Universitätsklinikum Hamburg-Eppendorf

Institut für Molekulare und Zelluläre Kognition

Prof. Dr. Dietmar Kuhl

The role of Arc/Arg3.1 in protein synthesis dependent and independent forms of synaptic plasticity

Dissertation

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Johanne Kläschen
aus Eutin

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Prüfungsausschuss, dritte/r Gutachter/in: Prof. Dr. G. Rune

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1 INTRODUCTION

The devastating effects of memory loss can be observed in neurocognitive diseases such as Alzheimers' Disease or Fragile X mental retardation. Dementia is not only accompanied by the loss of independence, but also leads to a disruption of self-perception and the patients' social context. Proper response to the environment of all species from invertebrates to humans relies on learning and memory, making these traits essential for survival.

Memory formation describes the ability of the mind to store and later recall information. It enables us to be aware of ourselves and to shape our behavior by learned and subsequently memorized information. One important feature of memory formation which cannot be found in regard to other cognitive functions is that formation of memory could be observed from the outside e. g. by behavioral testing of animals. Thus this endlessly fascinating mental process becomes accessible to investigation enabling scientists to combine behavioral studies in animals with cellular and molecular analysis. Subsequent progress in understanding the underlying cellular and molecular mechanisms of memory formation will pave the way for a deeper understanding of this fundamental function of the brain. By this mean also potential therapeutic targets for the treatment of neurological diseases involving dementia will be revealed.

1.1 LEARNING AND MEMORY

Specific forms of memories were distinguished by different characteristics such as their content or the time period in which they could be recalled. On the basis of their duration short and long term memories were distinguished. Short term memory describes the temporal storage of information for a period of seconds to minutes whereas long term memory describes the ability of information to be stored for extended periods of time ranging from hours to days and even lifelong.

Long term memory could be further differentiated into explicit and implicit memory (Squire 1987). Explicit memory is defined as conscious remembering and contains episodic, i. e. remembering of events in time, and semantic information, i. e. factual knowledge of the external world. Explicit memory is encoded in the

hippocampus and later represented in cortical brain areas using bidirectional cortical-hippocampal connections (Eichenbaum 2000). Studies of animals and humans using functional imaging or cortical lesions showed that information is represented in the cortical areas which are specialized for processing this particular kind of information. In contrast, implicit memory is the memory for perceptual and motor skills which are not intentionally recallable. It includes procedural memory, e. g. the knowledge how to ride a bicycle, based on rather subcortical areas as the striatum (Packard and Knowlton 2002) or cerebellum and emotional memory involving the amygdala (Bechara et al. 1995).

The definition of different types of memory systems and the understanding of the underlying neural mechanisms of memory formation in general made a big progress in 1957 by the studies of Brenda Milner. She studied a patient known by his initials as H.M. whose medial temporal lobe was bilaterally removed in the context of epilepsy surgery. Besides an amelioration of the seizures H.M. had a serve anterograde amnesia and a temporally graded retrograde amnesia. The storage of new, declarative information was severely impaired whereas his short term memory, personality and general intelligence remained intact. Brenda Milner concluded that *„whenever the hippocampus and hippocampal gyrus were damaged bilaterally, some memory deficit was found, but not otherwise.“* (Scoville and Milner 1957). This was the first evidence that the medial temporal lobe, a brain area containing the hippocampus and its adjacent cortex including the *entorhinal*, *perirhinal* and *parahippocapal cortices*, is essential for the encoding and consolidation of new explicit information. In contrast, retrieval of remote childhood memories and factual knowledge acquired early in life remained intact in H.M. suggesting that memories become independent of the medial temporal lobe at some time (Eichenbaum 2000). Furthermore his personality and procedural knowledge, e. g. skills and habits, appeared to be unchanged thereby supporting the theory that different memory systems exist in parallel specialized for different kinds of information processing. Consolidation of memories is thought to be established by two succeeding processes. First consolidation is thought to occur on a synaptic level lasting hours mediated by the hippocampus and neocortex. In parallel or later a much slower process of system consolidation lasting weeks creates a cortical representation by network activity is initiated (Dudai 2004, Frankland & Bontempi 2005). Thereby information retrieval initially dependent on the hippocampus becomes independent on the hippocampus

and retention is maintained by the cortical brain areas supporting this type of memory. This model of gradual consolidation of memory requiring different brain regions at different steps of consolidation could well account for phenomena like retrograde amnesia in H.M. (Squire & Alvarez 1995).

External information is thought to be represented in the brain by pattern activity in networks of neurons. It is agreed that events leading to modifications of neuronal networks in turn result in modifications of behavior. The engram of learning and memory is therefore thought to be found in the adaptive shape of neuronal networks formed through plastic modification of neural plasticity (Bliss & Collingridge 1993).

First insight into the cellular mechanisms underlying memory formation was gained by the study of the modification of a reflex by experience in invertebrates. In this way Eric Kandel studied the phenomena of sensitization, habituation and classic conditioning at the gill withdrawal reflex in the giant marine snail *Aplysia* 50 years ago (Kandel 2001). His studies revealed first molecular cascades employed in memory formation which were later shown to be conserved in mammals.

Phenomena, like sensitization and classic conditioning, were primarily constituted as psychological approaches by the behaviorists at the turn of the century and then later transferred to the developing field of neuroscience in biology. Nowadays the immense capacity and specificity of memory is thought to at least partly depend on the plasticity of neuronal networks. Distinct patterns of synaptic activation are thought to cause an increase or decrease in the efficacy of synaptic transmission between neurons, thereby shaping synaptic circuits and neuronal networks. This provides a mechanism by which experience-dependent shapes of neuronal networks might cause a modification of the output, i. e. the resulting behavior.

1.2 SYNAPTIC PLASTICITY

It is generally accepted that memory is formed and maintained by physical alterations of the properties of neurons and their connections in response to stimulation (Bliss & Collingridge 1993, Kandel 2001, Malenka & Bear 2004). Activity-dependent changes of synaptic strength result in a modification of neuronal networks thus modifying the processing of information in the brain.

In 1894, the famous neuroscientist Santiago Ramón y Cajal hypothesized that memory is stored in the growth of new connections between neurons (Cajal 1894). One hypothesis how this could be mediated was later introduced by Donald Hebb in 1949, who postulated that an increase in synaptic efficacy is based on the repeated and persistent stimulation of postsynaptic neuron by a presynaptic neuron: *"When an axon of cell A is near enough to excite cell B and repeatedly and persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A´s efficiency, as one of the cell firing B, is increased"* (Hebb 1949). This idea was later summarized by the term "synaptic plasticity" and shaped the idea of a coincidence-detection rule. Thus the term "synaptic plasticity" describes the capability of neurons to alter the strength of their synapses, i. e. how effectively they transfer information, in response to activity.

In 1973 Bliss and Lømo found indeed first experimental evidence for a long-lasting potentiation of synaptic transmission in the dentate gyrus in the anaesthetized rabbit (Bliss and Lømo 1973). This phenomenon, later termed long-term potentiation (LTP), was defined as a long-lasting increase in the postsynaptic response after stimulation. Today *in vivo* long term field recordings using chronically implanted electrodes in awake animals could demonstrate that LTP could last for months and even for a year (Abraham et al. 2002).

Especially because of its persistence, LTP attracted much interest as a candidate model for mammalian memory formation and has been a target of intensive research in neuroscience for the last four decades (Bliss & Collingridge 1993). This capacity of persistence actually led to the "synaptic plasticity and memory" (SPM) hypothesis that *"Activity-dependent plasticity is induced at appropriate synapses during memory formation, and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which the plasticity is observed"* (Martin et al. 2000).

Much research using *in vivo* and *in vitro* experiments focused on LTP as a model for synaptic plasticity and tried to link it to behavioral studies of learning in animals. Thereby further compelling parallels between LTP and memory were revealed. Memory and synaptic plasticity both consist of at least two distinct phases, short-term and long-term memory and the early and late phase of LTP, respectively. Information, once encoded, needs to be transformed from an initially labile state into a more stable state less susceptible to interference and extinction (Dudai 2004). As a prominent

characteristic shared by memory formation and synaptic plasticity this consolidation process requires a critical time of *de-novo* protein synthesis during and short after acquisition or induction, respectively (Nader et al. 2000, Kandel 2001). Consistent with the observation that learning is accompanied by structural changes, such as increases in spine density (Lamprecht & LeDoux 2004), corresponding changes were found in response to LTP induction (Engert & Bonhoeffer 1999, Bourne & Harris 2008). Morphological changes including modifications of spine size and shape and their corresponding postsynaptic densities (PSD) as well as the formation of new spines are assumed to be involved in the stabilization of long-lasting increases of synaptic efficacy (De Roo et al. 2008). Such forms of structural plasticity were also shown to be dependent on protein synthesis (Yang et al. 2008).

Further shared properties make it likely that LTP plays an important role in memory formation. Thus plenty evidence was found that at least in parts the same signaling cascades are involved in LTP and memory formation. For example both memory formation and synaptic plasticity were found to depend on N-methyl D-aspartate (NMDA)-type glutamate receptor activation (Morris et al. 1986, Davis & Butcher 1992). Furthermore activation of Ca^{2+}-dependent kinases, such as protein kinase A and mitogen activated protein kinase (MAPK, also known as extracellular regulated kinase (ERK)), was shown to be required for the induction of long-lasting LTP as well as memory (Abel et al. 1997, Kelleher et al. 2004). Moreover, similar modifications in the hippocampus were induced by behavioral paradigms (Whitlock 2011).

Activity-dependent plasticity may change synaptic strength in opposite directions, while LTP increases it, long-term depression (LTD) persistently weakens it. This decrease in synaptic efficacy is mainly thought to be mediated by an increase in AMPA receptor (α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA)-type glutamate receptor) endocytosis leading to a reduction of surface AMPA receptors. Different types of LTD, NMDA and metabotropic glutamate receptor (mGluR)-dependent LTD, were distinguished on basis of the receptors activated during induction (Massey & Bashir 2007). Thus bidirectional plasticity of synaptic transmission provides a proper mean by which integration within neuronal circuits might be shaped.

Besides synaptic plasticity, which is in the focus of this study, other forms of plasticity such as intrinsic or structural plasticity contribute to the plasticity of the

nervous system. Intrinsic plasticity describes the capability of neurons to alter their level of excitability as a homeostatic mechanism or in the context of metaplasticity. Structural plasticity describes the modification of the structure of a synapse, e. g. the size of the postsynaptic density or its spine morphology.
Dysfunction of synaptic plasticity was shown to be involved in a variety of neurological and neuropsychological disorders such as addiction, Alzheimer's disease and other mental disorders (Nestler 1997).

1.3 LONG-TERM POTENTIATION

The most extensively studied form of synaptic plasticity within the brain is long-term potentiation (LTP) in the *hippocampus*. Long-term potentiation is a collective term covering different forms of synaptic plasticity that are characterized by a lasting increase in synaptic strength following a conditioning stimulus. There is a variety of conditioning stimuli, e. g. electrical induction protocols such as high-frequency stimulation (HFS) and theta-burst stimulation or chemical agents such as the brain-derived neurotrophic factor (BDNF) and the cyclic adenosine monophosphate (cAMP) analog forskolin (Huang & Kandel 1994) that induce LTP.
Three basic properties were established for LTP: cooperativity, associativity and input specificity. First, "cooperativity" determines that a sufficient strength of the conditioning stimulus is required to induce LTP, i. e. a threshold for LTP induction. Second, "associativity" describes the phenomenon that if a weak input converging on the same neuron in time with a strong input, LTP will be induced in both pathways. Third, "input specificity" defines that modifications which increase synaptic efficacy in LTP are restricted to the synapse receiving the conditioning stimulus and are not disseminated to surrounding not potentiated synapses (Bliss & Collingridge 1993).
As is the case with behavioral memory, LTP could be divided into different phases (1) an early phase of LTP (e-LTP) which is thought to be mediated by post-translational modification of pre-existing proteins and is not affected by inhibitors of protein synthesis and (2) a late phase of LTP (l-LTP) which is assumed to require transcription and translation of new proteins by its susceptibility to drugs (Huang & Kandel 1994, Kelleher et al. 2004). Blockers of translation and transcription were shown to attenuate LTP maintenance at different time

points (Nguyen et al. 1994). The induction of these different forms of LTP, transient and long-lasting LTP, was shown to require different electrical stimulation protocols for induction. Early LTP which is reported to be transient, declining to baseline within one to three hours depending on the experimental conditions, is generally induced by a single train of high-frequency stimulation (1-HFS). Whereas late LTP which is reported to persist for many hours requires several repetitive HFS-trains (typically three) to be induced (Huang & Kandel 1994, Kelleher et al. 2004).

The induction of LTP is assumed to require (1) repetitive stimulation of presynaptic excitatory cells releasing glutamate into the synaptic cleft and (2) simultaneous depolarization of the postsynaptic cell. This could be attained by the activation of ionotropic AMPA receptor leading to Na^+ influx. If AMPA receptor mediated depolarization has led to removal of Mg^{2+} block of the NMDA receptor, glutamate binding to postsynaptic NMDA receptors causes opening of its Na^+ und Ca^{2+} permeable channel. The resulting increase in the concentration of intracellular calcium ($[Ca^{2+}]_i$) through NMDA receptors is in general accepted to be essential for the induction of some forms of both LTP and LTD (Launey et al. 1999).

Signaling pathways leading from the elevation of a postsynaptic increase in $[Ca^{2+}]_i$ to enhanced efficacy of synaptic transmission have been extensively studied. There is general consensus that LTP expression in the CA1 region of the hippocampus, is mainly mediated postsynaptically but there are still some arguments pointing to a presynaptic component of LTP expression (Errington et al. 2003). The rise in $[Ca^{2+}]_i$ leads to the activation of multiple kinases, like CamKII, PKA, MAPK and in consequence ERK and other, which in turn causes posttranslational modification of preexisting proteins (Huang & Kandel 1994).

An increase in synaptic strength is commonly thought to be mediated by changes in AMPA receptor number, type and properties, i. e. single channel conductance, since AMPA receptors are the principal ionotropic receptors mediating most of the excitatory postsynaptic transmission (for review Malinow & Malenka 2002, Shepherd & Huganir 2007). With regard to LTP it was reported that direct phosphorylation of the GluR1 subunit via CamKII increases single channel conductance (Derkach et al. 1999). Furthermore it was shown that high-frequency stimulation induces a rapid insertion of GluR1 containing AMPA receptors directly into the synapse or at extrasynaptic sites wherefrom lateral diffusion to the PSD occurs (Park et al. 2004). To maintain the AMPA receptor mediated increase in

synaptic strength AMPA receptors have to be stabilized in the PSD to prevent the subsequent reduction in AMPA receptors by endocytosis. Stabilization of AMPA receptors might be mediated either by anchoring proteins which prevent interaction with the endocytic machinery or by a replacement with constitutively expressed GluR2 and GluR3 containing AMPA receptors which are more stable (Anggono & Huganir 2012, Hayashi et al. 2000, Shi et al. 2001).

Besides a remodeling of the protein assembly located in the PSD, LTP was shown to be accompanied by structural alterations of spine size, shape and number (Engert & Bonhoeffer 1999, De Roo et al. 2008). One key element of morphological changes was shown to be the rapid reorganization of the actin cytoskeleton in spines (Fukazawa et al. 2003).

Since synaptic proteins were shown to be constantly trafficked in and out of synapses and are subjects to protein turnover, it is likely that the early phase of LTP will be reversed with time unless synaptic modifications will be somehow consolidated. Evidence obtained from *in vivo* and *in vitro* studies showed that this transformation form an initial labile modification into a long-lasting, stable remodeling of the synapse, i. e. late LTP, requires synthesis of new proteins (Krug et al. 1984, Frey et al. 1988). In addition, enduring alterations of spine structure were also found to require de-novo protein synthesis (Yang et al. 2008).

Thus signaling to the nucleus to initiate protein synthesis might provide new proteins necessary to stabilize the rearrangement of protein networks determining synaptic strength. One well described pathway coupling synaptic activity to gene expression is via the cAMP responsive element (CREB), which is activated in a [Ca^{2+}] and cAMP-dependent manner (Impey et al. 1996). The cAMP responsive element (CREB) binds to the cAMP responsive element (CRE) in the promotor of target genes.

It was shown that local protein synthesis for at least a subset of mRNAs could occur in dendrites (Ostroff et al. 2002, for review Sutton & Schuman 2006). Local protein synthesis was shown to be required for the expression of long-lasting LTP since the blockade of protein synthesis affected LTP maintenance in dendrites isolated from the cell bodies (Kang & Schuman 1996, Cracco et al. 2005). Thus an immediate part of LTP expression is thought to dependent on translation of preexisting mRNA, likely dendritic, later sustained by somatic transcription and translation (Kelleher al. 2004, Costa-Mattioli et al. 2009). As soma appears to be still critically involved in synaptic plasticity consolidation evidence on how synapse-specificity of LTP could be

conserved is not finally clarified. A hypothesis how synapse-specificity in LTP could be maintained although translation or at least transcription takes place in the soma was introduced by Frey and Morris in 1997 (Frey & Morris 1997). They proposed that a weak stimulation protocol which is sufficient to induce e-LTP leads in addition to setting of a plasticity tag. This tag enables the synapse to capture plasticity related proteins if a second stimulus strong enough to initiate l-LTP activates protein synthesis in the soma within a distinct time period. Thus synaptic specificity is thought to be preserved by a tag which marks recently activated synapses (Frey & Morris 1997).

Not only the control of translation appeared to be essential but also protein degradation appeared to play a substantial role in synaptic plasticity. Blockade of the proteasome system was shown to alternate formerly protein synthesis dependent LTP into protein synthesis independent LTP which is no longer affected by inhibitors of protein synthesis in its maintenance (Fonseca et al. 2006, Karpova et al. 2006) .

Since protein synthesis appeared to be essential for long-lasting changes of synaptic weight, the question remained open which genes are involved in the process of consolidation (Kelleher et al. 2004, Costa-Mattioli et al. 2009). Therefore the identification of genes regulated by activity and learning-related experience attracted interest of many researchers. The term „immediate early genes“ characterize genes which are activated “in first-line” in a transient and rapid manner in response to a variety of stimuli, e. g. seizure or behavioral tasks such as spatial learning and exploration (Guzowski et al. 2001). They differ from so called „late-response genes“ in the characteristic that their expression is independent of the initiation of *de-novo* protein synthesis. No transcription factors have to be synthesized in advance so that these genes are activated and transcribed in the first round of response after stimulation. Many genes belonging to the class of immediate early genes (IEG) were identified on basis of their rapid induction by pharmacological convulsive stimulation, e. g. c-fos, erg1/zif268, tPA, Arc/Arg3.1, Homer1a and other (Morgan et al. 1987, Nedivi et al. 1993, Qian et al. 1993, Link et al. 1995). These genes could be further distinguished into the classes of transcription factors, which regulate gene expression, and effector IEGs. The latter were found to belong to the classes of growth-factors, cytoskeleton proteins or signaling molecules and are directly involved in synaptic function.

Among these genes much research has been focused on Arc/Arg3.1 since it exhibits compelling properties and was found to play a unique role in synaptic plasticity and memory formation.

1.4 ARC/ARG3.1

One of the most extensively studied IEGs is Arc/Arg3.1 (for review Bramham et al. 2010, Shepherd & Bear 2011). It was discovered in the laboratories of professor Dietmar Kuhl and professor Paul Worley within the same year (Link et al. 1995, Lyford et al. 1995). In professor Kuhl´s laboratory the newly identified IEG was termed "activity-regulated gene of 3.1 kb transcription length" (Arg3.1) and in the laboratory of professor Worley "activity-regulated cytoskeleton-associated protein" (Arc).

Arc/Arg3.1 is rapidly induced in response to a variety of stimuli such as strong synaptic activity, e. g. seizure or synaptic plasticity inducing stimuli, or behavioral tasks, e. g. spatial learning or contextual fear conditioning (Steward et al. 1998, Kelly & Deadwyler 2002, Czerniawski et al. 2011). Interestingly, Arc/Arg3.1 expression in the *dentate gyrus* was shown to be activated by the same stimulation patterns which were shown to induce LTP in the measured neurons (Link et al. 1995, Lyford et al. 1995, Waltereit et al. 2001). Later it was shown that Arc/Arg3.1 is also increased in response to chemical (DHPG) or electrical stimulation used to evoke LTD (Park et al. 2008). Common to the LTP inducing stimuli which in parallel also induce Arg3.1/Arc expression is their activation of NMDA receptors. In addition, mGluR activation and BDNF application have been found to increase Arc/Arg3.1 expression (Waltereit et al. 2001, Park et al. 2008).

Arc/Arg3.1 mRNA was shown to be rapidly transported into dendrites where it is specifically located at recently activated dendritic sites (Link et al. 1995, Lyford et al. 1995, Kuhl & Skehel 1998). Steward et al. demonstrated in 1998 by application of HFS to the *medial perforant path* that Arc/Arg3.1 mRNA specifically accumulated in the dendritic segments on which the *medial perforant path* terminates in the *dendate gyrus* (Steward et al. 1998). Furthermore Arc/Arg3.1 is localized to the nucleus where it was shown to modulate GluR1 transcription (Korb et al. 2013).

The time course of Arc/Arg3.1 expression in response to synaptic activity is unique among activity-regulated genes: A basal level of Arc/Arg3.1 mRNA and protein detectable at synapses under baseline condition is increased following synaptic plasticity inducing stimuli in two steps. First there is an immediate increase in Arc/Arg3.1 protein as a result of local translation of Arc/Arg3.1 mRNA at the synapse. This is followed by a second increase occurring 5-30 min later when Arc/Arg3.1 protein and mRNA are delivered from the nucleus to the activated synapses. The amounts of available Arc/Arg3.1 mRNA and protein appear to be tightly regulated. The exact signaling cascades linking postsynaptic stimulation to Arc/Arg3.1 transcription are not yet elucidated. It was shown that, PKA and MAPK/ERK are critically involved in Arc/Arg3.1 induction (Waltereit et al. 2001), similar to their requirement in the induction of long-lasting LTP. Multiple transcriptional enhancer sites including serum response elements (SRE), synaptic-activity response elements (SARE) and a "zeste-like" element were identified in the Arc/Arg3.1 promotor region and shown to be involved in the activity-dependent regulation of Arc/Arg3.1 transcription (Waltereit et al. 2001, Pintchovski et al. 2009, Kawashima et al. 2008).

As in transcription, translation of the Arc/Arg3.1 mRNA appeared to be tightly regulated. On one hand Arc/Arg3.1 translation is regulated at the step of translation initiation, the step generally considered as the rate-limiting step in translation. A key component in the process of translation is the eukaryotic initiation factor 4E (eIF4E) which promotes via binding to the cap structure at the 5´end of the mRNA the formation of the preinitiation complex (Bramham et al. 2010). On the other hand the Arc/Arg3.1 mRNA was shown to contain an internal ribosomal entry site (IRES), a special sequence allowing translation initiation in a cap-independent manner. Thus IRES-dependent ribosome recruitment might allow translational control of a subset of mRNAs, such as Arc/Arg3.1, under conditions of reduced general protein synthesis (Costa-Mattioli et al. 2009). Translation is further modulated by neuronal activity at the step of peptide chain elongation via phosphorylation of elongation factor 2 (eEF2). Phosphorylation of eEF2 is thought to inhibit general protein synthesis in long-lasting synaptic plasticity, but paradoxically promote the translation of specific mRNAs such as Arc/Arg3.1 (Chotiner et al. 2003). This model suggests that inhibition of global translation favours the translation initiation of mRNAs, which are inefficiently translated under basal conditions, via an increase in the abundance of

rate-limiting translational factors (Park et al. 2008). Accordingly, Park et al. demonstrated that Arc/Arg3.1 translation in m-GluR LTD is critically dependent on eEF2 phosphorylation (Park et al. 2008).

Moreover the number of proteins from one mRNA is limited since Arc/Arg3.1 mRNA was shown to be subject to nonsense-mediated mRNA decay (Giorgi et al. 2007). The Arc/Arg3.1 protein itself is subject to rapid degradation by the ubiquitin and the UB3a pathway (Greer et al. 2010).

Not only the amount of protein appear to be tightly regulated but also the multitude of potent Arc/Arg3.1 elicitors and involvement of multiple signaling cascades suggest that Arc/Arg3.1 expression might be regulated in different, fine-tuned ways corresponding to the actual source of induction (Shepherd & Bear 2011). For example Arc/Arg3.1 was reported to be induced in response to both, LTP and LTD evoking stimulation paradigm, but the related time course of Arc/Arg3.1 expression differed significantly (Park et al. 2008).

Because of its rapid expression in response to different stimuli, Arc/Arg3.1 could be used as a marker of recently activated neurons thereby allowing the mapping of neuronal networks involved in information processing (Guzowski et al. 2001, Burke et al. 2005).

Most importantly, Arc/Arg3.1 appears to be essential for the formation of long-lasting memories. Plenty evidence obtained from behavioral studies suggest that Arc/Arg3.1 is implicated in the consolidation of hippocampus-dependent and independent explicit and implicit memory (Guzowski et al. 2000, Plath et al. 2006, Ploski et al. 2008). In a constitutive Arc/Arg3.1 knock-out mice model memory consolidation failed in a variety of behavioral tests, e. g. spatial learning, fear conditioning, conditioned taste aversion and object recognition, whereas short-term memory appeared to be intact as reported by Plath et al. (Plath et al. 2006). For example freezing during the acquisition phase in contextual fear conditioning was indistinguishable in Arc/Arg3.1 knock-out and wild-type mice. In contrast, during the test performed 24 hours later Arc/Arg3.1 knock-out mice exhibited significantly less freezing in the conditioning context compared to their wild-type littermates. These results demonstrated that Arc/Arg3.1 knock-out mice had a selective loss of long-term memory whereas short-term memory appeared unaffected (Plath et al. 2006). These deficiency in memory consolidation in spatial memory and Pavlovian fear conditioning was also reported after disruption of

Arc/Arg3.1 translation with antisense oligodeoxynucleotides (ODNs) (Guzowski et al. 2000, Ploski et al. 2008).

Consistent with the observed impairments in memory consolidation, associated deficits in the consolidation of synaptic plasticity were found (Guzowski et al. 2000, Plath et al. 2006). In the study of Plath et al. a fundamental biphasic alteration of hippocampal LTP was observed in the dentate gyrus *in vivo* and in area CA1 *in vitro*. The amplitude of LTP during e-LTP in Arc/Arg3.1 knock-out mice was enhanced by 50 % compared to wild-type mice. In addition LTP maintenance appeared to be impaired since fEPSP responses decayed back to baseline within 90 min. Besides these alterations Arc/Arg3.1 had no impact on basal synaptic transmission, neuronal structure, induction of synaptic plasticity or short-term memory per se (Plath et al. 2006). Qualitatively similar results were obtained by inhibiting Arc/Arg3.1 translation with antisense ODNs (Guzowski et al. 2000). Arc/Arg3.1 antisense ODNs were infused 1.5 hours before LTP induction and compared to treatment with scrambled ODNs in the contralateral hemisphere. LTP induced in the dentate gyrus of awake, freely moving rats after antisense infusion was shown to have a similar magnitude but declined significantly compared to control over the subsequent days. A further study using Arc/Arg3.1 antisense ODNs assessed different time points for inhibition of Arc/Arg3.1 translation and its dynamic contribution to LTP in the *dentate gyrus in vivo* (Messaoudi et al. 2007). They showed that application of Arc/Arg3.1 antisense ODNs before or immediately after LTP induction, evoked a transient inhibition of LTP expression which recovered with rehabilitated Arc/Arg3.1 synthesis. In contrast, antisense application two hours, but not four, after LTP induction completely reversed LTP suggesting that sustained Arc/Arg3.1 synthesis is required for LTP consolidation (Messaoudi et al. 2007).

Furthermore Arc/Arg3.1 was also found to be essential for long-lasting forms of LTD. Long-term depression depending on activation of metabotropic glutamate receptors (mGluR) induced by either the mGluR agonist DHPG or paired-pulse low-frequency stimulation protocol (PP-LFS) was shown to rapidly induce local Arc/Arg3.1 translation within five minutes. Moreover Arc/Arg3.1 translation was found to be also essential for mGluR LTD expression since stable LTD expression was impaired in Arc/Arg3.1 knock-out mice (Park et al. 2008). Another form of LTD depending on the activation of NMDA receptors was found to be attenuated in Arc/Arg3.1

knock-out mice in one study (Plath et al. 2006) but was reported to be intact in another study (Park et al. 2008).

Thus Arc/Arg3.1 was shown to be involved in both bidirectional changes of synaptic plasticity, i. e. LTP and LTD. With regard to LTD previous evidence suggests that Arc/Arg3.1 may be directly involved in the immediate expression of LTD by mediating a decrease of synaptic strength via endocytosis of surface AMPA receptors (Waung et al. 2008). But in addition, Arc/Arg3.1 was shown to be essential for the consolidation of both types of synaptic plasticity. The question how Arc/Arg3.1 is implicated in these consolidation processes of synaptic plasticity is still unsolved. Some evidence suggests that Arc/Arg3.1 has a functional role in the consolidation of structural changes in LTP by local stabilization of F-actin since l-LTP was shown to be attenuated by interference with actin polymerization (Fukazawa et al. 2003, Bramham 2008). These hypothesis is supported by the findings that (1) disruption of Arc/Arg3.1 translation induced dephosphorylation of cofilin, a major regulator of actin dynamics, and (2) infusion of the F-actin stabilizing drug jasplakinolide reversed LTP attenuation by Arc/Arg3.1 antisense ODNs (Messaoudi et al. 2007). Recent evidence demonstrated further involvement of Arc/Arg3.1 in structural plasticity. Confocal microscopy in primary hippocampal cultures showed that Arc/Arg3.1 overexpression increased spine density and the proportion of thin spines. In contrast, loss of Arc/Arg3.1 *in vivo* decreased spine density. Especially, thin spines have been reported to be more motile and have a greater capacity to enlarge and stabilize after LTP. In the later study the functional role of Arc/Arg3.1 in regulating spine morphology was linked to its ability to regulate AMPA receptor endocytosis (Peebles et al. 2010).

This coincidence of impairments in consolidation of memory as well as synaptic plasticity make it likely that deficits in synaptic plasticity might account for the failure in consolidating short-term memories into long-lasting late-memories.

Previous work suggested an involvement of Arc/Arg3.1 in AMPA-receptor trafficking in and out of the synapse by interacting with endophilin 2/3 and dynamin (Chowdhury et al. 2006). In this way it might be implicated in strengthening and weakening of synapses. Cycling of AMPA receptors was shown to be a fundamentally required for homeostatic plasticity. Homeostatic plasticity is proposed to occur in neurons to scale the neuronal output within a physiological range without affecting the relative weight of individual synapses (Turrigiano & Nelson 2004). By

this mean the average output of a neuron could be maintained which is essential for efficient information encoding in neuronal networks. Scaling of surface AMPA receptors allowed the adjustment of their number with respect to the recent history of the synapse, e. g. chronic inactivity (Shepherd et al. 2006). Shepherd et al. demonstrated that Arc/Arg3.1 is critically involved in homeostatic scaling since Arc/Arg3.1 knock-out provoked a strong impairment of scaling of surface AMPA receptors in response to chemical manipulations of the recent synaptic activity (Shepherd et al. 2006). Whether Arc/Arg3.1 mediated endocytosis is restricted to a specific subtype of AMPA receptors could not be clarified since previous results were pointing in different directions (Rial Verde et al. 2006, Shepherd et al. 2006).

Recently, Okuno et al. presented a hypothesis linking Arc/Arg3.1 function in AMPA receptor trafficking to its role in consolidation of long-term synaptic plasticity (Okuno et al. 2012). They proposed that Arc/Arg3.1 is targeted to inactive synapses which have been previously upregulated, a process termed “inverse tagging”. CaMKIIß present at inactive synapses is thought to mediate the tagging of Arc/Arg3.1 to inactive synapses in response to Arc/Arg3.1 inducing stimuli. Thus Arc/Arg3.1 might contribute to neuronal plasticity by selectively weakening previously potentiated synapses based on their present status of inactivity. Arc/Arg3.1 quantitatively determines GluR1 turnover in recently inactivated synapses thereby facilitating the contrast between weak and strong, enduring activated synapses. This scenario proposed by Okuno et al. reconciles the apparently contradiction between the weakening of synapses by Arc/Arg3.1 prompting AMPA receptor endocytosis on one hand and Arc/Arg3.1 requirement for plasticity consolidation on the other hand.

Besides this experimental data, disruption of intact Arc/Arg3.1 function was implicated in cognitive disorders, such as Fragile X mental retardation and Alzheimer’ Disease (Dickey et al. 2003, Zalfa et al. 2003).

In summary the reviewed data demonstrate that Arc/Arg3.1 is a key regulator of the consolidation step in both memory formation and synaptic plasticity. Moreover Arc/Arg3.1 appears to play an important role in homeostatic plasticity.

1.5 EXTRACELLULAR FIELD RECORDING

Neurons use electrical signals for information processing which could be detected using standard electrophysiological techniques. Recording from brain slice preparations was first developed by Yamamoto and McIlwain who showed that slices of the *prepyriform cortex* could be maintained *in vitro* and electrophysiological recordings can still measure electric activity comparable to activity of intact preparations (Yamamoto and Mcilwain 1966). Field extracellular postsynaptic potentials (fEPSP) could be recorded extracellularly in tissues exhibiting electric activity, e. g. brain or heart. They measure extracellular potential differences evoked by the coaction of a population of neurons in a network. Importantly, in comparison to intracellular recording, which measures the electric signals of individual neurons, fEPSPs are summated signals of groups of neurons. The fEPSP is evoked by extracellular currents flowing in response to synchronous activity of neurons. Therefore it could be used to assess the strength of synaptic transmission in a network of coactivated neurons. Extracellular field recordings are an elementary electrophysiological technique used to study synaptic transmission and related phenomena, e. g. LTP and LTD. Notably, LTP was first discovered by Bliss and Lømo using *in vivo* field recordings (Bliss and Lømo 1973). Since then field recordings, especially in the hippocampal brain slice preparation, were extensively used to study synaptic plasticity, the candidate cellular model of learning and memory formation.

1.6 THE HIPPOCAMPUS

The hippocampus is located in the medial temporal lobe and belongs to the *Allocortex*. It is composed of two gyri, the *Dentate Gyrus (DG)* and the *Hippocampus proper*, the *Cornu Ammonis (CA),* and the *Subiculum* as illustrated in Figure 1.1. The *Cornu Ammonis* is further divided into the three parts *CA3 - CA1*.

It is a brain region which has reciprocal connections to nearly all parts of the neocortex and many subcortical areas, e. g. *Amygdala*, *medial Septum*. Projections from neocortical areas terminate in the parahippocampal region, including the *perirhinal*, *parahippocampal* and *entorhinal cortices*.

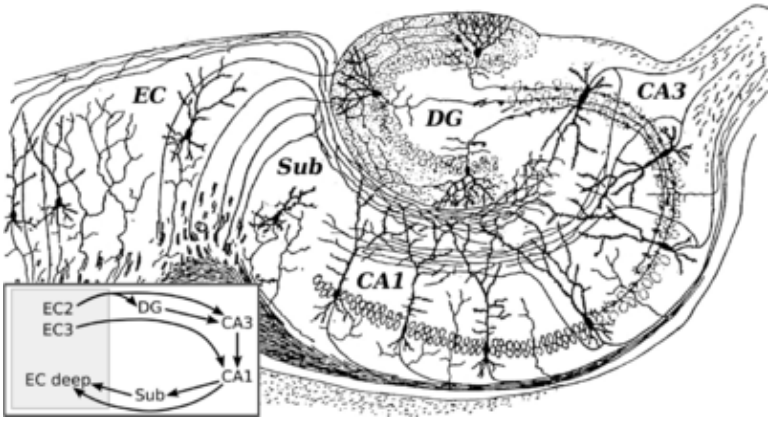

Figure 1.1: The Hippocampal Formation

Drawing of a transverse hippocampal section demonstrating the trisynaptic circuit, which is composed by the synaptic connections between the *Dentate Gyrus* (DG), *CA3* and *CA1*. The major excitatory input to the hippocampus is a projection from the adjacent parahippocampal region, which includes the *entorhinalen cortex* (EC). This major input, the *perforant path*, terminates on the *granule cell layer* in *DG* and *CA3*. Axons from *granule cells* of the *DG* in turn project to the *CA3* neurons, termed the *"mossy fibre pathway"*. Axons originating from *CA3 pyramidal cells* project to the *CA1* neurons located in both the *ipsilateral* and *contralateral hippocampus*. The *ipsilateral* projection, the *Schaffer collateral pathway*, terminates mainly on the apical dendrites in *Stratum Radiatum* and to a lesser extent on the basal dendrites in *Stratum Oriens*, whereas the smaller *commissural pathway* mainly terminates in the *Stratum Oriens*. Neuronal output leaves the *hippocampus* via the *subiculum* (Sub) and *entorhinalen cortex* (EC) (modified from Cajal).

