence and modulate dendritic signal processing in CA1 pyramidal cells of the hippocampus. More specifically, the main aim of the study was examining if corticosterone modulates the effect of noradrenaline.

The following questions were specifically addressed:

a) Does corticosterone modulate the effect of noradrenaline on signal processing?

b) How do noradrenaline and corticosterone influence summation and amplification of postsynaptic potentials, which have been shown to modulate synaptic plasticity?

c) How do noradrenaline and corticosterone influence the slow afterhyperpolarization that follows action potential firing and the effect this has on signal processing?

d) How does the activity of the HCN-channel mediate the modulatory effect of noradrenaline and corticosterone in regards to postsynaptic potentials and the generation of sag?

e) How noradrenaline and corticosterone influence the input resistance of the cells?

More knowledge on the electrophysiology of neurons in the hippocampus is needed since the hippocampus is involved in neurological and psychiatric disorders such as Alzheimer's, Schizophrenia and epilepsy. Under the influence of chronic stress, mental illnesses such as depression and posttraumatic stress disorder can develop. Understanding the normal function of the brain and hippocampus, might help researchers understand what changes occur when these diseases develop. In the long run, such understanding might allow us to find ways of preventing or reversing the onset of these diseases.

2 Materials and Methods

2.1 Animal model and approval

All experiments were performed in accordance to guidelines and criteria set by the responsible veterinarian (ethical committee) at the Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo. The experiments were in compliance with the statute regulating animal experimentations given by the Norwegian Ministry of Agriculture, 1996. All participants in the study had the FELASA (Federation for Laboratory Animal Science Associations) certification needed to perform animal research, as required by the Norwegian Food Safety Authority.

Male Wistar rats, 23-28 days post-natal, were used in all experiments, and they were ordered from Scanbur, Denmark. The animals were acclimated for at least six days before any experiments were conducted. The animals were housed with *ad libitum* access to food and water, in GR900 cages with a floor area if 904 cm^2. Nesting material and paper tubes were placed in the cage as toys. The animals were under a 12h light-dark cycle, with the lights turned on from 07.00 to 19.00. The temperature in the cage was kept at 23°C, with a relative humidity of 55%. The air inside the cage was exchanged 65 times per hour.

2.2 Slice preparation

Slices for whole-cell current-clamp experiments were prepared using male Wistar rats aged P23-28.

The rat was placed in a glass chamber and anesthetized with Suprane, (Baxter, Oslo, Norway). The animal was considered anesthetized, when it lost the righting reflex, and did not correct its position after being rolled over on its back. After being anaesthetized, the animal was decapitated with scissors, and the brain was removed and immediately placed in ice cold cutting solution containing (in mM) 87 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 25 glucose, 74.8 sucrose, 4 $MgCl_2$, and 1 $CaCl_2$. The solution was bubbled with carbogen gas (95 % O_2 and 5 % CO_2) before and during use. Horizontal slices, 400 µm, containing the hippocampus were prepared from the right hemisphere using a Leica VT1200 microtome. During slicing, the brain was submerged in ice cold cutting solution, but it was not bubbled

with carbogen gas (Figure 7, A). The slices were placed in cold cutting solution to slow down the physiological processes in the brain, such as neurotransmitter release and action potential propagation, and to increase viability.

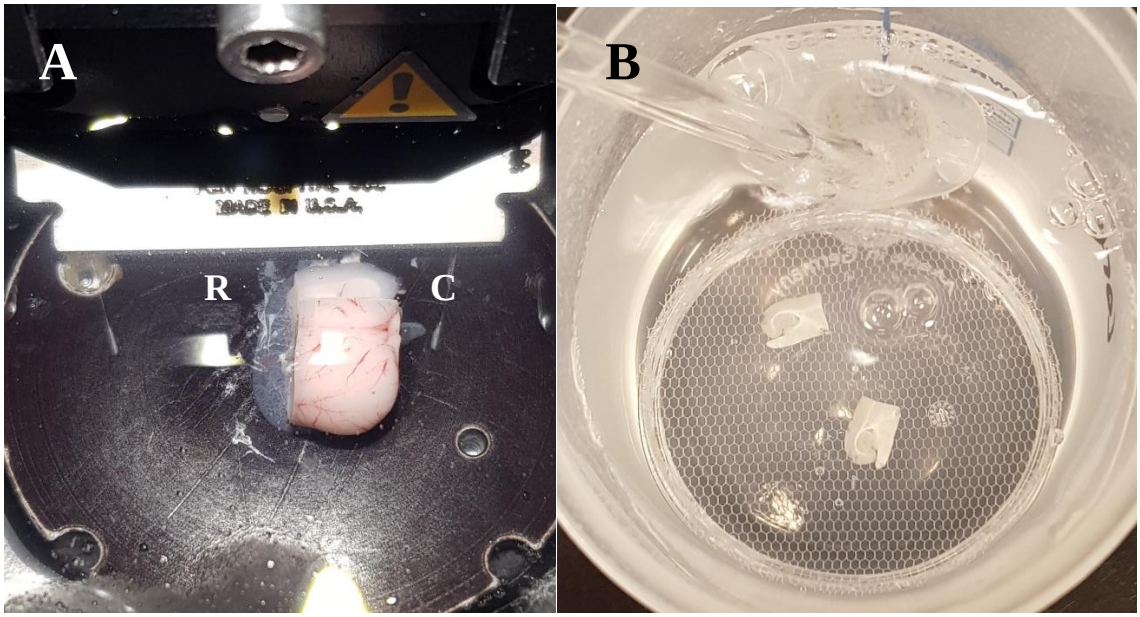

Figure 7: Slice preparation of the brain. A. The right hemisphere of the brain was cut into 400 µm slices using a microtome, R and C refer to the rostral and caudal direction. **B.** Slices were incubated in a special holding chamber while being bubbled with carbogen gas.

The slices were incubated for 30 min at 35 °C in a specially constructed holding chamber containing recording solution consisting of (in mM) 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 glucose, 1 $MgCl_2$, 2 $CaCl_2$ (Figure 7, B) (Table 3 in the Appendix). The recording solution was meant to imitate cerebrospinal fluid, and it was also bubbled with carbogen. The incubation allows the cells to recover from being sliced, and acclimatizes the slices to the recording solution and temperature. Additionally, the incubation in a warmer temperature allows the cells to reactivate their metabolic processes again, resulting in normal electrophysiological activity during the subsequent patch clamp recording. After the incubation, the holding chamber is kept at room temperature, 25 °C, to slow down the metabolic processes slightly, so the slices can be kept healthy longer (Booker et al., 2014). The slices were used within 6 h after starting the incubation.

2.3 Whole-cell patch clamp setup and recording

All recordings in this study were performed in current clamp mode. During the experiments the temperature of the recording solution was kept between 31.5-32.5 °C, and continuously bubbled with carbogen gas. Slices where the primary apical dendritic branch of the CA1 pyramidal cells were parallel to the surface of the slice were used. This was done in order to prevent recording from cells where the dendrites were cut and damaged during slicing.

2.3.1 Current clamp setup

The whole-cell patch clamp setup used in this study consisted of a microscope, a camera, a recording chamber, two micromanipulators, an isolator, a pump, two pipette holders, an amplifier, a digitizer and a computer with software for recording and analyzing data (Figure 8).

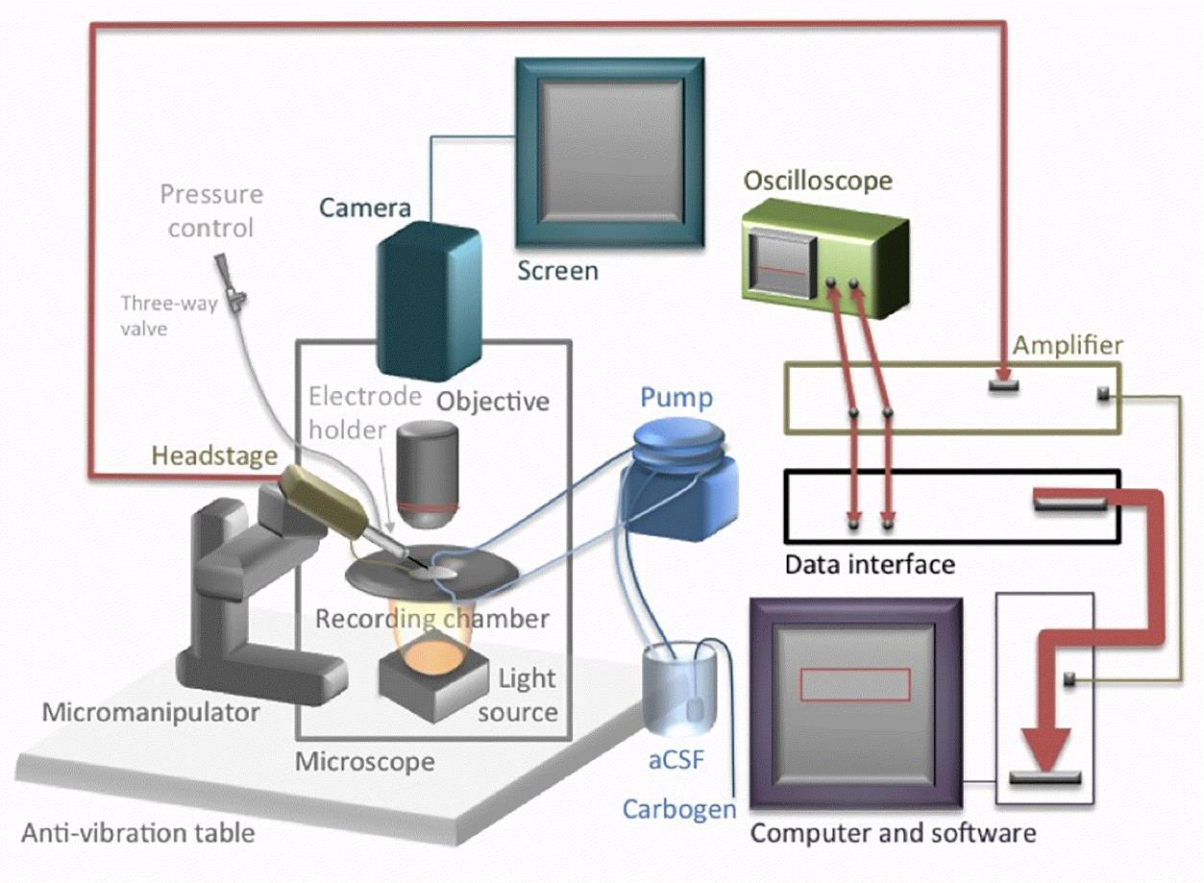

Figure 8: Whole-cell patch clamp setup. Source: axolbio

A microscope (Olympus Bx51W1, Olympus Corporation, Tokyo, Japan) with 4x and 40x objective lenses and an infrared filter was used to visualize the cells in the slices. An amplifier (Dagan BVC-700A, Dagan Corporation, MN, USA) to amplify the signal before it was sent to the digitizer (Digidata 1322A, Axon Instruments, CA, USA) which converts the electrical data recorded into digital output. Data recording was done using pCLAMP 10 (Axon Instruments, CA, USA). The micromanipulators were from Luigs and Neumann (Feinmechanik und elektrotechnik GmbH, Ratingen, Germany), and the pipette holders were from G23 Instruments (G23 Instruments, London, UK).

During electrophysiological recordings noise can be introduced in the form of vibrations and electromagnetic interference. In order to reduce noise, the experiments were performed on a Newport anti-vibration table placed in a Faraday cage. The amplifier, digitizer, screen, micromanipulators, computer and software were placed outside of the cage and instruments were grounded appropriately.

2.3.2 Procedure for obtaining a recording from a CA1 pyramidal cell

Before recording, 5 µM SR 95531 (Gabazine) and 50 µM D-2-Amino-5-phosphonopentanoic acid (APV) were added to the recording solution. All concentrations mentioned are final concentrations in the recording solution. For experiments in which the slices were incubated in corticosterone prior to recording, 100 nM corticosterone was added to the recording solution as well. Gabazine, a $GABA_A$ receptor antagonist was added to the recording solution in the setup in order to block inhibitory postsynaptic currents (Booth et al., 2014). APV is an NMDA receptor antagonist. APV was added to the recording solution to ensure that the recorded changes in synaptic potential were directly caused by the addition of noradrenaline, and not by noradrenaline modulating NMDA receptor activity through activation of the β-adrenergic receptor, and to prevent causing LTP due to repeated stimulation (Marzo et al., 2009, Burgard et al., 1989, Lin et al., 2003).