Hence the parahippocampal region serves as a convergence site for cortical input passing information forward to the hippocampus. Thus different modes of information could be integrated and associated in the hippocampus before the processed information will be returned to the neocortical areas via the parahippocampal region. This remarkable connectivity of the hippocampus early led to the idea that the hippocampus may play an important role in the processing of external information like it occurs during learning and memory formation. Indeed in succession of the pioneering studies of Brenda Milner much research focused on the involvement of the hippocampal formation in learning and memory formation. These studies revealed that the Hippocampus appeared to be essential for the formation of newly acquired memories (for review Eichenbaum 2000, Andersen 2006). The hippocampus appears to play a special role in the formation of spatial memory. The observation that lesions of the hippocampus interfere with the formation of spatial memory led to the

hypothesis that within the hippocampus a spatial map is generated representing the extracellular space. Using *in vivo* electrophysiological recordings in rats performing spatial tasks it was discovered that individual pyramidal neurons will fire only if the head of the animal is in a specific part of the environment, the cell´s place field (O'Keefe & Nadel 1979). Thereby the firing pattern of the neurons encode the animals place in space, leading to the term "place cells". Later the firing pattern of hippocampal neurons could be extended to the coding of further behavioral parameters (Wiener et al. 1989).

Moreover the hippocampus is part of the limbic system, a network of interconnected brain areas assumed to play an important role in emotion.

2 AIMS OF THE STUDY

Arc/Arg3.1 is an immediate early gene which is tightly linked to neuronal activity in its induction and was shown to be essential for the consolidation of memory and synaptic plasticity (Guzowski et al. 2000, Plath et al. 2006, Messaoudi et al. 2007, Park et al. 2008). Previous reports concerning the role of Arc/Arg3.1 in synaptic plasticity *in vivo* or *in vitro* mainly examined the role of Arc/Arg3.1 in protein synthesis dependent forms of LTP (Guzowski et al. 2000, Plath et al. 2006, Messaoudi et al. 2007). To investigate the role of Arc/Arg3.1 in the maintenance of synaptic plasticity in more detail, I sought to establish two stimulation protocols which induce either transient or long-lasting and protein synthesis-dependent LTP in wildtype brain slices. I then compared these transient and long-lasting forms of LTP in wildtype and in Arc/Arg3.1 knock-out mice to study the role of Arc/Arg3.1. Using a transgenic mouse line in which dendritic Arc/Arg3.1 translation is disrupted I tested whether locally and somatically translated Arc/Arg3.1 serve different functions in LTP. In addition, the function of Arc/Arg3.1 in basal synaptic transmission was studied via the detection of the PS-threshold, IO-curves and Paired-Pulse Facilitation.

SPECIFIC GOALS

I) Establishing stimulation protocols which allow the selective induction of transient and long-lasting LTP in wildtype mice.

II) Investigate the role of local synaptic translation of Arc/Arg3.1 in the expression and consolidation of synaptic plasticity.

III) Examine LTP in the presence of protein synthesis inhibitors to test whether Arc/Arg3.1 is specifically linked to protein synthesis dependent forms of plasticity.

3 MATERIALS & METHODS

3.1 ANIMALS AND HUSBANDRY

The animal care, maintenance and experimental procedures were performed in accordance with the Ministry of Science and Public Health of the City State of Hamburg, Germany.

Mice were raised under similar conditions and kept in plastic cages under standard housing conditions (rodent provender and water *ad libitum,* nesting material provided).

Adult mice aged 2-6 months, were used in all experiments. In this age, network organization and plasticity have reached a stable mature status (Palmer et al. 2004).

Mice of both sexes are included in the experiments at balanced numbers.

3.2 GENOTYPES AND BREEDING SCHEMES

The aim of this study was to examine the role of Arc/Arg3.1 in synaptic plasticity. For this purpose three different mouse lines were studied:

I. Mouse line E8KO.

   These are conventional knock-out mice, in which the full gene locus of Arc/Arg3.1 was deleted from the germline. These mice lack Arc/Arg3.1 gene throughout their entire development. This line was previously described by Plath et al. (Plath 2006).

   This Arc/Arg3.1 knock-out mouse line was raised in a C57Bl/6J background. Heterozygous Arc/Arg3.1 mice (Arc/Arg3.1$^{+/-}$) were crossed to generate WT (Arc/Arg3.1$^{+/+}$), heterozygous (Arc/Arg3.1$^{+/-}$) and KO (Arc/Arg3.1$^{-/-}$). WT and knock-out mice of this line were later used in experiments presented in chapter 4.1 and 4.2. Heterozygous mice for breeding were generated by back-crossing heterozygous mice with C57Bl/6J mice to obviate the development of substrains.

II. To study the functional role of dendritic Arc/Arg3.1 mRNA, a Tg line was engineered and referred to as TgMXS*B6. In transgenic mice the 3' UTR of

the Arc/Arg3.1 encoding region was replaced by the 3' UTR of Zif268. The mutated sequence was re-introduced into the conventional knock-out line via a P1 derived artificial chromosome (PAC) as described in Mao (2008). This mutation was shown to prevent the transport of the Arc/Arg3.1 mRNA from the nucleus to the dendrites while Arc/Arg3.1 protein is still present in synapses Mao (2008). This mouse line was used in experiments presented in chapter 4.3, 4.4 and 4.5.

3.3 SLICE PREPARATION

Animals were anesthetized with volatile anesthesia (Trifluorethane) and decapitated using scissors soon after disappearance of the toe pinch reflex. Skin and skull were cut using a micro scissor along the *sutura sagittalis* and *sutura coronalis*. *Bulbi olfactorii* and the *cerebellum* were cut off with a scalpel and the brain was removed using a small spatula and immersed in ice-cold and carbogenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (Schmitz aCSF: 119 mM NaCl, 2.5 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 1.3 mM $MgSO_4$, 10 mM Glucose, 2.5 mM $CaCl_2$; pH: 7.4 (gassed)). All chemicals were purchased by Sigma Aldrich (Steinheim), if not otherwise denoted. Artificial cerebrospinal fluid was cooled down by frozen aCSF to minimize cellular metabolism during preparation. The brain was cut into its two hemispheres by scalpel and the apical poles of the halves were cut off. The two hemispheres were glued with their cut surface onto the object-mounting platform of the microtom (HM650V, Microm) and immediately placed in the microtom chamber which had been filled with ice-cold, carbogenated aCSF.

Transversal slices of the hippocampus were prepared at 350 µm and left to recover in an incubating chamber filled with aCSF placed in a water bath at 36°C for 30 min. Afterwards slices were incubated for further 30-45 min at room-temperature.

A piece of mouse tail was stored at -18°C for regenotyping.

3.4 ELECTROPHYSIOLOGY

All electrophysiological recordings were performed at 34 to 36°C under submerged conditions in a multi-slice field recording system (synchroslice, Lohmann Research Equipment, Castrop-Rauxel). With this system up to four slices could be recorded simultaneously (Figure 3.1).

It enables a direct comparison of the effects of different stimulation protocols or drugs on slices derived from the same mouse. Thus influencing factors, such as the genetic background or the experiments history e. g. the conditions during the preparation of the mouse, could be minimized. Perfusion with aCSF was constant at a flow-rate of 2 ml/min to minimize mechanical disruption of the recording.

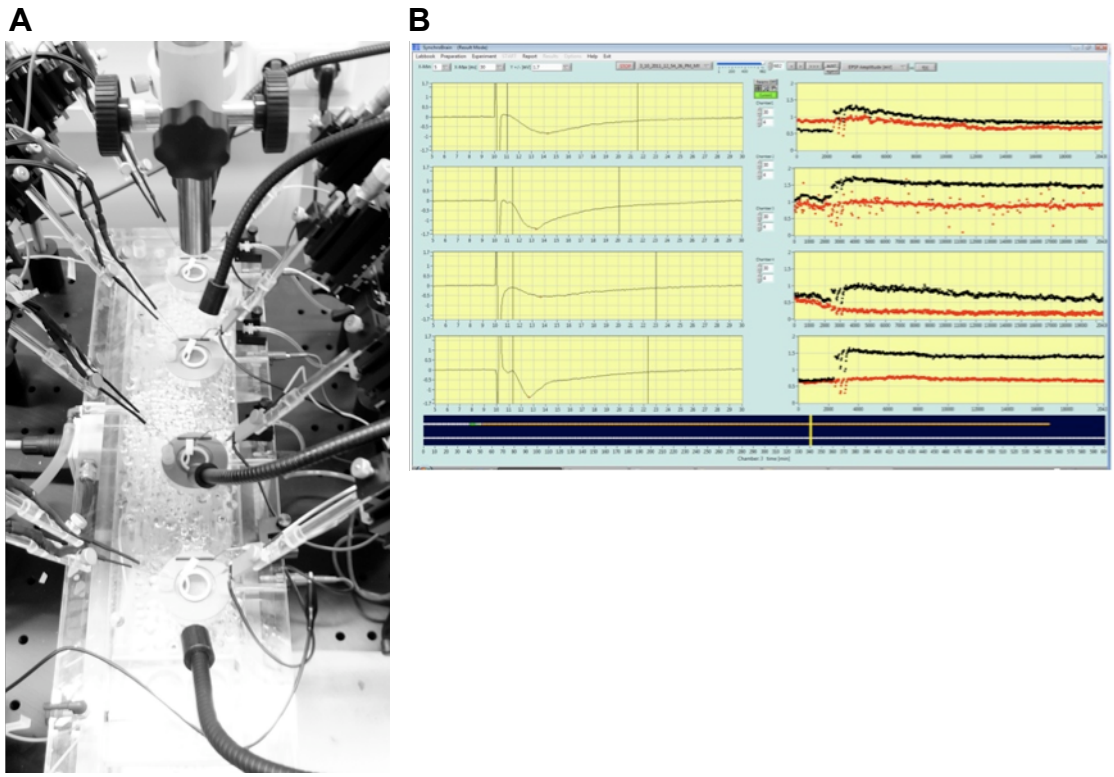

Figure 3.1: The multi-slice field recording system synchroslice

(A) Photograph of recording chambers and electrodes of the multi-slice field recording system. **(B)** Screen-shot of a running LTP-experiment in Synchrobrain: In the left part of the screen the ongoing fEPSP trace and on the right side the time course of the corresponding experiments in chamber 1-4 (top-down) are shown.

Synaptic transmission and plasticity were measured in the *Stratum radiatum* of the *CA1* region of the hippocampus as demonstrated in Figure 3.2. This region contains mainly the synapses between the *Schaffer collaterals* coming from the *CA3* neurons and the apical dendrites of the *CA1 pyramidal neurons*.

Concentric, bipolar stimulating electrodes (stainless steel, contact diameter 0.35 mm, impedance 0.1 MΩ) and platinum/tungsten electrodes (impedance 0.5 - 0.8 MΩ) were used for stimulation and recording, respectively. In most experiments a second stimulating electrode was placed on the opposite (*subicular*) side of the recording electrode with approximately the same distance to the *Stratum pyramidale* and to the recording electrode as the first stimulating electrode. With this arrangement, called two-pathway protocol, the stimulation electrodes activated two independent sets of afferent *Schaffer-collateral* fibers converging on the same population of neurons.

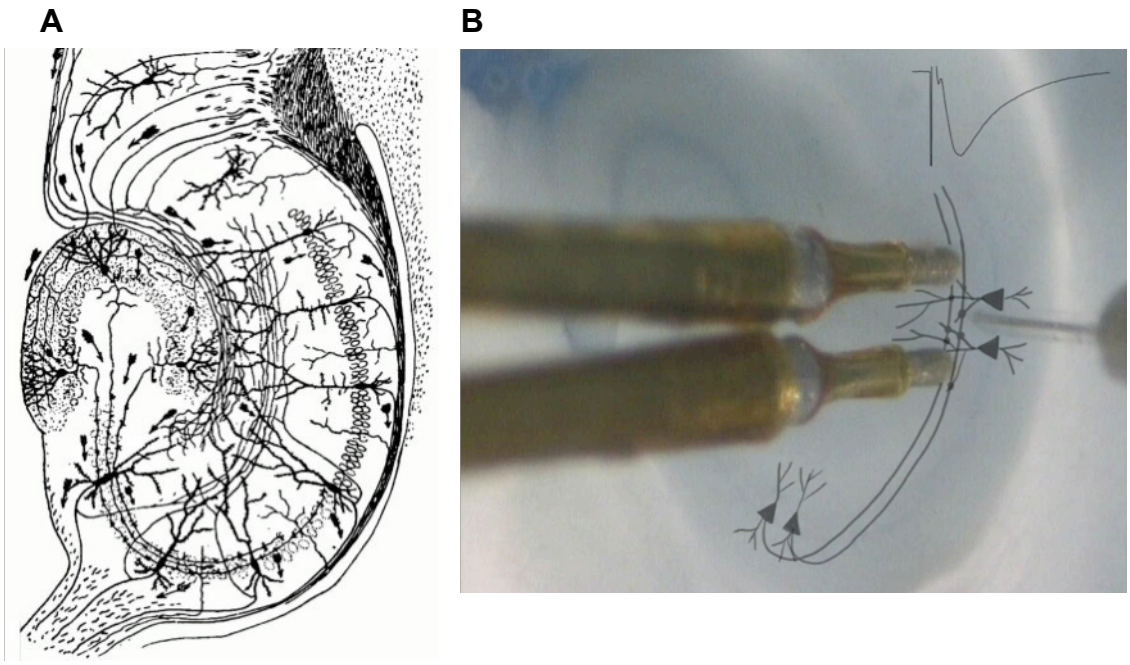

Figure 3.2: Experimental setting in the hippocampal brain slice preparation

(A) drawing of a transverse section of the rat hippocampal formation showing nerve cells and their projections (Cajal 1911) **(B)** Experimental setting: Example photograph showing the positioning of stimulating electrodes (left side) and the recording electrode (right side) on the hippocampal slice preparation. Superimposed drawings indicate *pyramidal neurons* of the *CA3* and *CA1* region (triangles) and the *Schaffer collateral pathway* (lines). Inset shows example fEPSP recorded in *Stratum radiatum*.

The second stimulated pathway served as a within slice-control for slice viability and recording stability. Furthermore it allowed to control for heterosynaptic effects of the conditioning protocol.

Pathway independence was tested utilizing paired-pulse stimulating protocols in an input-specificity test. Two-pathway protocol and input-specificity test are explained in detail in chapter 3.4.3 in context of paired-pulse facilitation. Data acquisition, electrical stimulation and perfusion were controlled via the software "Synchrobrain" (Lohmann Research Equipment, Castrop-Rauxel). On- and offline analysis of field potentials (fEPSP) was performed with the same program. Field EPSP recordings were sampled at 10 kHz and filtered with a 16 channel filter amplifier with a fixed bandwidth of 1 Hz to 3 kHz. After on-line A/D conversion data was stored on a personal PC.

Routinely cleaning of recording electrodes, chambers and associated tubings was done using 5 % H_2O_2 and 0.5 M HCL solutions and protease. Tubings were replaced after mechanical abrasion. Cleaning and replacement procedures were controlled to not influence the recordings.

3.4.1. Stimulation paradigms

The time-scale of experiments is illustrated in Figure 3.3.

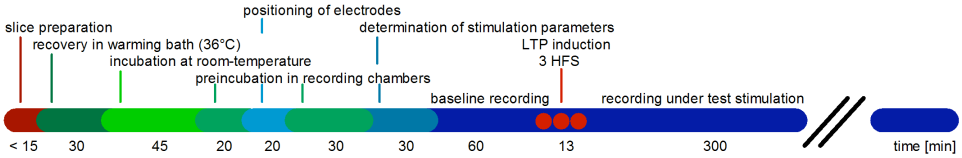

Figure 3.3: Time-scale of experiments

Slices were prepared in ice-cold aCSF and left to incubate in a warming bath (30 min) and subsequently at room-temperature (45 min). Afterwards slices were transferred to the synchroslice system and let to adapt to the recording chamber (t=34-36°C) for further 20 min. Electrodes were positioned in *Stratum Radiatum* and I/O-curves and paired-pulse facilitation tests were conducted to determine stimulation intensity and pathway independence after a further adaptation time of 30 min, respectively. Baseline recording was obtained for 60 min before HFS was delivered to the stimulated pathway. Test stimulation frequencies were applied for the following five hours.

Slices were always prepared between 09:00 and 10:00 AM. After preparation slices rested for at least 1.5-3 hours before preliminary recordings were taken, and no high-frequency stimulation (HFS) was delivered until 3-4 hours after dissection.

The preincubation period was used to enable repairing and adaptation processes following slice preparation and to establish metabolic stability (Ho et al. 2004, Sajikumar et al. 2005, Whittingham et al. 1984).

Field EPSPs were evoked by bipolar stimuli with a pulse-duration of 200 µs. If not otherwise stated the stimulus intensity (SI) was chosen that evoke 30 % or 50 % of the maximum fEPSP before a population spike (PS) would be generated in mice of mouse line E8KO and TgMXS*B6, respectively. This was determined on the basis of the IO-curves, as explained in chapter 3.4.2. Stimulation parameters were held constant during the entire experiment. LTP experiments were always conducted in the same manner as illustrated in Figure 3.4. A two-pathway protocol was used in which electrode 1, later referred to as the stimulated pathway (stim), received the LTP inducing stimulus (HFS), whereas electrode 2 served as a control pathway. The control pathway (con) received test stimulation, as applied during baseline recording, throughout the entire experiment.

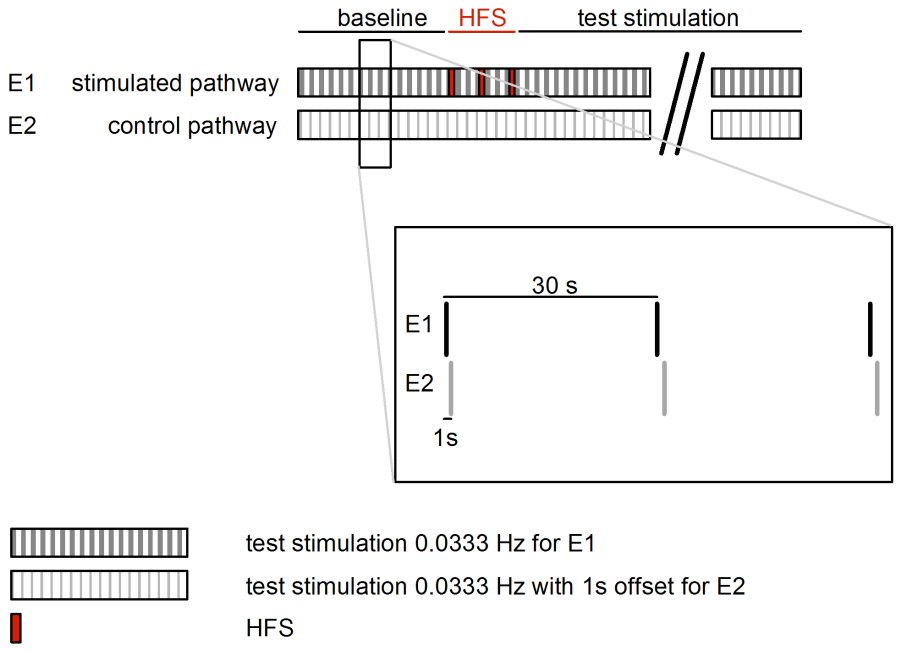

Figure 3.4: Timeline for stimuli of electrodes

Timeline of stimuli for electrode 1 (E1) and electrode 2 (E2) are shown. Different stimulation paradigms are indicated by pattern as explained in the legend.

Input specificity of the two pathways was established using input specificity tests as explained in chapter 3.4.3. Basal synaptic transmission was monitored by low-frequency test stimulation in the two separate pathways.

Responses were evoked alternately by stimulation at a frequency of 0.0333 Hz. This means that the control pathway was activated with an offset of 1 sec with respect to the activation of the stimulated pathway. Before LTP induction baseline responses were recorded for at least 40 min. Long-term potentiation was induced by either 1 or 3-HFS, as described in detail in chapter 3.4.4. Afterwards test stimulation, as during baseline recording, was again applied to both pathways to monitor changes in fEPSP response for at least 5 hours post-HFS.

3.4.2. Input-output curves

To study basal synaptic transmission at the *Schaffer collateral-CA1* synapses the efficiency of synaptic transmission was assessed by construction of input-output curves (IO-curves). At least seven stimuli of randomly chosen intensities were applied to the *Schaffer collaterals* and the fEPSP amplitude measured in response. With increasing stimulation intensity both FV and fEPSP amplitude increased due to activation of additional fibers (Figure 3.5 A). PS will be evoked in the postsynaptic neurons by further increased stimulation intensity (Figure 3.5 B).

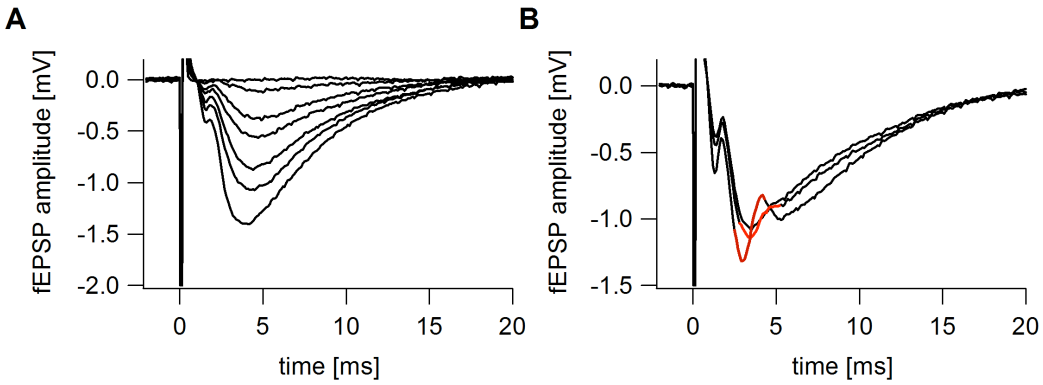

Figure 3.5: Field EPSPs recorded in response to increasing stimulation intensity

fEPSPs recorded in response to increasing stimulation intensity. Data obtained from two different slices **(A)** Stimuli were delivered with 159 µA, 440 µA, 710 µA, 764 µA, 1070 µA, 1265 µA and 1508 µA. **(B)** Increasing stimulation intensity evoked a PS ($SI_{threshold}$). The incidence of a PS is indicated by the positive deflection in the decaying phase of the fEPSP, marked in red. A further increase in SI evoked an even pronounced PS which now determined the amplitude of the fEPSP waveform.

The incidence of a PS is not only related to the degree of synaptic transmission but also influenced by the excitability of the neurons. Therefore the stimulation intensity needed to evoke a PS, later referred to as threshold intensity ($SI_{threshold}$), was determined for each slice in advance. The incidence of a PS was indicated by a positive deflection in the decaying phase of the dendritically measured fEPSP shape. This was hereafter named PS threshold. It could be related to the amount of injected current needed to make a sufficient number of neurons fire action potentials or to the fEPSP amplitude or slope reflecting the synaptic transmission needed to initiate action potentials in the postsynaptic cells. Thereby it is a measure of the excitability within the network of recorded neurons.

Usually, IO-curves were conducted at intensities ranging from subthreshold to the PS-threshold intensities. Thereby fEPSPs recorded in IO-curves represent pure synaptic responses. Accordingly, the maximum fEPSP was defined as the fEPSP without signs of PS appearance.

The response to increasing SI was measured by the fEPSP amplitude and plotted against the SI to construct an IO-curve. The distribution of the measured values usually followed a monotonously increasing function of a sigmoid shape. A sigmoid curve fit was performed using Igor PRO 6.06, as illustrated in Figure 3.6.

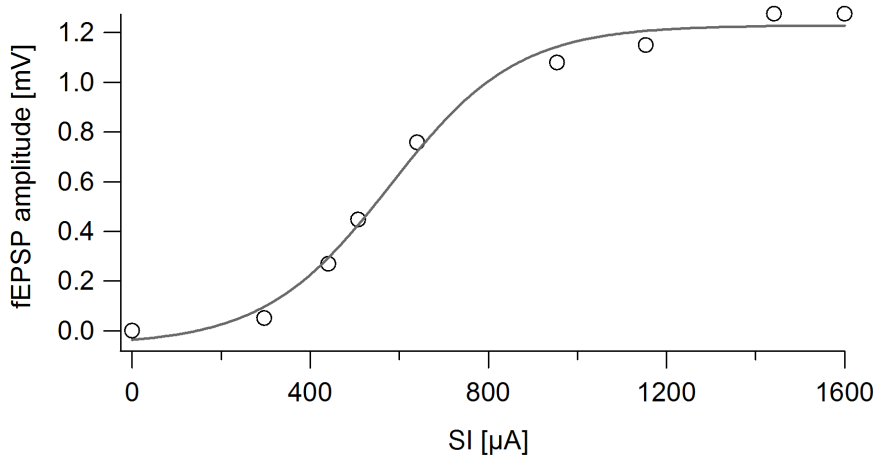

Figure 3.6: Example IO-curve derived from a WT slice

Illustration of the relationship between fEPSP amplitude and SI as an IO-curve. fEPSP amplitude is plotted against SI. Data derived from the experiment is indicated by open circles. Line represents the fit-curve generated with sigmoid curve fitting in Igor PRO 6.06.

Two values were added for this procedure, one with the coordinates (0/0) and another repeating the maximum value at 1600 µA, to obtain fit waves of a comparable length for different slice preparations. Curves generated by sigmoid fits for each slice were averaged and summary curves were generated for each genotype. Within the bounds of this IO-curve presentation, the threshold SI needed to evoke a PS in individual slices could not be expressed.

Two characteristic parameters could be derived from summary IO-curves, the threshold fEPSP ($fEPSP_{threshold}$) and the half-fEPSP ($fEPSP_{50\%}$).

Recordings of extracellular field potentials had two separable components, as shown in Figure 3.7. Faster than the fEPSP a small component occurred, reflecting the action potentials in the activated presynaptic fibers, called the fiber volley (FV). The amplitude of the fiber volley, which is proportional to the number of presynaptic neurons recruited by the stimulation, could be used to estimate the strength of afferent inputs.

To obtain more accurate information about basal synaptic transmission at single synapses the ratio of the fEPSP amplitude divided by the FV amplitude could be computed and presented as a function of SI. This was performed if significant differences in IO-curves were found. If no differences were found in IO-curves just fEPSP amplitude / FV quotients were computed for the $fEPSP_{threshold}$ and $fEPSP_{50\%}$ to exclude that the total number of synapses is different.

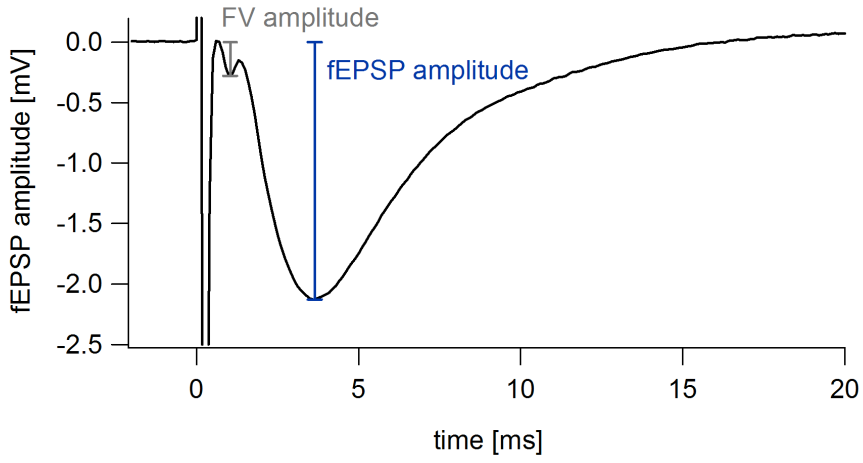

Figure 3.7: Field EPSP trace depicting FV and fEPSP amplitude

The sharp signal reflecting the stimulus artifact is followed by a small negative wave, the FV, and thereafter the fEPSP.

3.4.3. Paired-pulse facilitation

The excitatory synapses of the CA1 neurons naturally display paired-pulse facilitation. The term „paired-pulse facilitation“ (PPF) describes the phenomenon that the delivery of two shortly spaced stimuli, e. g. at an interstimulus interval (ISI) of 50 ms, elicits an increase in the response to the second stimulus.

It is well established that paired-pulse facilitation is a form of short-term plasticity which is mainly based on presynaptic mechanisms. Therefore it could be used to control for changes in presynatic function.

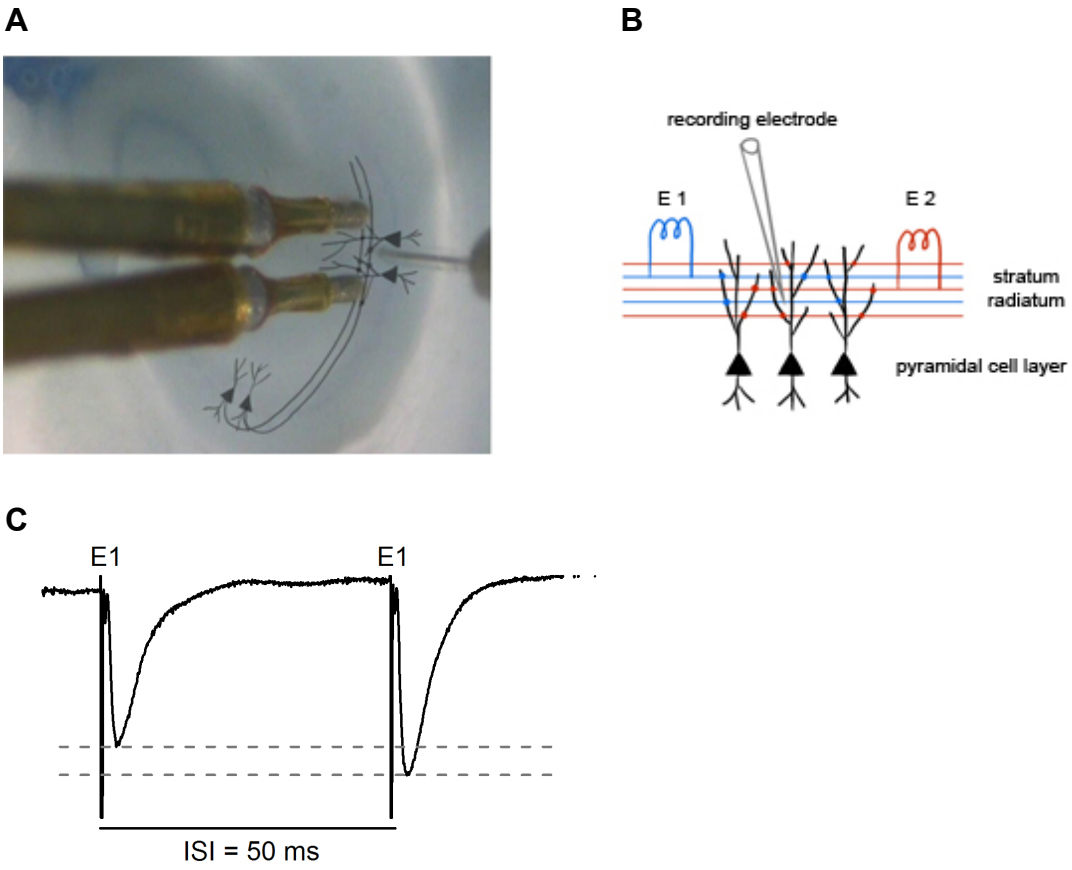

Figure 3.8: Paired-pulse facilitation

(A) Example photograph illustrating positioning of electrodes in a two-pathway protocol. Superimposed drawings indicate pyramidal neurons of the *CA3* and *CA1* region (triangles) and the Schaffer collateral pathway (lines). **(B)** Schematic representation depicting the independent but converging inputs on CA1 neurons. This two-pathway protocol allowed to record two different synaptic inputs, representing either the stimulated or control pathway. Stimulation of electrode 1 (E1) and electrode 2 (E2) activate different sets of presynaptic fibers as illustrated in black and red respectively. These two pathways converge on the same population of *CA1* neurons. **(C)** Example fEPSP trace illustrating the response to paired-stimuli delivered to E1 recorded in a WT slices. The second fEPSP is increased compared to the first fEPSP.

Facilitation could be explained by the addition of Ca^{2+} influx during the second stimulus to residual Ca^{2+} from the first. The increased Ca^{2+} concentration in the presynaptic terminal causes an increase in probability of transmitter release.

The magnitude of facilitation is inversely related to the initial probability of release based on the assumption that the higher the probability of release, the less scope there is for presynaptically mediated facilitation and the smaller the degree of PPF (McNaughton 1982). Paired-pulse facilitation was investigated in *Schaffer collateral CA1 synapses* in a two-pathway setting on the hippocampal slice preparation, as illustrated in Figure 3.8 A and B. Two stimuli of equal SI were delivered to one pathway with an ISI of 50 ms, as shown in Figure 3.8 C. This protocol was shown to be sufficient to induce PPF.

Paired-pulse facilitation was calculated as a facilitation ratio, i. e. the amplitude of the second response relative to the first. Averaged data from three consecutive PPF-tests were used to determine PPF.

Since PPF is based on the fact that the same set of fibers was stimulated twice, PPF could be used to test for input specificity within a two-pathway protocol. After testing PPF in each pathway, one pathway was activated 50 ms after the other to test whether PPF also occurred in this setting, as shown in Figure 3.9.

If normal PPF was detected when pulses were delivered to the different pathways, it could be expected that at least partly the same presynaptic fibers were recruited by both stimuli. In contrast, the absence of PPF confirmed pathway independence.

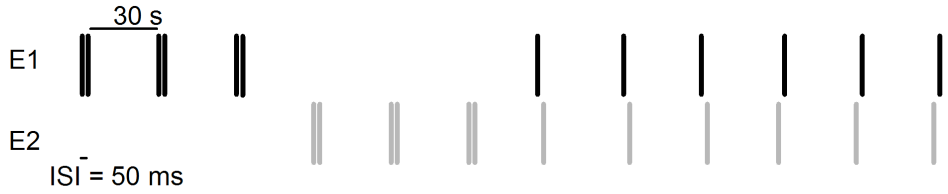

Figure 3.9: Timeline of stimuli during an input-specificity test

PPF in one pathway was tested by delivery of two shortly spaced pulses (paired pulses with an inter-pulse interval (ISI) = 50 ms). Paired-pulses were delivered three times to each electrode, here labeled E1 and E2. First PPF was examined in the pathway of E1. Afterwards PPF was examined in pathway of E2. After this a stimulus was delivered to E1 and in rapid succession with the same ISI as used during PPF to E2 and vice versa. Besides the measurement of PPF for each of the two pathways, the second half of the protocol test for input specificity as described in the text.

3.4.4. LTP induction protocols

LTP was induced electrically by two different stimulation paradigms.

Previous studies reported that a weak conditioning stimulus, one high-frequency train (100 pulses at 100 Hz; 1-HFS), produced a form of LTP, that decayed back to baseline values within three hours. In contrast, the application of a stronger stimulation protocol, consisting of three high-frequency trains (3-HFS), was found to evoke a stable, long lasting form of LTP, which depended on protein synthesis (Huang & Kandel 1994, Kelleher et al. 2004).

We used 1-HFS and 3-HFS with inter-train intervals of 5 min for LTP induction to examine different forms of LTP.

To the control pathway test stimulation was constantly applied during the entire experiment.

3.4.5. Drugs

Protein synthesis was inhibited by perfusion with 80 µM cycloheximide (CHX) or 40 µM anisomycin diluted in aCSF throughout the entire experiment (Capron et al. 2006; U. Frey et al. 1988). Cycloheximide and anisomycin were once prepared as a 500 × and 100 × stock solution dissolved in H_2O respectively, aliquoted and stored at - 18°C (CHX) or in the refrigerator (anisomycin). These stocks were diluted daily in aCSF to achieve the final concentration.

For any given drug, control experiments were conducted in parallel thereby reducing effects of genetic background or experience of the mouse, preparation or recording conditions.

Protein synthesis inhibitors were added to the perfusion at the beginning of the experiments. Thus slices were incubated with protein synthesis inhibitors after they were transferred to the recording chamber. Approximately 20 min of incubation had passed before parameters of basal synaptic transmission were assessed allowing drug penetration and blockade of protein synthesis by binding to ribosomes. Thus protein synthesis inhibitors are assumed to be present at the time of LTP induction. This was shown to be the critical time period for treatment with protein synthesis inhibitors to blockade late LTP expression (Frey et al. 1988).

3.4.6. Rejection criteria

Experiments had to satisfy specific criteria to be included in the final analysis. The following rejection criteria were established in advance: Experiments were excluded from analysis if (1) no stable baseline was established over the last 20 min of baseline recording, (2) the control pathway decayed more than 30% below baseline within five hours (3) changes in fiber volley (FV) amplitude did exceeded 50% or (4) instability of the recording was evident.

The main reason for rejection was instability of the recording indicated by parallel disruptions of both pathways presumably as a result of changes in slice perfusion levels or deficits in slice viability.

3.4.7. Data analysis and statistics

The slope of the fEPSP was measured to assess synaptic strength within the population of recorded synapses. The slope was defined as a linear fit to the descending part of the fEPSP (10-30%). The slope was used as a parameter for synaptic strength instead of the fEPSP amplitude since a population spike (PS) occurred in most cases after LTP induction. Due to the rapid transduction of the potential difference the PS is superimposed on the fEPSP. The PS itself is subjected to another form of plasticity, EPSP-Spike potentiation, which is based on changes in excitability instead of changes in synaptic strength.

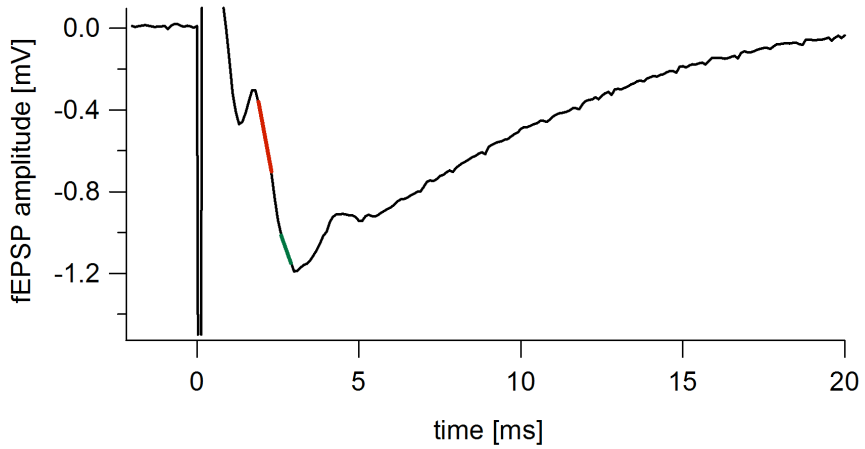

Figure 3.10: Slope of the fEPSP and the PS are distinguishable

The slope of the fEPSP and PS are depicted in red and green, respectively.

Therefore the PS complicated the measurement of the pure synaptic component of synaptic transmission by the fEPSP amplitude. The slope of the fEPSP was unaffected by PS appearance and appeared to be distinguishable from the slope of the PS (Figure 3.10).

LTP was considered as an increase in fEPSP slope of more than 115 % compared to baseline values which remained elevated above baseline for more than half an hour.

Field EPSP slopes were normalized to the average response of the last 15 min of baseline recording before delivery of HFS and divided into five minute data bins.

In LTP experiments time courses of individual representative experiments as well as time course summary graphs are presented for each genotype. The fEPSP slope is normalized to pre-HFS baseline and plotted against time. Time is depicted relative to HFS. Summary data are presented as group means ± standard error of the mean (S.E.M.). fEPSP traces are shown to present raw data and illustrate measurements. For all experiments both the number of slices, indicated by "n", and the number of animals, indicated by "N", were given. Male and female mice were used both in balanced proportions and no differences in plasticity have been observed among sexes.

LTP could be divided into different components, its early and late phase (e-LTP and l-LTP), as explained in chapter 1.3. To quantify these periods bar graphs were created. The peak slope, representing the magnitude of LTP, was always recorded in the early phase of LTP immediately after conditioning stimulation. Accordingly, the averaged response of the first 15 minutes after the last train of HFS was quantified as a measure for LTP magnitude and displayed in bar graphs to characterize e-LTP. As a measure for the maintenance of LTP the response during the last hour of recording (240-300 min post-HFS) was averaged and afterwards used as a representation for the l-LTP.

Mean values of time periods ± S.E.M. were plotted as bar graphs.

The stability of late-LTP was measured by comparing the level of potentiation during the last hour of recording (t = 240-300 min) to (1) 15 min pre-tetanus baseline recording of the same pathway and to (2) the simultaneously recorded control pathway to take systemic changes into consideration.

Analysis of basal synaptic properties took place in advance of each experiment. In this context the differentiation between stimulated and control pathways was dispensed. Since later division in stimulated versus control pathways should have no effect on

prior recordings of basal synaptic transmission slices were treated equally for this analysis.

Statistical analysis was performed using SPSS. Each slice was considered as an individual "n". Statistical comparison of data derived from two groups was made by *Student´s t-test* and *analysis of variance (ANOVA)* if more than two groups were compared, e. g. for presentation in bar graphs. The non-parametric tests Mann-Whitney-U-test was used if the data was not normally distributed. Results of time course graphs were analyzed using univariate, multi-way *analysis of variance* (ANOVA). Genotype (WT, Arc/Arg3.1 knock-out and, if relevant, transgenic mice), stimulus (3-HFS and 1-HFS or CHX), pathway (stimulated and control pathway), and time were included as fixed factors. Post-hoc pairwise comparisons were performed using no adjustments. In time-course graphs significance was depicted by bars if significance level was $p < 0.05$.

Higher levels of significance are specified in bar graphs by ** ($P < 0.01$) and *** ($P < 0.001$).

During experiments WT, KO and Tg mice were interleaved. The experimenter was blind to the mice genotype until completion of experiments and analysis to ensure that measures of performance were unbiased.

For the study of basal synaptic transmission it was differentiated between slices with stable control pathways during LTP recordings and slices later excluded from analysis. We checked for any statistical significant differences in parameters determining basal synaptic transmission which might influence and thereby predict the stability of subsequent recordings. If no statistical differences were detected, data was pooled for analysis.

Data-acquisition and offline-analysis were performed with "synchrobrain" (Lohmann Research Equipment, Castrop-Rauxel), Igor Pro 6.06 (WaveMetrics, Portland) and Excel (Microsoft, Redmond).

4 RESULTS

4.1 ESTABLISHMENT OF EXPERIMENTAL CONDITIONS

We wanted to study the functional role of Arc/Arg3.1 in transient and long-lasting forms of LTP. To investigate these different components we sought to establish two different stimulation protocols previously shown to dissect these two phases (Huang & Kandel 1994, Kelleher et al. 2004).

First, an appropriate stimulation strength to induce either transient or long-lasting LTP was wanted. The chosen stimulation intensity had to be sufficient and strong enough to activate the processes required for LTP induction. This phenomenon termed "cooperativity" is one of the basic properties of LTP which describes the intensity threshold for LTP induction (Bliss & Collingridge 1993). The amplitude of the stimulation intensity applied to the slice determines the recruitment of presynaptic fibers but might further influence the degree of inhibition in the network by an activation of inhibitory interneurons. Both factors are putatively involved in the process of LTP induction. In addition to this, the amplitude of stimulation intensity is critically influencing LTP duration as demonstrated by Huang and Kandel (Huang & Kandel 1994).

To obtain a stimulation intensity which enabled us to evoke transient and long-lasting LTP selectively, we tested two different stimulation intensities: On one hand a stimulation intensity yielding 30 % of $fEPSP_{max}$ (without PS, later referred to as SI type 1) and on the other hand a stimulation intensity yielding 50 % of $fEPSP_{max}$ including PS (later referred to as SI type 2).

Both stimulation intensities (mean SI type 1: 424 ± 20 µA, n = 32; mean SI type 2: 580 ± 26 µA, n = 34; $p < 0.001$) were sufficient to induce LTP in slices from WT mice (Figure 4.1). While baseline fEPSP amplitudes were slightly larger in response to the stronger stimulation (SI type 1: 0.79 ± 0.07 mV, n = 32; SI type 2: 0.93 ± 0.02 mV, n = 34; $p = 0.121$, n. s.), the magnitude and duration of LTP were similar in response to the two different stimuli.

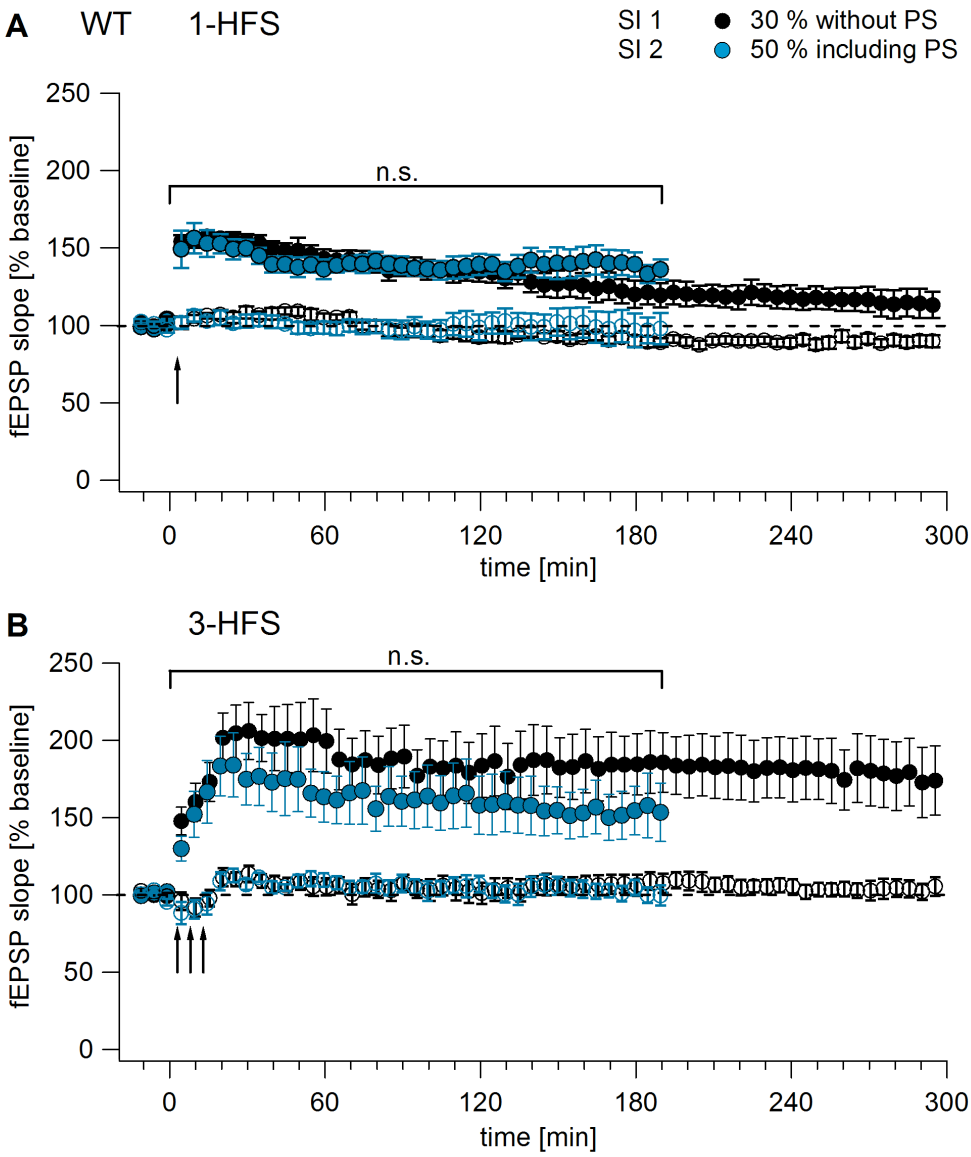

Figure 4.1: Variation of SI neither influence LTP magnitude or duration significantly

Summary graph: fEPSP slope values are plotted against time to illustrate LTP time courses. **(A)** LTP induced in WT by 1-HFS using SI type 1 (black circles, n = 10) or SI type 2 (blue circles, n = 8). **(B)** LTP induced in WT by 3-HFS using SI type 1 (black circles, n = 8) or SI type 2 (blue circles, n = 9). Control pathways are represented by open circles in the corresponding coloring.

The peak amplitude reached with SI type 1 and 2 respectively was not significantly different. This applied to LTP in WT slices after 1-HFS (SI type 1: 157 ± 4 % n = 10; SI type 2: 156 ± 10 %, n = 8; p = n. s.) and 3-HFS (SI type 1: 206 ± 18 %, n = 8, SI type 2: 184 ± 19 %, n = 9; p = n. s.). Similar results were obtained in KO slices after 1-HFS (SI type 1: 181 ± 15 %, n = 6; SI type 2: 172 ± 10 %, n = 5; p = n. s.) and 3-HFS (SI type 1: 201 ± 15 %, n = 9, SI type 2: 193 ± 26 %, n = 7; p = n. s.). The remaining potentiation of the fEPSP recorded 180 min after HFS was also not

significantly different between WT (1-HFS: SI type 1*:* 120 ± 9 %, n = 10; SI type 2: 139 ± 8 %, n = 8; p = n. s.; 3-HFS: SI type 1: 185 ± 20 %, n = 8, SI type 2: 155 ± 16 %, n = 9; p = n. s.) and KO slices (1-HFS: SI type 1: 138 ± 8 %, n = 6; SI type 2: 132 ± 11 %, n = 5; p = n. s.; 3-HFS: SI type 1: 160 ± 16 %, n = 9, SI type 2: 155 ± 15 %, n = 7; p = n. s.).

LTP in response to 3-HFS in WT tended to be smaller in experiments using a higher SI. This trend was not significant but might be explained by an effect of saturation. As LTP was displayed as a percentage increase of the fEPSP, saturation of the fEPSP response might occur. To test this hypothesis absolute fEPSP values were compared. The analysis showed that the absolute fEPSP amplitude after 3-HFS was 1.01 ± 0.11 mV with SI 1 and 1.19 ± 0.19 mV with SI 2 (p = n. s.). Thus no differences in maximum fEPSP amplitude were be detected. Nevertheless, the statistic power might be to small to test for such small differences.

In consequence, an intermediate stimulation intensity yielding 50 % of the $fEPSP_{max}$ without PS was used for the experiments in mouse line TgMXS*B6. I chose this SI to increase the signal-to-noise ratio by larger fEPSPs compared to SI 1 without the risk of saturation effects (as discussed for SI 2). In turn recorded fEPSPs will be highly sensitive to changes in both directions. Increases in fEPSP slope will be detected more sensitively since fEPSPs have more room to grow until saturation of synapses.

Secondly, experiments were further used to determine the reasonable duration of recording required for differentiation of transient and long-lasting LTP. In the first set of experiments (SI type 1) 1-HFS LTP was still significantly increased compared to baseline after three hours (Figure 4.1 A). Thus we extended the recording time post HFS to five hours (SI type 2) and observed a full decay of LTP to baseline levels four to five hours after LTP induction. In contrast to 1-HFS, five hours after the 3-HFS stimulus LTP was still strongly and significantly increased. We concluded that a post-HFS recording time of five hours was required to assure distinction of transient and long-lasting LTP under these recording conditions.

4.2 LTP IN MOUSE LINE E8KO

To investigate whether Arc/Arg3.1 is required in e-LTP as well as l-LTP, we examined LTP induced by either 1-HFS or 3-HFS in WT and KO mice.

4.2.1. LTP in WT mice (E8KO)

In this chapter the results of LTP experiments in WT mice of the mouse line E8KO are presented.

LTP was induced by 1-HFS and 3-HFS in hippocampal brain slice preparations as described in chapter 3.4.

Individual experiments are shown in Figure 4.2 to illustrate representative LTP time courses recorded following 1-HFS and 3-HFS. After recording stable field excitatory postsynaptic potentials (fEPSPs) in both pathways for at least 30 min LTP was induced in one pathway, meanwhile the other pathway continuously received baseline stimuli. Field EPSPs recorded in the stimulated pathway showed an immediate increase in slope in response to both stimulation protocols. Following 1-HFS the fEPSP subsequently declined to baseline. In contrast, following 3-HFS the fEPSP remained elevated for five hours after HFS. Corresponding fEPSP traces to the presented experiments are displayed in Figure 4.2 A and B above the time course graphs. The traces show an increase in the amplitude and the slope of the fEPSP in the stimulated pathway 15 min post-HFS. Following 1-HFS in trace 3 the slope of the waveform decreased to pre-HFS baseline indicating a reduction in synaptic strength to baseline levels. However, an enhancement in excitability persisted, as shown by the population spike (PS) still observable in trace 3. In contrary, following 3-HFS the increase of the fEPSP persisted for five hours.

Importantly, the FV amplitude, as far as detectable, was unchanged. This indicated that the increase in the fEPSP rather reflected an increase in synaptic strength than an enhancement of presynaptic fiber recruitment. Field EPSP traces recorded in the within-slice control pathways in both experiments remained unchanged throughout the entire experiment.

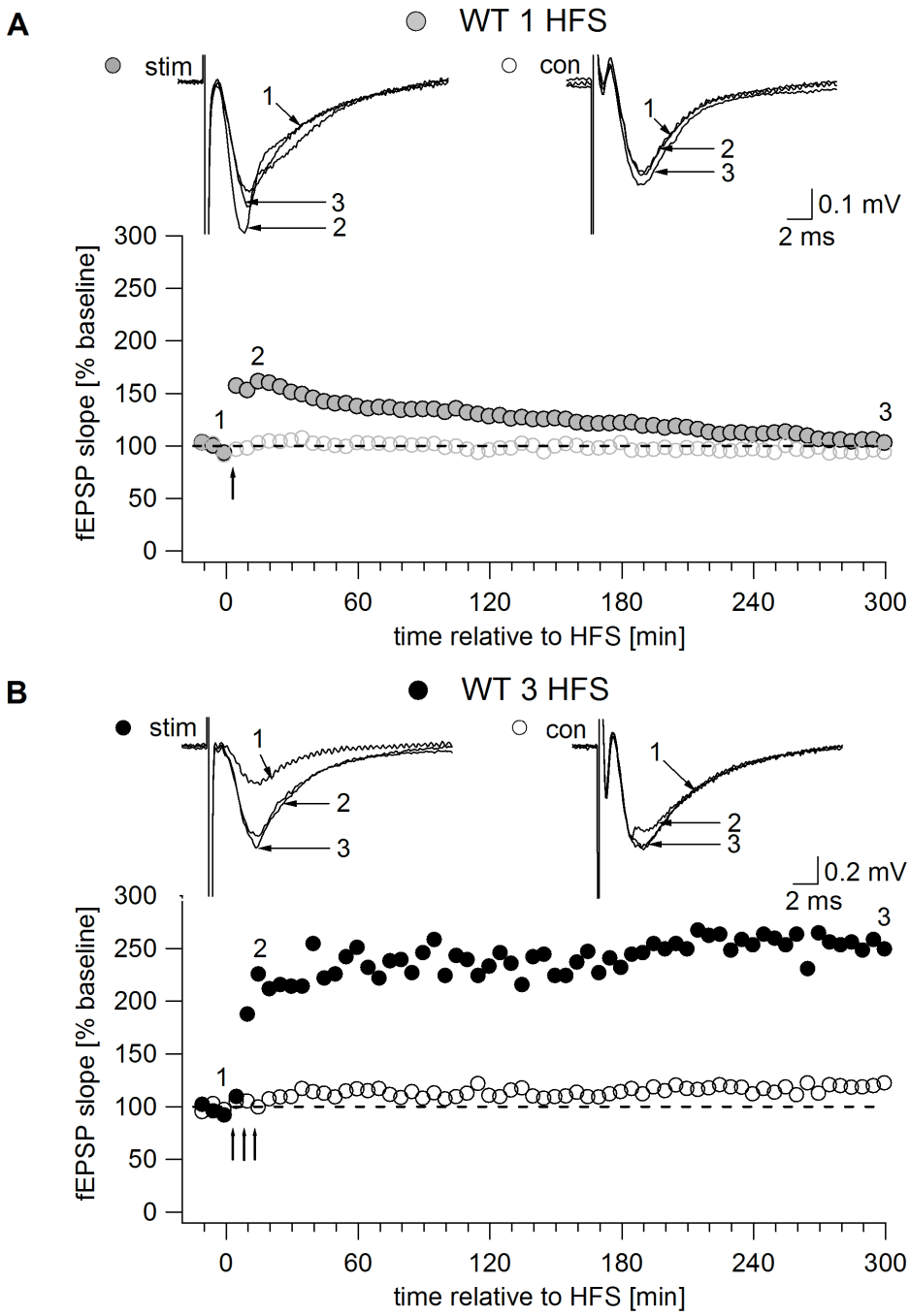

Figure 4.2: LTP in WT mice in response to 1-HFS and 3-HFS

Single experiments showing representative time courses of LTP induced by either 1-HFS (A) or 3-HFS (B). Normalized fEPSP slopes are plotted against time. **(A)**, **(B)** LTP induced by 1-HFS or 3-HFS in one pathway is indicated by filled grey or black circles respectively (referred to as stim). The corresponding within-slice control pathways are indicated by open grey or black circles (referred to as con). Insets show fEPSP traces (average of 10 consecutive sweeps) taken from the presented experiment (1) during baseline stimulation, (2) 15 min after the last train of HFS and (3) 5 hours after delivery of HFS as indicated in the time course graph. fEPSPs of the stimulated and control pathway are displayed on the left and right side, respectively.

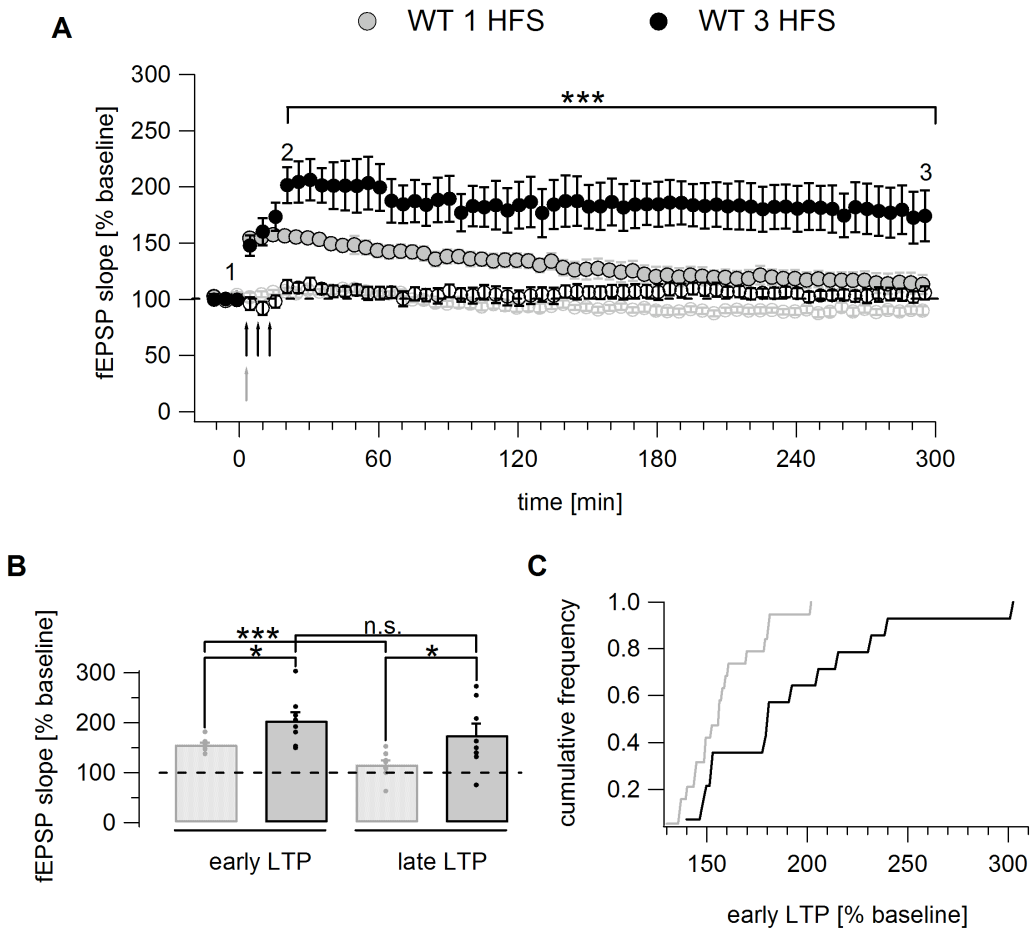

Figure 4.3: 1-HFS and 3-HFS induce transient and long-lasting LTP in WT slices, respectively

(A) Summary graph: LTP evoked by 1-HFS (n = 10, N = 9) and 3-HFS (n = 8, N = 8) is indicated by filled grey or black circles respectively. The within-slice control pathways (open circles) are illustrated in corresponding colors. Asterisks indicate significance in the post-hoc ANOVA comparison between the stimulated pathway following 1-HFS and 3-HFS ($P < 0.001$). **(B)** Bar graph: Comparison of the averaged response during early (first 15 min post-HFS) and late LTP (240-300 min post-HFS). Bars represent mean, error bars describe S.E.M., and dots indicate values for individual experiments. **(C)** Cumulative histogram: Cumulative frequencies of the maximum potentiation recorded during early LTP after stimulation with 1-HFS (n = 19) or 3-HFS (n = 14) are displayed in grey and black lines respectively.

Averaged data obtained from WT mice receiving either 1-HFS (n = 10 slices, N = 9 mice) or 3-HFS (n = 8 slices, N = 8 mice) is presented as a summary graph (Figure 4.3).

The fEPSP slope was reliably potentiated and the enhancement of the fEPSP slope was significant compared to pre-HFS baseline values (baseline: 100 ± 3 %; post-HFS: 1-HFS: 157 ± 4 %, n = 10; 3-HFS: 206 ± 18 %, n = 8; $p < 0.001$). Induction of long-term potentiation only failed in 2 out of 68 WT slices corresponding

to the criteria explained in chapter 3.4.6 (~ 97 % successful LTP inductions). This indicated that both 1-HFS and 3-HFS reliably induced LTP in WT slices.

The peak increase in fEPSP slope in WT slices was significantly higher in response to 3-HFS compared with 1-HFS (1-HFS: 157 ± 4 %, n = 10; 3-HFS: 206 ± 18 %, n = 8; $p < 0.001$). The initially potentiated fEPSP slope in LTP induced by 1-HFS subsequently decreased and returned to baseline levels. Statistical analysis of individual time points using ANOVA showed that the pathway receiving 1-HFS was no longer significantly different from baseline or its within-slice control pathway after 230 min post-HFS ($p = 0.077$). This was consistent with our expectation that 1-HFS is only sufficient to induced a transient form of LTP which declines back to baseline values (5 hrs post-HFS: 113 ± 9 % compared to pre-HFS baseline 101 ± 1 %, n = 10; $p = 0.177$).

In contrast, LTP induced by 3-HFS was larger and lasted throughout the experiment (5 hrs post-HFS: 180 ± 22 %, n = 8). Following 3-HFS LTP remained stably high (e-LTP vs. l-LTP following 3-HFS: $p = 0.172$; l-LTP vs. baseline or control pathway: $p < 0.001$). Statistical analysis using ANOVA showed that LTP induced by 3-HFS was significantly enhanced compared to LTP following 1-HFS throughout the entire experiment ($p < 0.001$ after 20 min).

The HFS (1 or 3) stimuli had no effect on the control pathway other than a short-lasting heterosynaptic depression during the delivery of the stimulation protocol. Furthermore, control pathways remained stable and no difference were detected between the control pathways derived from the two different conditioning experiments (1 vs. 3 HFS; $p = 0.372$). This indicated that the form of LTP induced in these experiments was confined to the synapse receiving HFS and was therefore input specific.

In Figure 4.3 B a bar graph presenting the quantified data for early and late LTP is displayed. It shows that e-LTP evoked by 1-HFS was significantly smaller compared to the e-LTP induced by 3-HFS (1-HFS: 156 ± 4 %, n = 10; 3-HFS: 204 ± 17 %, n = 8; $p = 0.028$). Late-LTP was significantly larger in the 3-HFS compared to the 1-HFS experiments (1-HFS: 116 ± 8 %, n = 10; 3-HFS: 205 ± 17 %, n = 8; $p = 0.02$). In the 1-HFS experiments e-LTP and l-LTP also differed significantly (e-LTP: 156 ± 4 %, n = 10; l-LTP: 116 ± 8 %, n = 10; $p < 0.001$) reflecting the time dependent decline of LTP. After 3-HFS l-LTP was not significantly different from e-LTP (e-LTP: 204 ± 17 %, n = 8; l-LTP: 179 ± 21 %, n = 8; $p = 0.381$).

To examine whether slices stimulated with 3-HFS generally exhibited higher amplitudes during e-LTP, a cumulative histogram was generated presenting the frequency distribution of the maximal potentiation achieved in individual experiments (Figure 4.3 C). For this analysis all experiments were included in which the recordings were stable during the first 90 minutes post-HFS. The number of analyzed slices increased to n = 19 (1-HFS) and n = 14 (3-HFS). The cumulative distribution of 3-HFS e-LTP was broader and shifted to the right compared with 1-HFS e-LTP. This demonstrates that in all experiments 3-HFS yielded larger LTP amplitudes than 1-HFS.

In brief these results demonstrate:

(1) 1-HFS and 3-HFS induced two different forms of LTP.

(2) 3-HFS induced higher e-LTP amplitudes than 1-HFS in WT slices.

(3) Late-LTP was only maintained after 3-HFS whereas LTP after 1-HFS returned to baseline levels.

4.2.2. LTP in KO mice (E8KO)

LTP was induced in KO slices using the same induction protocols as described for experiments conducted in WT mice.

Individual experiments showing representative LTP time courses are shown in Figure 4.4. In response to 1-HFS as well as 3-HFS a robust enhancement of synaptic transmission was observed which remained elevated throughout the five hours post HFS. The control pathways remained unchanged through the experiment, demonstrating input specificity of LTP in these experiments. Furthermore no detectable alterations in FV amplitudes were found indicating constant presynaptic fiber recruitment.

I repeated these experiments in six slices derived from six mice stimulated with 1-HFS and nine slices derived from nine mice stimulated with 3-HFS (Figure 4.5). Both conditioning protocols, 1-HFS and 3-HFS, induced a significant increase in synaptic transmission. LTP was reliably induced since only 1 out of 40 slices failed to induce LTP.

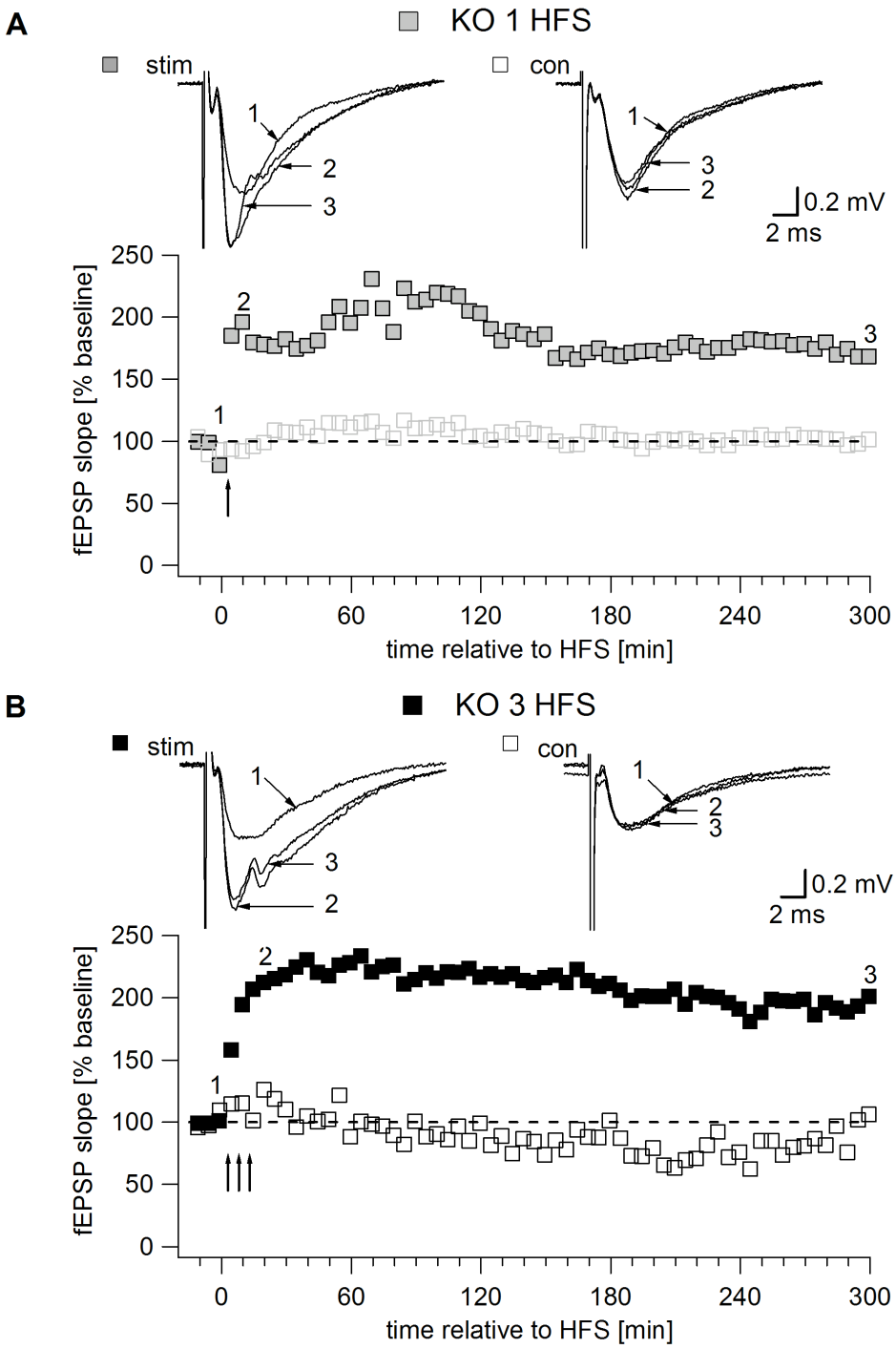

Figure 4.4: LTP in KO mice in response to 1-HFS and 3-HFS

Single experiments showing representative time courses of LTP induced by either 1-HFS (A) or 3-HFS (B) respectively. The normalized fEPSP slope is plotted against time. **(A)**, **(B)** LTP induced by 1-HFS or 3-HFS in one pathway is indicated by filled grey or black squares respectively. Insets show fEPSP traces (average of 10 consecutive sweeps) taken from the presented experiment (1) during baseline stimulation, (2) 15 min after the last train of HFS and (3) 5 hours after delivery of HFS.