Whole-cell current-clamp recordings were made from CA1 pyramidal cells in the hippocampus. Only slices where the dendrites of the CA1 pyramidal cells ran parallel to the surface of the slice were used. A slice was transferred from the holding chamber to the recording chamber in the patch-clamp setup. The slice was held in place and submerged in the solution by a platinum ring with thin strands of fishing line across it.

In order to simulate the CA1 pyramidal cells receiving input from the entorhinal cortex trough the perforant path, a stimulating pipette was placed in the stratum lacunosum-moleculare. The stimulating pipette was pulled from a borosilicate glass capillary (Sutter Instruments, CA, USA) using a pipette puller (PP-830, Narishige, Japan). The pipette was filled with 1 M NaCl, and had a resistance between 6-9 MΩ. It was placed in the stratum lacunosum-moleculare using one of the micromanipulators.

To patch a pyramidal cell in the CA1 area of the hippocampus, a recording pipette was pulled from a borosilicate glass capillary (Sutter Instruments). The resistance of the pipette was between 6-8 MΩ and it was filled with intracellular solution. The intracellular solution consisted of (in mM) 120 Kgluconate, 20 KCl, 2 NaATP, 3 $MgCl_2$, 10 HEPES, and 0.4 NaGTP.

Positive pressure, 70-90 mbar, was applied to the recording pipette before using a micromanipulator to bring the pipette to a CA1 pyramidal cell. After approaching the cell, the pipette was pressed gently against the cell membrane in order to produce an indentation. After the indentation was observed the pressure was released from the pipette and -65 mV of holding voltage was applied. Additionally, negative pressure was used to create a seal between the pipette and the cell membrane, the resistance of which exceeded one giga-ohm (GΩ). After obtaining the giga-ohm seal short pulses of negative pressure (i.e. suction) was used to break into the cell, obtaining the whole-cell configuration. During the recording the neurons were held at -65 mV, by passing current through the recording electrode.

2.3.3 Current clamp recording

Prior to the start of the study, some criteria were set as quality indicators that needed to be fulfilled in order to initiate recording. First, the dendrites of the cells had to run parallel with the slice, in order to decrease the changes that the dendrites were cut during the making of the slices. Secondly, only cells with a round and smooth surface were selected, as this reduced the chance of patching an apoptotic cell. After breaking in, only cells with a resting membrane potential more negative than -55 mV were selected for recording.

Before any recording was started, the capacitance and bridge-balance were adjusted and compensated for. When the membrane potential is recorded through an electrode, as is the

case during a current clamp recording, the resistance of the electrode can cause artifacts. When current is injected into the cell it will cause a voltage drop across the electrode resistance. This voltage error is corrected for by the amplifier, by injecting current across the electrode so no voltage drop is observed. This is called adjusting the bridge balance. The electrode resistance can change throughout the recording, so the bridge balance was constantly monitored and adjusted.

Three different protocols were used when performing current clamp recordings; 1) current-voltage protocol, 2) threshold & 2x threshold protocol, 3) time course protocol.

Current-voltage protocol: The current-voltage protocol applies a series of square current pulses of fixed-duration which are increasing in amplitude to the cell and measures how the membrane potential and/ or action potential firing frequency changes in response. The current-voltage protocol used in this study consisted of current steps from -500 pA to 900 pA with 100 pA increments. The current was applied for 1000 ms (Figure 9). This protocol was performed both before and after an experimental intervention, e.g. adding noradrenaline,

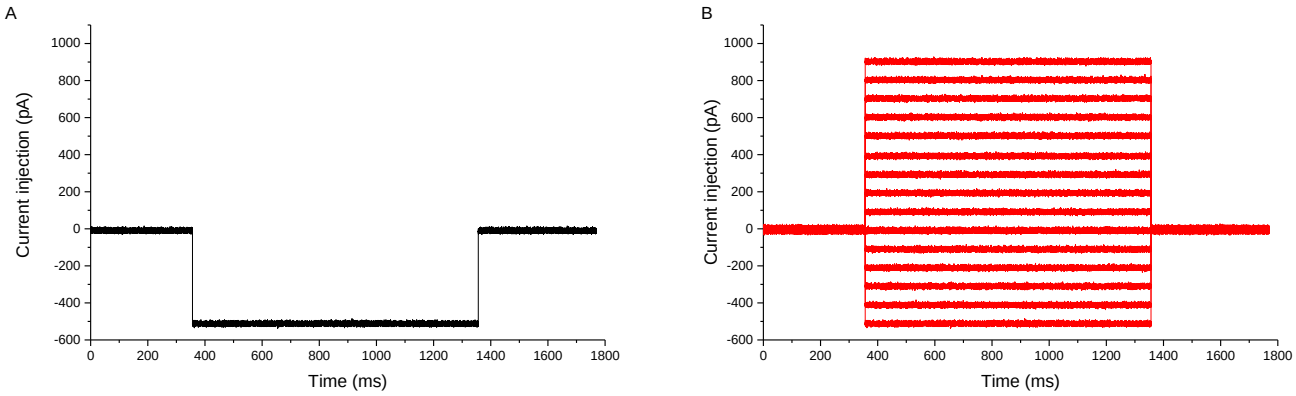

Figure 9: Current-voltage protocol. A. The first sweep during the protocol with a square negative current pulse of -500 pA. B. An overlay of all 15 current pulses used during the protocol.

Time course: The time course protocol consisted of four different elements that allowed for the investigation of changes in electrophysiological properties in response to stimulation and current injection (Figure 10).

1) The protocol contained five excitatory postsynaptic current (EPSC) pulses, delivered extracellularly by the stimulation pipette, with a frequency of 50 Hz, which were used to measure summation of EPSPs (Figure 10 B.)

2) A 200 ms depolarizing square current pulse was used to elicit eight action potentials, followed by a sAHP. The size of the depolarizing current was adjusted during the recording so it always produced eight action potentials (Figure 10 A.).

3) A 1 s hyperpolarizing current step of -200 pA was used to measure the amplitude and ratio of the sag that results from the opening of HCN-channels.

4) Five 200 ms hyperpolarizing pulses of -30 pA were applied at a 2,5 Hz rate to measure the input resistance of the cell.

One sweep in the recording lasted 10 s and included all elements listed above. In between each recorded sweep, 5 sweeps of the depolarizing step were performed, so the amplitude of depolarization could be adjusted to elicit eight action potentials.
During a whole time course recording 6 min of baseline activity was recorded before adding 10 µM Noradrenaline to the recording solution. After adding noradrenaline, the cell was recorded for another 19 min for a total recording time of 25 min, with a total of approx. 70 sweeps.

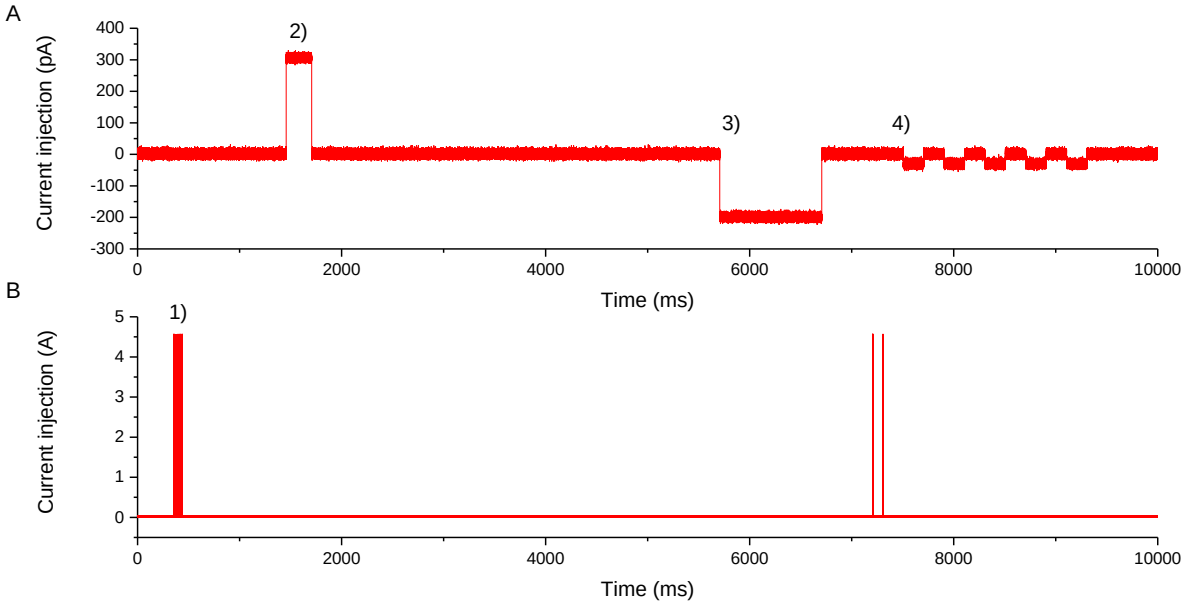

Figure 10: Time course protocol. Numbers 1-4 refer to the different element described in the text above. A. Square current pulses during the time course protocol. B. Extracellular stimulation during the time course protocol.

For each cell, the current-voltage protocol was performed prior to and after the time course protocol, in order to study the effect of treatment with a neuromodulator (Figure 11).

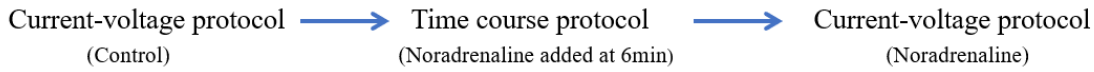

Figure 11: Workflow of the protocols performed when recording from a cell. 10 µM noradrenaline was added 6 min into the time course recoding, after obtaining a stable baseline.

2.3.4 Neuromodulators

Different neuromodulators were used in this study (Table 1). Gabazine and APV were added to the recording solution in the patch clamp setup at least 30 min before recording, and were thus uniformly circulating during the recording. 10 µM noradrenaline was added to the recording solution approximately 6 minutes into the time course protocol, the recording solution took 45 sec to reach the recording chamber. In half of the recordings, the slices were incubated in corticosterone for 1 h prior to recording, and corticosterone was also added to the recording solution. During the recordings with both corticosterone and noradrenaline, ascorbic acid was added to prevent oxidation of noradrenaline.

All compounds listed in Table 1 were stored as aliquots in a -18°C freezer.

Table 1: Overview of medium composition during recording. + indicates that the compound was present in the medium, - indicates that the compound was absent in the medium.

Compound	Solvent	Supplier	Final concentration	NA medium	NA + CORT medium
Noradrenaline	dH_2O	Abcam, Cambridge, UK	10 µM	+	+
Corticosterone	70 % ethanol	Sigma Aldrich, USA	100 nM	-	+
Gabazine	dH_2O	Tocris, Bristol, UK	5 µM	+	+
APV	0,1 M NaOH	Tocris, Bristol, UK	50 µM	+	+
Ascorbic acid*	dH_2O	Tocris, Bristol, UK	100 µM	-	+

*Ascorbic acid was only added during the noradrenaline and corticosterone recordings

2.4 Data- and statistical analysis

Data was recorded using Clampfit 10.7 (Molecular Devices LLC, CA, USA). Data analysis was performed using Origin (OriginLab Corporation, MA, USA) and Clampex 10.7 (Molecular Devices LLC, CA, USA), while statistical analysis was performed in Origin. To assess whether the electrophysiological properties were significantly different between control conditions and after adding noradrenaline, 'pair-sample t-tests' were performed in Origin. To quantify and significant differences between the control cells and cells incubated in corticosterone 'two-sample t-tests' were performed. In both cases $P < 0,05$ was considered significant. For reference of the statistical significance the following symbols are used:
* $p<0,05$, ns $p>0,05$.

2.4.1 Data filtering

The amplifier had a 10 K Vm filter. During recording the Vm had a lowpass filter of 1 kHz and a highpass filter of 1 Hz, with a sampling rate of 20 kHz. Additionally, the bridge balance and capacitance of the cell were adjusted before recording.

2.4.2 Cell inclusion criteria for analysis

For a recorded cell to be included in the analysis, the recording had to be stable. Cells with a large change in bridge balance (>10 MΩ) were excluded. So were cells that did not have a stable baseline during the recording, for example due to unexpected depolarizations. Lastly, during some recordings the cells failed to produce proper action potentials during the last current-voltage protocol, and these cells were also excluded.

2.4.3 Measurement of peak EPSP amplitude

During the time course 5 EPSP train pulses were delivered at a frequency of 50 Hz in order to stimulate the summation of EPSPs. The peak amplitude of the EPSPs was measured as the highest increase in membrane potential relative to the RMP, regardless of which EPSP in the train had the highest amplitude.

2.4.4 Measurement of slow afterhyperpolarization

Slow afterhyperpolarizations that follow a square positive current pulse can last up to several seconds. In this study, sAHP was measured as the absolute value of the Vm between 100-300 ms after the current pulse. The average membrane potential 150 ms before the positive current pulse were used as baseline (Figure 12).

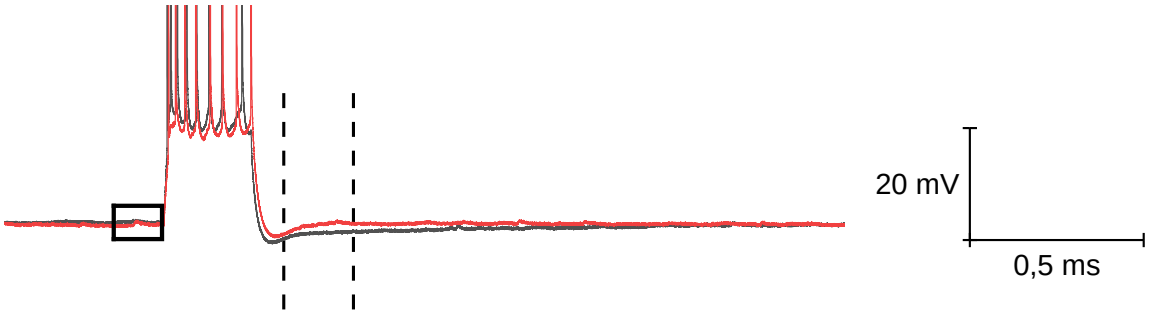

Figure 12: Measurement of slow afterhyperpolarization. The black box indicates where the baseline measurement was taken. The two vertical dotted lines indicate 100-300 ms after the depolarizing current pulse, and indicates the window in which the sAHP was measured.

A significant correlation between the number of action potentials elicited during the positive square current injection and the sAHP amplitude (Pillai et al., 2014). This is why it was important to regulate the positive current injection, so it always elicited eight action potentials during the time course.

2.4.5 Measurement of sag

Both the current-voltage protocol and the time course protocol included a hyperpolarizing current injection which was used to measure the sag caused by I_h. Sag was measured as the difference between steady-state membrane potential at the end of the hyperpolarizing current injection relative to the peak hyperpolarization at the beginning of the current injection (Figure 13).

For both the peak hyperpolarization and the steady-state hyperpolarization the value was measured in a range of 20 ms, and the average value within this range was taken. This was done in order to normalize the value and avoid measuring artifacts in the recording.

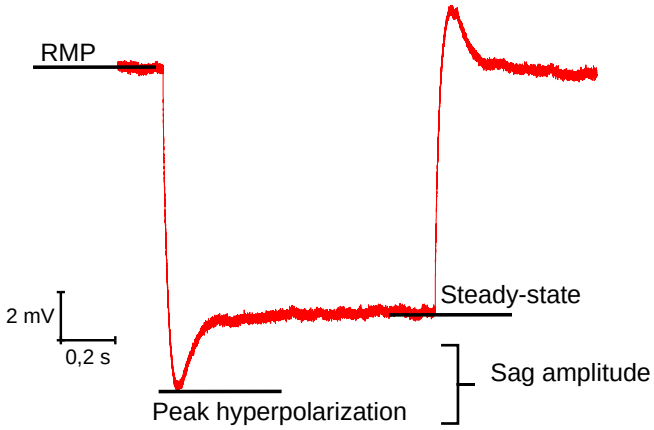

Figure 13: Measurement of sag. Black lines indicate the voltage at which the average RMP, maximum hyperpolarization and steady-state measurements were taken.

2.4.6 Measurement of input resistance

During the time course, five -30 pA square pulses lasting 200 ms each were applied at a frequency 2,5 Hz to measure the input resistance of the cell. The mean value of the last 50 ms of each pulse was used as the change in membrane potential (ΔV). The average membrane potential 200 ms before the first pulse were used as a baseline.

The input resistance during each of the five pulses was calculated using Ohm's law:

$$Input\ resistance\ M\Omega = \frac{\Delta V\ mV}{-30\ pA} * 1000$$

The final input resistance of the cell was calculated as the average from the five current pulses during each sweep.

2.4.7 Normalization of data

Where it was appropriate, the data was normalized for a more accurate representation and comparison between different recordings. The data from each recording was normalized in origin using the following formula before averaging for all the cells:

$$Normalized\ value = \frac{Measured\ value}{Average\ baseline}$$

The average baseline was calculated from the first 5 minutes of the time course before adding noradrenaline.

3 Results

More than 180 successful recordings were made during the course of the study. Most of these recordings were made to get used to the patch clamp technique and current clamp recordings, testing different neuromodulators, and optimizing the recording protocols. 20 of the recordings are presented here. These recordings were made towards the end of the study with suitable neuromodulators and an optimized protocol.

10 recordings were made from cells with 5 µM gabazine and 50 µM APV in the recording solution, with 10 µM noradrenaline added approx. 6 min into the time course recording. These cells are referred to as control cells and data from these recordings are referred to as control and noradrenaline.
The remaining 10 recordings were made from cells with 5 µM gabazine, 50 µM APV, and 100 nM corticosterone in the recording solution, with 10 µM noradrenaline and 100 µM ascorbic acid added approx. 6 min into the time course recording. These cells are referred to as cells in corticosterone and recordings from these cells are referred to as control (corticosterone) and noradrenaline (corticosterone).

3.1 Excitatory postsynaptic potentials

In this study postsynaptic potentials were studied in CA1 pyramidal cells of the hippocampus. A train of 5 stimulating pulses was delivered at 50 Hz to axons in the stratum lacunosum-moleculare in order to produce postsynaptic potentials in the CA1 pyramidal cells. The intensity of the stimulus was adjusted arbitrarily for each cell to obtain an adequate level of summation that was detectable against background noise. The stimulation intensity was this different for cells within the same group. 5 µM gabazine was used to block inhibitory postsynaptic potentials, so that only EPSPs remained, and the amplitude of their summation is what we measured in this study.

Figure 14 shows example traces of the EPSP during the time course recording for the control cells and cells in corticosterone in the absence and presence of noradrenaline.

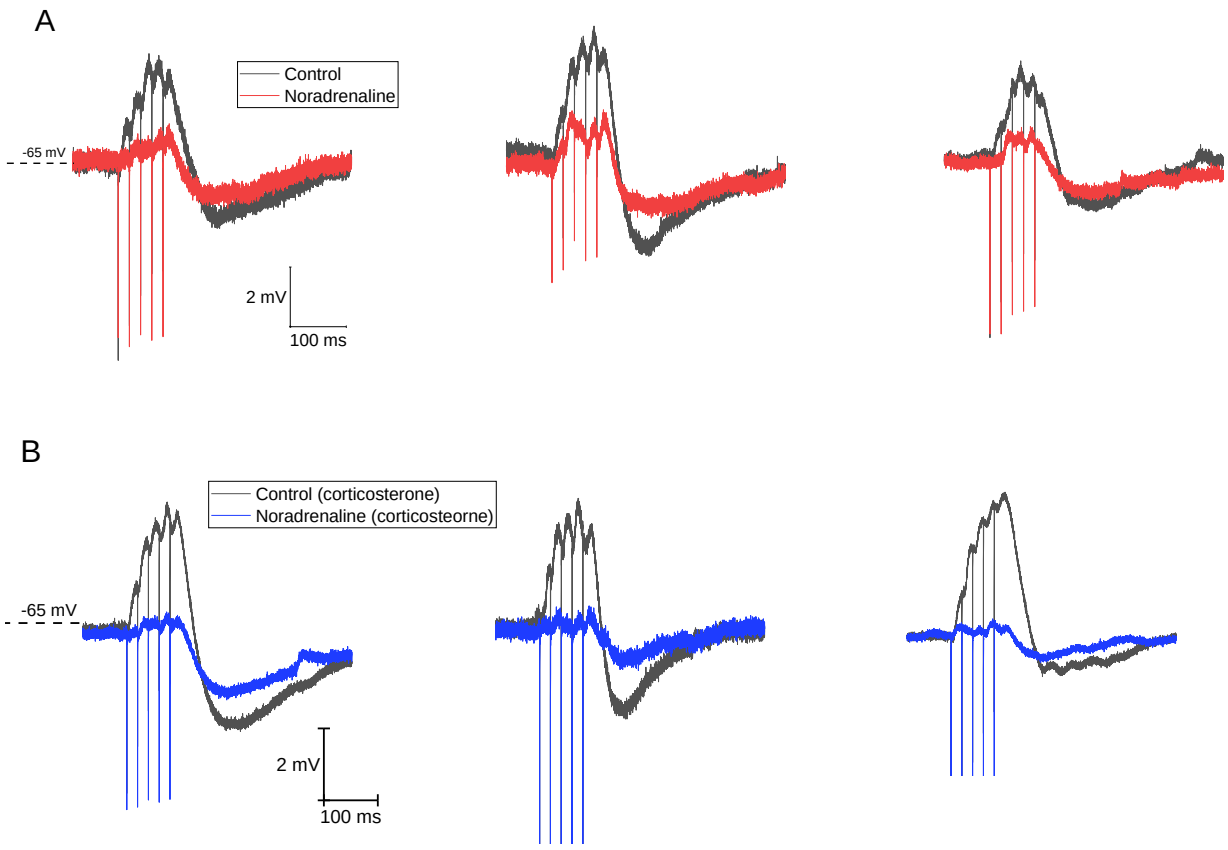

Figure 14: Example traces of the EPSP summation from cells where the EPSP amplitude decreased after the addition of noradrenaline. EPSP amplitude was measured in CA1 pyramidal cells of the hippocampus during stimulation of the stratum lacunosum-moleculare. All cells were held at a RMP of -65 mV. **A.** Traces from three different control cells where the EPSP amplitude decreased after adding 10 µM noradrenaline. Black line: EPSP summation during control conditions, Red line: EPSP summation after adding noradrenaline. **B. T**races from three different cells incubated in corticosterone where the peak EPSP amplitude decreased after adding 10 µM noradrenaline. Gray line: EPSP summation during control conditions, Blue line: EPSP summation after adding noradrenaline.

To investigate how the addition of noradrenaline changed the EPSP amplitude over time the average EPSP amplitude was plotted. Figure 15 illustrates how the addition of noradrenaline decreased the average peak EPSP in control cells and cells incubated in corticosterone during the time course recording.

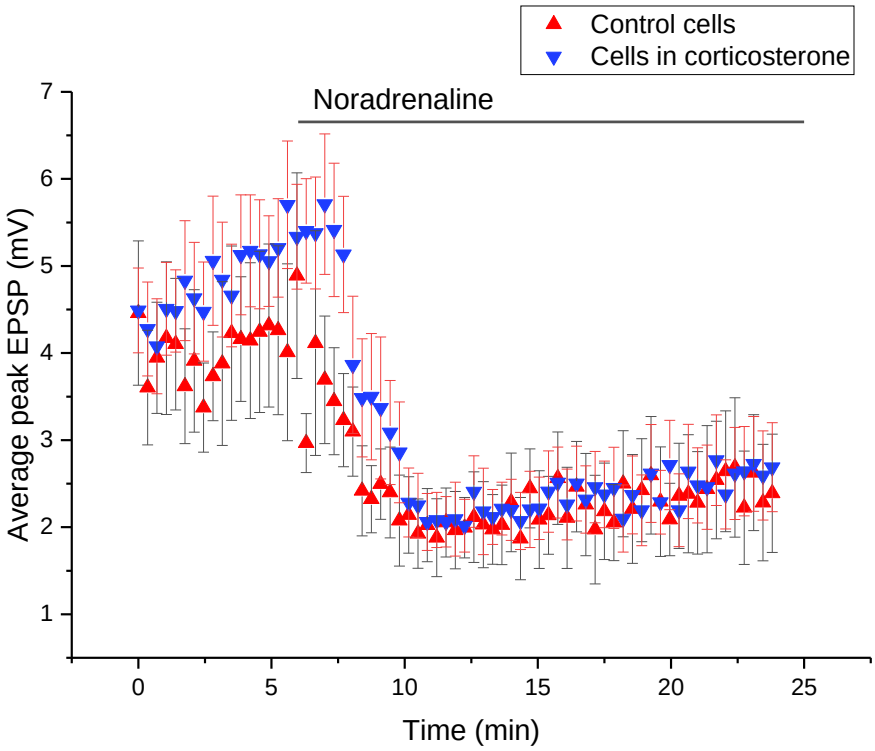

Figure 15: Change in average peak EPSP amplitude over the time course. Average peak EPSP amplitude during the time course recording measured compared to RMP (-65 mV). 10 µM noradrenaline was added 6 min into the time course. Red triangles: control cells (n = 10). Blue triangles: cells incubated in 100 nM corticosterone (n = 10). Error bars represent the SEM.