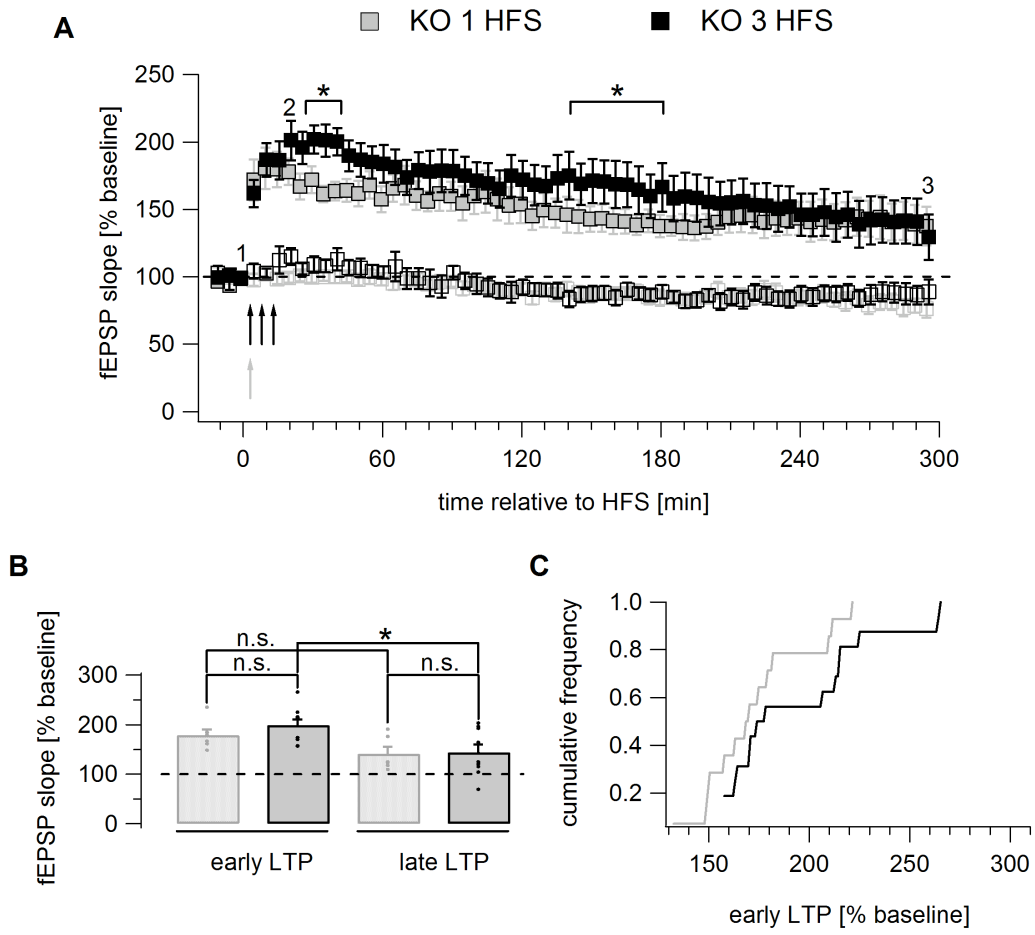

Figure 4.5: Loss of graded LTP in KO mice

(A) Summary graph: LTP induced by 1-HFS (grey squares; n = 6, N = 6) and 3-HFS (black squares; n = 9, N = 8) in KO slices. Open squares illustrate within-slice control pathways. Asterisks indicate significance in the post-hoc ANOVA comparison of stimulated pathways. **(B)** Bar graph: Comparison of the averaged response during early and late LTP. Bars represent mean, error bars describe S.E.M. and dots values for individual experiments. **(C)** Cumulative histogram: Cumulative frequency of the maximal potentiation recorded during early LTP after application of 1 (n = 14) or 3-HFS (n = 16) is displayed in a grey and black respectively.

Field EPSP were significantly potentiated in comparison to baseline in response to both 1-HFS and 3-HFS stimuli (Figure 4.5 A: 1-HFS: 181 ± 15 %, n = 6; 3-HFS: 201 ± 15 %, n = 9; $p < 0.001$). The amplitude of e-LTP was similar between the stimuli ($p = 0.087$).

In response to both stimuli, LTP reached its maximum within 30 min after HFS (e-LTP) and diminished thereafter. Comparison of the remaining fEPSP potentiation five hours post-HFS to pre-HFS baseline recordings showed that fEPSPs were still significantly elevated following 1-HFS (5 hrs post-HFS: 133 ± 12 %, n = 6,

pre-HFS baseline 99 ± 4 %, n = 6; p = 0.042) as well as 3-HFS (5 hrs post-HFS: 141 ± 17 %, n = 9, pre-HFS baseline: 98 ± 3 %, n = 9; p = 0.049). Statistical post-hoc comparison of the stimulated pathways at all time points found no significant differences in LTP induced by either 1-HFS or 3-HFS except for two short phases (25.5 - 40.5 min & 140.5 – 180.5 min post-HFS).

Control pathways of both conditions tended to decline but this decline was only significant for 1-HFS (control fEPSP slope 5 hrs post-HFS: 79 ± 15 %, n = 6, p = 0.02) whereas control fEPSPs remained unchanged for 3-HFS (control fEPSP slope 5 hrs post-HFS: 86 ± 22 %, n = 9, p = 0.355). Thus input-specificity of LTP was at least maintained during the first two hours post-HFS in Arc/Arg3.1 deficient mice.

For quantification and direct comparison of e-LTP and l-LTP pooled data of the specific time periods is displayed in bar graphs (Figure 4.5 B). Analysis of pooled data, representing e-LTP and l-LTP, demonstrated that e- LTP tended to be slightly - but not significantly - higher following 3-HFS (1 HFS: 179 ± 12 %, n = 6; 3 HFS: 199 ± 12 %, n = 9; p = 0.258). Late-LTP was not significantly different between the stimuli (1-HFS: 141 ± 14 %, n = 6; 3-HFS: 144 ± 16 %, n = 9; p = 0.865). Hence, in contrast to WT, different strengths of LTP induction stimuli (1-HFS vs. 3-HFS) caused no great modification of the resulting LTP.

As illustrated in Figure 4.5 A, the initially potentiated fEPSP slightly decayed following both conditioning protocols. To test the significance of this observation we compared e-LTP and l-LTP over all experiments. Following 3-HFS l-LTP was significantly reduced compared to e-LTP (e-LTP: 199 ± 12 %, n = 9; l-LTP: 144 ± 16 %, n = 9; p = 0.013). After 1-HFS the slight decrease in LTP was not significant (e-LTP: 179 ± 12 %, n = 6; l-LTP: 141 ± 14 %, n = 6; p = 0.064).

Cumulative histograms of e-LTP amplitudes in response to 1-HFS and 3-HFS are given in Figure 4.5 C. Both distributions start at a high e-LTP amplitude and have similar half-maximal values, but the 3-HFS distribution is slightly right-shifted towards larger amplitudes.

In summary:

(1) The amplitude of e-LTP was similar in response to 1-HFS and 3-HFS.

(2) 1-HFS and 3-HFS LTP was induced which remained elevated compared to baseline, albeit at a declining rate.

4.2.3. Comparison of LTP in WT and KO mice (E8KO)

Next we wanted to examine how the complete deletion of the Arc/Arg3.1 gene affected LTP expression in response to 1-HFS and 3-HFS. For this reason the data obtained in LTP experiments in WT and KO was compared with respect to the genotype (Figure 4.6).

In response to 1-HFS KO slices exhibited larger LTP throughout the entire recording period. This difference was statistically significant shortly during e-LTP (WT: 157 ± 4 %, n = 10; KO: 181 ± 15 %, n = 6; p = 0.019), an intermediate phase (55 – 110 min: p < 0.048) and l-LTP (WT: 113 ± 9 %, n = 10; KO: 138 ± 14 %, n = 6; p = 0.007). In WT slices the potentiation of the fEPSP after 1-HFS decreased to baseline (fEPSP slope 5 hrs post-HFS 113 ± 9 %, n = 10; 0.177). In contrast to this, 1-HFS LTP in KO slices remained significantly elevated for five hours (fEPSP slope 5 hrs post-HFS: 138 ± 14 %, n = 6; p < 0.001).

A cumulative histogram comparing the magnitude of LTP is presented in Figure 4.6 C. The distribution of peak LTP amplitudes in KO was shifted to the right in comparison to WT, indicating that LTP was larger in each of the KO slices recorded.

A comparison of LTP in response to 3-HFS (Figure 4.6 B) shows that e-LTP was similar in WT and KO slices (WT: 206 ± 18 %, n = 8; KO: 201 ± 15 %, n = 9; p = 0.543). However, the late phase of LTP in KO slices was reduced compared to WT slices at four hours post-HFS (p = 0.046). Five hours after LTP induction the increase in fEPSP slope was maintained at 180 ± 22 % in WT (n = 8), whereas the remaining fEPSP slope potentiation recorded in KO was 141 ± 17 % (n = 9) (p = 0.02).

No significant differences between the control pathways were be found using *ANOVA* to compare individual time points (p ≥ 0.218).

The overlapping curves in the cumulative histogram for WT and KO slices after 3-HFS indicate that not only the average e-LTP was similar but also the distribution of data obtained from individual experiments appeared similar (Figure 4.6 C).

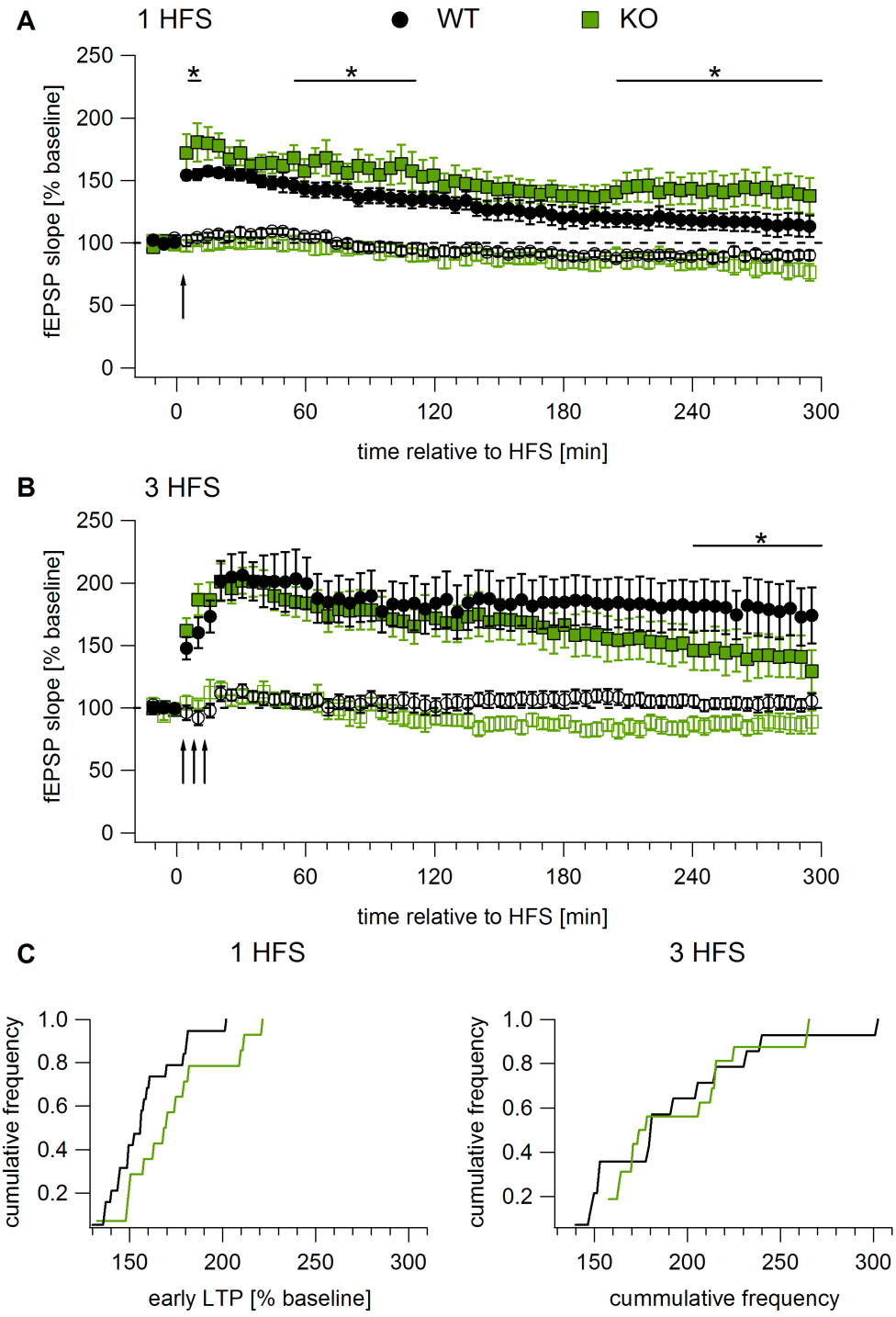

Figure 4.6: Comparison of LTP expression in KO and WT mice

LTP induced in WT (black circles) and KO (green squares). **(A)** 1-HFS (WT: n = 10, N = 9; KO: n = 6, N = 6). **(B)** 3-HFS (WT: n = 8, N = 8; KO: n = 9, N = 8). **(C)** Cumulative histogram: Cumulative frequencies of the maximal potentiation recorded during early LTP after application of 1-HFS (WT: n = 19; KO: n = 14) or 3-HFS (WT: n = 14; KO: n = 16) are displayed in black and green respectively.

Taken together these results suggested:

(1) In response to weak stimulation protocols, i. e. 1-HFS, LTP was of both higher amplitude and a more stable maintenance in KO compared to WT slices.

(2) 3-HFS LTP in KO slices appeared to be attenuated compared to WT.

In KO slices the remaining level of potentiation five hours post-HFS was still elevated compared to baseline but in comparison to WT slices receiving the same stimulation paradigm an impairment of LTP maintenance became obvious.

4.2.4. Basal synaptic transmission in mouse line E8KO

I further asked whether LTP expression might be related to differences in basal synaptic properties. Therefore we tested basal synaptic transmission and paired-pulse facilitation to detect differences in pre- or postsynaptic function. Basal synaptic transmission was assessed at *Schaffer-Collateral* projections to the apical dendrites of the *CA1* neurons, the same synaptic connections in which LTP measurements took place. The fEPSP amplitude was measured in responses to single-pulse stimulation. First the amount of injected current needed to evoke a population spike in the recorded neurons was measured and defined as the PS threshold.

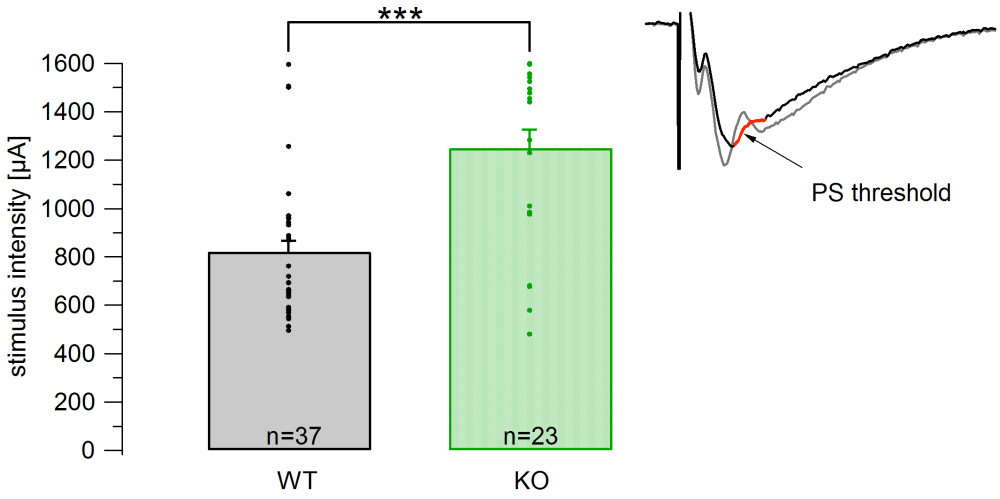

Figure 4.7: KO mice require higher stimulation intensities to reach the PS threshold

Bar graph: Threshold intensity required to evoke a population spike in WT (black bar; n = 37) and KO (green bar: n = 23)). Inset illustrates the PS threshold within the fEPSP trace as indicated by the positive deflection in the fEPSP trace (highlighted in red). Underlying grey fEPSP trace demonstrates the fully developed PS.

This analysis showed that a significantly higher stimulation intensity ($SI_{threshold}$) was needed to evoke a PS in KO slices compared to WT slices (Figure 4.7: WT: 821 ± 26 µA, n = 37; KO: 1250 ± 77 µA, n = 23; P < 0.001). Despite the fact that a higher stimulation intensity was needed to evoke a PS, the maximum amplitude of the fEPSP recorded at the threshold ($fEPSP_{threshold}$) was similar in WT and KO slices (WT: 1.5 ± 0.09 mV, n = 37; KO: 1.62 ± 0.17 mV, n = 23; p = 0.548).

To further examine basal synaptic transmission IO-curves were generated (Figure 4.8). Construction of IO-curves was carried out as explained in chapter 3.4.2. Although both genotypes reached a comparable maximum fEPSP, the slope of the IO-curve generated for KO was less steep. To assess whether the difference in slope was significant the SI needed to evoke a fEPSP yielding 50 % of the maximum fEPSP ($fEPSP_{50\%}$) was compared using *student´s t-test*.

The SI required to evoke the $fEPSP_{50\%}$ was higher in KO slices (WT: 479 ± 19 µA, n = 37; KO: 544 ± 26 µA, n = 23; p = 0.041). This suggests that synaptic transmission in KO slices was less responsive to afferent stimulation but owns the same capability to generate a comparable postsynaptic response.

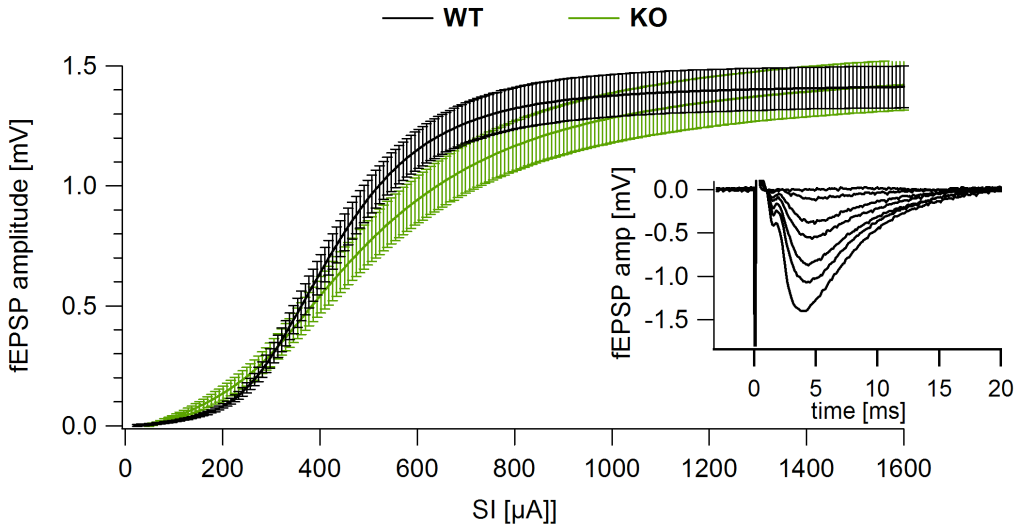

Figure 4.8: IO-curves in KO are less steep but reach a similar $fEPSP_{max}$ compared to WT

IO-curves derived from 37 WT slices (N = 11) and 23 KO slices (N = 10). Inset shows example recording of fEPSP in response to increasing SI.

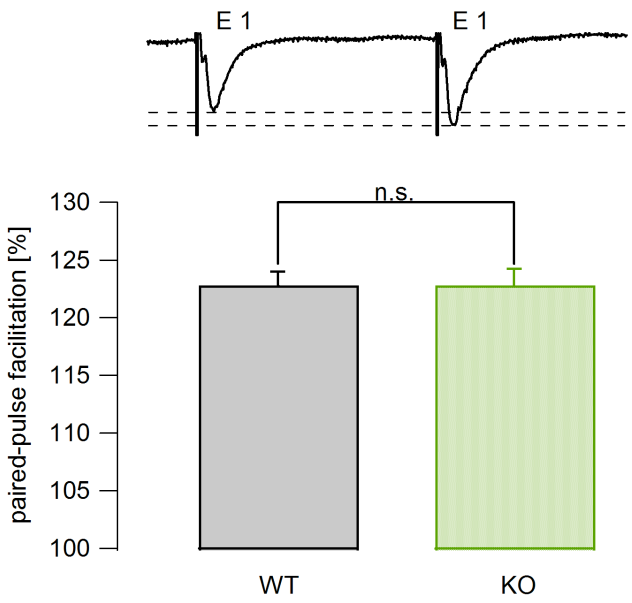

Figure 4.9: PPF is similar in WT and KO mice

PPF is presented as the facilitation ratio, i. e. amplitude of the second response relative to the first. WT (n = 68) and KO (n = 57) are illustrated in black and green respectively. Inset shows example paired-pulse fEPSP trace.

The degree of PPF was assessed in WT and KO slices to test for differences in presynaptic function as described in chapter 3.4.3. Paired pulse facilitation was similar in WT and KO slices (Figure 4.9: WT: 122.8 ± 1.2 %, n = 68; KO: 122.8 ± 1.47 %, n = 57; p = 0.996).

In brief, examination of basal synaptic transmission showed similar preconditions in WT and KO slices regarding the maximum fEPSP which could be attained and presynaptic function. Nevertheless, KO mice appeared to be less excitable.

4.3 LTP IN MOUSE LINE TGMXS*B6

I next asked whether the different translation modes or kinetics of Arc/Arg3.1 expression had a specific function in LTP consolidation. To study the role of locally translated Arc/Arg3.1 I included transgenic Arc/Arg3.1 mice (Tg) into my analysis. To avoid any inequalities and to directly compare the effect of dendritic Arc/Arg3.1 translation, Tg mice were compared to WT and KO mice derived from the same mouse line (TgMXS*B6).

Data from LTP experiments in mouse line TgMXS*B6 is presented in the following chapters.

4.3.1. LTP in WT mice (TgMXS*B6)

Long-term potentiation was recorded in WT slices in response to 1-HFS and 3-HFS (Figure 4.10).

Field EPSP responses recorded in slices exhibited an immediate increase in response to both conditioning protocols. The peak increase in LTP amplitude was just slightly, but significantly higher in WT receiving 3-HFS compared to 1-HFS (1-HFS: 162 ± 10 %, n = 9; 3-HFS: 186 ± 8 %, n = 8; p = 0.001). After 1-HFS fEPSP immediately started to decline but stabilized after three hours at a potentiation level of 121 - 135 % of baseline fEPSP amplitude.

In contrast, after 3-HFS the initial potentiation continuously decayed during the remaining 5 hours. As a result of this decline the amplitude of 3-HFS LTP was similar to 1-HFS LTP at 260 min post-HFS (fEPSP potentiation 5 hrs post HFS: 1-HFS: 121 ± 12 %, n = 9; 3-HFS: 123 ± 8 % n = 8, p = 0.116). Thus 1-HFS and 3-HFS LTP was maintained to the same degree in WT slices.

The remaining potentiation five hours after HFS in response to 1-HFS and 3-HFS returned to levels not significantly different from pre-HFS baseline values (1-HFS: 5 hrs post-HFS: 121 ± 12 %, n = 9; p = 0.125; 3-HFS: 5 hrs post-HFS: 123 ± 8 %, n = 8; pre-HFS baseline: 106 ± 3 %, n = 17; p = 0.141).

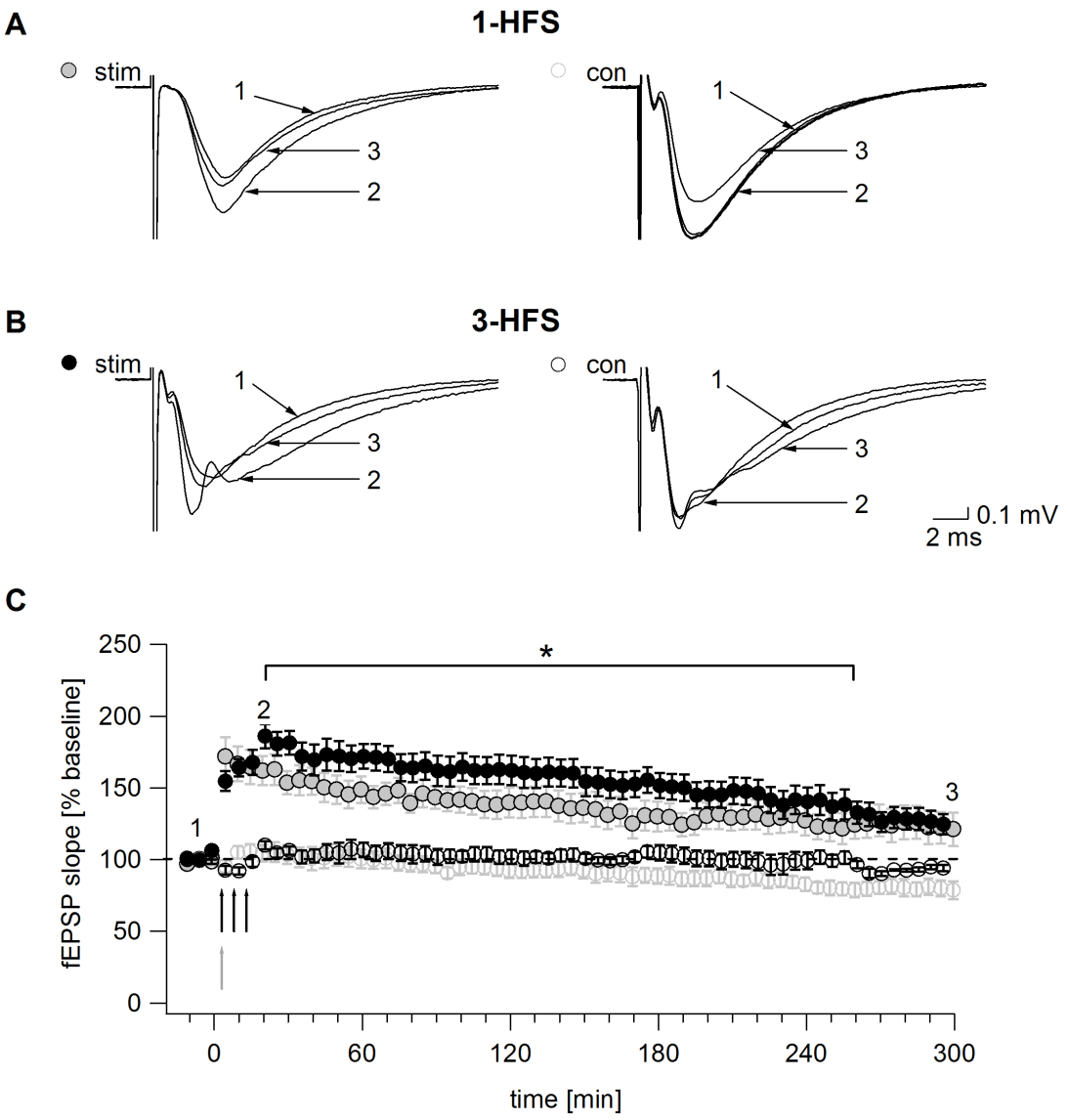

Figure 4.10: Transient LTP in WT in response to 1-HFS and 3-HFS (TgMXS*B6)

(A), **(B)** Representative fEPSP traces illustrating the fEPSP development during LTP. Recordings were made in response to 1-HFS (A) and 3-HFS (B) during (1) baseline stimulation, (2) 15 min after the last train of HFS and (3) 5 hours after delivery of HFS. **(C)** Summary graph: LTP evoked by 1-HFS (n = 9 ; N = 6) and 3-HFS (n = 8; N = 6) in WT as indicated by filled grey and black circles respectively. The within-slice control pathways (open circles) are illustrated in corresponding coloring. Asterisk indicated significance in the post-hoc ANOVA comparison between stimulated pathways (1-HFS vs 3-HFS).

I noticed, however, that the control pathways in these experiments showed a small but steady decline of the fEPSP slope in 1-HFS (pre-HFS: 100 ± 2 %; 5 hrs post-HFS: 79 ± 15 %, n_{con} = 5; p = 0.025) and 3-HFS experiments (pre-HFS: 99 ± 3 %; 5 hrs post-HFS: 93 ± 3 %, n_{con} = 7; p = 0.007).

It could not be excluded that the decline observed in the control pathway was a global, unspecific effect reflecting a general decline of synaptic transmission which affected the whole slice. To account for this general decline, a second comparison of the fEPSP slope in the stimulated pathway with its control pathway was performed. Field EPSPs recorded five hours post-HFS in the pathways receiving the conditioning stimuli were still potentiated compared to their within-slice control pathways after 1-HFS (stim: 121 ± 12 %, n = 9; con: 79 ± 15 %, n = 5; $p < 0.001$) as well as 3-HFS (stim: 123 ± 8 %, n = 8; con: 93 ± 3 %, n = 7; $p = 0.001$).

Taken together this data demonstrate that 1-HFS and 3-HFS can induce synapse-specific LTP in WT slices from TgMXS*B6 mice. However, this 3-HFS LTP declined over time in contrast to the high and stable 3-HFS LTP evoked in WT slices of the E8KO mouse line. Furthermore it has to be noticed, that a general diminishment of synaptic transmission occurred in these experiments as indicated by the significant decline observed in the control pathway.

4.3.2. LTP in KO mice (TgMXS*B6)

To examine LTP in the total absence of Arc/Arg3.1 LTP experiments were conducted in KO mice of mouse line TgMXS*B6 (Figure 4.11).

In these experiment both 1-HFS and 3-HFS again evoked a significant enhancement of synaptic transmission compared to pre-HFS baseline or the within-slice control pathways ($p < 0.001$).

These experiments show an enhancement of the initial magnitude of LTP in KO slices after 3-HFS. In response to 1-HFS fEPSP were potentiated to a peak slope amplitude of 179 ± 8 % (n = 7), whereas the peak increase following 3-HFS reached significantly higher levels (213 ± 8 %, n = 8; $p = 0.01$). In response to both conditioning protocols a decline of the initially robust LTP was observed. Two hours after HFS, 3-HFS LTP decayed to the same level as 1-HFS LTP (115 min post-HFS: 1-HFS: 132 ± 4 %, n = 7; 3-HFS: 164 ± 14 %, n = 8; p = n. s.). Five hours after HFS fEPSPs decayed to baseline level in response to 1-HFS as well as 3-HFS (1-HFS: 114 ± 10 %, n = 7; $p = 0.174$; 3-HFS: 126 ± 11 %, n = 8; p = n. s.). In 3-HFS experiments a significant decline of the control pathway was detected (pre-HFS: 103 ± 3 %; 5 hrs post-HFS: 78 ± 8 %, n = 6; $p = 0.007$).

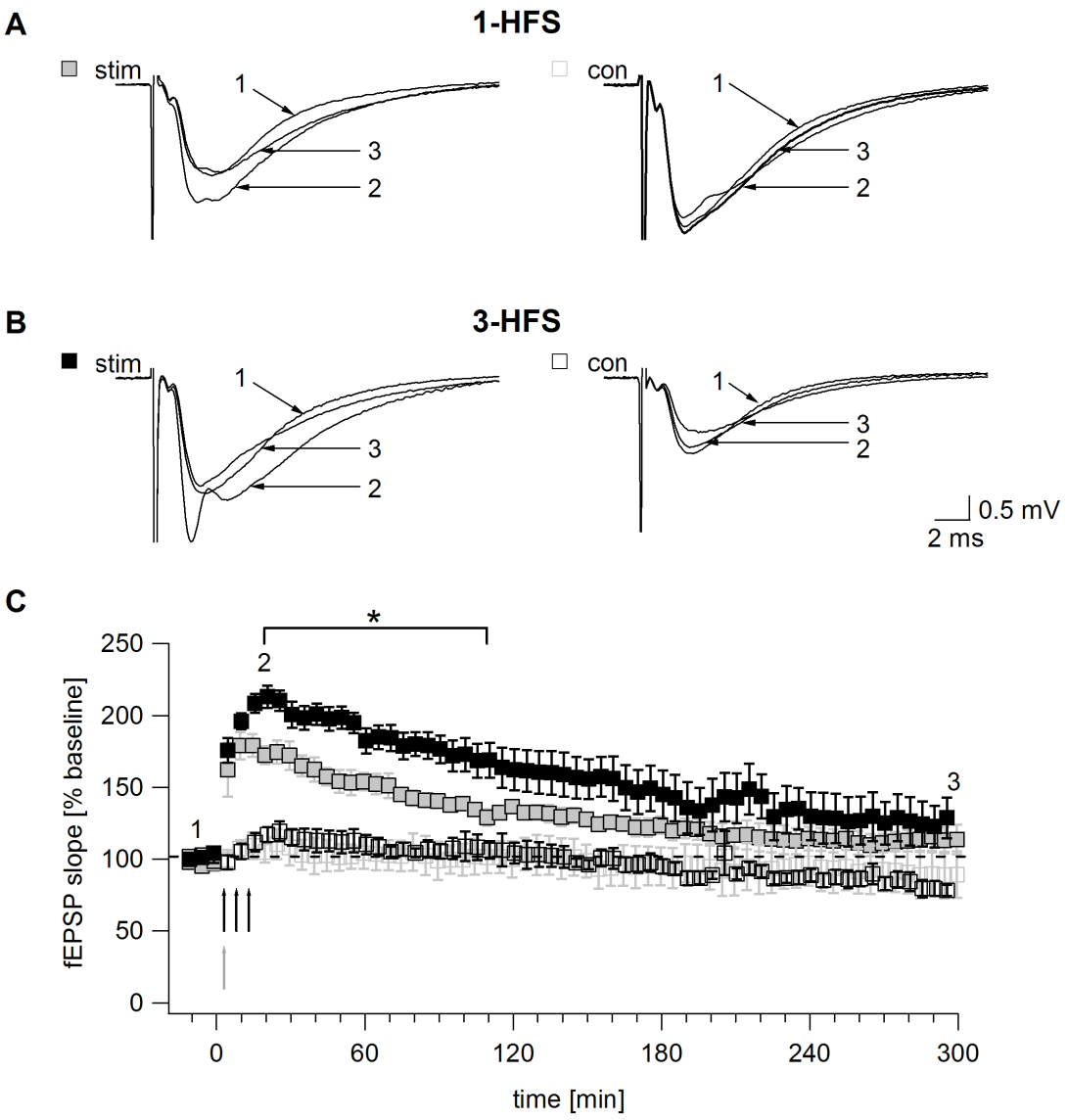

Figure 4.11: Transient LTP in KO mice in response to 1-HFS and 3-HFS (TgMXS*B6)

(A), **(B)** Representative fEPSP traces illustrating the fEPSP development during LTP. Recordings were made in response to 1-HFS (A) and 3-HFS (B) during (1) baseline stimulation, (2) 15 min after the last train of HFS and (3) 5 hours after delivery of HFS. **(C)** summary graph: LTP evoked by 1-HFS (n = 7; N = 3) and 3-HFS (n = 8; N = 3) in KO is indicated by filled grey or black squares respectively. The within-slice control pathways (open squares) are illustrated in corresponding coloring. Asterisks indicate significance in the post-hoc ANOVA comparison of the pathways receiving the conditioning protocol (1-HFS vs 3-HFS).

With regard to the decline observed in the 3-HFS control pathway, as second comparison of the remaining fEPSP slope five hours after 3-HFS showed that fEPSP were still significantly potentiated compared to control (stim: 126 ± 11 %, n = 8; con: 78 ± 8 %, n = 6; $p < 0.001$).

Taken together these experiments demonstrate that 1-HFS and 3-HFS LTP in KO slices was maintained to the same degree and declined to baseline.

4.3.3. LTP in Tg mice (TgMXS*B6)

Next I examined LTP expression in Tg mice to study the role of locally translated Arc/Arg3.1 in synaptic plasticity (Figure 4.12). Field EPSPs increased immediately after 1-HFS and 3-HFS. In response to 3-HFS e-LTP was increased compared to 1-HFS and remained significantly larger for 10 min post-HFS (1-HFS: 171 ± 10 %, n = 10; 3-HFS: 217 ± 19 %, n = 11; p = 0.011). The initially strong 3-HFS LTP subsequently decayed and was no longer significantly different from 1-HFS 30 min after HFS (1-HFS: 166 ± 11 %, n = 10; 3-HFS: 200 ± 19 %, n = 11; p = 0.086).

Field EPSPs of stimulated pathways were no longer significantly different to control pathways after 265 min (1-HFS; p = 0.54) and 260 min (3-HFS; p = 0.064) post-HFS. Five hours after HFS fEPSP in the pathway receiving 1-HFS were maintained at 132 ± 15 % of pre-HFS baseline (n = 10), a level not significantly different from pre-HFS baseline (p = 0.178). Similarly, fEPSPs following 3-HFS returned to baseline levels (5 hrs post-HFS: 129 ± 11 %, n = 11; p = 0.066).

Representative fEPSP traces are shown illustrating changes in fEPSP after 1-HFS and 3-HFS (Figure 4.12 A). Field EPSPs show an increase in amplitude and slope in response to both conditioning protocols. Population spikes developed after the enhancement of synaptic transmission indicating that the firing threshold in the postsynaptic neurons now discharging action potentials was reached. Field EPSP traces recorded in control pathways remained stable during the whole recording. Furthermore FV amplitudes remained constant in all recorded pathways.

Taken together these experiments showed LTP in Tg slices was instable, likewise returning to baseline levels after 1-HFS and 3-HFS. A small, but significant difference in e-LTP amplitudes (3-HFS > 1-HFS) was detected.

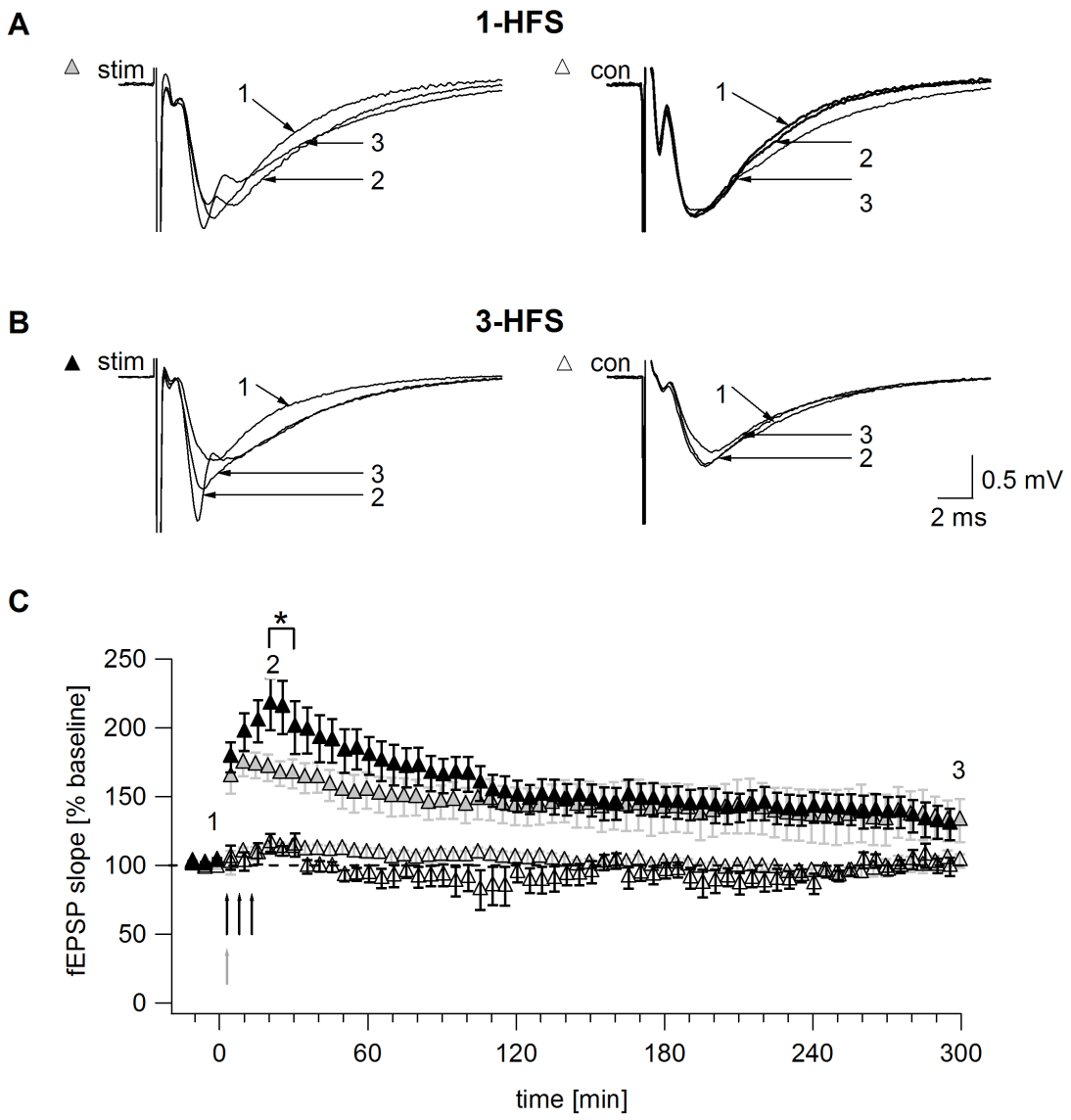

Figure 4.12: Transient LTP in Tg mice in response to 1-HFS and 3-HFS (TgMXS*B6)

(A), **(B)** Representative fEPSP traces illustrating the fEPSP development during LTP. Recordings were made in response to 1-HFS (A) and 3-HFS (B) during (1) baseline stimulation, (2) 15 min after the last train of HFS and (3) 5 hours after delivery of HFS. **(C)** Summary graph: LTP evoked by 1-HFS (n = 10; N = 5) and 3-HFS (n = 11; N = 6) is indicated by filled grey or black triangles respectively. The within-slice control pathways (open triagles) are illustrated in corresponding coloring. Asterisks indicate significance in the post-hoc ANOVA comparison of stimulated pathway (1-HFS vs 3-HFS).

4.3.4. Comparison of LTP in mouse line TgMXS*B6

To directly investigate the functional role of Arc/Arg3.1, I compared 1-HFS and 3-HFS LTP between the genotypes.

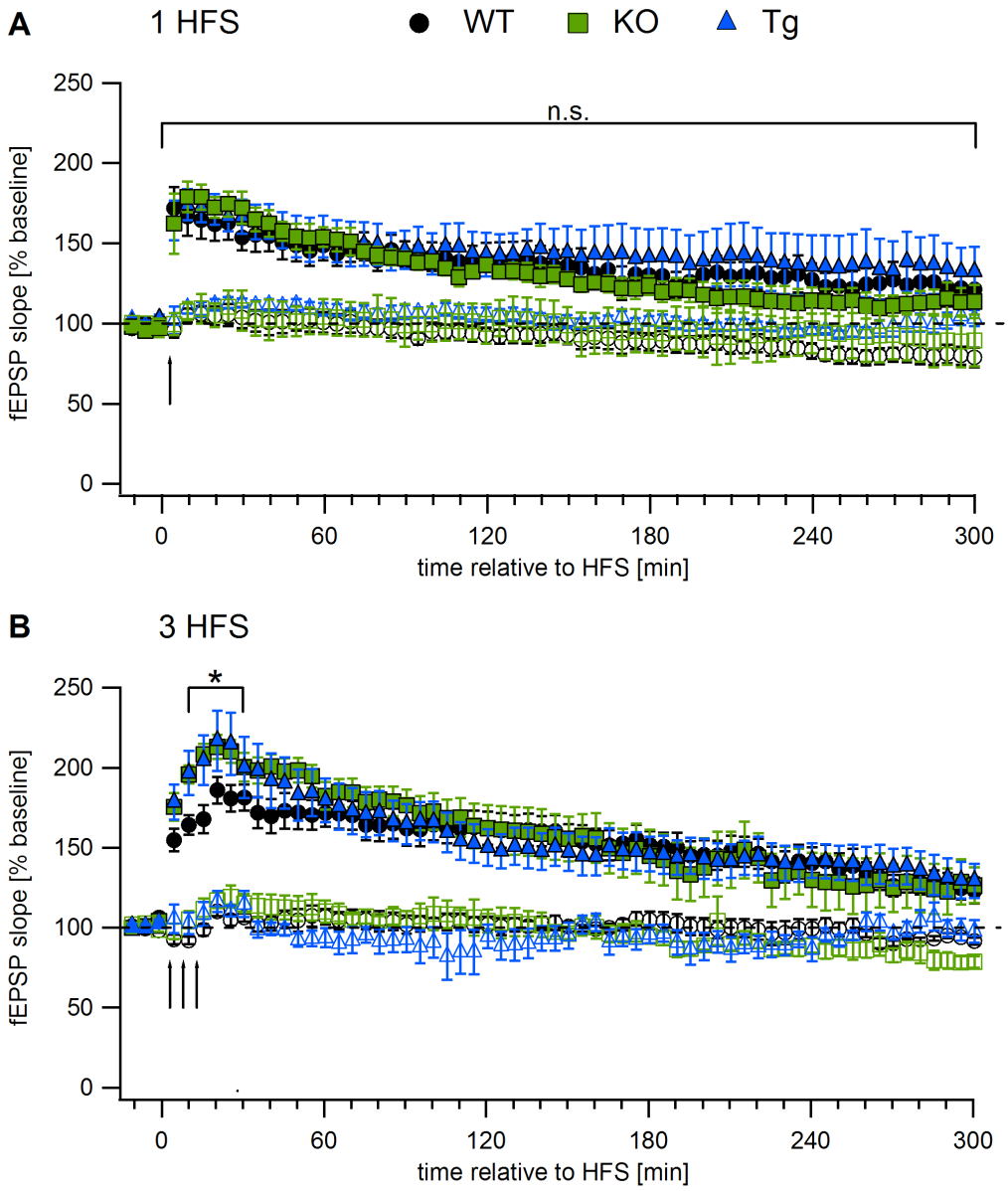

Figure 4.13: 1-HFS and 3-HFS are only sufficient to induce transient LTP in all genotypes but e-LTP was significantly increased in response to 3-HFS in KO and Tg mice

(A), **(B)** Comparison of LTP induced by either 1-HFS (A) or 3-HFS (B) in hippocampal slices of WT (A: n = 9; N = 6; B: n = 8; N = 6), KO (A: n = 7; N = 3; B: n = 8; N = 3) and Tg mice (A: n = 10; N = 5; B: n = 11; N = 6). Stimulated pathways receiving the conditioning protocol are depicted by black circles (WT), green squares (KO) and blue triangles (Tg). Control pathways are illustrated by open symbols in the corresponding colors. Asterisks indicate significance in the post-hoc ANOVA comparison of stimulated pathways in KO and Tg slices compared to WT slices.

LTP evoked by 1-HFS was similar amongst all three genotypes (Figure 4.13 A). One HFS induced LTP in slices of Tg and KO mice of a magnitude comparable to WT slices (WT: 162 ± 10 %, n = 9; KO: 179 ± 8 %, n = 7; Tg: 171 ± 10 %, n = 10; p = n. s.). Moreover, no significant alterations of the subsequent decay phase of LTP were found. Consequently, fEPSP slope in Tg and KO were maintained at a level similar to WT five hours post-HFS (WT: 121 ± 12 %, n = 9; KO: 126 ± 11 %, n = 7; Tg: 132 ± 15 %, n = 10; p = n. s.). Control pathways showed no significant alterations (overall decline after five hours: WT: 81 ± 5 %, n = 5; KO: 89 ± 16 %, n = 4; Tg: 103 ± 5 %, n = 7; p = n. s.).

LTP induced by 3-HFS was compared (Figure 4.13 B). The amplitude of e-LTP was similar in Tg and KO slices ($p_{Tg/KO}$ = n. s.) but was significantly higher in comparison to WT slices (WT: 186 ± 8 %, n = 8; KO: 213 ± 8 %, n = 8; Tg: 217 ± 19 %, n = 11; $p_{WT/KO} = 0.013$, $p_{WT/Tg} = 0.026$). The initially enhanced LTP rapidly decreased and potentiated fEPSP slopes of KO and Tg were no longer significant from WT 15 minutes after HFS. An overall decay of the initially increased fEPSP was observed in all genotypes. Five hours post-HFS fEPSPs were maintained to a comparable degree (WT: 123 ± 8 %, n = 8; KO: 114 ± 10 %, n = 8; Tg: 129 ± 11 %, n = 11; p = n.s).

In summary, a transient form of LTP was similarly induced by 1-HFS in all genotypes. Early-LTP in KO and Tg slices was enhanced compared to WT slices after 3-HFS. 3-HFS induced LTP of increased amplitude compared to 1-HFS, but remained transient and was not sufficient to induce long-lasting l-LTP in these experiments. Since no enduring late phase of LTP was induced even in WT slices, it remains unclear whether differences in l-LTP maintenance exist amongst KO, Tg and WT slices.

4.3.5. Basal synaptic transmission in mouse line TgMXS*B6

The stimulation intensity required to evoke a PS was significantly higher in KO slices than in WT and Tg slices (WT: 902 ± 59 µA; KO: 1323 ± 58 µA; Tg: 962 ± 61 µA; $p_{WT/KO} < 0.001$, $p_{WT/Tg}$ = n. s., $p_{KO/Tg} < 0.001$).

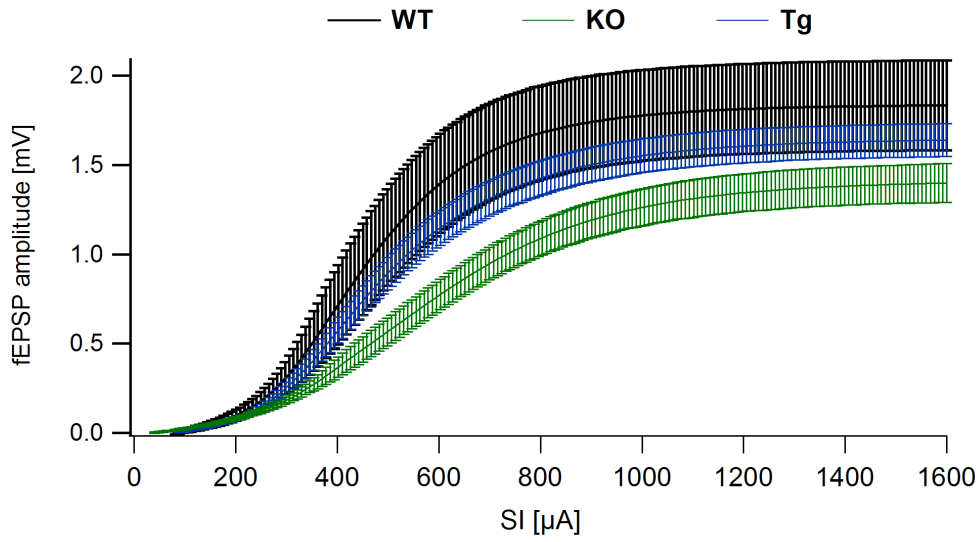

Figure 4.14: Constitutive Arc/Arg3.1 is involved in basal synaptic transmission in KO mice

IO-curves derived from WT slices (black, n = 33, N = 7), KO slices (green, n = 32, N = 5) and Tg slices (blue, n = 40, N = 7).

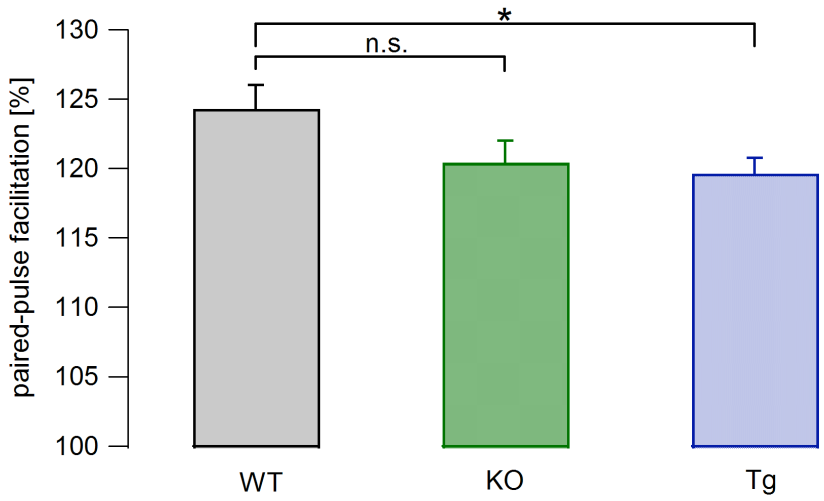

Figure 4.15: Paired-pulse facilitation ratio

Reduced PPF (in percent) in KO (green; n = 32) and Tg slices (blue; n = 40) compared to WT (black; n = 33).

Furthermore differences in the maximum fEPSP recorded at the PS threshold were found (WT: 1.82 ± 0.12 mV; KO: 1.41 ± 0.09 mV; Tg: 1.64 ± 0.09 mV; $p_{WT/KO} = 0.01$, $p_{WT/Tg}$ = n. s., $p_{KO/Tg}$ = n.s). As illustrated in Figure 4.14 fEPSP in WT slices were significantly increased compared to KO slices over the whole range of stimulation intensities. Field EPSPs recorded in Tg slices were of intermediate amplitude, not significantly different to WT or KO slices.

Paired-pulse facilitation was tested to assess presynaptic function. The PPF ratio was lower in KO and Tg slices compared to WT, but this differences only proved to be significant for Tg slices (Figure 4.15: WT: 124.29 ± 1.73 %; KO: 120.35 ± 1.61 %; Tg: 119.56 ± 1.23 %; $p_{WT/KO}$ = n.s, $p_{WT/Tg} = 0.047$, $p_{KO/Tg}$ = n.s).

4.4 LONG-LASTING LTP AND ITS DEPENDENCE ON PROTEIN SYNTHESIS

It is well established that long-lasting forms of synaptic plasticity require activity-induced protein synthesis (Krug et al. 1984, Frey et al. 1988). Late-LTP is, by definition, characterized by its dependence on de novo protein synthesis. Therefore we wanted to examine whether 3-HFS LTP induced in our experiments met this conditions. If the molecular mechanisms underlying its maintenance were altered in Arc/Arg3.1 KO has not yet been determined. For this reason we tested whether 3-HFS LTP expressed by WT, KO and Tg mice is dependent on protein synthesis. Mice used in the following experiments were derived from mouse line TgMXS*B6.

To investigate this question cycloheximide (CHX) or anisomycin (ANI) were applied to the aCSF during the experiment to block synthesis of new proteins. Cycloheximide interferes with the translocation at the ribosome and anisomycin is an inhibitor of the peptidyl transferase. Thus both inhibitors are reversible blockers of the elongation step during translation (Obrig et al. 1971). Cycloheximide and anisomycin were applied at a concentration of 80 µM and 40 µM respectively. Cycloheximide and anisomycin were bath-applied throughout the entire experiment.

4.4.1. Effect of cycloheximide on LTP in WT mice

We first investigated the protein synthesis dependence of 3-HFS LTP in WT slices (TgMXS*B6). Long-term potentiation was induced by 3-HFS in either standard recording medium or in the presence of 80 µM CHX.

Three-HFS caused a robust enhancement of synaptic transmission in control as well as in CHX containing solution as shown by fEPSP traces and corresponding time-course (Figure 4.16).

In the slice exposed to control aCSF, LTP persisted at a high level for five hours (Figure 4.16 A). In contrast, in the slice exposed to CHX-containing aCSF the initial LTP persisted for about 30 min and then declined to baseline level (Figure 4.16 B). The fEPSP shape recorded five hours after LTP induction in CHX was not different from the baseline fEPSP shape.

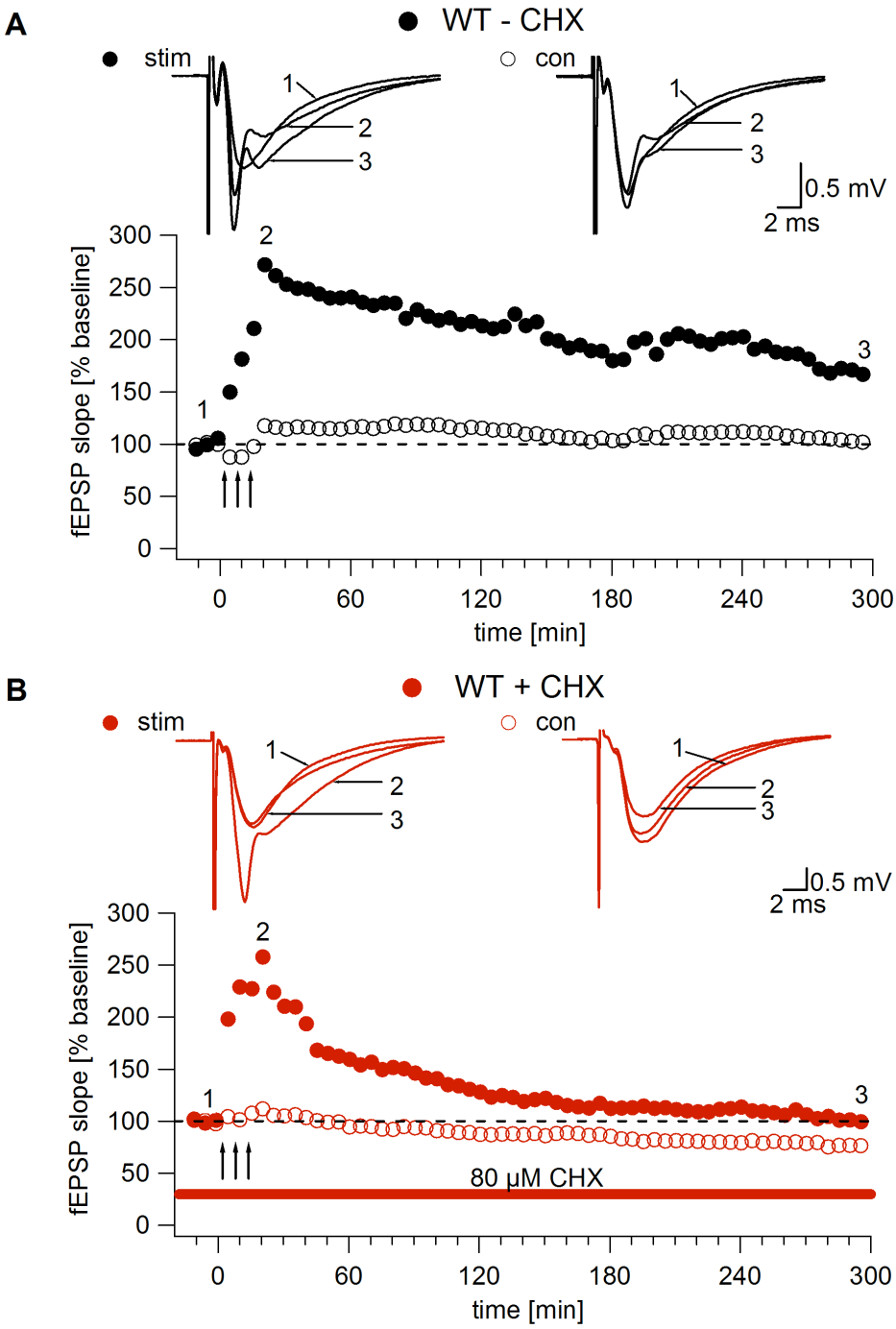

Figure 4.16: LTP in WT mice treated +/- CHX

Single experiments illustrating representative time courses of 3-HFS LTP induced either (B) under regimes of reduced protein synthesis (80 µM CHX) or (A) under control conditions. Normalized fEPSP slopes are plotted against time. **(A)**, **(B)** Stimulated pathways are indicated by filled black (control conditions;- CHX) and red circles (CHX treatment;+ CHX) respectively. Corresponding within-slice control pathways are indicated by open black or red circles. Insets show fEPSP traces taken from the presented experiment (1) during baseline stimulation, (2) 15 min after the last train of HFS and (3) 5 hours after delivery of HFS as indicated in the time course graph. Field EPSP traces are displayed on the left (stimulated pathway) and right side (control pathway).

In this example experiment, the within-slice control pathway of the experiment without CHX treatment developed a PS after 3-HFS indicating a change in excitability. However, this phenomenon was not observed in other experiments and could therefore be considered as an individual case. Control pathways and FV amplitude in both pathways were unaffected by delivery of 3-HFS indicating stability of the recording. Input specificity of LTP was maintained also in slices exposed to CHX aCSF. The control pathway recorded in a CHX treated slice slightly decreased mirroring the decrease in fEPSP slope observed in LTP time courses.

To investigate whether these results are representative the number of CHX treated slices was increased to nine and compared to eight control slices (Figure 4.17 A). Consistent with our previous experiments 3-HFS induced an immediate increase of the fEPSP compared to pre-HFS baseline ($p < 0.001$). LTP induction was unaffected by CHX since 3-HFS reliably induced LTP in all slices examined under control and drug conditions in WT (- CHX: 14/14; + CHX: 13/13). Moreover, the peak increase in amplitude the fEPSP slope was similar under both conditions (WT - CHX: 223 ± 16 %, n = 8; WT + CHX: 199 ± 12 %, n = 9; $p = 0.587$). This peak amplitude of LTP was reached after the same time in both cases (20 min post-HFS, i. e. 5 min after the delivery of the last train of HFS). This data indicates that LTP induction and its early phase were unaffected by CHX treatment.

In control aCSF the maximum LTP slightly declined but stabilized at a high level for the rest of recording (5 hrs post-HFS: stim: 155 ± 26 %, n = 8, con: 100 ± 3 %, n = 4; $p = 0.004$). In contrast, in CHX treated slices e-LTP continued to decline. The declining LTP under CHX-aCSF became significantly different from LTP under control aCSF at 75.5 min post-HFS, suggesting that the onset of protein synthesis requirement as a for LTP started approximately one hour post-HFS (75.5 min post-HFS: $p = 0.027$; after 190 min post-HFS: $p < 0.001$). Field EPSPs were diminished to baseline levels and were similar control fEPSPs after 125.5 min post-HFS (p = n. s.). Five hours post-HFS fEPSPs returned to baseline levels (5 hrs post-HFS: 86 ± 7 %, n = 9; p = n. s.).

In the presence of CHX fEPSPs recorded in control pathways significantly declined over time (pre-HFS: 100 ± 2 %, 5 hrs post-HFS: 79 ± 12 %, n = 6; $p = 0.015$). However, this decline was not significant at any time point if compared to the control pathway recorded in control-aCSF ($p > 0.32$).

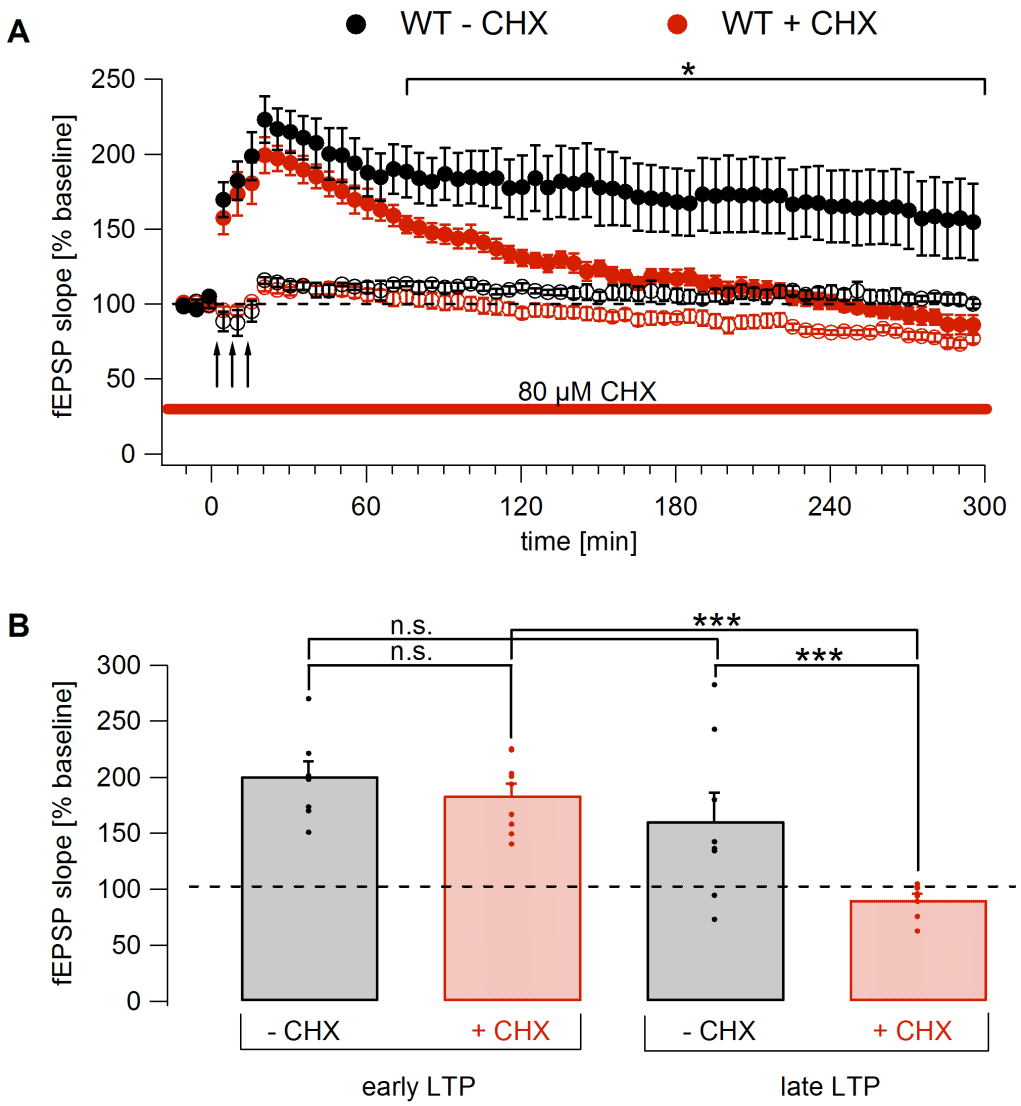

Figure 4.17: Late-LTP is dependent on protein synthesis in WT mice

(A) Summary graph: LTP (3-HFS) in WT treated with 80 µM CHX (filled red circles; n = 9; N = 6; red bar) compared to untreated control slices (filled black circles; n = 8; N = 6). Open circles represent control pathways. Asterisks indicate significance in the post-hoc ANOVA comparison between stimulated pathways of CHX treated and untreated control slices ($p < 0.05$) **(B)** Bar graph: Comparison of the e-LTP and l-LTP in CHX-treated and control slices of WT mice.

Therefore CHX exclusively affected LTP at the stimulated synapses but did not impair synaptic transmission in the control pathway.

To directly compare e-LTP and l-LTP the maximum e-LTP and the average l-LTP from all experiments were pooled (Figure 4.17 B). Similar to the presentation in LTP time course graphs, e-LTP was not influenced by CHX (WT - CHX: 218 ± 14 %,

n = 8; WT + CHX: 197 ± 9 %, n = 9; p = n. s.). In contrast, the maintenance of LTP was strongly affected since l-LTP in CHX treated slices differed significantly from l-LTP in untreated WT slices (WT - CHX: 161 ± 25 %, n = 8; WT + CHX: 91 ± 5 %, n = 9; p = 0.001). Indeed, in all slices treated with CHX the fEPSP declined to near-baseline level. No evidence of sustained l-LTP occurred in any of the examined slices treated with CHX. Early and late LTP in CHX treated slices differed significantly reflecting the abolishment of LTP (WT + CHX: 197 ± 9 %, n = 9; WT + CHX: 91 ± 5 %, n = 9; p < 0.027). In contrast, l-LTP in control aCSF was significantly enhanced compared to the control pathway (p = 0.024). Enhancement of synaptic transmission in control aCSF was stable (e-LTP: 218 ± 14 %, n = 8; l-LTP: 161 ± 25 %, n = 8; p = 0.073).

In summary, this data showed that CHX did not influence e-LTP expression and induction but completely prevented its stabilization and late phase in WT slices.

4.4.2. Effect of cycloheximide on LTP in KO mice

I next asked whether the consolidation of LTP was also dependent on protein synthesis in KO slices (TgMXS*B6).

Individual LTP experiments accompanied with corresponding fEPSP traces are shown in Figure 4.18. As illustrated by the fEPSP traces and the time course graphs, CHX did not affect LTP induction since fEPSPs increased strongly under both conditions. A slight diminishment of LTP was observed over time but it remained highly elevated above baseline until the end of recording under both conditions. Thus, surprisingly, CHX treatment was ineffective in KO mice.

Control pathways and FV amplitudes of both pathways remained constant throughout the experiment indicating that basal synaptic transmission was stable and presynaptic fiber recruitment was unaffected by CHX treatment. Furthermore this confirmed that LTP was input-specific under these conditions. To verify the striking insensitivity to protein synthesis inhibitors in KO slices, the number of examined slices was increased.

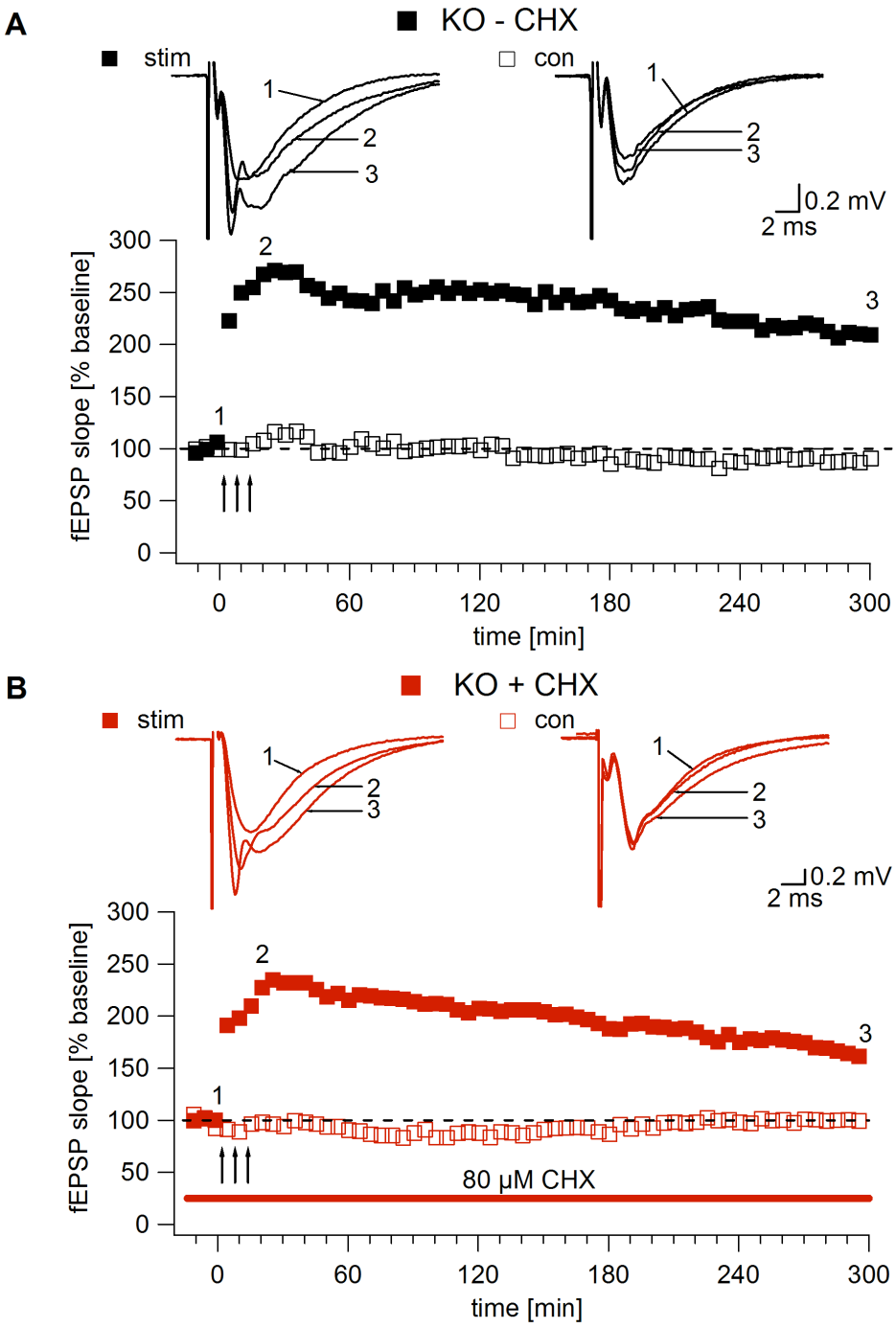

Figure 4.18: LTP in KO mice +/- CHX

Single experiments showing representative time courses of 3-LTP induced either in the presence of 80 µM CHX (B) or under control conditions (A). Normalized fEPSP slopes are plotted against time. **(A)**, **(B)** Stimulated pathways are indicated by filled black or red squares respectively. Corresponding within-slice control pathways are indicated by open black or red squares. Insets show fEPSP traces (average of 10 consecutive sweeps) taken from the presented experiment (1) during baseline stimulation, (2) 15 min after the last train of HFS and (3) 5 hours after delivery of HFS as indicated in the time course graph. fEPSPs of the stimulated and control pathway are displayed on the left and right side, respectively.