In Figure 15 the average peak amplitude of the EPSP during the first 5 min of recording was 3.99 ± 0.08 mV for control cells and 4.72 ± 0.09 mV for cells in corticosterone. During the last 5 min of the recording, in the presence of noradrenaline, the average peak EPSP decreased to 2.42 ± 0.04 mV for control cells and 2.53 ± 0.05 mV for cells in corticosterone. This means that the average peak EPSP amplitude decreased 39.82 % for the control cells, and 46.40 % for the cells incubated in corticosterone.

In the control cells the peak EPSP amplitude significantly decreased in 9/10 cells and remained unchanged in 1/10 cells after adding 10 µM noradrenaline during the time course recording. When the cells were incubated in 100 nM corticosterone for prior to recording, the peak amplitude of the EPSP decreased in 8/10 cells and remained unchanged in 2/10 cells after adding noradrenaline (Appendix).

Since the amplitude of the stimulation was adjusted arbitrarily for each cell, the data had to be normalized in order to accurately compare the effect of noradrenaline on peak EPSP amplitude between the two cell groups (Figure 16 A). In order to quantify the variability

between the control and noradrenaline conditions both for the control cells and the cells in corticosterone a box and whisker plot was constructed (Figure 16 B.)

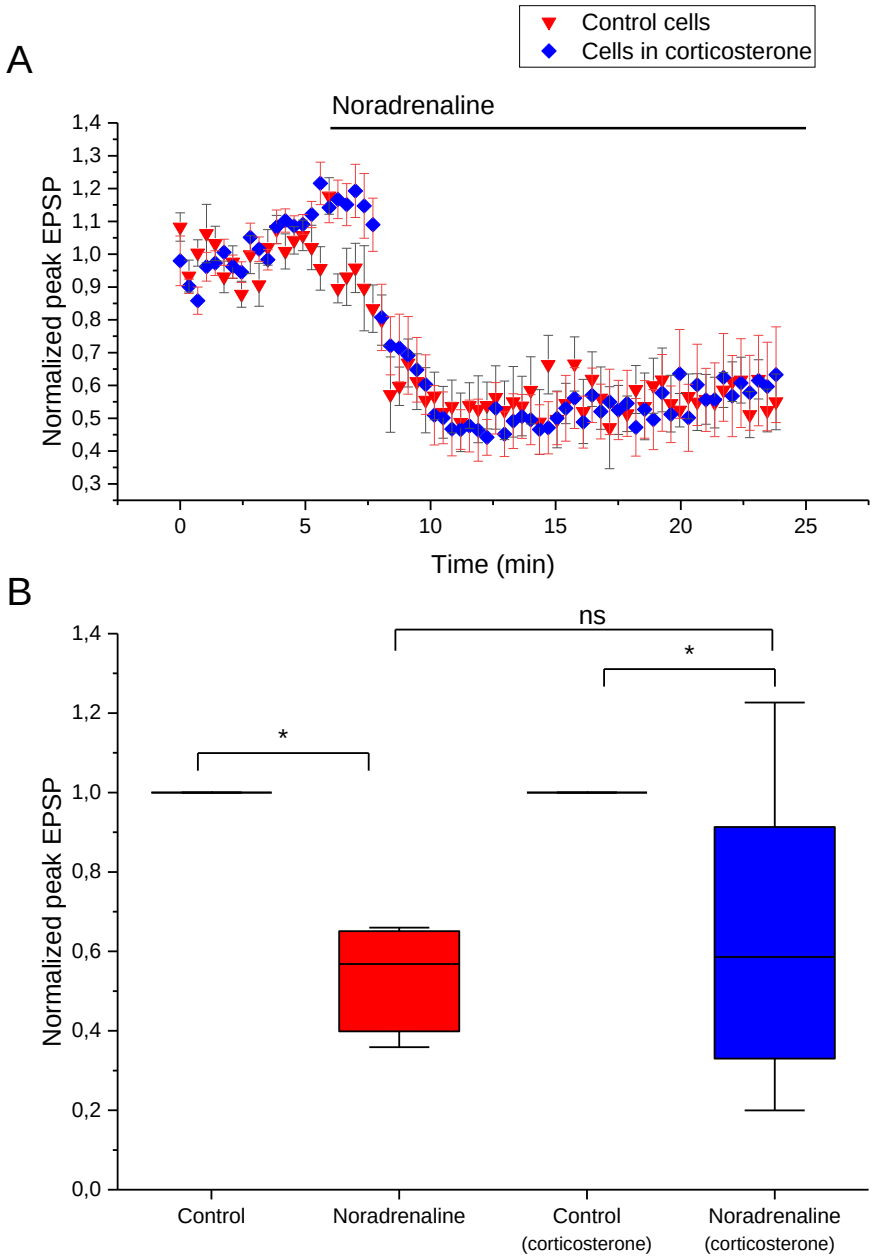

Figure 16: Normalized and averaged change in average peak EPSP amplitude over the time course. A. Normalized average peak EPSP amplitude during the time course 10 µM noradrenaline was added 6 min into the recording. Red triangles: control cells (n = 10). Blue squares: cells incubated in 100 nM corticosterone (n = 10). Error bars represent the SEM. **B.** Quantification of the difference in average peak EPSP during the first 5 min (control) and last 5 min (noradrenaline) of the time course between control cells and cells in corticosterone. Boxes represent the first to third quartile, the line in the box represents the mean value, and the whiskers represent the lower and higher 1,5 inter quartile range. * $p < 0.05$, ns $p > 0.05$.

Figure 16 A. illustrates that after the peak EPSP values have been normalized, there is no significant difference between the control cells and the cells in corticosterone. This is further confirmed in Figure 16 B, where there was no statistically significant difference in the normalized peak EPSP after adding noradrenaline between the two cell groups ($p = 0.8953$). As for the difference between the control and noradrenaline conditions there was a significant difference both for the control cells ($p = 0.0001$), and the cells incubated in corticosterone ($p = 0.0059$).

3.2 Slow afterhyperpolarization

As mentioned in section 1.5.3, sAHP plays a role in regulating the excitability and repolarization of neurons after they fire APs. A positive square current pulse was applied to the cells to elicit a train of APs followed by a sAHP in the time course protocol. Since it has been found that the number of APs influences the amplitude of sAHP (Pillai et al., 2014), the amplitude of the positive current was adjusted manually to consistently elicit eight APs.

Figure 17 shows example traces of the sAHP amplitude during the time course for both the control cells and cells in corticosterone.

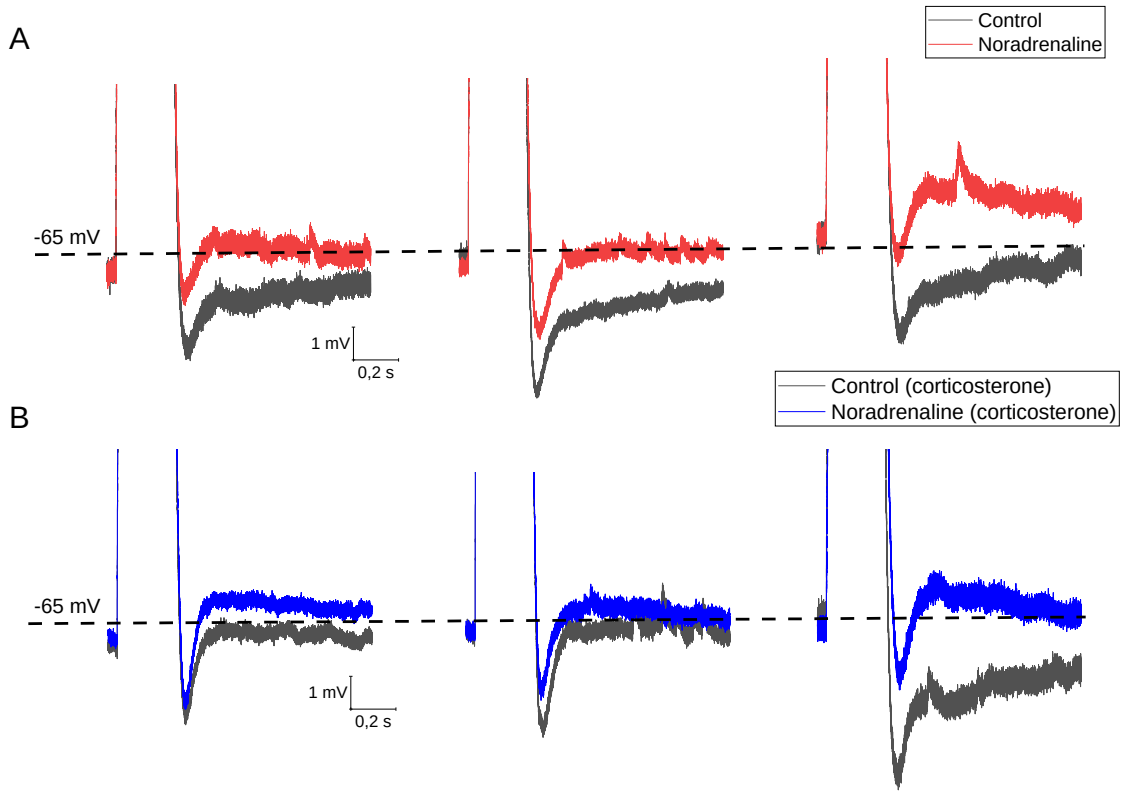

Figure 17: Example traces of the sAHP from cells where sAHP decreased after the addition of noradrenaline. All cells were held at -65 mV. **A.** Traces from three different control cells where the sAHP decreased after adding 10 µM noradrenaline. Black line: sAHP during control conditions, Red line: sAHP after adding noradrenaline. **B.** Traces from three different cells incubated in corticosterone where the sAHP decreased after adding 10 µM noradrenaline. Gray line: sAHP during control conditions, Blue line: sAHP after adding noradrenaline.

There was a significant block, or decrease, in sAHP in 9/10 of the control cells after the addition of noradrenaline, with an increase in sAHP observed in the remaining cell. For the cells incubated in corticosterone the sAHP was significantly decreased in 10/10 cells.

Figure 18 A. shows that for both the control cells and cells incubated in corticosterone the sAHP decreased (became more positive) after noradrenaline was added to the recording solution. For the control cells the sAHP decreased from -0.83 ± 0.02 mV to 0.58 ± 0.01 mV. For the cells in corticosterone the sAHP decreased from -0.18 ± 0.03 mV to 0.94 ± 0.03 mV. For the cells incubated in corticosterone, the sAHP overall is more depolarized compared to the control cells.