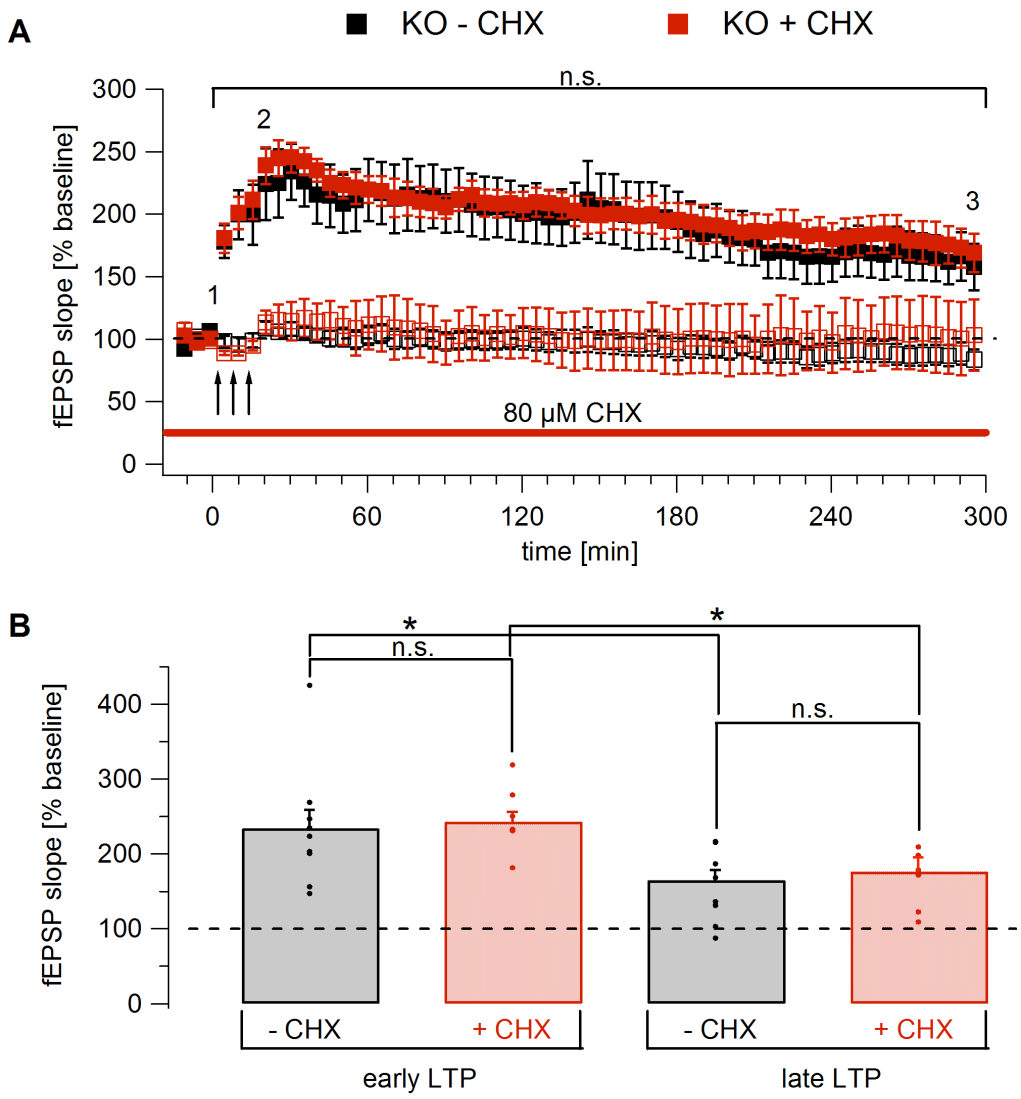

Figure 4.19: Late-LTP in KO mice is insensitive to CHX treatment

(A) Summary graph: 3-HFS LTP in KO treated with 80 µM CHX (filled red squares; n = 10; N = 7; red bar) compared to untreated control slices (filled black squares; n = 10; N = 7). Open squares represent control pathways. **(B)** Bar graph: Comparison of the early and late LTP in CHX-treated and control slices of KO mice.

Summary data obtained from KO slices treated with CHX (n = 10, N = 7) and untreated control slices (n = 10, N = 7) is presented in Figure 4.19 A.

Three-HFS induced similar e-LTP in CHX treated and untreated KO slices (- CHX: 225 ± 27 %, n = 10; + CHX: 245 ± 15 %, n = 10; p = n. s.). Under both conditions a slight decline of LTP was observed over time. Nevertheless, fEPSPs recorded under both conditions remained significantly potentiated compared to within-slice control pathways five hours post-HFS (p = 0.003). Under both conditions

LTP was maintained to the same degree (5 hrs post-HFS: - CHX: 158 ± 19 %, n = 10; + CHX: 169 ± 15 %, n = 10; p = n. s.).

As in stimulated pathways, no differences in synaptic transmission in the control pathways were observed (- CHX: 84 ± 8 %, n_{con} = 9; + CHX: 98 ± 34 %, n_{con} = 5; p = n. s.).

Early-LTP (- CHX: 234 ± 18 %, n = 10; + CHX: 243 ± 18 %, n = 10; p = 0.736) and l-LTP quantification (- CHX: 167 ± 17 %, n = 10; + CHX: 179 ± 19 %, n = 10; p = n. s.) showed similar amplitudes under both conditions (Figure 4.19 B).

Comparison of the averaged e-LTP and l-LTP indicated a significant reduction in l-LTP compared to e-LTP under both conditions (- CHX: p = 0.008; + CHX: p = 0.011). Nonetheless, l-LTP under both conditions was significantly different to corresponding data of the control pathway (- CHX: p = 0.002; + CHX: p = 0.011) demonstrating that fEPSPs were still potentiated.

Taken together, this data showed that blockade of de-novo protein synthesis did not affect the LTP evoked in KO slices.

4.4.3. Effect of cycloheximide on LTP in Tg mice

In slices of transgenic mice a normal level of basal Arc/Arg3.1 expression and an intact delivery of the protein from the soma to the dendrites are expected. But local Arc/Arg3.1 translation immediately after stimulation at activated synaptic sites is disrupted (Mao 2008). I used these mice to examine the role of dendritic Arc/Arg3.1 mRNA translation for the rescue of LTP under conditions of reduced protein synthesis as I had observed in KO slices. Three-HFS LTP was induced in either control aCSF (n = 8; N = 5) or in the presence of CHX (n = 8, N = 5) as shown in Figure 4.20.

Long-term potentiation was robustly induced by 3-HFS under both conditions (Figure 4.20 A: - CHX: 209 ± 11 %, n = 8; + CHX: 195 ± 12 %, n = 8; p = 0.293). However, LTP subsequently declined to baseline levels irrespectively whether protein synthesis inhibitors were applied or not (5 hrs post-HFS: - CHX: 109 ± 11 %, n = 8; + CHX: 106 ± 8 %, n = 8; p = 0.536).

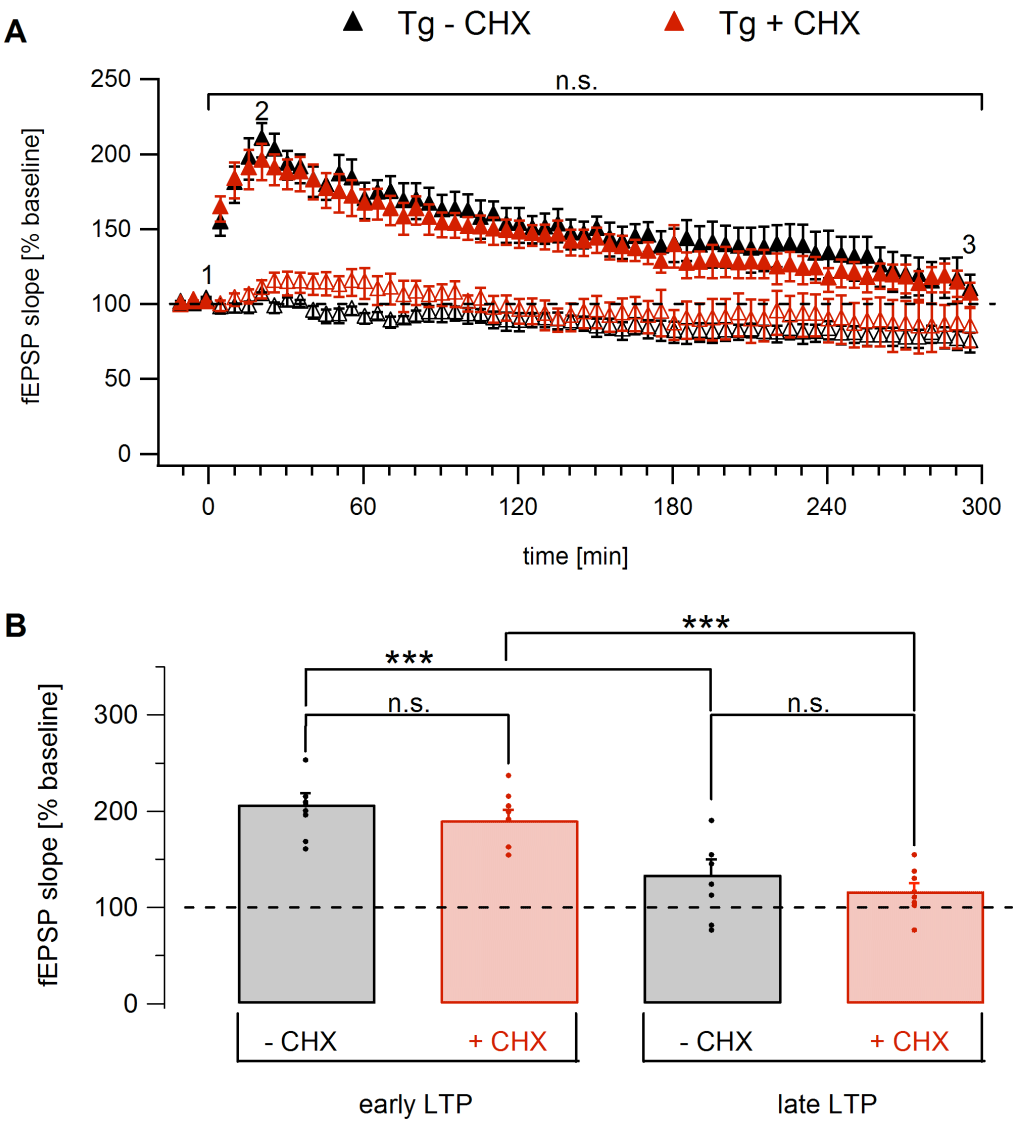

Figure 4.20: 3-HFS induce protein synthesis independent, transient LTP in Tg mice

(A) Summary graph: 3-HFS LTP in Tg treated with 80 µM CHX (filled red triangles; n = 8; N = 5; red bar) compared to untreated control slices (filled black triangles; n = 8; N = 5). Open triangles represent control pathways. **(B)** Bar graph: Comparison of the early and late LTP in CHX-treated and control slices of Tg mice.

This showed that 3-HFS was not sufficient to induce stable, long-lasting LTP in Tg slices at any conditions (this chapter and chapter 4.3.3). *De-novo* protein synthesis was found to be not critically involved in this transient and declining form of LTP since no differences in LTP time courses were observed under both conditions (p = n. s. at all time points). Both control pathways showed a decrement in the control pathway but no additional difference between these control pathways was found

(5 hrs post-HFS: p = 0.585). Cycloheximide treatment had no unspecific effect on synaptic transmission under baseline conditions.

Quantification of the early and late phase of LTP found no differences between the two conditions with regard to e-LTP (- CHX: 189 ± 9 %, n = 8; + CHX: 188 ± 11 %, n = 8; p = n. s.) and l-LTP (- CHX: 124 ± 14 %, n = 8; + CHX: 117 ± 9 %, n = 8; p = n. s.) as shown in Figure 4.20 B. A significant decrease of the fEPSP during LTP was observed in CHX treated (p < 0.001) as well as untreated control slices (p = 0.002).

4.4.4. Comparison of LTP in the different mouse lines under reduced protein synthesis

To assess the effect of Arc/Arg3.1 on LTP maintenance and its protein synthesis dependence, LTP recorded in WT, KO and Tg slices was examined.

First, LTP recorded in control aCSF was compared (Figure 4.21 A). The peak amplitude of fEPSP potentiation in response to 3-HFS was similar in Wt, KO and Tg slices (WT: 223 ± 16 %, n = 8; KO: 225 ± 27 %, n = 10; Tg: 220 ± 13 % n = 8; p = n. s.). The remaining potentiation was similarly elevated compared to pre-HFS baseline in WT and KO (5 hrs post-HFS WT: 155 ± 26 %, n = 8; KO: 158 ± 19 %, n = 10; p = n. s.). Statistical analysis of individual time points found no differences between these genotypes throughout the entire experiment (p = n. s.).

In contrast, field EPSP values recorded in Tg slices decreased over time and reached significantly smaller values compared to WT and KO slices in the last 10 min of recording (5 hrs post-HFS WT: 155 ± 26 %, n = 8; KO: 158 ± 19 %, n = 10; Tg: 109 ± 11 %, n = 8; p = n. s.). Between KO and Tg slices a further time period was significantly different as indicated in Figure 4.21 A (140 - 180 min post-HFS). Input specificity of LTP was observed in all genotypes. Field EPSPs of control pathways showed no difference between the genotypes (p > 0.05).

Secondly, the effect of a blockade of *de-novo* protein synthesis on LTP maintenance was compared in the different genotypes (Figure 4.21 B).

Here, CHX treatment had a different effect on the expression of l-LTP in WT and in KO slices. Under conditions of reduced protein synthesis the peak amplitude of LTP differed significantly between these two groups (WT: 199 ± 12 %, n = 9; KO: 245 ± 15 %, n = 10; p = 0.039).

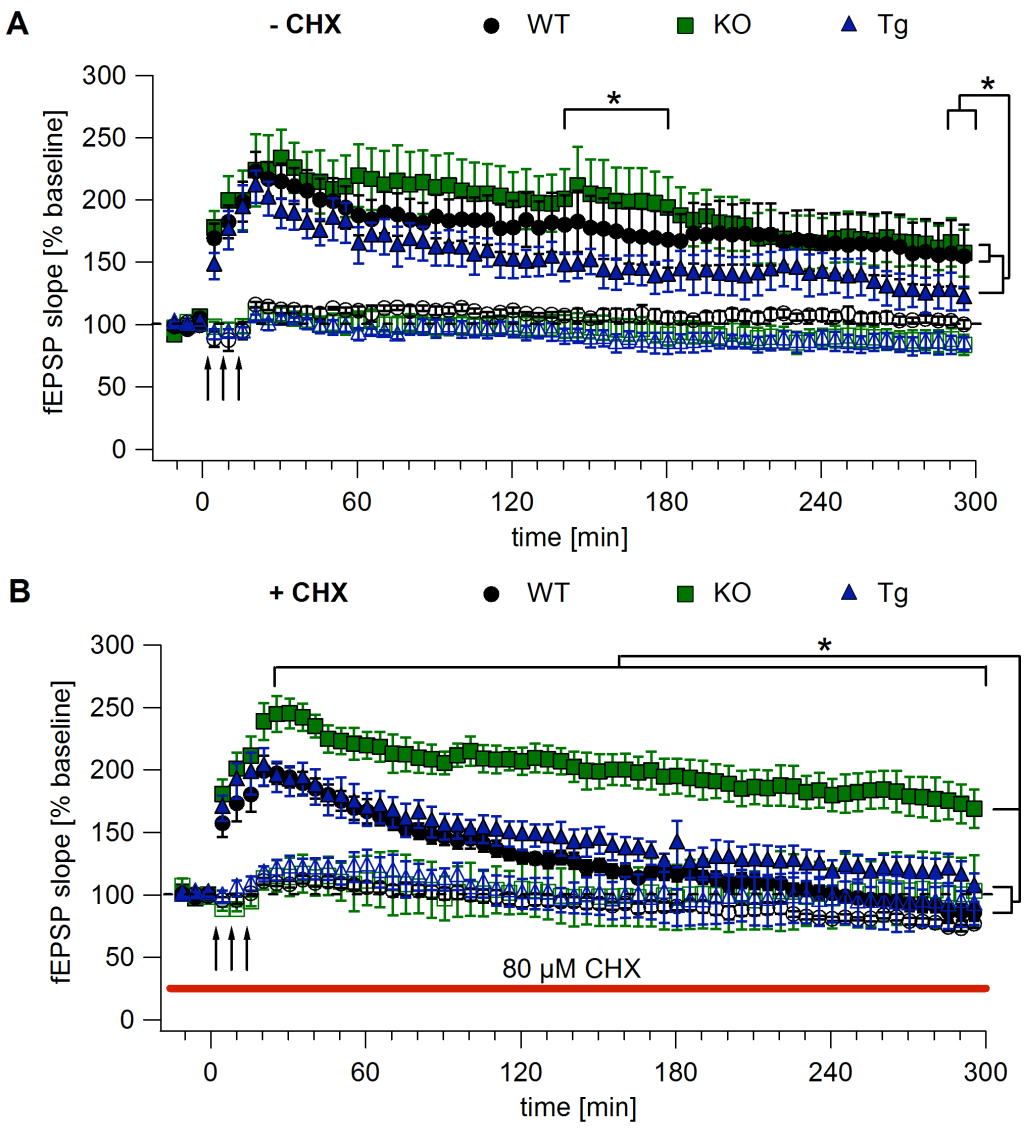

Figure 4.21: Long-lasting LTP and its dependence on protein synthesis in WT, KO and Tg mice

(A) LTP induced with 3-HFS under control conditions in WT (black circles: n = 8; N = 6), KO (green squares: n = 10; N = 7) and Tg slices (blue triangles: n = 8; N = 5). **(B)** LTP induced with 3-HFS in presence of CHX in WT (n = 9; N = 6), KO (n = 10; N = 7) and Tg slices (n = 8; N = 5). Asterisks indicate significance in the post-hoc ANOVA comparison between stimulated pathway of WT, KO and Tg slices ($P < 0.001$).

This initial potentiation was maintained in KO, whereas CHX completely abolished l-LTP in WT (5 hours post-HFS WT: 86 ± 7 %, n = 9, KO: 169 ± 15 %, n = 10; $p < 0.001$). Thus in the presence of CHX LTP was significantly different in WT and in KO slices revealing a striking difference in the mechanisms underlying LTP expression.

Transgenic slices differed fundamentally from both WT and KO slices. Both HFS stimuli were only capable of inducing declining transient forms LTP in Tg slices. This form of LTP was shown to be independent of protein synthesis and could thereby be considered as e-LTP. Long-term potentiation in Tg slices was similar to LTP recorded in WT slices in presence of protein synthesis inhibitors - no matter if they were conducted in the presence or absence of CHX (e-LTP: WT: 199 ± 12 %, n = 9, Tg: 196 ± 12 %, n = 8, p = n. s.; l-LTP: WT: 86 ± 7 %, n = 9, Tg: 106 ± 8 %, n = 8, p = n. s.). This again demonstrated that in transgenic slices just transient LTP could be induced.

4.4.5. Effect of anisomycin on LTP in WT and KO mice (TgMXS*B6)

To rule out the possibility that the presented results were due to some particularity of CHX, e. g. special drug side-effects, we performed another series of experiments using the translational inhibitor anisomycin instead of CHX to inhibit protein synthesis (Figure 4.22).

In contrast to the effect of CHX in WT slices, LTP amplitude decreased immediately after HFS in presence of anisomycin (- ANI: 272 ± 26 %, n = 10, + ANI: 212 ± 6 %, n = 6; $p < 0.001$). Long-term potentiation in WT slices under control conditions declined for 3.5 hours post-HFS before stabilization at a similar level as in the slices recorded in the previous set of experiments (chapter 4.4.1). In presence of anisomycin LTP showed a constant decline of the initially potentiated fEPSP slopes. In anisomycin treated slices LTP was significantly smaller than in control aCSF as indicated in Figure 4.22 A. Compared to untreated control slices, LTP was of less amplitude in anisomycin treated slices. This difference was found to be significant as indicated in Figure 4.22 A.

Late-LTP in anisomycin treated slices declined strongly and was no longer different from control pathway at 225 min post-HFS (5 hrs post-HFS: con: 85 ± 6 %, n = 6, + ANI: 123 ± 4 %, n = 6; p = n. s.). In contrast, fEPSPs of untreated WT remained constantly enhanced compared to control pathway (con: 105 ± 6 %, n = 10, - ANI: 155 ± 20 %, n = 10; $p < 0.001$).

Different effects were observed in KO slices, the peak amplitude of e-LTP was significantly larger under anisomycin compared to control aCSF (Figure 4.22. : - ANI: 239 ± 13 %, n = 6, + ANI: 290 ± 28 %, n = 9; p = 0.009).

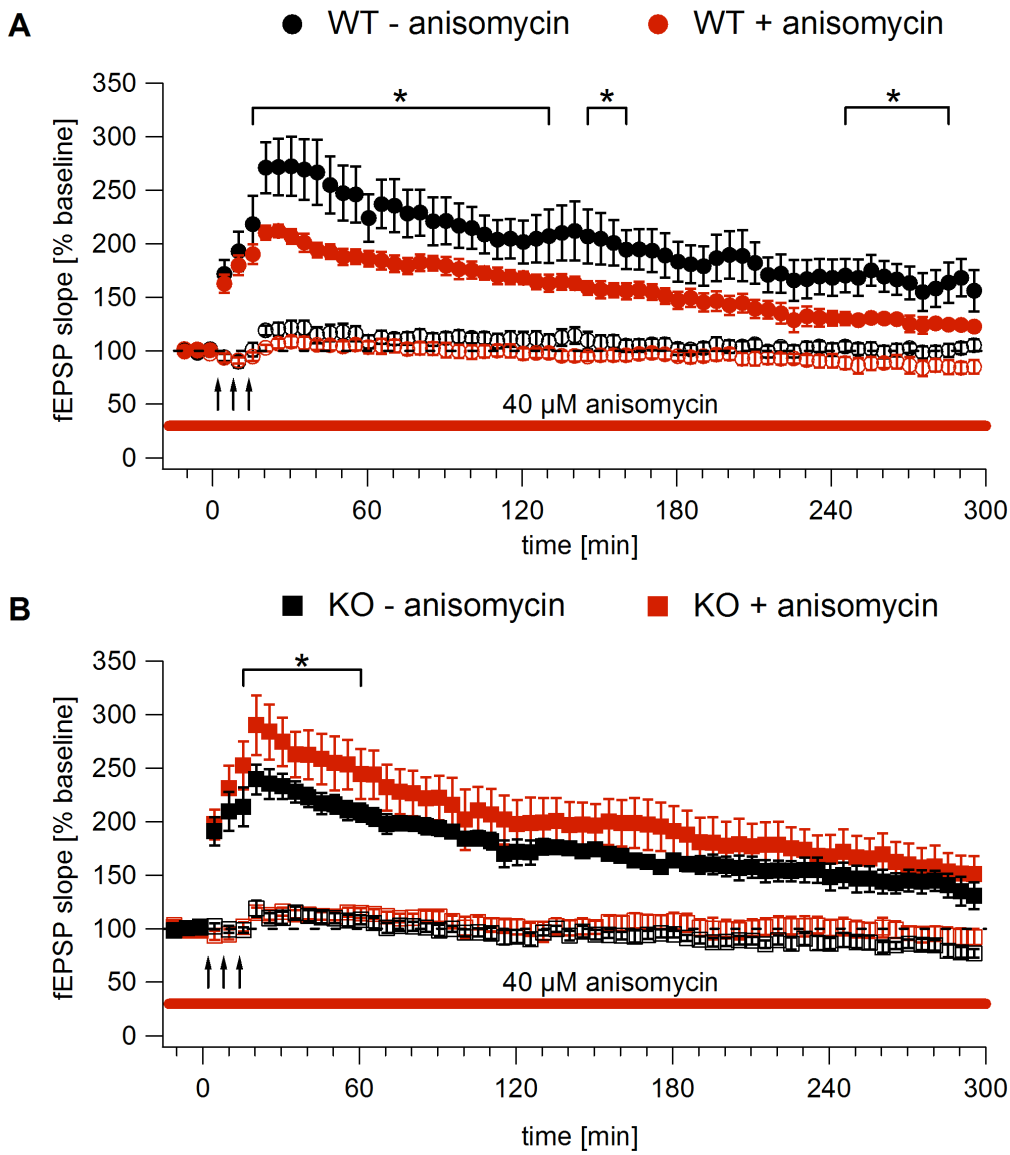

Figure 4.22: Anisomycin treatment qualitatively reproduced the effect of CHX

(A) Summary graph: LTP (3-HFS) in WT mice treated with 40 µM anisomycin (filled red circles; n = 6; N = 4) compared to untreated control slices (filled black circles; n = 10; N = 5). Open circles represent control pathways. Bars indicate statistical significance between stimulated pathways. **(B)** Summary graph: LTP (3-HFS) in KO mice treated with 40 µM anisomycin (filled red squares; n = 9; N = 6) compared to untreated control slices (filled black squares; n = 6; N = 5). Open squares represent control pathways. Asterisks indicate significance in the post-hoc ANOVA comparison between stimulated pathways.

At later time points LTP in the anisomycin treated slices tended to be larger than in control-aCSF but this difference was not significant beyond the first 60 min after HFS (5 hrs post-HFS: - ANI: 131 ± 11 %, n = 6, + ANI: 150 ± 17 %, n = 9; p = 0.303). Thus LTP maintenance again was shown to be insensitive to protein synthesis inhibition as observed under CHX treatment. KO slices showeded LTP lasting at least for five hours ($p < 0.001$).

In the next section e-LTP and l-LTP of the CHX and ANI series of experiments is presented and compared in bar graphs (Figure 4.23).

Early LTP and late LTP recorded in control aCSF in the two series of experiments varied in magnitude but were not significantly different in neither WT (e-LTP in $control_{CHX}$: 218 ± 14 %, n = 8, e-LTP in $control_{ANI}$: 263 ± 22 %, n = 10, p = 0.119; l-LTP in $control_{CHX}$: 161 ± 25 %, n = 8, l-LTP in $control_{ANI}$: 169 ± 13 %, n = 10, p = n. s.) nor KO slices (e-LTP in $control_{CHX}$: 234 ± 24 %, n = 10, $e\text{-}LTP_{ANI}$ in control: 236 ± 13 %, n = 6, p = 0.954; $l\text{-}LTP_{CHX}$ in control: 165 ± 18 %, n = 10, $l\text{-}LTP_{ANI}$ in control: 148 ± 11 %, n = 6, p = n. s.).

However, e-LTP in control WT slices of the ANI-series tended to be enhanced compared to WT slices of the CHX-series but this effect was not significant as shown above. Since no statistically significant differences were observed in LTP of untreated control slices between the two sets of experiments (CHX and ANI) the data of these experiments was pooled together and compared to the effects of CHX and anisomycin treatments. As shown in Figure 4.23 A, both protein synthesis inhibitors impaired LTP. Early LTP was slightly reduced under protein synthesis inhibition, an effect that was statistically significant for CHX but not for anisomycin (aCSF: 241 ± 14 %, n = 16, + CHX 197 ± 9 %, n = 9, + ANI 210 ± 6 %, n = 6; $p_{aCSF/CHX} = 0.016$, $p_{aCSF/ANI}$ = n. s.). Both protein synthesis inhibitors caused a strong and statistically significant reduction of l-LTP (aCSF: 164 ± 14 %, n = 16 + CHX 91 ± 5 %, n = 9, + ANI 129 ± 4 %, n = 6; $p_{aCSF/CHX} < 0.001$, $p_{aCSF/ANI} = 0.03$).

While the effect of anisomycin compared to CHX on LTP maintenance was quantitatively reduced, there was, however, still a qualitative and significant decrement observable in Arc/Arg3.1 WT slices which was again not detectable in KO slices.

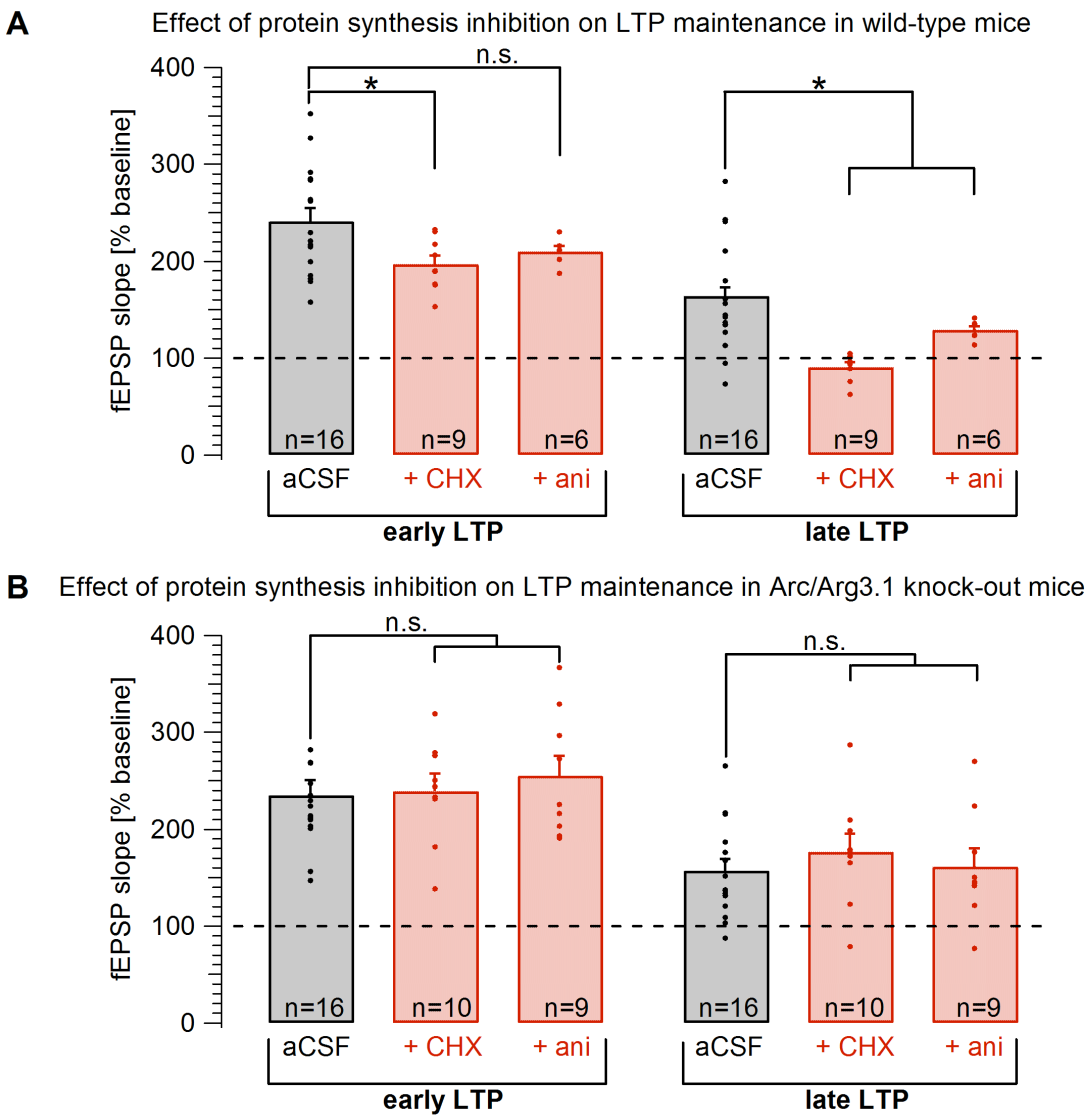

Figure 4.23: Comparison of CHX and ANI effects on e-LTP and l-LTP in WT and KO mice

Bar graph comparing e-LTP and l-LTP in CHX- and ANI-treated to untreated control slices recorded in drug-free aCSF. In this graph control slices recorded in aCSF of both series of experiments were pooled.

In contrast to WT, LTP in KO slices was surprisingly insensitive to both CHX and anisomycin and neither e-LTP nor l-LTP were sensitive to the inhibitors (e-LTP: aCSF: 235 ± 16 %, , n = 16; + CHX: 243 ± 13 %, n = 10; + ANI: 255 ± 21 %, n = 9; $p_{aCSF/CHX}$ = n. s., $p_{aCSF/ANI}$ = n. s.; l-LTP: aCSF: 157 ± 12 mV, n = 16; + CHX; 179 ± 15 %, n = 10; + ANI; 161 ± 19 %, n = 9; $p_{aCSF/CHX}$ = n. s., $p_{aCSF/ANI}$ = n. s.).

Taken together the crucial finding that LTP maintenance in WT was sensitive to CHX, while LTP in the Arc/Arg3.1 KO slices was insensitive to CHX, was reproduced by anisomycin treatment.

4.4.6. Effect of protein synthesis inhibition on basal synaptic transmission in WT, KO and Tg mice

4.4.6.1. Basal synaptic transmission in WT mice (TgMXS*B6)

Inhibitors of protein synthesis could, in theory, have effects on basal synaptic transmission which might in turn affect LTP. To examine this possibility we tested and compared basal synaptic transmission in the presence and absence of CHX and anisomycin.

The PS threshold in slices exposed to inhibitors of protein synthesis and control slices was compared as an indication for changes in excitability (Figure 4.24). The PS threshold was not shifted by CHX-treatment (- CHX: 907 ± 76 µA, n = 23; + CHX: 979 ± 79 µA, n = 17; p = n. s.) or anisomycin-treatment (- ANI: 937 ± 75 µA, n = 24; + ANI: 1054 ± 56 µA, n = 14; p = n. s.). Moreover, control data derived from untreated WT of both experimental sets showed similar results regarding the PS threshold (p = n. s.).

For further analysis of basal synaptic transmission IO-curves comparing treated and untreated control slices were generated (Figure 4.25).

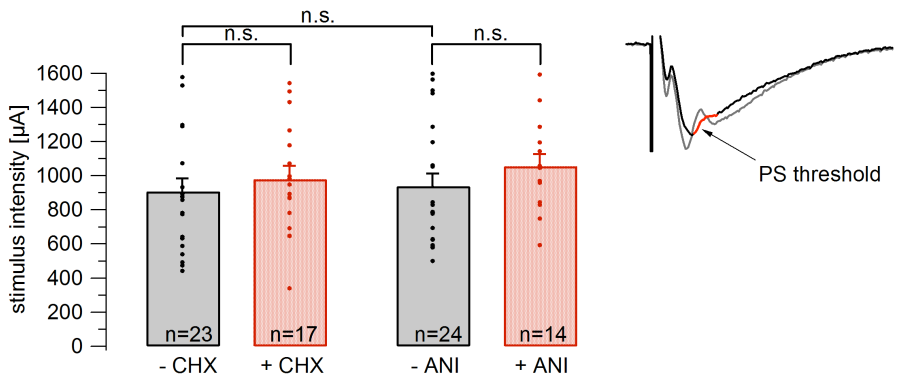

Figure 4.24: PS threshold is not shifted by CHX or anisomycin-treatment

Bar graph: Threshold intensity required to evoke a population spike in slices treated with protein synthesis inhibitors (black bars; CHX: n = 17, ANI: n = 14) and untreated control slices (red bars; CHX: n = 23, ANI: n = 24). Inset illustrates the PS threshold within the fEPSP trace as indicated by the positive deflection in the fEPSP trace (highlighted in red). Underlying grey fEPSP trace demonstrates the fully developed PS.

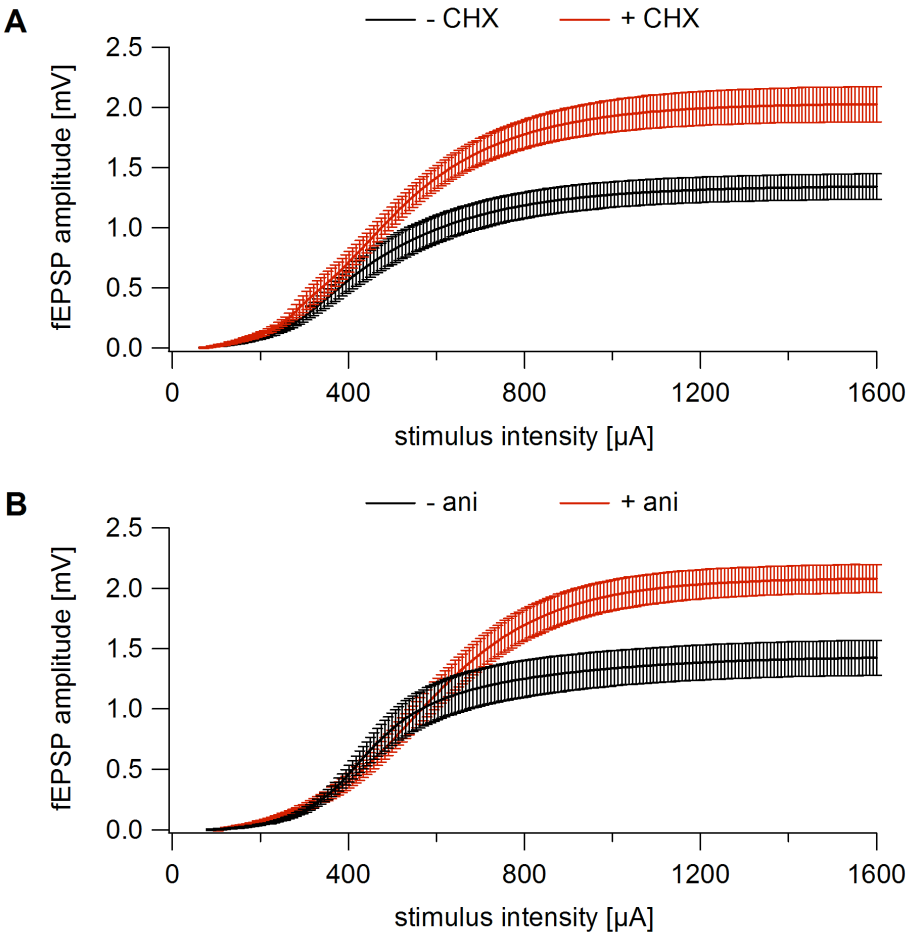

Figure 4.25: Treatment with protein synthesis inhibitors enhances basal synaptic transmission in WT slices

Field EPSP responses were plotted against increasing stimulation intensity and displayed as IO-curves. Data derived from WT control slices (A: n = 23; B: n = 24) are illustrated by black lines and compared to data from slices incubated in **(A)** either 80 µM CHX (n = 17) or **(B)** 40 µM anisomycin (n = 14).