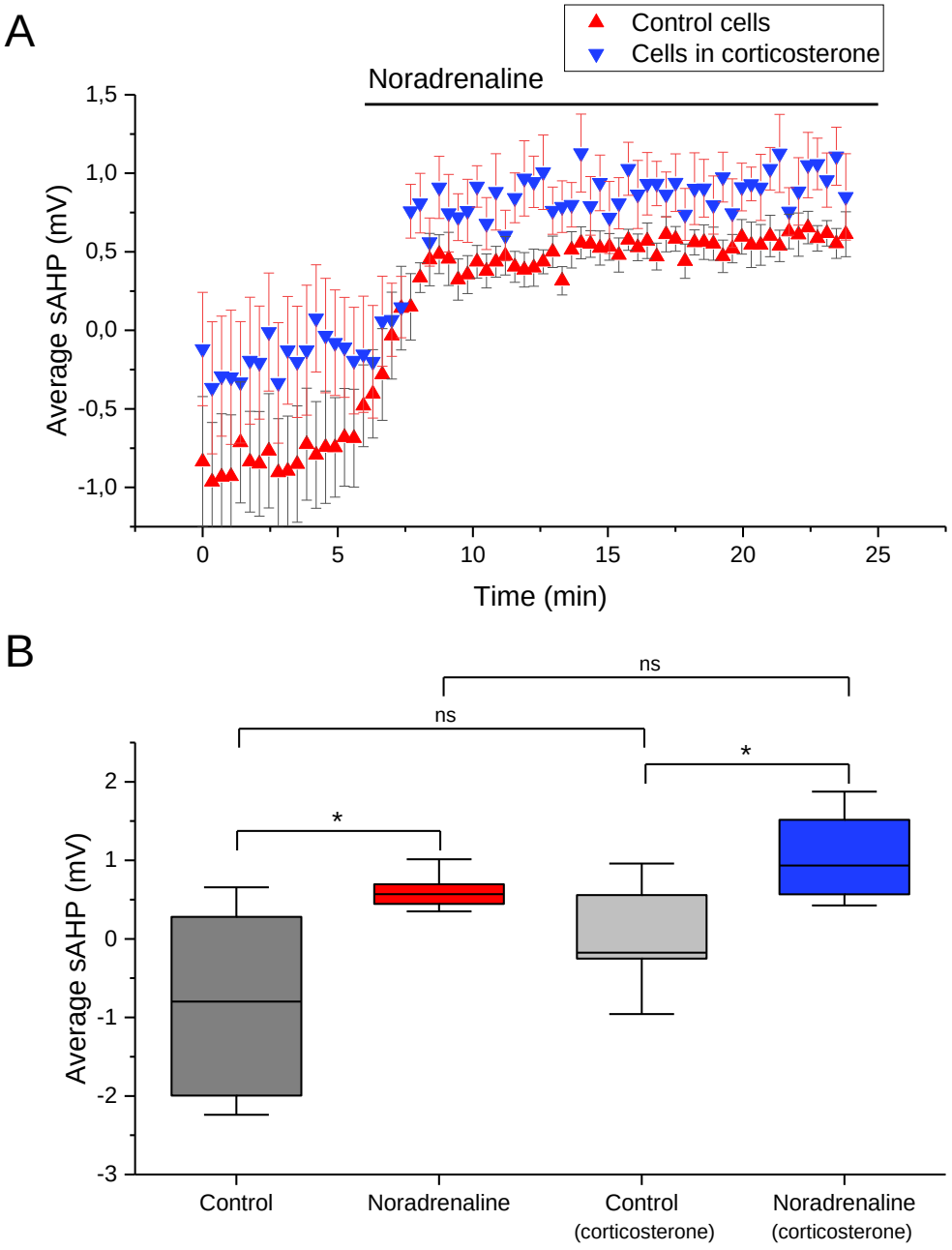

Figure 18: Change in average sAHP after adding noradrenaline for control cells and cells incubated in corticosterone. A. Average sAHP measured compared to RPM (-65 mV). 10 µM noradrenaline was added 6 min into the recording. Red triangles: control cells (n = 10). Blue triangles: cells incubated in corticosterone (n = 10). Error bars represent the SEM. **B.** Quantification of the difference in average sAHP during the first and last 5 min of the time course recording. The two boxes to the left represent the control cells, the two boxes to the right represent cells in corticosterone. * $p < 0.05$, ns $p > 0.05$.

In Figure 18 B. it can be seen that after noradrenaline was added to the recording solution there was a significant difference in the average sAHP for both the control cells ($p = 0.0025$)

and the cells incubated in corticosterone (p = 0.0046). There was no significant difference between the control conditions (p = 0.2255) nor between the noradrenaline conditions (p = 0.0666).

3.3 Sag amplitude

Sag is caused by the opening of HCN channels during a hyperpolarization, which allows entry of Na^+ and K^+, to depolarize the cell. The HCN channel is activated by hyperpolarization, but its activity is voltage dependent; channel opening and prolonged activity becomes more likely as the membrane potential gets more negative. The amplitude of the sag is a measure of the amount of HCN channels that are open, as a larger sag amplitude generated by the same level of hyperpolarization indicates that more HCN channels are open.

As seen from the traces in Figure 19, the sag amplitude increased for both the control cell and cell in corticosterone in the presence of noradrenaline. The traces in Figure 19 are taken from the current-voltage protocol where the peak hyperpolarization for the control and noradrenaline conditions were at the same level. It can be seen that in the presence of noradrenaline the steady state amplitude is decreased in the noradrenaline condition, meaning that the sag amplitude is increased.

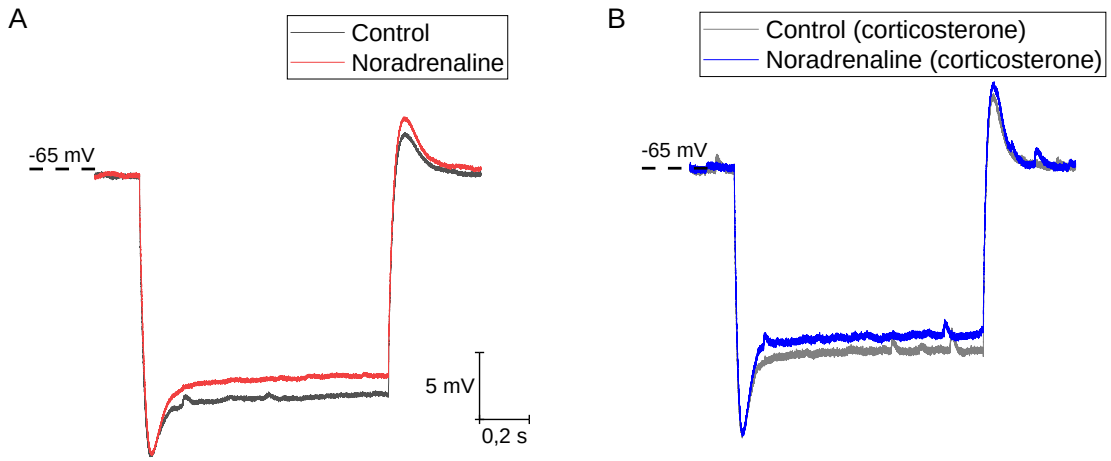

Figure 19: Traces of the sag from the current-voltage protocol. All traces are from the -500 pA current pulse during the current-voltage protocol. The cells were held at -65 mV. **A.** Traces of the hyperpolarization and sag from a control cell. Black trace: control condition, Red trace: noradrenaline condition. **B.** Traces of the hyperpolarization and sag of a cell incubated in corticosterone. Grey trace: control condition, Blue trace: noradrenaline condition.

The average changes in sag amplitude during the time course protocol are plotted in Figure 20 A, and the changes in sag amplitude between the control cells and corticosterone incubated cells are quantified in Figure 20. B.

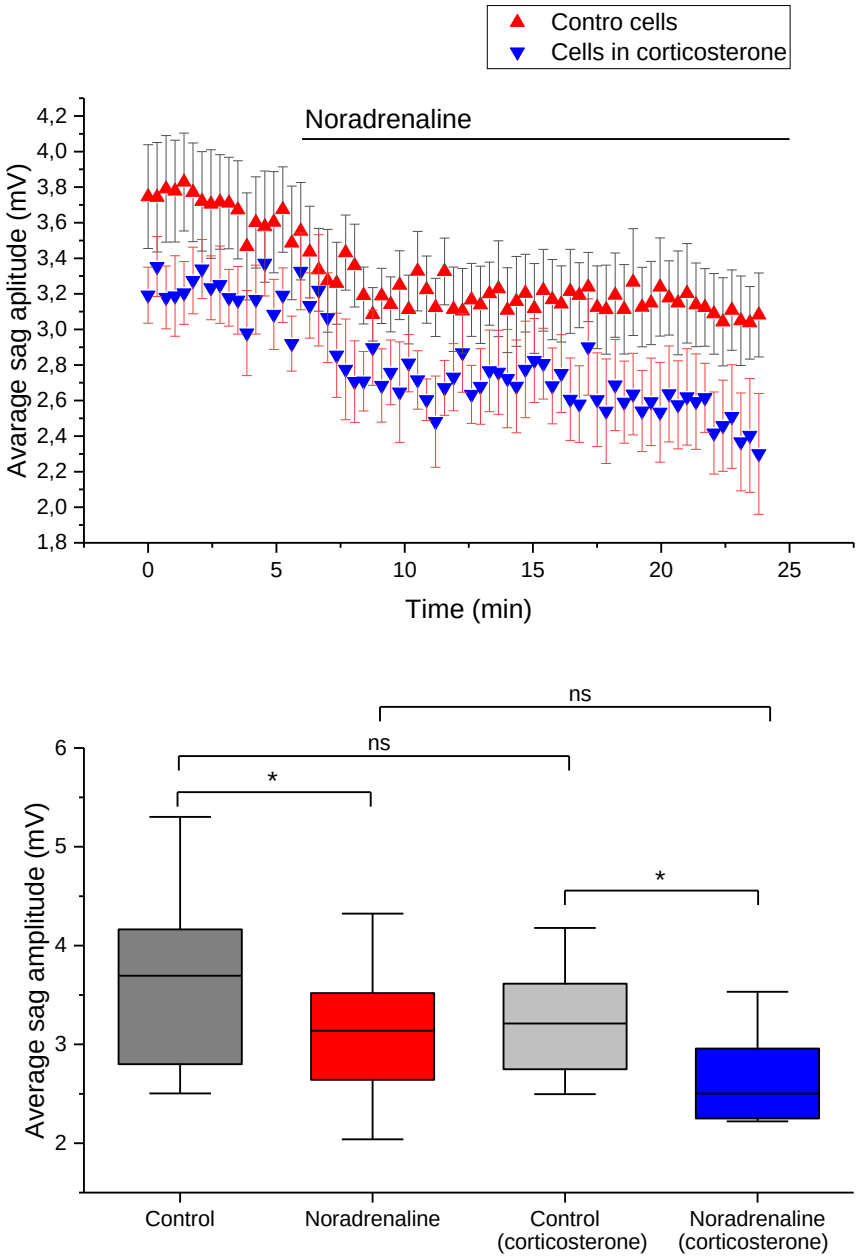

Figure 20: Average changes in sag amplitude after adding noradrenaline for control cells and cells incubated in corticosterone. A. Average sag amplitude in response to a -200 pA current pulse during the time course. 10 µM noradrenaline was added 6 min into the recording. Red triangles: control cells (n = 10). Blue triangles: cells incubated in corticosterone (n = 10). Error bars represent the SEM. **B.** Quantification of the difference in average sag amplitude during the first and last 5 min of the time course. The two boxes to the left represent the control cells. The two boxes to the right represent the cells incubated in corticosterone. * $p < 0.05$, ns $p > 0.05$.

During the time course recording the sag amplitude decreased significantly in 10/10 control cells after adding noradrenaline. For the cells incubated in corticosterone the sag amplitude decreased significantly in 8/10 cells, and did not change significantly in 2/10 cells after noradrenaline was added.

As can be seen from figure 20 A. the sag amplitude in response to a -200 pA current pulse decreased for both control cells and cells incubated in corticosterone after noradrenaline was added during the time course recording. The overall sag amplitude appears to be lower for the cells incubated in corticosterone, but statistical analysis indicated that it was not significant as seen in Figure 20 B..

The average sag amplitude during the first 5 min of the recording was 3.70 ± 0.02 mV for the control cells and 3.21 ± 0.03 mV for the cells in corticosterone. During the last 5 min of the recording the sag amplitude had increased to 3.14 ± 0.02 mV for the control cells and 2.52 ± 0.03 mV for the cells in corticosterone. This means that the sag amplitude decreased 15.14 % for the control cells and 22.12 % for the cells in corticosterone. From Figure 20 B. we see that there is a significant difference in sag amplitude after adding noradrenaline for both the control cells ($p = 0.0024$) and cells incubated in corticosterone ($p = 0.0070$). There was no significant difference between the two groups during the control condition ($p = 0.1547$) nor after adding noradrenaline ($p = 0.0895$).

As mentioned in section 1.5.2, sag is voltage dependent, as well as being dependent on the activity of HCN channels. Since I_h and sag are voltage-dependent, the amplitude of the sag generated at a range of membrane potentials was measured, in order to correct for the effect of input resistance on the data. Sag amplitude was plotted against the absolute peak hyperpolarization (Figure 21 A. and B.) from the current-voltage recordings (Figure 9).

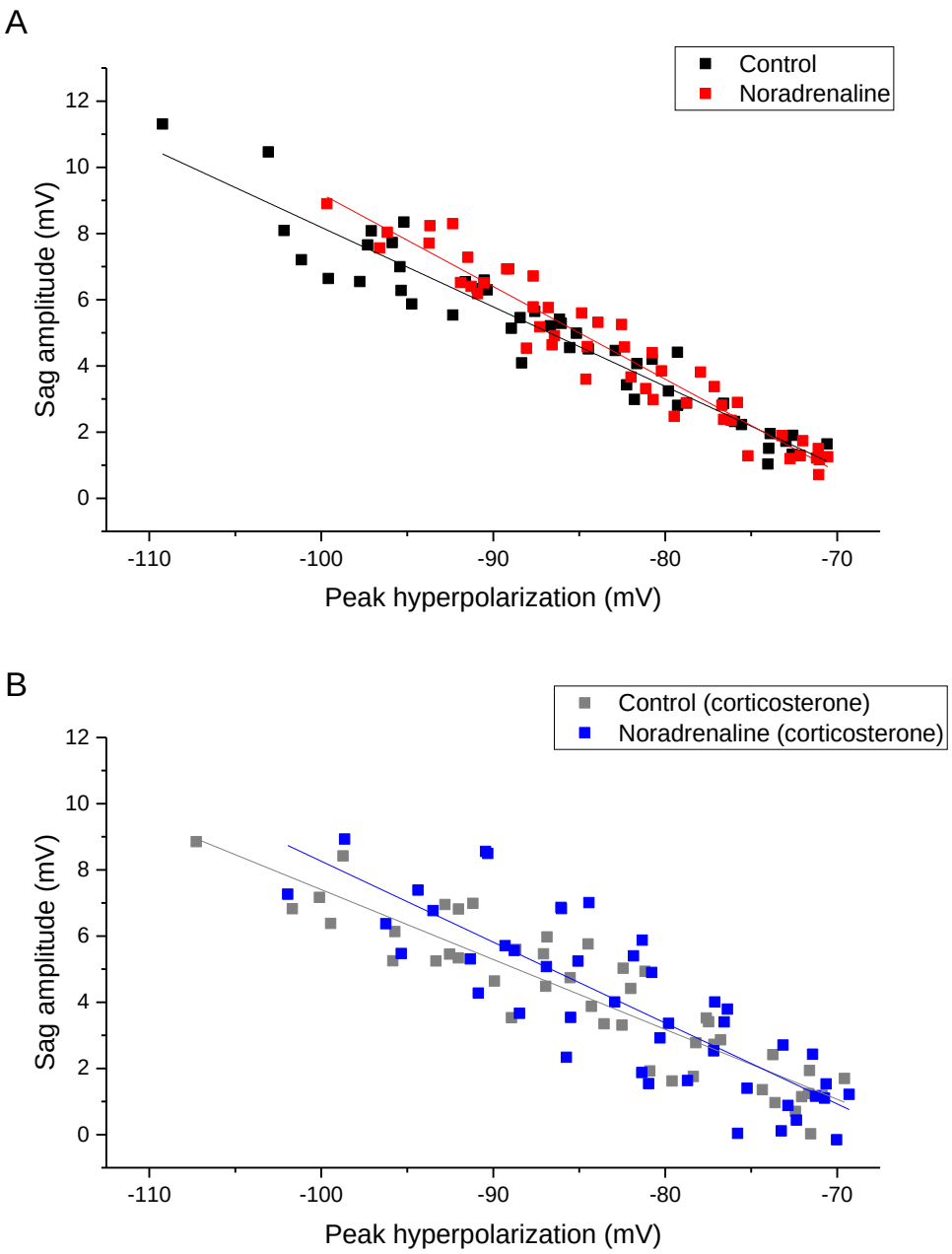

Figure 21: Change in sag amplitude in relation to the absolute peak hyperpolarization. Data was taken from each of the five negative current steps in the current-voltage protocol, and a linear fit line was added in Origin. **A.** Sag amplitude plotted against the absolute peak hyperpolarization for the control cells (n = 10). Black squares: control conditions. Red squares: noradrenaline conditions. Black line: linear fit for the control conditions, slope: -0.2403, R^2: 0.9256. Red line: linear fit for the noradrenaline condition, Slope: -0.2799, R^2: 0.9328. **B.** Sag amplitude plotted against the absolute peak hyperpolarization for the cells incubated in corticosterone (n = 10). Gray squares: control conditions. Blue squares: noradrenaline conditions. Grey line: linear fit for the control conditions, Slope: -0.2112, R^2: 0.8349. Blue line: linear fit for the noradrenaline condition, Slope: -0.2445, R^2: 0.6871.

From the trend lines in Figure 21 it can be seen that in the presence of noradrenaline a smaller peak hyperpolarization is needed to produce a sag with the same amplitude than before the addition of noradrenaline. This is true for both the control cells and cells incubated in corticosterone. In both Figure 21 A. and B. the slope of the fitted line is steeper after the addition of noradrenaline.

3.4 Input resistance

As mentioned in section 1.5.4 input resistance is a measure of how much current needs to be applied to the cell to change the voltage potential of the membrane. When the input resistance is high it means that less current in needed to change the membrane potential.

The input resistance has a statistically significant decrease in 9/10 of the control cells, with a significant increase in 1/10 cells after the addition of noradrenaline. For the cells incubated in corticosterone there was a significant decrease in input resistance in 10/10 cells in the presence of noradrenaline.

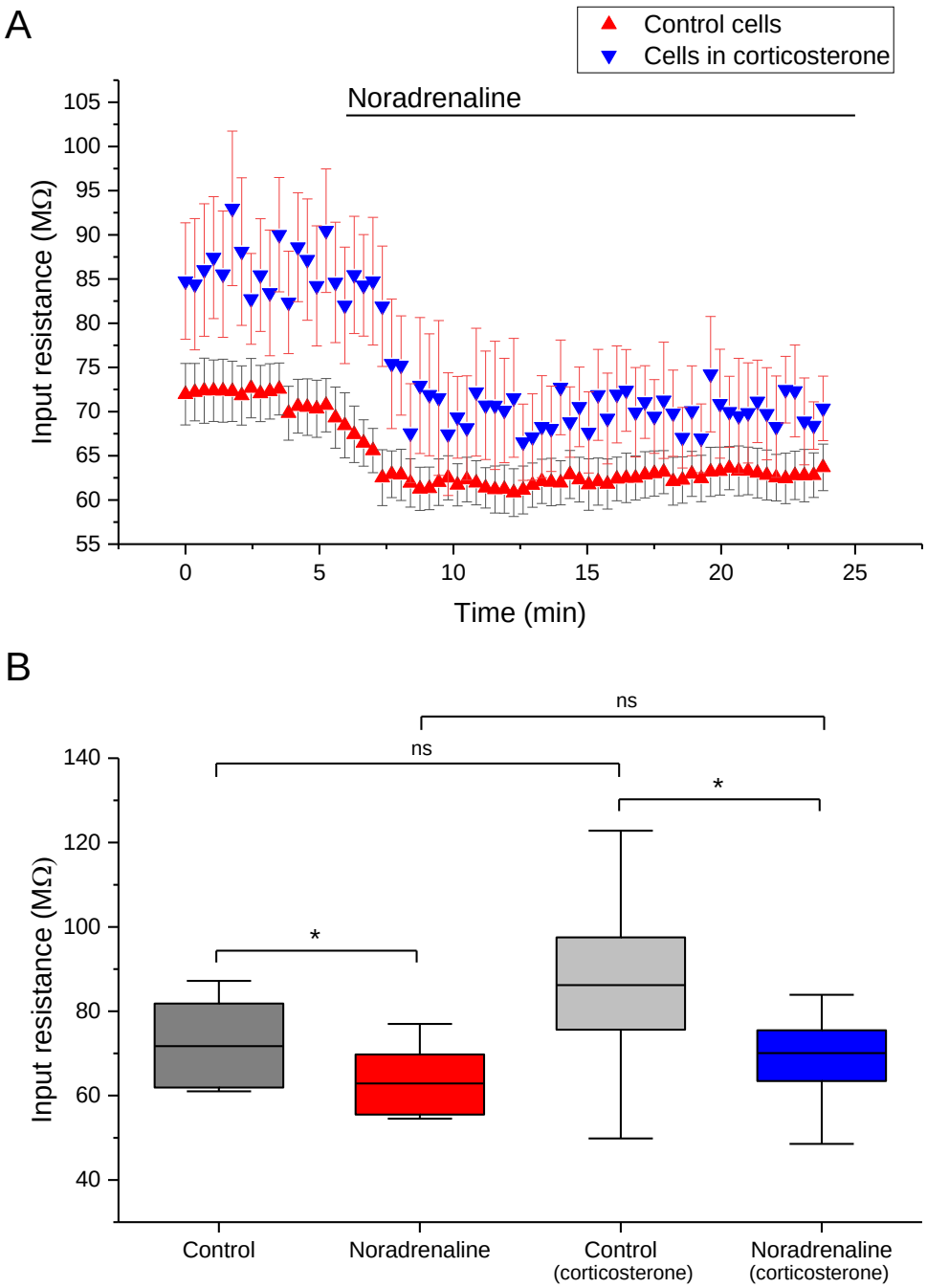

Figure 22: Average changes in input resistance after adding noradrenaline for control cells and cells incubated in corticosterone. A. Average sag amplitude during the time course. 10 µM noradrenaline was added 6 min into the recording. Red triangles: control cells (n = 10). Blue triangles: cells incubated in corticosterone (n = 10). Error bars represent the SEM. **B.** Quantification of the difference in average input resistance during the first and last 5 min of the time course. The two boxes to the left represent the control cells. The two boxes to the right represent the cells incubated in corticosterone. Boxes represent the first to third quartile, the line in the box represents the mean value, and the whiskers indicate the SEM. * $p < 0.05$, ns $p > 0.05$.

Figure 22 A. shows that for both the control cells and the cells incubated in corticosterone the input resistance decreased in the presence of noradrenaline. In the control cells the input resistance decreased from 71.74 ± 0.24 MΩ to 62.99 ± 0.10 MΩ. For the cells incubated in corticosterone the input resistance decreased form 86.22 ± 0.75 MΩ to 70.21 ± 0.47 MΩ. This means that the input resistance decreased 12.36 % for the control cells and 18.78 % for the cells in corticosterone. The input resistance decreased slightly more after the addition of noradrenaline for the cells incubated in corticosterone. Overall, the input resistance was higher in the cells incubated in corticosterone throughout the entire time course.

From figure 22 B. we see that there is a significant difference in input resistance after adding noradrenaline for both the control cells ($p = 0.0035$) and cells incubated in corticosterone ($p = 0.0006$). There was no significant difference between the two groups during the control condition ($p = 0.0620$) nor after adding noradrenaline ($p = 0.1668$).

3.5 Cell depolarization

During a recording session the RMP of the cell was noted before and after any recordings were made. For both the control cells and the cells in corticosterone the RMP was depolarized after recording, when there was noradrenaline present, Table 2.

Table 2: Comparison of the average RMP before and after a recording.

	Average RMP in control conditions	Average RMP after adding noradrenaline
Control cells (n = 10)	-62.2 ± 1.00 mV	-59.9 ± 1.19 mV
Cells in corticosterone (n = 10)	-63.7 ± 1.25 mV	-62.2 ± 1.23 mV

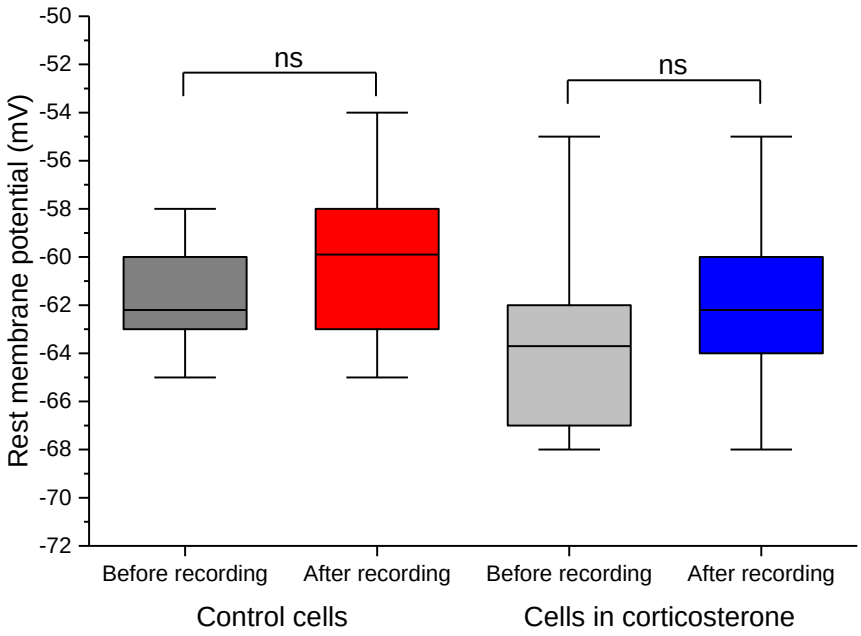

Figure 23: Quantification of the difference in average RMP before and after the addition of noradrenaline. The two boxes to the left represent the control cells. The two boxes to the left represent the cells incubated in corticosterone. Boxes represent the first to third quartile, the line in the box represents the mean value, and the whiskers indicate the SEM. * $p < 0.05$, ns $p > 0.05$.

The difference in RMP for the control cells before and after adding noradrenaline was not found to be significant ($p = 0.0938$), similarly the difference for the cells in corticosterone was also not found to be significant ($p = 0.2235$) (Figure 23).