Comparison of the two conditions found that the response to single stimuli in WT slices treated with both protein synthesis inhibitors was increased over the whole range of stimulation intensities compared to untreated control slices.

In accordance with our expectations IO-curves from WT mice recorded under control conditions were similar regarding the maximum fEPSP ($Control_{CHX}$: 1.34 ± 0.10 mV, n = 23; $Control_{ANI}$: 1.59 ± 0.14 mV, n = 24; p = n. s.) and the half-maxmium fEPSP ($Control_{CHX}$: 0.65 ± 0.05 mV, n = 23; $Control_{ANI}$: 0.76 ± 0.07 mV, n = 24; p = n. s.) indicating constant conditions of basal synaptic transmission.

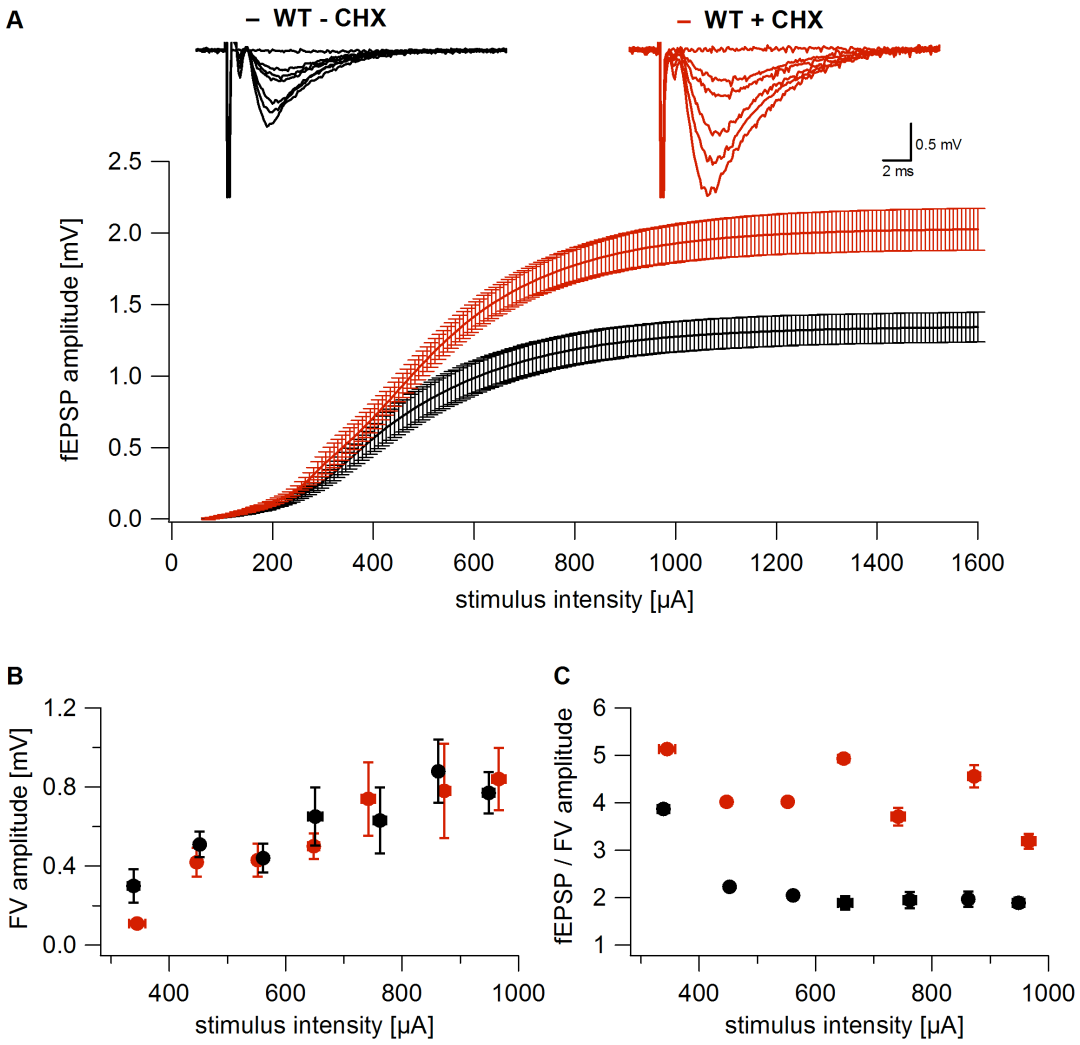

Figure 4.26: Enhancement of basal synaptic transmission by CHX occurs at single synapses

(A) Input-output curves: fEPSP amplitude of CHX-treated (red line; n = 17) and untreated WT control slices (black line; n = 23) is plotted against stimulus intensity. Insets show superimposed fEPSPs derived from representative experiments. **(B)**, **(C)** FV amplitude and fEPSP/FV amplitude ratio (WT - CHX: black circles; WT + CHX: red circles) are displayed against stimulation intensity, respectively. Markers depict means of 100 µA bins ± S.E.M.

I next asked whether this differences in response to single stimuli could be rather explained by pre- or postsynaptic mechanisms. The results obtained from the CHX experiments are presented above (Figure 4.26).

Using extracellular field recording the FV, a small component preceding the fEPSP, could be detected which is reflecting the action potentials in the stimulated presynaptic axons. The FV amplitude, which is proportional to the number of presynaptic neurons recruited by stimulation, could be used to estimate the strength of

the afferent input. In this way synaptic input/output values could be compared more directly.

With increasing stimulation intensity the number of activated presynaptic rose almost linearly (Figure 4.26 B.). The number of activated presynaptic fibers appeared to be similar in CHX treated and untreated control slices.

To obtain an estimate of the postsynaptic EPSP amplitude, the ratio of the fEPSP amplitude divided by the FV amplitude was computed and presented as a function of the injected current (Figure 4.26 C). The figure shows that for all the tested stimulation intensities, that the fEPSP/FV ratio was higher in CHX-treated slices. In anisomycin treated slices similar effects were observed.

Secondly, PPF was assessed as a measure of the release probability in control-aCSF slices and compared to slices treated with either CHX or anisomycin. Paired-pulse facilitation was not significantly influenced by the application of protein synthesis inhibitors (Figure 4.27). Control aCSF-treated and CHX-treated slices expressed PPF at a comparable degree (- CHX: 124.85 ± 2.16 %, n = 29; + CHX: 123.91 ± 1.62 %, n = 23; p = n. s.). Anisomycin treated slices exhibited similar PPF to their control aCSF-treated slices (- ANI: 119.82 ± 1.20 %, n = 29; + ANI: 120.58 ± 1.35 %, n = 18; p = n. s.).However, the degree of PFF varied between the experimental sets.

An altered balance of inhibition and excitation within the slice might also influence IO-curves and synaptic plasticity by a negative feedback via inhibitory interneurons. To examine whether inhibition is altered in CHX treated slices, the half-width of the fEPSP was measured.

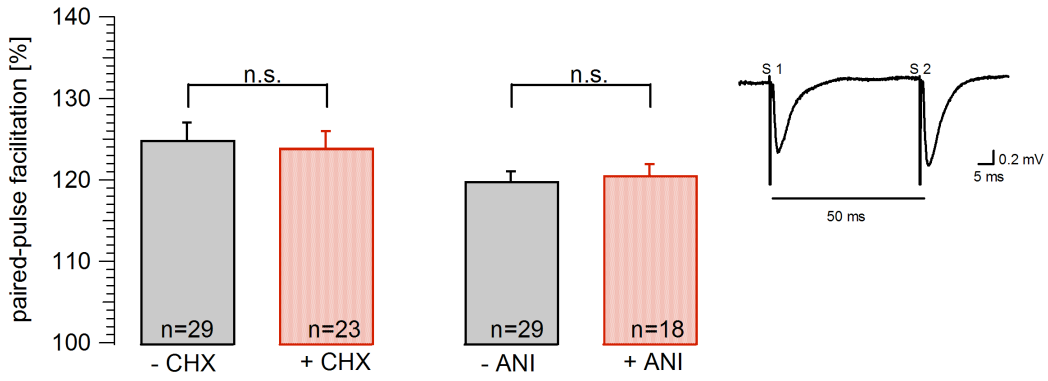

Figure 4.27: PPF was not influenced by protein synthesis inhibition in WT slices

PPF is presented as the facilitation ratio, i. e. amplitude of the second response relative to the first. Inset shows example paired-pulse fEPSP sweep.

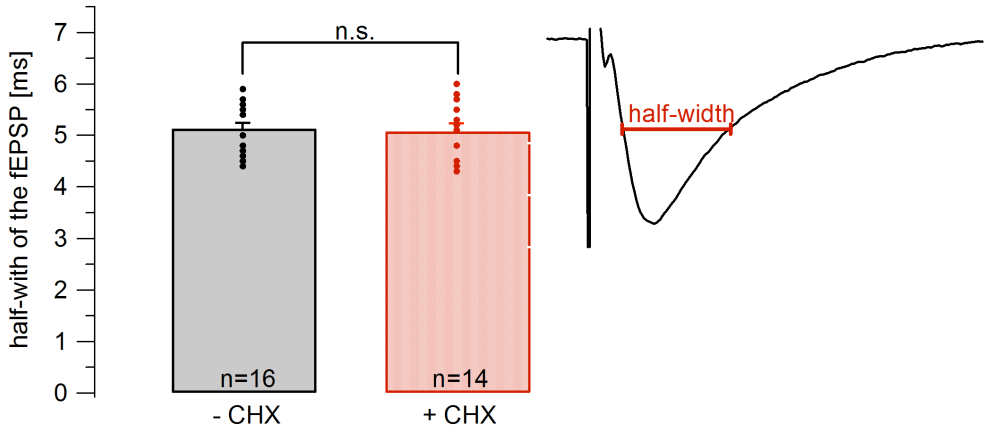

Figure 4.28: Field EPSP half-width was not altered by CHX-treatment in WT slices

Bar graph: fEPSP half-width of CHX-treated (red bar, n = 14) and control slices (black bar, n = 16). Inset shows example fEPSP trace.

A representative example of the half-width measurement is presented in Figure 4.28. The half-width was not significantly different between CHX-treated and control slices ($p = 0.786$), suggesting that CHX did not modify the rapid inhibitory feedback of the low-frequency fEPSPs.

4.4.6.2. Basal synaptic transmission in KO mice (TgMXS*B6)

Basal synaptic transmission in KO was assessed. The stimulation required to evoke a PS in the postsynaptic neurons was not shifted by treatment with neither CHX

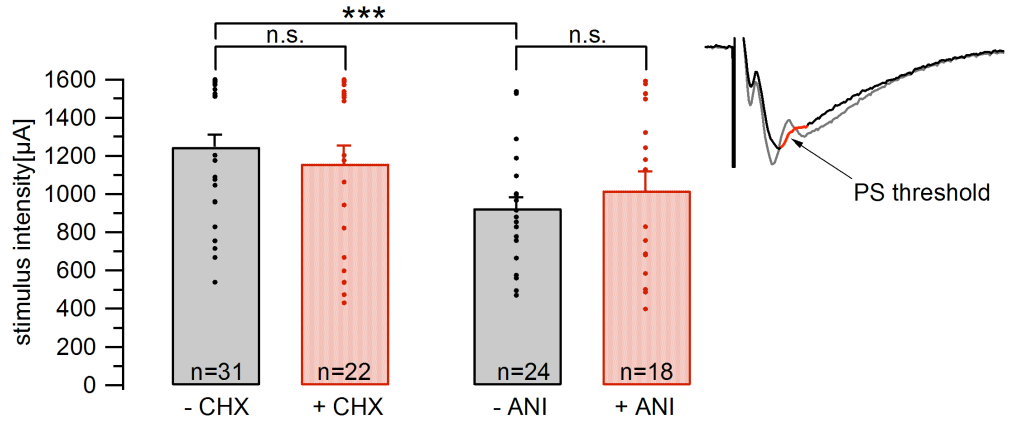

Figure 4.29: PS threshold is not affected by inhibition of protein synthesis in KO slices

Stimulation intensity required to evoke PS in the postsynatic neurons. PS thresholds were reached at similar stimulation intensities in slices exposed to inhibitors of proteins synthesis (+ CHX: n = 22; + ANI: n = 18) and their corresponding control experiments (- CHX: n = 31; - ANI: n = 24).

(- CHX: 1245 ± 66 µA, n = 31; + CHX: 1159 ± 95 µA, n = 22; p = n. s.) nor anisomycin (- ANI: 926 ± 58 µA, n = 24; + ANI: 1019 ± 100 µA, n = 18; p = n. s.) as shown in Figure 4.29.

Analysis of IO-curves is displayed in Figure 4.30. Strikingly, the response to stimulation pulses of increasing intensity was not altered in KO slices exposed to protein synthesis inhibitors.

Neither CHX nor anisomycin significantly influenced the maximum fEPSP amplitude (- CHX: 1.3 ± 0.09 mV, n = 31, + CHX: 1.36 ± 0.07 mV, n = 22, p = n. s.; - ANI: 1.92 ± 0.17 mV, n = 24, + ANI: 1.92 ± 0.19 mV, n = 18, p = n. s.)

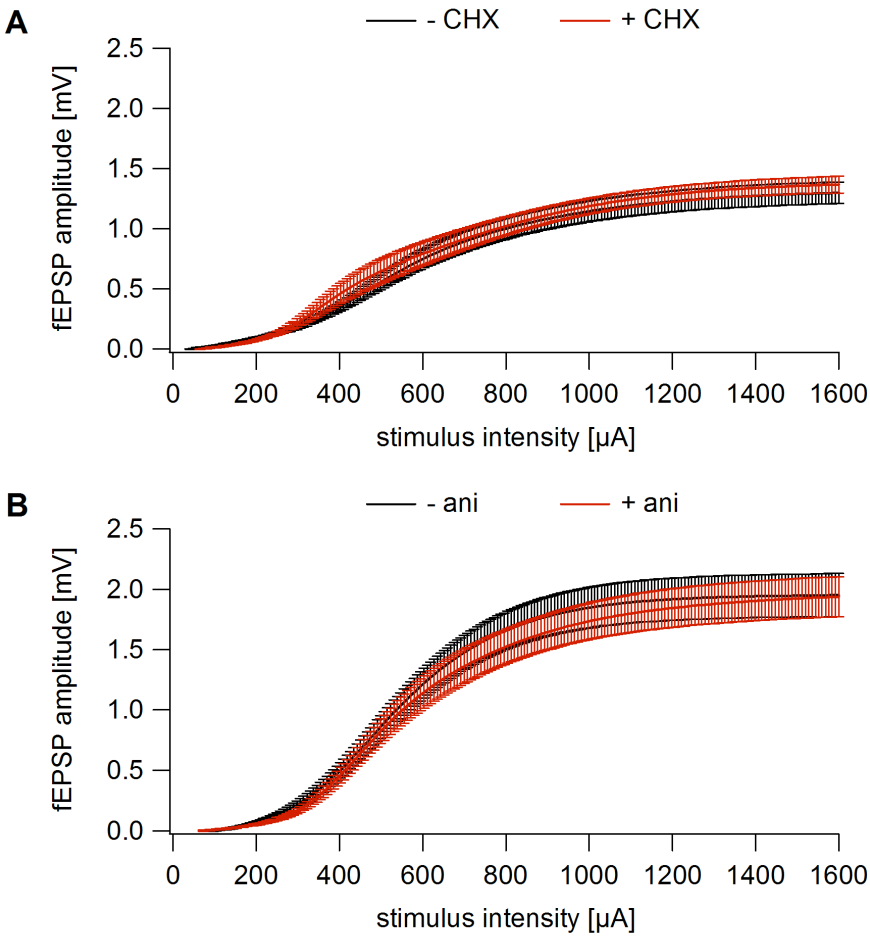

Figure 4.30: IO-curves are not shifted in KO mice in presence of protein synthesis inhibitors

Field EPSP responses were plotted against increasing stimulation intensity and displayed as IO-curves. Data derived from untreated KO control slices (A: n = 31; B: n = 24) are illustrated by black lines and compared to data from KO slices incubated in **(A)** either 80 µM CHX (n = 22) or **(B)** 40 µM anisomycin (n = 18).

or the stimulation intensity needed to evoke the half maximum fEPSP (- CHX: 606 ± 26 µA, + CHX: 566 ± 54 µA, p = n. s.; - ANI: 545 ± 27 µA, + ANI: 593 ± 40 µA, p = n. s.).

Since postsynaptic parameters were shown to be unaffected by protein synthesis inhibition in KO slices, I further asked whether this also accounts for presynaptic function (Figure 4.31). A statistically significant increase in PPF of slices exposed to CHX was found (- CHX: 121.25 ± 1.59 %, n = 33; + CHX: 129.16 ± 1.95 %, n = 22; p = 0.003). Paired-pulse facilitation in untreated slices was not significantly different in the two experimental series (p = n. s.). Anisomycin also caused a slight, albeit statistically insignificant increase in PPF (- ANI: 120.39 ± 1.27 %, n = 28; + ANI: 123.89 ± 1.54 %, n = 28; p = n. s.).

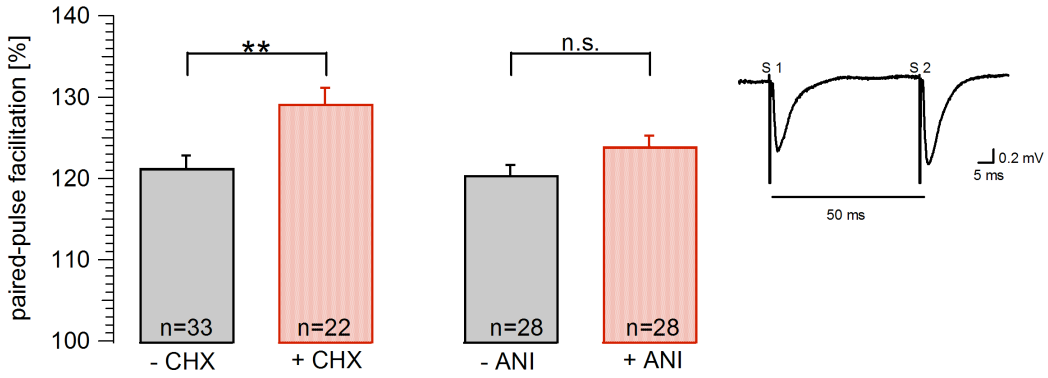

Figure 4.31: PPF is increased by CHX in KO slices

PPF is presented as the facilitation ratio, i. e. amplitude of the second response relative to the first. Inset shows example paired-pulse fEPSP sweep.

4.4.6.3. Basal synaptic transmission in Tg mice (TgMXS*B6)

The effect of CHX treatment on basal synaptic transmission in transgenic slices was examined (Figure 4.32). The stimulation intensity required to reach the PS threshold was not significantly different between control- and CHX-aCSF treated slices as demonstrated in Figure 4.32 A (- CHX: 687 ± 101 µA, n = 19 + CHX: 813 ± 105 µA, n = 17; p = n. s.). As shown in Figure 4.32 C fEPSPs were smaller in CHX treated slices over the whole range of stimulation intensities but comparison of the maximum fEPSP (- CHX: 1.61 ± 0.17 mV, n = 19; + CHX: 1.4 ± 0.13 mV, n = 17; p = n. s.) and the stimulation intensity required to evoke half maximum fEPSP (- CHX: 473 ± 37 mV, n = 19; + CHX: 505 ± 27 mV, n = 17; p = n. s.) showed no

significant differences. In the Tg mice, PPF was significantly reduced in slices exposed to CHX (Figure 4.32 B: - CHX: 124.4 ± 1.38 %, n = 23; + CHX: 119.11 ± 1.64 mV, n = 18; p = 0.019).

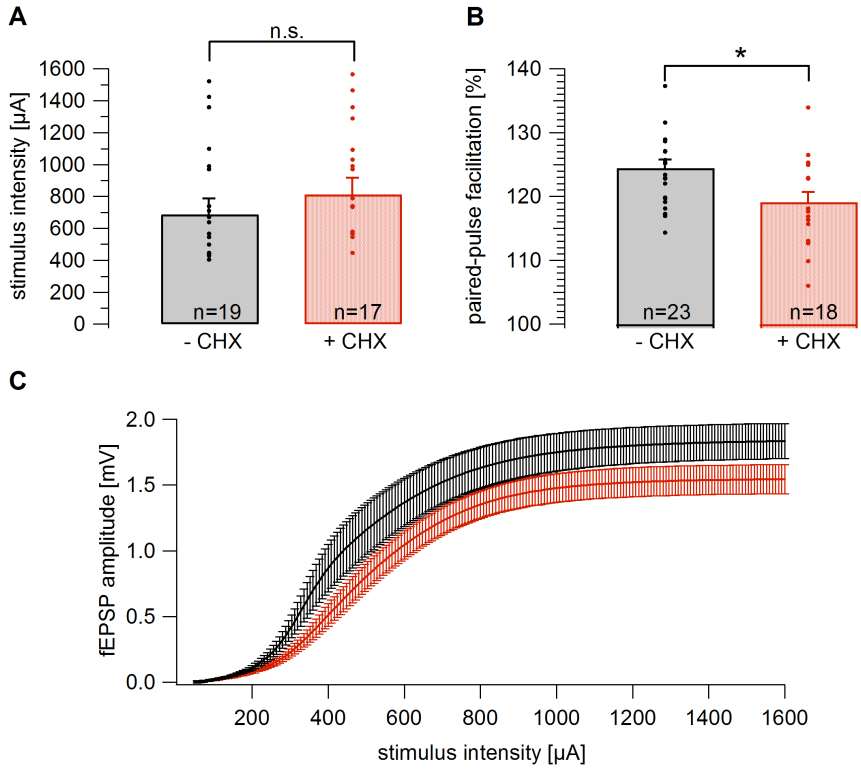

Figure 4.32: CHX-effect on basal synaptic transmission in Tg mice

(A) Bar graph showing the stimulation intensity required to reach the PS threshold in Tg slices treated with CHX (red bar) or untreated control slices (black bar). **(B)** Bar graph showing paired-pulse facilitation ratio obtained by PPF stimulation in Tg slices treated with CHX (red bar) or untreated control slices (black bar). **(C)** IO-curves obtained from CHX treated (red; n = 17) and untreated control slices (black; n = 19).

4.5 POST-HOC ANALYSIS OF DIFFERENCES IN 3-HFS LTP IN WT MICE

This study included two sets of experiments examining 3-HFS LTP in mouse line TgMXS*B6. First, 3-HFS LTP was examined in comparison to 1-HFS in WT, KO and Tg slices (chapter 4.3.1), hereafter referred to as Set-1. Secondly, 3-HFS LTP in WT was examined as control in CHX-experiments (chapter 4.4.1), hereafter referred to as Set-2.

The results from the two sets differed in few important ways: In Set-1, 3-HFS was insufficient to induce stable, enduring l-LTP in all genotypes. In addition, e-LTP was enhanced in KO and Tg slices compared with WT slices. In Set-2, 3-HFS reliably induced l-LTP in WT and KO slices but e-LTP was similar in all genotypes.

An explanation for these differences would be that the slices under examination differed in their basal synaptic properties or their viability, parameters that could strongly influence LTP. This was tested by comparing parameters of basal synaptic transmission in WT slices of these different experimental sets. The stimulation intensity required to evoke a PS was similar between the experiments sets (WT_{Set-1}: 902 ± 59 µA (n = 33); WT_{Set-2}: 907 ± 76 µA (n = 23); p = n. s.) indicating a similar status of neuronal excitability in the slices. Maximum fEPSPs were significantly larger in the first set of experiments compared to control slices recorded in set-2 ($fEPSP_{max}$: WT_{Set-1}: 1.82 ± 0.12 mV (n = 33), WT_{Set-2}: 1.34 ± 0.10 mV (n = 23), p = 0.004).

To test possible relationship between the fEPSP amplitude and LTP, e-LTP amplitude was plotted against the baseline fEPSP amplitude of all the experiments in SET-1 and SET-2 together (Figure 4.33 A). This plot showed a negative correlation as indicated by the linear regression (r = -0.629, p = 0.01). This suggests that slices with smaller baseline fEPSP were more likely to exhibit LTP of greater magnitude. This might explain, why in SET-1, in which the baseline fEPSPs were larger, the LTP was smaller compared to SET-2. To test whether the remaining l-LTP amplitude five hours after HFS was related to e-LTP, l-LTP was plotted against baseline fEPSP amplitude (Figure 4.33 B: r = 0.715, p = 0.01).

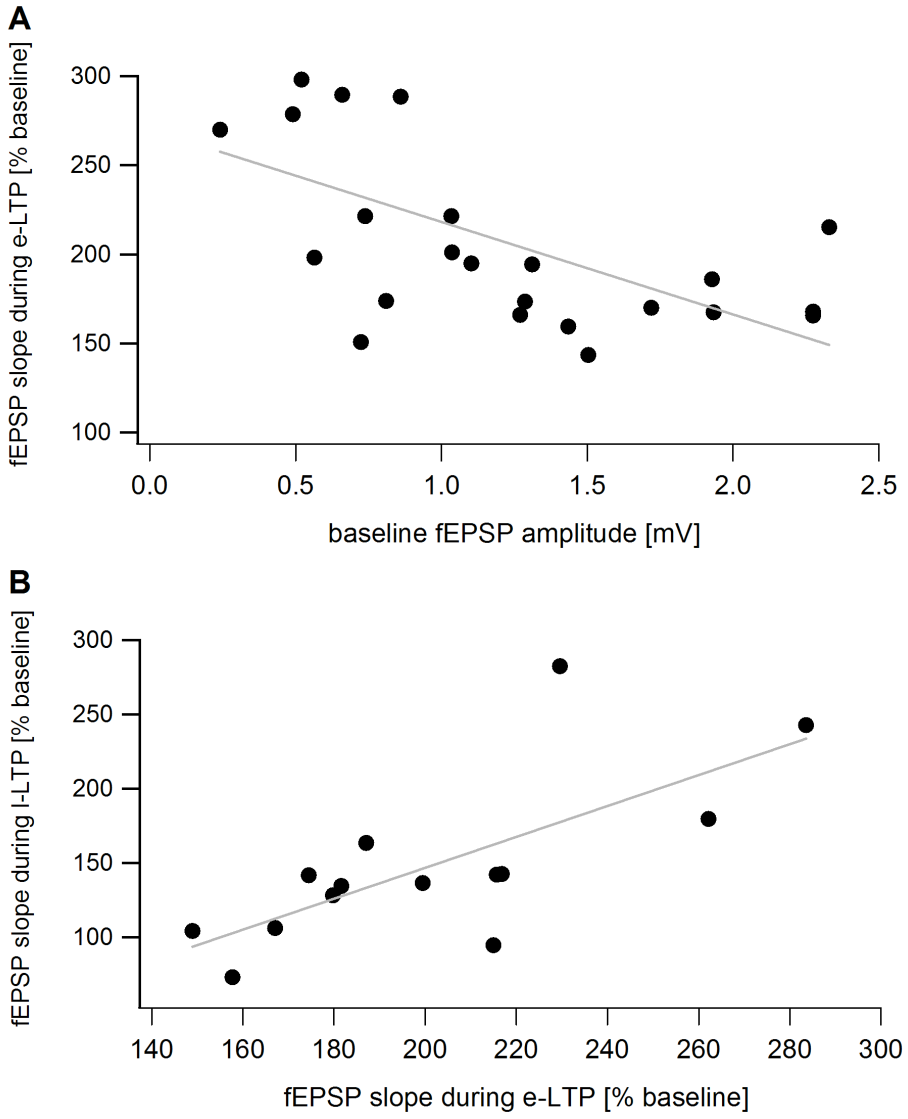

Figure 4.33: Correlation between baseline fEPSP amplitude and LTP magnitude

Maximum fEPSP slope measured during e-LTP in all TgMXS*B6 WT slices receiving 3-HFS (n = 24) as a function of baseline fEPSP amplitude.

A second explanation would be that the ability to induce stable and enduring LTP is related to the slice viability. If this hypothesis is correct than it could be expected that slices recorded in the first set of experiments were for some reason of lower quality. To test this hypothesis the fEPSP/FV ratio was measured to assess slice viability. The fEPSP/FV ratio was 9 ± 3 (n = 8) in Set-1 and 10 ± 3 (n = 12)in Set-2. Thus slice viability as indicated by the fEPSP/FV ratio appeared to be unaltered between the experimental sets (p = n. s.).

Although no differences in fEPSP/FV ratio could be detected, it is notable that control pathways of WT and KO slices recorded in Set-1 showed a significant decline in

contrast to control slices recorded in Set-2. This observation might hint to a general, unspecific attenuation of slice conditions during recording.

5 DISCUSSION

The mechanisms underlying memory formation have been the focus of studies in the recent decades. The immediate early gene Arc/Arg3.1 was shown to exhibit several features that make it a central regulator of memory formation. Previous studies demonstrated that disruption of Arc/Arg3.1 function critically impaired memory formation (Guzowski et al. 2000, Plath et al. 2006, Ploski et al. 2008). Linked to its role in memory, Arc/Arg3.1 was also shown to be required for the consolidation of various forms of synaptic plasticity, a key mechanism hypothesized to underlie memory formation (Guzowski et al. 2000, Plath et al. 2006, Shepherd et al. 2006, Messaoudi et al. 2007, Park et al. 2008). Arc/Arg3.1 transcription and translation are tightly regulated by synaptic activity and behavior. Notably, Arc/Arg3.1 mRNA is specifically delivered from the soma along the dendrites to stimulated synapses, where it can be locally translated quickly upon stimulation. The significance of this local synaptic translation of Arc/Arg3.1 remains entirely unknown (Link et al. 1995, Lyford et al. 1995). To investigate the mechanisms by which Arc/Arg3.1 affects the consolidation of synaptic plasticity, I examined its role in protein synthesis dependent and independent forms of LTP. Furthermore, the role of local Arc/Arg3.1 synthesis in dendrites versus somatically synthesized Arc/Arg3.1 in LTP was examined by using a mouse model lacking dendritic targeting of Arc/Arg3.1 mRNA.

5.1 ARC/ARG3.1 FUNCTION IN TRANSIENT AND LONG-LASTING LTP (MOUSE LINE E8KO)

5.1.1. Transient and long-lasting LTP in WT slices

The first aim of this study was to establish stimulation protocols which induce transient and long-lasting LTP to investigate the specific role of Arc/Arg3.1 in these forms of plasticity. After testing several parameters I decided to use 1-HFS to induce transient LTP and 3-HFS in 5 min intervals to induce long-lasting LTP. In all experiments the stimulus intensity was set to yield 50 % of the maximum fEPSP (without pop spike) to enable a maximum range for LTP expression.

In wildtype slices e-LTP was reliably induced by both conditioning protocols. However, the potentiation of the fEPSP in response to 1-HFS declined back to baseline values within four hours, resembling early LTP, whereas 3-HFS LTP was of higher amplitude and showed an enduring and stable late phase, resembling late LTP.
These results are consistent with the findings of Huang and Kandel in 1994, who showed that these different stimulation protocols elicited transient and long-lasting LTP at hippocampal CA3-CA1 synapses (Huang & Kandel 1994). In agreement with their results 3-HFS increased the amplitude as well as the duration of LTP compared to 1-HFS. A discrepancy between their data and this study is the difference in duration of e-LTP, which lasted longer in my experiments. Recent studies demonstrated that the decay time of e-LTP is related to the synaptic usage (Fonseca et al. 2006).
Furthermore, the effect of the conditioning protocol was restricted to the stimulated synapses, indicating that input-specificity of LTP was conserved in these experiments.

5.1.2. Arc/Arg3.1 function in 1-HFS and 3-HFS LTP

Since previous work demonstrated the pivotal role of Arc/Arg3.1 in memory formation and synaptic plasticity, we focused on Arc/Arg3.1 function in transient and long-lasting LTP in the presented study. To examine Arc/Arg3.1 function in either transient or long-lasting LTP, 1-HFS and 3-HFS LTP was induced in Arc/Arg3.1 deficient mice. First, both conditioning protocols induced LTP in knock-out slices as reliably as in wildtype slices as reported by previous studies (Guzowski et al. 2000, Messaoudi et al. 2007, Plath et al. 2006). In accordance with these previous studies input-specificity was conserved in knock-out slices.
However, the amplitude of e-LTP and the stability of the l-LTP differed between the wildtype and knock-out slices, indicating that Arc/Arg3.1 might play a role in these two different phases of LTP. In wildtype slices, 3-HFS induced larger e-LTP than 1-HFS. In contrast, e-LTP in knock-out slices was of equivalent magnitude in response to both 1-HFS and 3-HFS. Thus in absence of Arc/Arg.3.1 e-LTP was no longer graded. Several reasons might account for the loss of graded e-LTP in the knock-out mice: Early-LTP results mainly from posttranslational modifications of the AMPA receptor density, type and properties resulting in an increase in AMPA receptor mediated excitatory current (for review Malinow & Malenka 2002,

Shepherd & Huganir 2007). (1) These modifications require the activity of several kinases such as CaMKII, PKA and ERK. Although Arc/Arg3.1 is not a kinase itself, it might affect the activity of CaMKIIa and CaMKIIb by binding to them in an activity dependent manner (Okuno 2012). Thus Arc/Arg3.1 might indirectly limit posttranslational modifications in wildtype mice. (2) A rapid insertion of AMPA receptors into the PSD could contribute to e-LTP (Shi et al. 2001, Malinow & Malenka 2002, Shepherd & Huganir 2007). Rapid removal of AMPA receptors from the PSD could counterbalance this insertion and lead to weaker e-LTP. Arc/Arg3.1 was shown to mediate the endocytosis of GluR1 containing AMPA receptors during slow homeostatic synaptic plasticity. Hence it could be hypothesized that Arc/Arg3.1 has a similar effect on AMPA receptors after insertion during e-LTP (Shepherd et al. 2006). In the latter case e-LTP in the knock-out slice would be larger than in wildtype slice due to the lack of Arc/Arg3.1-mediated AMPA receptor endocytosis.

The tight control and balance of synaptic strength has been shown to be critical for the encoding of information as required in memory formation. This tight control of the degree of synaptic potentiation and depression within the neuronal network would be disrupted in knock-out mice by the loss of graded LTP in response to different stimulation. Thus the loss of graded LTP could contribute to the impairment of memory as observed in Arc/Arg3.1 knock-out mice.

Also long-lasting LTP depended on Arc/Arg3.1 expression in these experiments. In response to 3-HFS, knock-out slices exhibited large e-LTP - however this potentiation significantly decayed within the following five hours, in contrast to wildtype slices which exhibited stable l-LTP exceeding five hours.

These differences in LTP expression could be suspected to be related to differences in basal synaptic properties caused by the deficiency of Arc/Arg3.1. Analysis of basal synaptic properties demonstrated that knock-out mice appeared to be less responsive to afferent stimulation as indicated by an increase in threshold intensity and the flat IO-curves. Despite this, pre- and postsynaptic function as characterized by PPF and the $fEPSP_{max}$ appeared unaltered, indicating that the neuronal networks own the same capability to increase their response. Differences in slice excitability and the PS threshold could affect LTP induction. Nonetheless, no differences in the frequency of LTP induction have been observed. In addition, I regularly observed PS in most slices without significant differences between the genotypes after LTP induction. Thus it

appears unlikely that differences in LTP maintenance were influenced by an alteration of excitability.

The role of Arc/Arg3.1 in synaptic plasticity and memory formation has been in the focus of many research projects linking electrophysiological data and behavioral studies. In these studies Arc/Arg3.1 function was either studied in a constitutive Arc/Arg3.1 knock-out mouse model, as in the presented experiments, or via disruption of Arc/Arg3.1 translation with antisense oligodeoxynucleotides (ODNs) resulting in a decrease in Arc/Arg3.1 protein of approximately 40 - 50 percent. These previous studies reported consistently that Arc/Arg3.1 was essential for the maintenance of LTP and initially potentiated fEPSPs rapidly decline to baseline within one to three hours depending on the type and site of recording. Also, the dynamic role of Arc/Arg3.1 in different phases of LTP have already been in the focus of the studies presented by Messaoudi et al. (Messaoudi et al. 2007). They specified Arc/Arg3.1 function *in vivo* in the *dentate gyrus* by delivery of antisense ODNs at different time points after LTP induction.