4 Discussion

The results show that the presence of noradrenaline influences the electrophysiological properties in neurons *in vitro*, as expected. However, there was no significant difference between the control cells and the cells incubated in corticosterone, indicating that corticosterone did not affect or modulate information processing in the CA1 pyramidal cells of the hippocampus.

As mentioned in Section 1.6, when noradrenaline is released during stress it binds to β-adrenergic receptors which activate a second messenger cascade, resulting in the production of cAMP. cAMP can then bind to HCN-channels, facilitating their opening and increasing I_h. The increase in I_h can explain the decrease in peak EPSP, blocked sAHP, increased sag amplitude, decreased sag ratio and decreased input resistance observed in this study.

4.1 Methodological issues that might affect the results obtained

4.1.1 Whole-cell patch clamp recordings

In this study, the whole-cell patch clamp configuration was used, which is an invasive technique and can affect the electrophysiological properties of a cell. When the patching pipette breaks through the cell membrane the intracellular environment of the cell and pipette become contiguous. The intracellular solution in the pipette is meant to closely resemble the intracellular solution in the cell, however, it will still lack some of the components found in a cell's cytosol. Over time, the solutions will diffuse and mix, slightly changing the intracellular composition of the cell, which can affect the studied properties.

Similarly, the recording solution that the slices are submerged in is a simplified version of a neuron's extracellular environment (cerebrospinal fluid), so the *in vitro* recordings made during this study will not be an exact representation of the *in vivo* effects noradrenaline and corticosterone has on modulation of information processing.

4.1.2 Influence of electrophysiological property interactions

Adding neuromodulators such as noradrenaline and corticosterone during a recording will cause a change in the electrophysiological properties of the cell. However, it can be difficult to determine whether the neuromodulator directly influenced a property, or if an observed change was the cause of another property changing. In section 1.5.2 it was mentioned that the sag amplitude is voltage dependent, and thus indirectly dependent on input resistance. Similarly, the input resistance will also influence the EPSP amplitude and the sAHP. Additionally, both EPSPs and the sAHP have been shown to modulate each other (section 1.5.3).

4.1.3 Intracellular solution and recording solution

Ideally, when investigating the effect of a compound on neuromodulation, only one parameter should change so that one can be sure that an observed change is caused by the added compound.

As mentioned in section 2.3.4 50 µM ascorbic acid was added to the recording solution together with noradrenaline, to act as an antioxidant and prevent the oxidation of noradrenaline. Ascorbic acid was only added during the recordings of cells incubated in corticosterone, and not during the control cell recordings. The reason for this was that the oxidation of noradrenaline was not raised as a concern before the end of the study, so there was no time to obtain new recordings for the control cells with noradrenaline and ascorbic acid. However, studies have reported that when noradrenaline is oxidized a brownish color is formed (Lamden and Harris, 1950), and this was not observed during any of the recordings. Additionally, noradrenaline showed a strong effect in the control cells as well, strengthening the notion that noradrenaline was not oxidized to an extent that affected the recordings.

Similarly, corticosterone was aliquoted in 70 % ethanol, so for the cells incubated in corticosterone both the ethanol or the corticosterone could have caused any observed changes. To prevent this, recordings were made prior to the corticosterone incubation where only 70 % ethanol was added during the time course recording. No significant changes were observed in any of the electrophysiological parameters after adding ethanol (data not shown), suggesting that any changes found in the presence of corticosterone are caused by corticosterone and not ethanol.

4.1.4 Temperature

Lastly, the recordings made in this study were performed ranging from 31.5 – 32.5 °C, which is different from a rat's physiological temperature of 3.,9 – 37.5 °C. For the whole-cell recording, the temperature was kept lower than the physiological temperature to slow down metabolic processes and keep the slices healthy longer (Booker et al., 2014). Changes in temperature have been reported to affect properties of neurons. Brain temperature has a larger effect on synaptic transmission than it has on action potential generation (Andersen and Moser, 1995).

As a consequence of the lower temperature used during the recordings the measured neuronal properties might deviate from what they would be under physiological temperature. The overall effect of noradrenaline and corticosterone should however be the same.

4.2 Noradrenaline decreases EPSP summation

For the control cells the peak EPSP amplitude decreased in 9/10 cells after noradrenaline was added to the recording solution. For the cells incubated in corticosterone the peak EPSP amplitude decreased in 8/10 cells after noradrenaline was added. For the remaining cells from both groups, the peak EPSP amplitude did not change. From Figure 24 in the Appendix, it can be seen that for the two cells incubated in corticosterone where the EPSP amplitude remained unchanged the EPSP amplitude initially decreased in the presence of noradrenaline, but slowly went back to baseline during the remainder of the time course. Since the first and last 5 min of the time course recording were compared, no significant change was reported from these cells. For the control cells the average peak EPSP decreased 39.82 %, and for the cells in corticosterone it decreased 46.40 % (Figure 15).

After normalizing the peak EPSP data for the control cells and cells in corticosterone, to account for the arbitrary stimulation amplitude used to elicit EPSPs, there was no statistically significant difference in the average peak EPSP summation between control cells and cells in corticosterone. For both groups the peak EPSP amplitude decreased after adding noradrenaline. For the control cells the normalized average EPSP amplitude decreased from 1.00 ± 0.02 mV to 0.56 ± 0.01 mV, and the cells in corticosterone decreased from 1.00 ± 0.02 mV to 0.58 ± 0.01 mV, which were not statistically different (Figure 16).

The change in peak EPSP amplitude can be attributed to noradrenaline increasing I_h. Some HCN-channels will be open at a cells RMP, and these channels help stabilize the RMP. As mentioned in section 1.5.2 the HCN channel has dual activation by membrane voltage and cAMP (Wahl-Schott and Biel, 2009). When noradrenaline is added the cAMP concentration increases so more HCN channels to open and the I_h increases (Marzo et al., 2009). The I_h causes the influx of Na^+ and K^+ ions that help depolarize the membrane potential towards RMP during a negative current pulse. It can therefore be counterintuitive that an increase in I_h decreases the EPSP amplitude.

The decrease in EPSP amplitude observed when I_h is increased can be attributed to a decrease in input resistance (Magee, 1998, Fan et al., 2005) and the theorized modulation of other ionic currents by I_h (Migliore and Migliore, 2012). A significant change in the input resistance following noradrenaline administration was observed for both the control cell and the cells in corticosterone (Figure 22). One of the ionic currents that is modulated by I_h is the M-current caused by the opening of voltage-gated K^+ channels, also called delayed rectifier K^+ channels (Kv7/ KNCQ) (George et al., 2009). George et al. found that when the M-current was active in CA1 pyramidal cells, an increase in I_h had an inhibitory effect an decreased the EPSP amplitude. This effect was not observed when the M-current was blocked. Additionally, the depolarization of the RMP that happens when HCN-channels open was showed to enhance the M-current (George et al., 2009). Interestingly, the voltage-gated K^+ channel is also modulated by cAMP, where the presence of cAMP increases its activity (Schroeder et al., 1998). It has also been found that blocking I_h prevents the decrease in EPSP amplitude (Fan et al., 2005, George et al., 2009).

Similarly, since corticosterone has been found to increase the intracellular cAMP concentration through the activation of β-adrenergic receptors (see Section 1.6), a decrease in EPSP amplitude was also expected for the cells incubated in corticosterone.

Even though 5 µM gabazine was used in the recording solution to block inhibitory synaptic $GABA_A$ transmission, some $GABA_B$ dependent IPSPs remained. Figure 14 shows that the presence of noradrenaline decreased the amplitude of the IPSP that followed the EPSP. As mentioned in section 1.5.1 noradrenaline has been shown to decrease synaptic inhibition (Madison and Nicoll, 1988). Lancaster et al. also observed that in the presence of noradrenaline the hyperpolarization following EPSPs was reduced, and they proposed that this might be due to a reduced I_{sAHP} (Lancaster et al., 2001).

4.3 Noradrenaline blocked the slow afterhyperpolarization with no significant effect observed from corticosterone

Noradrenaline significantly decreased the sAHP that follows an AP train. As mentioned in section 1.5.3 the sAHP is a Ca^{2+}-activated K^+-current (I_{sAHP}). It is known that noradrenaline and cAMP block I_{sAHP} (Lancaster et al., 2001, Madison and Nicoll, 1986b), while incubation with corticosterone increases the amplitude of the sAHP (Pillai et al., 2014, Joels and de Kloet, 1989).

Noradrenaline is known to decrease I_{sAHP} through the activation of β-adrenergic receptors, cAMP and PKA which block the Ca^{2+}-activated K^+-current (Madison and Nicoll, 1986b, Pedarzani and Storm, 1993, Lancaster et al., 2001).

Corticosterone on the other hand, has been shown to increase I_{sAHP} by enhancing Ca^{2+}-currents. Calcium entry into a neuron during AP firing is essential for opening the Ca^{2+}-activated K^+-channels that underly sAHP (Pillai et al., 2014). In the study by Pillai et al., the delayed, genomic effect of corticosterone during stress was stimulated by incubating hippocampal slices in 100 nM corticosterone, and recording from the slices 1-4 h after incubation.

Noradrenaline and corticosterone thus have opposite effects on the sAHP. Little is known about the interaction of noradrenaline and corticosterone with regards to the sAHP. The amplitude of the sAHP itself is influenced by several neurotransmitter and neuromodulators, and it is the interaction between these that determine the ultimate outcome on the sAHP. A study performed by Joels and de Kloet found evidence that glucocorticoids, such as corticosterone, could reduce the excitability of cells caused by neurotransmitters like noradrenaline (Joels and de Kloet, 1989).

Since noradrenaline and corticosterone have opposing effects, one would expect an increased sAHP amplitude in the cells incubated in corticosterone, compared to the control cells, even in the presence of noradrenaline. Surprisingly, a decrease, rather than an increase in sAHP amplitude was observed (Figure 18).

As shown in Figures 17 and 18, the sAHP amplitude decreased in both control cells and cells in corticosterone after adding noradrenaline. Unexpectedly, the average sAHP amplitude was

much smaller for the cells in corticosterone (-0.18 ± 0.03 mV) compared to control cells (-0.83 ± 0.02 mV). After adding noradrenaline, the sAHP was blocked and actually turned into a slow afterdepolarization (sADP) in both groups. Again, surprisingly the sADP was larger for the cells in corticosterone (0.94 ± 0.03 mV), compared to the control cells (0.58 ± 0.01 mV). From these results it seems like corticosterone increased, rather than reduced, the cell effect of noradrenaline. However, after performing two-sample t-test, no significant difference was found between the control cells and the cells in corticosterone.

Pillai et al. also found a correlation between sAHP amplitude and the number of APs elicited during the depolarizing current step. More APs corresponded to a stronger sAHP. During the recording the positive current injection was monitored and adjusted to produce eight action potentials. This was, however, technically difficult, so sometimes seven or nine action potentials were elicited. This happened relatively infrequently, so it should not have had a large effect on the observed and measured sAHP.

As mentioned in 4.1.2 several properties can influence sAHP, such as EPSP, RMP and input resistance. Previous studies have not found corticosterone to have an effect on input resistance or RMP (Pillai et al., 2014, Joels et al., 2008). Noradrenaline, on the other hand has been found to depolarize the RMP and decrease input resistance (Joels et al., 2008, Marzo et al., 2009). For the control cells and the cells incubated in corticosterone there was not a significant difference in average RMP (Table 2), nor in input resistance (Figure 22 B.), so this can not explain the observation that corticosterone did not significantly change the effect of noradrenaline.

Another explanation might be the corticosterone incubation time. In this study, cells were incubated in 100 nM corticosterone for 1h prior to recording. From similar studies studying the effect of corticosterone on sAHP amplitude a wide range of corticosterone incubation times have been reported; ranging from 20 min (Joels and de Kloet, 1989) to over 4 (Pillai et al., 2014). A longer incubation time will make sure that any effect of corticosterone on the sAHP is caused by its genomic actions, and not by any fast, non-genomic actions. Future experiments could investigate the relationship between corticosterone incubation time and sAHP amplitude, as well as the modulatory effect of corticosterone incubation time on the action of noradrenaline.

Lastly, as mentioned in section 1.5.3 the EPSP summation and sAHP influence each other. The decrease in EPSP amplitude following noradrenaline administration was attributed to an increase in I_h. I_h has been found to affect mAHP (Gu et al., 2005), but no clear effect has been found on sAHP, so this could be interesting to study in the future.

4.4 Noradrenaline decreased sag amplitude

Sag is caused by the opening of HCN channels in CA1 pyramidal cells during a hyperpolarization of the cell's membrane. Noradrenaline indirectly affects the sag by increasing the intracellular cAMP concentration, which activates HCN channels.

As seen from Figure 20, noradrenaline decreased the sag amplitude in both control cells and cells in corticosterone, which is contradictory to the previously discussed view that noradrenaline increases I_h. This can be explained by the decrease in input resistance that was observed following the addition of noradrenaline (Figure 24). Since the negative current step that hyperpolarized the membrane and caused the sag remained the same, the decrease in input resistance resulted in a decreased hyperpolarization, and therefore a decreased sag. No significant difference in sag amplitude was observed between the control cells and the cells in corticosterone (Figure 20).

In Figure 21 the sag amplitude was plotted against the peak hyperpolarization to cancel out the effect of input resistance. In both Figure 21 A. and B. the slope of the linear fit for the cells in the presence of noradrenaline is steeper, meaning that a smaller peak hyperpolarization is needed to produce a sag of the same amplitude. In other words, at the same peak hyperpolarization, in the presence of noradrenaline, the sag amplitude would be increased.

A curious observation is that the measured sag amplitude was increased in the current-voltage recording that was taken after adding noradrenaline (Figure 19). The decrease in input resistance that was observed during the time course was also seen in the current-voltage data (data not shown). One would therefore expect the sag amplitude to be decreased here as well, not increased. An explanation could be that the negative current step was larger (-500 pA compared to -200 pA), meaning that the increased current could dominate the effect of the decreased input resistance and increase the membrane potential, resulting in an increased sag.

4.5 Noradrenaline decreased input resistance

As expected, the input resistance decreased following the administration of noradrenaline in both the control cells and cells incubated in corticosterone (Figure 22). Many studies have shown the excitatory effect of noradrenaline, which is in part due to a decrease in input resistance (Joels et al., 2008, Marzo et al., 2009). As mentioned in 4.4.1 the decrease in input resistance might explain why the sag amplitude decreased during the time course recording.

No statistical difference was found in input resistance between the control cells and cells in corticosterone, neither in the absence or presence of noradrenaline (Figure 22).

4.6 Noradrenaline had no significant effect the resting membrane potential of cells

As seen from Figure 23, noradrenaline did not significantly change the average resting membrane potential of the cells. As mentioned before, noradrenaline is an excitatory neurotransmitter which has been shown to significantly depolarize the RMP of CA1 pyramidal cells. Noradrenaline depolarizes the resting membrane potential through activation of the β-adrenergic receptor, which increases the cAMP concentration and opens more HCN channels and increases I_h, even when the membrane potential is at rest. The I_h causes the influx of Na^+ and K^+ ions that depolarizes the RMP (Grzelka et al., 2017, Marzo et al., 2009).

The reason why a statistically significant effect on the RMP after noradrenaline administration was not observed might be that the variation within each of the cell groups was quite large, compared to the number of cells (n = 10) in each group. In the study by Grzela the membrane potential significantly decreased by 3.4 ± 0.3 mV (n = 11), while in this study the membrane potential decreased by 2.3 ± 1.2 mV. The greater variation in RMP obtained in this study can be a result of experience, the patch clamp technique is quite difficult to master, and had only been practiced for a little over a year, as well as small variations that might have occurred during the preparation of the brain slices which could have affected the viability of the cells and their RMP.

4.7 There was no significant difference between the control cells and the cells incubated in corticosterone

In all of the electrophysiological properties measured, no significant difference was found between the control cells and the cells incubated in corticosterone, indicating that corticosterone did not modulate the effect of noradrenaline for the properties investigated.

Corticosterone has previously been found to enhance the effect of noradrenaline during stress (section 1.6), but this was the non-genomic, short term action of corticosterone. In this study, the focus was mainly on the interaction between the delayed genomic effect of corticosterone on modulation of noradrenaline.

As mentioned in section 1.4.2, both noradrenaline and corticosterone influence synaptic plasticity during stress, and corticosterone mediates the effect of the β-adrenergic receptor, increasing the cAMP concentration (Zhou et al., 2012, Marzo et al., 2009), so corticosterone was hypothesized to modulate the action of noradrenaline.

As stated in section 4.6, the variability between cells within the same group (control cells or cells in corticosterone) was quite high. This can clearly be seen by the large error bars in the time course overviews and box plots. The variability between cells might have been caused by slight differences when preparing the slices which might have affected the viability of the cells.

Lastly, due to time constraint as well as difficulties mastering the patch clamp technique, only a small number of recordings were made that could be used in this study. Given more time, additional recordings could be made which could significantly decrease the within cell variability and might show an effect of corticosterone on modulation of the actions of noradrenaline.

5 Conclusion

The results from this study suggest that corticosterone has no significant effect on the action of noradrenaline in regards to the electrophysiological parameters that were measured.

For both the control cells and the cells incubated in corticosterone the EPSP summation decreased, the sAHP decreased, the sag amplitude decreased, and the input resistance decreased during the time course of each whole-cell recording.

Both the effect of noradrenaline on EPSP summation, sAHP and input resistance were as expected, but the effect on sag amplitude was opposite of the expected response. This could however, be explained by the change in input resistance that was observed.

It was also observed that corticosterone did not significantly influence the action of noradrenaline on the sAHP. Corticosterone and noradrenaline have previously been shown to have opposite effects on sAHP amplitude. An explanation for why no significant effects were observed might be the corticosterone incubation time.

The results seem to suggest that corticosterone does not significantly modulate the action of noradrenaline, but this conclusion might have changed if more recordings were made and included in the data, decreasing the variability and error within the cell groups.

6 Future perspectives

In future experiments, several factors may be considered in order to further investigate the interaction between corticosterone and noradrenaline during stress, and their effect on dendritic signaling processing.

Firstly, different incubation times for corticosterone can be tested, to ensure that it has a near maximal effect on the sAHP before performing any recordings, as proposed in section 4.3.

Additionally, recordings including a washout after the application of noradrenaline could be interesting, to see if the effect of noradrenaline on the electrophysiological properties returns to baseline, or if the effect is lasting. Since noradrenaline is a neurotransmitter, the effect on signal processing should subside quickly after noradrenaline is washed out. A longer lasting effect in the corticosterone incubated cells could hint further at a plausible interaction between noradrenaline and corticosterone.

Lastly, as stated in section 4.6 and the conclusion, due to the time constraint only a limited number of recordings for the control cells and cells in corticosterone were made. Continuing the investigation and making more recordings will hopefully decrease the observed variability within the two different cell groups, and elucidate clearer differences between the groups.

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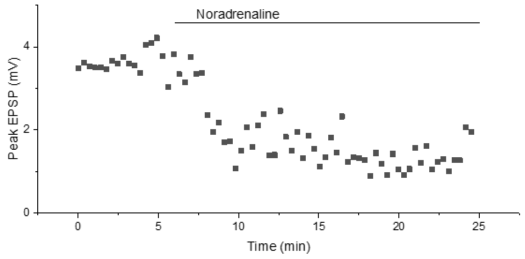
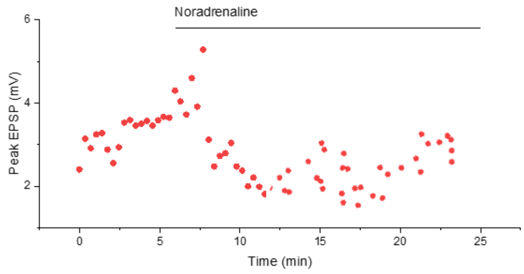
8 Appendix


Noradrenaline
Peak EPSP (mV)
Time (min)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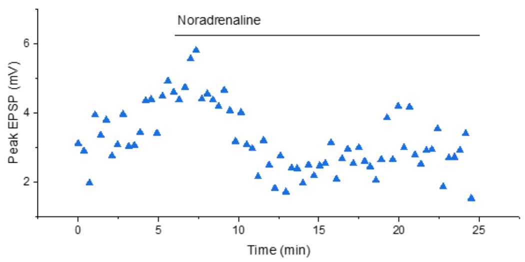
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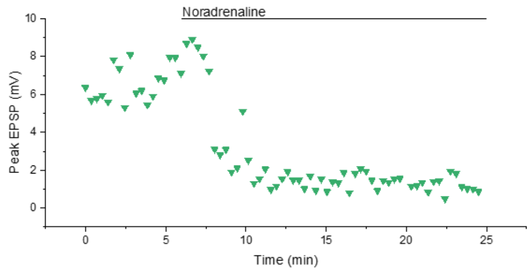
Peak EPSP (mV)
Time (min)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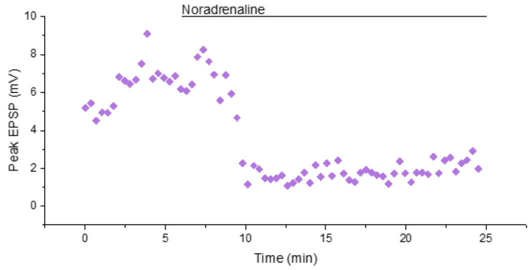
Time (min)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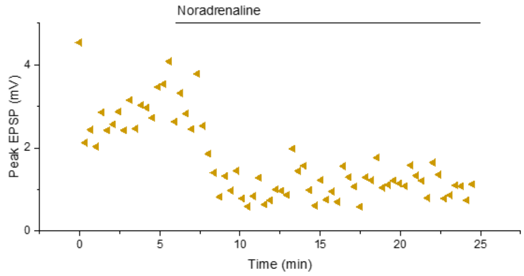
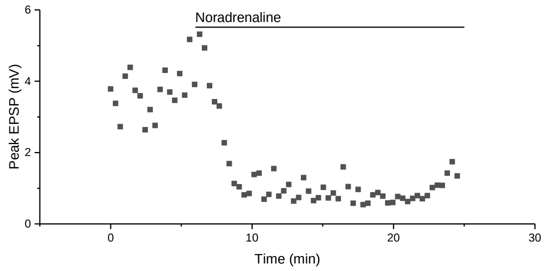
Peak EPSP (mV)
Time (min)


Noradrenaline
Peak EPSP (mV)
Time (min)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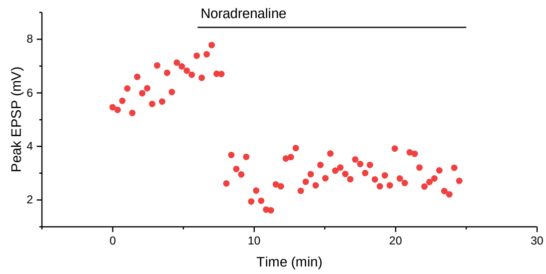
Peak EPSP (mV)
Time (min)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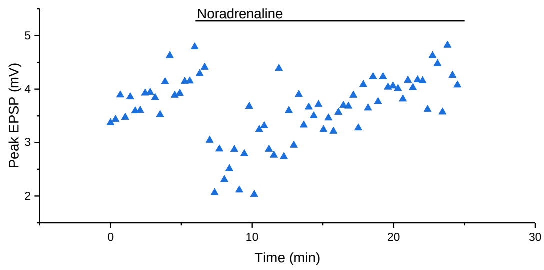
Time (min)


Noradrenaline
Peak EPSP (mV)
Time (min)

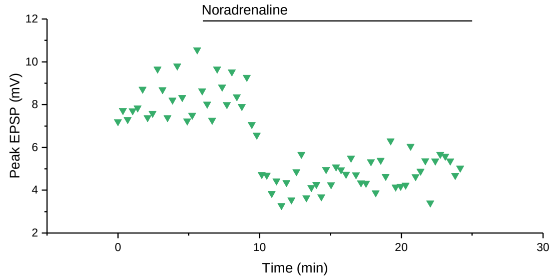

Noradrenaline
Peak EPSP (mV)
Time (min)

Figure 24: Overview of the peak EPSP time course for each individual cell incubated in corticosterone (n = 10). The peak EPSP decreased in 8/10 cells. In the remaining 2/10 cells (indicated by red boxes) the peak EPSP initially decreased but then went back to baseline, so no significant statistical difference was observed when comparing the peak EPSP amplitude during the first and last 5 min of the time course recording.

Table 3: Components of the cutting solution and the recording solution. The chemicals were dissolved in ddH_2O

Cutting solution		**Recording solution**	
Chemical	**Concentration (mM)**	**Chemical**	**Concentration (mM)**
NaCl	87	NaCl	125
$NaHCO_3$	25	$NaHCO_3$	25
KCl	2.5	KCl	2.5
NaH_2PO_4	1.25	NaH_2PO_4	1.25
Glucose	25	Glucose	10
Sucrose	74.8	$MgCl_2$	1
$MgCl_2$	4	$CaCl_2$	2
$CaCl_2$	1		