The findings in this study are partially in agreement with these previous findings. In the current study it was demonstrated that in knock-out slices LTP maintenance was impaired compared to wildtype slices but a complete decline to baseline has not been observed. Thus the deficit in LTP consolidation was milder than reported previously.

The influence of Arc/Arg3.1 on LTP maintenance might be influenced by a number of factors. Possible reasons for these inconsistencies include differences in experimental conditions, i. e. (1) whether experiments were conducted *in vivo* or *in vitro*, (2) whether single synapses or a whole population of neurons were stimulated, (3) whether inhibitors of GABAergic-transmission were applied, or (4) differences in test pulse rate. For instance it is known that LTP in the CA1 region requires different conditions than in the *dentate gyrus*. Experimental data acquired in either region could not be generalized to the other without limitations. The region of interest in recordings focusing on LTP in presence of Arc/Arg3.1. ODNs was the *dentate gyrus* (Messaoudi et al. 2007, Guzowski et al. 2000). Furthermore these experiments have been conducted *in vivo*. In contrast to the more native state during *in vivo* recordings, the deafferentation and separation of neuronal connections occurring during preparation led to unequal conditions, i. e. the loss of spontaneous background activity occurring in acute hippocampal slices. The damage during slicing might

further provoke changes in metaplasticity, e. g. the release of neurotransmitters, growth factors and other substances potentially altering synaptic function.

The only study focusing on LTP in CA1 under similar *in vitro* conditions has been conducted in the same mouse line by Plath et al. (Plath et al. 2006). In contrast to the current study, Plath et al. investigated pairing-induced LTP in small synaptic populations and single cells. Results of the current study were obtained using field recordings measuring the synchronous activity of a large population of neurons. Experimental results obtained from populations of synapses and populations of neurons might differ for a number of reasons. Recordings from stimulated synapses exclusively monitor changes at activated synaptic sites and therefore no interactions between the neurons were monitored. In addition, Plath et al. used inhibitors of GABA-receptor mediated synaptic transmission in their experiments. It could not be excluded that an altered balance of excitation/inhibition changes LTP conditions.

Furthermore recent studies emphasized that the frequency of baseline test stimulation significantly influences the maintenance of LTP and its dependence on *de novo* protein synthesis (Fonseca et al. 2006). Assuming that synaptic activity increases the turnover of proteins required for LTP maintenance and in consequence its stability, differences in baseline stimulation rate might account for differences in LTP decay times between the experiments. For example Plath et al. used a higher rate of stimulation (0.1 Hz) in comparison to the current study (0.033 Hz) which might result in a pronounced turnover of protein required for LTP or pronounced trafficking of AMPA receptors, thereby prompting LTP decay.

5.2 THE FUNCTION OF SOMATIC AND DENDRITIC ARC/ARG3.1 SYNTHESIS IN LTP (TGMXS*B6)

In a second series of experiments the function of local Arc/Arg3.1 translation at dendritic sites in synaptic plasticity was examined. In response to plasticity inducing stimuli Arc/Arg3.1 induction has a unique time-course. Arc/Arg3.1 expression was found to be increased in two steps. First local translation of dendritic Arc/Arg3.1 mRNA extended the amount of Arc/Arg3.1 protein available at synapses. A second increase in Arc/Arg3.1 occurs when newly transcribed Arc/Arg3.1 mRNA and protein

are delivered from the soma (Mao, unpublished data). This transport of Arc/Arg3.1 mRNA to dendritic sites and its on-site translation are a unique feature of Arc/Arg3.1.

In a previous study, Messaoudi et al. (2007) investigated the function of Arc/Arg3.1 at specific time points after BDNF-LTP induction. The authors used antisense ODNs to temporarily and partially knock-down Arc/Arg3.1 translation. They reported that early Arc/Arg3.1 synthesis was required for early BDNF-LTP expression, whereas sustained Arc/Arg3.1 synthesis was necessary to consolidate these modifications. This study indicated different roles of Arc/Arg3.1 translation during the induction- and maintenance-phases of LTP, but did not examine the role of synaptic (local) and global (somatic) Arg3.1 translation.

In our laboratory a new transgenic mouse line in which the Arc/Arg3.1 mRNA was modified in a way that prevents its delivery to dendrites, thereby abolishing dendritic/synaptic translation. The Arc/Arg3.1 mRNA of this transgenic mice is properly translated in the soma after strong activation and the Arc/Arg3.1 protein is rapidly transported to the stimulated dendrites (Mao 2008). This mouse line provided us with the opportunity to test the role of local Arg3.1/Arc translation in LTP.

LTP induced in wildtype mice of mouse line TgMXS*B6 was different from LTP in wildtype mice of the E8KO line. The major difference was that in wildtype TgMXS*B6 mice 3-HFS induced only slightly higher early LTP than 1-HFS. After both stimuli LTP was not maintained at a stable level and declined back to baseline levels within five hours.

A comparison of LTP between wildtype, knock-out and transgenic slices of mouse line TgMXS*B6 in the first set of experiments (chapter 4.3, for summary Figure 4.13) raised the following conclusions: (1) 1-HFS and 3-HFS induced transient LTP of indistinguishable magnitude and time course in all genotypes suggesting that this LTP was independent of Arc/Arg3.1. It could be speculated that Arc/Arg3.1 might not even be induced by this type of weak stimulation since the threshold for the induction of IEG was shown to be associated with l-LTP induction as previous studies correlating LTP persistence and IEG induction proposed (Abraham et al. 1993). However, the basal level of Arc/Arg3.1 present at synapses appeared to have no influence on transient LTP magnitude and duration. (2) In response to 3-HFS, e-LTP was larger in knock-out and transgenic slices compared with wildtype slices. A similar enhancement of e-LTP in knock-out slices was previously reported in both CA1 and DG (Plath et al. 2006). These data suggest that the increased e-LTP was

most likely mediated by local Arc/Arg3.1 translation rather than by constitutively expressed basal Arc/Arg3.1. Accordingly, Tg mice lacking local Arc/Arg3.1 translation resembled knock-out mice during this phase. Furthermore, estimated rates of somatic protein synthesis and transport of proteins to activated synaptic sites require more time (Ouyang et al. 1999).

In a subsequent series of experiments 3-HFS LTP was again tested in wildtype, knock-out and transgenic slices (chapter 4.4, for summary Figure 4.21 A). In this second series stable LTP of equivalent magnitude and duration was induced in wildtype and knock-out slices, demonstrating that in this mouse line even long-lasting LTP was induced in Arc/Arg3.1 knock-out slices. Until now it has been reported that Arc/Arg3.1 is essential for long-term memory as well as long-lasting synaptic plasticity (Guzowski et al. 2000, Plath et al. 2006, Shepherd et al. 2006, Messaoudi et al. 2007, Park et al. 2008). In my experiments though, stable and long-lasting LTP was observed in Arc/Arg3.1 knock-out slices. A possible model for this observations is given and illustrated in the next chapter (Figure 5.1).

In contrast to wildtype and knock-out slices, in transgenic slices 3-HFS was only sufficient to induce transient LTP in all slices examined in both sets of experiments (Figure 4.12, Figure 4.21 A and B). Previous experiments showed that the only difference between knock-out and transgenic mice is the absence of locally translated Arc/Arg3.1 mRNA which presumably results in a delayed increase in Arc/Arg3.1 protein at stimulated synapses since Arc/Arg3.1 has afore been delivered from the soma (Mao 2008). This remarkable difference between wildtype, knock-out and transgenic slices in the ability to induce long-lasting LTP demonstrates that Arc/Arg3.1 serves different functions depending on its destination, i. e. locally versus somatically translated Arc/Arg3.1. The absence of local Arc/Arg3.1 translation completely prevented l-LTP induction, whereas in the total absence of Arc/Arg3.1 l-LTP was unaffected. Although no differences in the structure und composition of locally and somatically translated Arc/Arg3.1 have yet been shown they appear to strikingly differ in their function at the synapse. One reason for this difference could be the different timescale of presence at synaptic sites. Locally translated Arc/Arg3.1 is present at synapses before somatically translated Arc/Arg3.1 is transported from the soma. Due to the delayed arrival of somatically translated Arc/Arg3.1 at synaptic sites it might interact with a different set of proteins. This may explain the observed differences in the function.

One explanation for the loss of long-lasting LTP in transgenic slices could be that locally translated Arc/Arg3.1 serves as a synaptic tag necessary to capture newly synthesized proteins which were delivered from the soma to consolidate long-lasting LTP. In consequence, tagging of recently activated synapses would be disrupted in transgenic slices and protein synthesis dependent consolidation of synaptic plasticity could not take place and LTP would decline.

The failure to induce long-lasting LTP in the first experiments (chapter 4.3) is likely to result from experimental conditions specific to this set of experiments, since stable LTP was induced by 3-HFS in this mouse line in a second set of experiments (control experiments chapter 4.4 and 4.5). The ability to induce enduring LTP might be related to slice conditions, e. g. slice viability and parameters of basal synaptic transmission, which might be compromised by several reasons. Furthermore, the duration of LTP was shown to be sensitive to various technical conditions, e. g. modifications of recovery conditions (interface vs. submerged) were shown to significantly influence the subsequently induced LTP (Capron et a. 2006). Post-hoc comparison of my data showed that one possible explanation is that the baseline fEPSPs were significantly larger in experiments with instable LTP which were in general correlated with reduced l-LTP amplitudes.

Examination of basal synaptic transmission in this mouse line showed that in the complete absence of Arc/Arg3.1 - as in knock-out mice - led to an increased PS threshold compared to wildtype and transgenic mice. This indicated that the presence of Arc/Arg3.1 increased the response to afferent stimulation.
In contrast to mouse line E8KO, the absence of Arc/Arg3.1 at synapses resulted in a depression of synaptic responses over the whole range of stimulation intensities as observed in IO-curves of Arc/Arg3.1 knock-out mice. This effect could be considered to be mediated by constitutive Arc/Arg3.1 expression since IO-curves are assumed to not induce processes of synaptic plasticity. Furthermore, an involvement of dendritic Arc/Arg3.1 translation could be excluded since transgenic slices which specifically lack dendritic Arc/Arg3.1 translation mirrored the results obtained in IO-curves of wildtype mice.
Taken together, present results add to previous findings that locally and somatically translated Arc/Arg3.1 serve different functions in LTP because transgenic slices only

exhibited transient LTP whereas in wildtype and knock-out slices long-lasting LTP could be induced. The data presented here demonstrates for the first time that also in knock-out slices stable, long-lasting LTP was observed for at least five hours indicating that some forms of l-LTP might still exist in the absence of Arc/Arg3.1.

5.3 LTP AND ITS DEPENDENCE ON PROTEIN SYNTHESIS

One characteristic property shared by both long-term memory consolidation and long-term plasticity is the dependence on *de novo* protein synthesis (Nader et al. 2000, Kandel 2001). To test whether the long-lasting LTP I observed was indeed protein synthesis dependent and to investigate the residual LTP in knock-out slices, I performed 3-HFS LTP experiments in the presence of protein synthesis blockers in wildtype, knock-out and transgenic slices.

To inhibit *de novo* protein synthesis cycloheximide and anisomycin were used to achieve a general inhibition of translation by global blockade of peptide chain elongation. Numerous studies have demonstrated that cycloheximide and anisomycin block LTP maintenance (Krug et al. 1984, Frey et al. 1988). Whether this unspecific blockade of translation prevents or reduces activity dependent Arc/Arg3.1 translation remains unknown. Previously, Park et al. reported a blockade of Arc/Arg3.1 translation by high dose cycloheximide (Park et al. 2008). However, the latter study was performed on cultured neurons under different experimental conditions to the one I employed. Cap-independent translation initiation via internal ribosomal entry sites (IRES) as occurring in Arc/Arg3.1 translation might render Arc/Arg3.1 translation independent of cycloheximide and anisomycin since cycloheximide was even shown to induce an enhancement of IRES-mediated translation initiation which offsets the inhibition of elongation (Costa-Mattioli et al. 2009, Fernandez et al. 2005). In consequence activity dependent Arc/Arg3.1 induction might still be active in the presence of protein synthesis inhibitors. Furthermore, Arc/Arg3.1 and other preformed proteins present at synapses were not inhibited.

First the effect of protein synthesis inhibition by cycloheximide treatment on LTP maintenance in wildtype slices was examined. Consistent with previous reports, cycloheximide treatment selectively blocked the late phase of LTP, whereas the induction and early phase of LTP remained intact (Krug et al. 1984, Frey et al. 1988).

Thus the presented data is in agreement with earlier reports that *de-novo* protein synthesis is not required during e-LTP.

The finding that cycloheximide treatment blocked long-lasting LTP in the stimulated pathway but did not affect basal synaptic transmission in the control pathway suggests that *de-novo* protein synthesis was critical only when additional or new proteins were required to consolidate changes in synaptic structure or function and not for baseline synaptic transmission. This is in accordance with previous studies (Krug et al. 1984, Frey et al. 1988).

Experiments were conducted twice using the both inhibitors to ensure that results were not caused or influenced by confounding drug variables, e. g. unspecific drug side-effects. Protein synthesis blockade by anisomycin also blocked l-LTP expression. The potentiation of the fEPSP diminished to baseline levels within four hours in the presence of anisomycin. Qualitatively, similar results to cycloheximide were obtained, yet the extent of l-LTP blockade was smaller and its decay time constant longer. Because slices were incubated with both protein synthesis inhibitors for at least 1.5 hours prior to LTP induction, different diffusion coefficients in the slice or cell penetration are unlikely to account for the differences between the blockers. A more likely explanation is that the efficacy of protein synthesis blockade differed between the inhibitors. In this context it has been previously shown that the amount of plasticity related proteins still available under conditions of reduced protein synthesis determines the degree of decline (Fonseca et al. 2004, Fonseca et al. 2006). An incomplete block of protein synthesis would explain the delayed decline observed in anisomycin treated slices.

The effect of protein synthesis inhibition in wildtype mice is contrasted by the lack of effect in knock-out mice. Field EPSP recordings during LTP remained significantly potentiated compared to pre-HFS baseline or within-slice control pathway. Furthermore no differences in LTP time courses in presence of cycloheximide or under control conditions have been detected. This result pointed to the surprising possibility that *de novo* protein synthesis is no longer required to maintain LTP in Arc/Arg3.1 deficient mice. This striking result was reproduced under anisomycin treatment, indicating that the effect was not related to a particular inhibitor.

No additional effect of cycloheximide treatment was observed in transgenic mice, suggesting that transgenic mice were not capable of sustaining protein synthesis dependent long-lasting LTP. This is further supported by the observation that LTP in

wildtype mice with protein synthesis inhibitors versus LTP in transgenic mice did not differ. This observation is in agreement with the hypothesis that dendritic Arc/Arg3.1 synthesis is required for synaptic tagging and the disrupted tagging would prevent the capture of plasticity related protein. Possible explanations are given later in this text.
Notably, the input specificity of LTP was conserved under cycloheximide treatment in all genotypes suggesting that mechanisms engaged in this process are independent of *de novo* protein synthesis.

To summarize, the presented results demonstrated the following important points:

(1) Inhibition of *de novo* protein synthesis prevented long-lasting LTP expression in wildtype slices.

(2) The requirement of *de novo* protein synthesis for the consolidation of LTP was dependent on the presence of Arc/Arg3.1 in synapses since in knock-out slices LTP was maintained in the presence of protein synthesis inhibitors.

(3) Impairment of local Arc/Arg3.1 translation at the synapse prevented long-lasting LTP expression in transgenic slices. LTP recorded in transgenic slices mirrored LTP in wildtype slices under conditions of reduced protein synthesis.

The dependence on protein synthesis is regarded as the key property of long-lasting LTP and of long-term memory formation itself. Nonetheless, the conventional view that protein synthesis is required to maintain LTP has been questioned in recent times by the results of different studies reporting long-lasting LTP in the absence of protein synthesis under special conditions (Vicker et al. 2012).
Huber et al. demonstrated that mice lacking the fragile X mental retardation protein (FMRP) exhibit mGluR-dependent LTD which was shown to be insensitive to protein synthesis inhibition (Huber et al. 2002, Hou et al. 2006, Nosyreva & Huber 2006). Normally FMRP acts as a repressor of translation in the brain. Protein levels of Arc/Arg3.1, CaMKII, Microtubule-associated protein 1B (MAP1B) and others have been reported to be enhanced in mice lacking FMRP. This led to the hypothesis that enhanced basal protein levels might be sufficient to provide the amount protein required to maintain mGluR LTD (Park et al. 2008).
Pang et al. demonstrated that the exogenous application of BDNF was able to rescue LTP induced in the presence of anisomycin and concluded that BDNF might be the

key regulator prompting structural and functional changes underlying long-lasting changes (Pang et al. 2004).
It was also demonstrated that *de novo* protein synthesis and protein degradation are co-regulated in synaptic plasticity. Stimuli inducing LTP also activate proteasome-mediated protein degradation, suggesting that LTP maintenance involves a regulated level of proteins at the synapse. This might be achieved by balancing *de-novo* protein synthesis and counteracting changes in protein degradation (Karpova et al. 2006). Accordingly, Fonseca et al. showed that concomitant inhibition of protein synthesis and protein degradation leaves LTP unaffected (Fonseca et al. 2006). Protein synthesis during LTP might be required to replenish LTP stabilizing proteins which have been depleted during LTP maintenance or have been subject to protein turnover. This hypothesis is supported by the finding that LTP was shown to be completely unaffected by protein synthesis inhibition when no test stimuli were delivered during protein synthesis inhibition (Fonseca et al. 2006).

Taken together these studies demonstrate that LTP consolidation does not depend on protein synthesis *per se*, since certain conditions were shown to render *de novo* protein synthesis unnecessary. Until now, interference or disruption of Arc/Arg3.1 function has been reported to completely prevent protein synthesis dependent long-lasting LTP. Experiments presented here instead pointed out that Arc/Arg3.1 might play a key role in determining whether *de novo* protein synthesis is required for plasticity consolidation. So far, different conditions were shown to render LTP independent of *de novo* protein synthesis.

My observation of the loss of protein synthesis dependence of LTP in knock-out slices might be explained by several reasons:

(1) Proteins required for LTP maintenance are present in amounts sufficient to mediate and maintain LTP before blockade of protein synthesis. This hypothesis would postulate that Arc/Arg3.1 is involved in the control of general protein synthesis.
(2) Inhibition of *de-novo* protein synthesis might be counteracted by changes in the rate of protein degradation controlled by Arc/Arg3.1.

(3) Alterations in AMPA receptor trafficking in Arc/Arg3.1 deficient mice might render LTP consolidation independent of plasticity-related proteins.

From the presented data it could not be determined why protein synthesis is only required for LTP maintenance when Arc/Arg3.1 is present. In the following paragraphs the above mentioned hypothesis will be discussed in more detail and experiments required to test them are given.

LTP is thought to be a multistep process mediated by posttranslational modifications of preexisting proteins on one hand and modifications of the synaptic protein composition at synapses regulated by alterations of gene expression on the other hand. The presence and composition of proteins at synapses can be influenced by either *de novo* protein synthesis, protein trafficking or protein degradation. Some evidence exists that LTP might be maintained under proteins synthesis inhibition. (1) The proteins required to maintain LTP would be present in a sufficient amount, either due to overexpression or changed balance between protein replenishment and degradation (Fonseca et al. 2006, Park et al. 2008). This would raise the possibility that in knock-out slices preexisting proteins are available in a sufficient amount to induce and maintain stable LTP. A potential influence of Arc/Arg3.1 on protein synthesis or degradation has not yet been investigated. Such an influence would be reflected by differences in protein amount and protein composition between wildtype and knock-out slices. Plath et al. reported that basal levels of AMPA or NMDA receptors, proteins which are essential for synaptic plasticity, were not altered in the Arc/Arg3.1 knock-out mice. *In vitro* intracellular recording of AMPA and NMDA receptor mediated excitatory postsynaptic currents (EPSC) showed no differences in baseline synaptic properties and in surface AMPA/NMDA receptor ratio in wildtype and Arc/Arg3.1 knock-out mice (Plath et al. 2006). On the contrary, in highly-active primary neuronal cultures GluR1 levels were increased in Arc/Arg3.1 knock-out neurons and a recent study linked this reduction to a repressive effect of Arc/Arg3.1 on GluR1 transcription (Korb et al. 2013). Thus, Arc/Arg3.1 might affect transcription/translation of synaptic proteins following neural activation, but not in a constitutive manner.

(2) and (3) The trafficking of AMPA receptors regulating the number of surface AMPA receptors by exocytosis, endocytosis, recycling and degradation is likely to be the principal regulator in stabilizing synaptic plasticity (Abraham & Williams 2003).

It remains possible that differences in AMPA receptor trafficking in wildtype and knock-out slices might account for differences in the maintenance and protein synthesis dependence of LTP which I observed.

Activity-induced changes in synaptic plasticity such as LTP are commonly expected to be regulated by modifications of AMPA receptor mediated synaptic transmission. Thus the regulated trafficking of AMPA receptors during LTP is a key element of synaptic plasticity (Anggono & Huganir 2012, Malinow & Malenka 2002, Shepherd & Huganir 2007). Previous studies demonstrated that Arc/Arg3.1 is implicated in AMPA receptor endocytosis in synaptic scaling during homeostatic plasticity and LTD (Beique et al. 2011, Chowdhury et al. 2006, Park et al. 2008, Plath et al. 2006, Rial Verde et al. 2006, Shepherd et al. 2006). The level of postsynaptic AMPA receptors which determines the strength of synaptic transmission is thought to be tightly controlled by two different processes (Anggono & Huganir 2012, Shepherd & Huganir 2007). (1) Constitutive recycling and replacement of receptors takes place in an activity independent manner. (2) Activity-dependent insertion of AMPA receptors during synaptic strengthening is counteracted by activity-dependent endocytosis.

Changes in synaptic strength as LTP or LTD could therefore be hypothesized to be mediated by changes in the rate of AMPA receptor trafficking: (1) Transient LTP is mediated by the insertion of new AMPA receptors into the PSD. (2) The transformation of e-LTP into l-LTP has to be accompanied by a stabilization of newly inserted AMPA receptors in the PSD to prevent internalization during receptor cycling. This cycling might be either activity-dependent or constitutive.

Since l-LTP is commonly thought to be dependent on de novo protein synthesis it could be assumed that newly synthesized proteins, so-called plasticity related proteins (PRP), might be critically involved in stabilizing AMPA receptors in the PSD after LTP induction. Plasticity related proteins might either function as AMPA receptor anchoring proteins or they might inhibit subsequent endocytosis by direct interaction with the endocytic machinery.

Based on the following assumptions a model of Arc/Arg3.1 function in plasticity is introduced:

(1) LTP is mediated by an increase in AMPA receptor number in the PSD.

(2) Consolidation of long-lasting LTP requires the stabilization of newly inserted AMPA receptors in the PSD to prevent subsequent endocytosis and recycling.

(3) The process of AMPA receptor stabilization depends on plasticity related proteins therefore requiring *de-novo* protein synthesis.

The model is further based on the following assumptions:

(4) Arc/Arg3.1 synthesis is not completely disrupted in the presence of cycloheximide and anisomycin. Since I did not test whether an unspecific blockade of *de-novo* protein synthesis indeed reduces Arc/Arg3.1 translation or not, it could be postulated that Arc/Arg3.1 synthesis remained unaffected. This hypothesis is supported by the finding that cap-independent translation was even enhanced in the presence of protein synthesis inhibitors as described above (Costa-Mattioli et al. 2009, Fernandez et al. 2005).

(5) Locally and somatically translated Arc/Arg3.1 have different functions at synaptic sites. Locally translated Arc/Arg3.1 in dendrites serves as a synaptic tag of recently activated synapses to capture plasticity related proteins whereas Arc/Arg3.1 protein delivered from the soma is involved in the endocytosis of AMPA receptors in an activity-dependent manner when these receptors have not been stabilized by PRPs beforehand. This difference is indicated by the different phenotypes in electrophysiological recordings and behavioral experiments (unpublished data).

Based on these requirements I developed a model explaining my results which is presented in Figure 5.1.

The initial increase in e-LTP is mediated by post-translational modifications provoking the insertion of new AMPA receptors into the PSD. Strong LTP inducing stimuli are sufficient to additionally induce local Arc/Arg3.1 synthesis in dendrites and global protein synthesis. Locally synthesized Arc/Arg3.1 functions as a synaptic tag marking the recently activated synapses or rather the recently inserted AMPA receptors. This tag is required to subsequently capture PRPs delivered from the soma to stabilize the AMPA receptors in the PSD.

In contrast to dendritic Arc/Arg.3.1, somatically synthesized Arc/Arg3.1 is involved in an activity-dependent endocytotic process reducing the postsynaptic level of

AMPA receptors to normal levels when these receptors have not been stabilized before. In wildtype slices PRPs stabilize AMPA receptors in the PSD by either direct interaction or inhibition of elements of the endocytic machinery, thereby preventing Arc/Arg3.1 mediated endocytosis. Thus LTP is consolidated and persistent. Under conditions of reduced protein synthesis no PRPs are delivered from the soma to stabilize receptors in the PSD and prevent subsequent removal. Arc/Arg3.1 synthesis still takes place under protein synthesis inhibition prompting AMPA receptor endocytosis. Consequently, LTP declines to baseline.

In Arc/Arg3.1 knock-out slices LTP is persistent and independent of protein synthesis. The loss of Arc/Arg3.1 would lead to a disrupted activity-dependent AMPA receptor endocytosis. Even under conditions of reduced protein synthesis the deficiency of PRP mediated AMPA receptor stabilization is compensated by the disruption of removal. Thus AMPA receptor surface levels remain increased, thereby maintaining LTP at a potentiated level. When activity-dependent decrease of synaptic transmission is inhibited like in knock-out slices, delayed constitutive endocytic and recycling processes might reduce the number of AMPA receptors. This general protein turnover might lead to a degradation of synaptic AMPA receptors and a delayed decline of LTP. This latter process could take place hours later. Thus LTP is expected to decline at some point in time, which is determined by the rate of synaptic usage as suggested by Fonseca et al. (Fonseca et al. 2006). This could also explain (1) why in experiments using higher baseline stimulation rates a decay of LTP in Arc/Arg3.1 knock-out mice was observed within the recorded time and (2) why memory deficits tested after days were still detectable in the knock-out mice, including the ones I used for experiments (Mao, unpublished data).

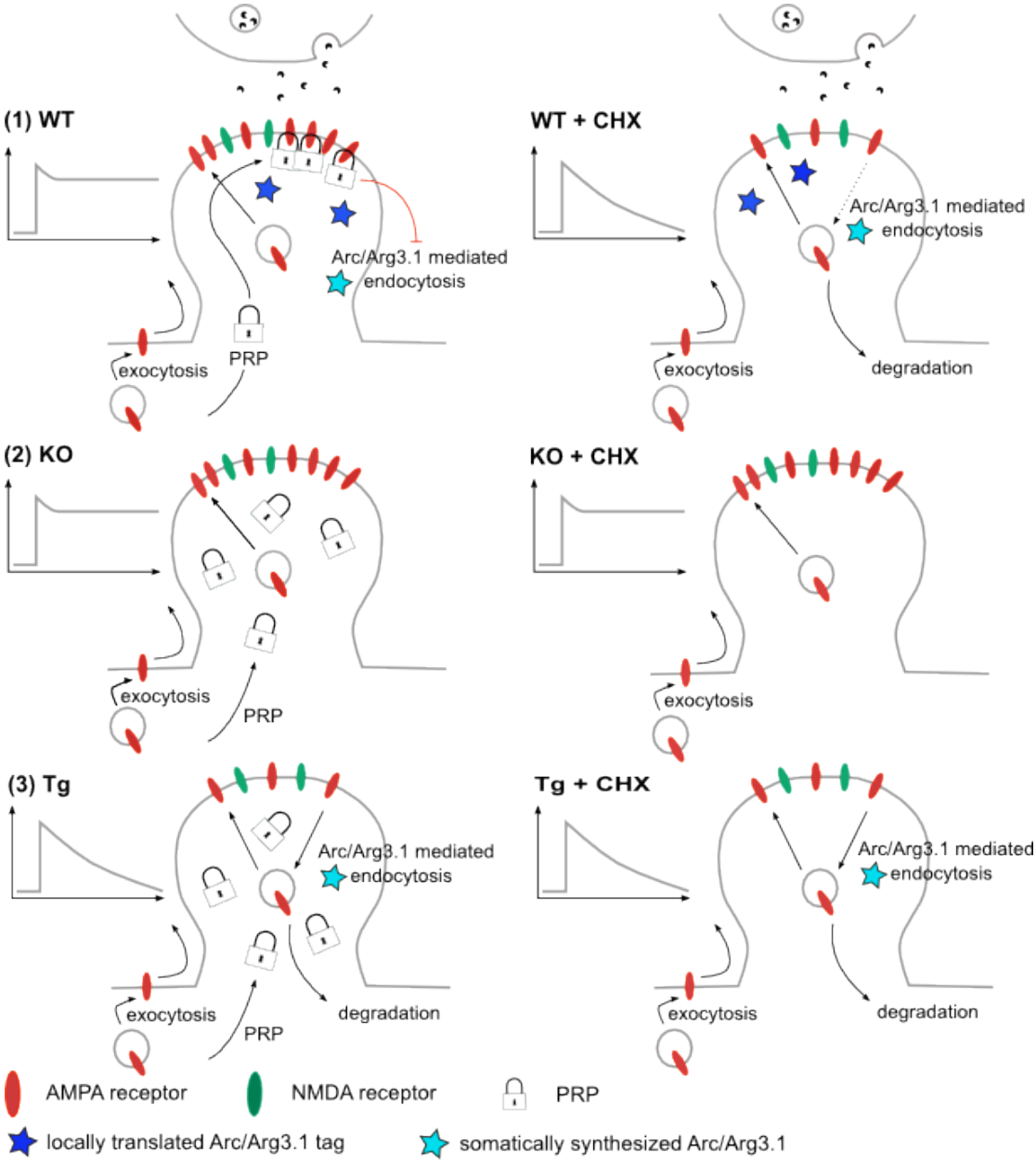

Figure 5.1: Model of Arc/Arg3.1 function in AMPA receptor trafficking during LTP

(1) WT: To transform transient-LTP into long-lasting LTP a stabilization of newly inserted AMPA-R in the PSD is required to counteract Arc/Arg3.1 dependent endocytosis of AMPA-R. This stabilization process is mediated by PRPs delivered from the soma and captured by locally translated Arc/Arg3.1 which functions as a tag in recently activated synapses. **WT + CHX:** The synthesis of PRPs is reduced. AMPA-R are not stabilized and are subject to endocytosis. **(2) KO +/- CHX:** Although no locally translated Arc/Arg3.1 tags capture AMPA-R stabilizing PRPs, AMPA-R numbers remain increased since Arc/Arg3.1-dependent endocytosis is also disrupted. **(3) Tg +/- CHX:** The process of local Arc/Arg3.1-dependent synaptic tagging and capture of PRPs is disrupted. The increase in AMPA-R number is reduced by Arc/Arg3.1-dependent endocytosis since PRP-dependent stabilization of AMPA-R does not occur.

In transgenic slices LTP is severely impaired due to the loss of local Arc/Arg3.1 synthesis. Regarding to my model locally translated Arc/Arg3.1 functions as a synaptic tag of recently inserted AMPA receptors, which is required to capture PRPs to stabilize them. Consequently, the loss of synaptic AMPA receptor tagging prevents the transformation and consolidation of transient into long-lasting LTP. The availability of PRPs has no further effect on LTP since PRPs are not guided to their site of action. No transformation of transient into long-lasting LTP takes place in transgenic slices. LTP declines under both conditions.

This putative model might explain the presented results but has not yet been verified. To test this hypothesis AMPA receptor trafficking has to be examined in more detail, the identity of proteins stabilizing AMPA receptors in the PSD and their orchestrated interaction with Arc/Arg3.1 and other proteins putatively involved AMPA receptor removal has to be investigated. Differences between locally and somatically translated Arc/Arg3.1 as indicated by my results have to be further examined. Most importantly the whole model is based on the assumption that Arc/Arg3.1 is translated in an amount sufficient to mediate endocytosis in the presence of cycloheximide or anisomycin. This has to be verified.

5.3.1. Basal synaptic transmission under conditions of reduced protein synthesis

The effects of protein synthesis inhibitors on basal synaptic transmission were tested in this study. Previous studies employing these inhibitors have rarely reported such effects (Cracco et al. 2005, Fonesca et al. 2006, Stanton et al. 1984). Surprisingly, analysis of basal synaptic transmission in my experiments, showed that the protein synthesis inhibitors cycloheximide and anisomycin influenced basal synaptic transmission in wildtype slices. I have often observed an increase in the fEPSP amplitude within the first 20 min of incubation in the presence of protein synthesis inhibitors in wildtype slices. This effect was quantified by comparing the input output (IO) curves of slices incubated in either aCSF alone or in the same aCSF containing protein synthesis inhibitors. Analysis of fEPSP/FV ratio showed that postsynaptic changes in synapse number or size were likely to account for the observed difference while presynaptic parameters such as FV and PPF were unchanged. This enhancement of synaptic strength or density under conditions of

reduced protein synthesis appears counterintuitive at first glance. Such phenomena have not been reported so far. One explanation might be that under control conditions receptor internalization and constitutive protein turnover counterbalance the insertion of new receptors into synapses. This process might be accentuated during slice recovery. Non-physiological global stimuli such as slicing and the concomitant deafferentiation could provoke an upregulation of receptors and sprouting of new spines (Kirov et al. 1999). During slice recovery spine retraction and receptor internalization might compensate this process. Proteins with relatively high turnover rates are affected first by inhibition of *de novo* protein synthesis. The smaller fEPSPs under control conditions could be explained when assuming that the process of spine retraction and receptor internalization requires *de novo* protein synthesis.

In contrast to wildtype slices, no changes in IO-curves were detected in knock-out slices. This might indicate that molecular processes occurring during slicing and recovery in wildtype mice do not occur in knock-out mice. As reported by Shepherd et al., knock-out mice exhibit deficits in homeostatic plasticity which control synaptic strength after periods of increased synaptic activity or inactivity (Shepherd et al. 2006). It is therefore plausible that Arc/Arg3.1 might also be essential for adaptive processes after slicing.

The Paired-pulse ratio was higher in knock-out slices in the presence of protein synthesis inhibitors. This might indicate a change in presynaptic functions caused by the protein synthesis inhibitors, for instance a reduction of probability of vesicle recruitment to the active zone during repetitive firing.

Since Arc/Arg3.1 knock-out mice were shown to exhibit LTP independent on protein synthesis on one hand, and seem to express no adaptive processes after slicing on the other hand, one might propose that the same mechanisms might be implicated in these processes. It is likely that AMPA trafficking is involved in adaptive processes as well as plasticity processes and Arc/Arg3.1 might be implicated in protein synthesis dependent processes controlling AMPA receptor stability at the PSD.

5.4 CONCLUSIONS

In this study the role of Arc/Arg3.1 in protein synthesis dependent and independent forms of synaptic plasticity was investigated. Weak (1-HFS) and strong stimulation protocols (3-HFS) were applied to trigger transient and long-lasting LTP, respectively, using two different mouse models. First Arc/Arg3.1 had no function in transient LTP because no differences were detected in wildtype and knock-out slices during transient LTP (Figure 4.6 A and Figure 4.13 A and B). The transient form of LTP is commonly assumed to be protein synthesis independent. Nevertheless, Arc/Arg3.1 affected the e-LTP amplitude of transient LTP, as observed in mouse line E8KO by the loss of LTP gradedness and in mouse line TgMXS*B6 by the enhancement in e-LTP amplitude. Since an increase in e-LTP amplitude was found in knock-out as well as transgenic slices I concluded that this enhancement is caused by the lack of locally translated Arc/Arg3.1.

Long-lasting LTP was surprisingly stable in knock-out slices. In mouse line E8KO the maintenance of long-lasting LTP was reduced compared to wildtype slices (Figure 4.6 B). However, in mouse line TgMXS*B6 no difference in l-LTP amplitude or duration were found between knock-out and wildtype slices (Figure 4.21 A). One could infer from these experiments that in mouse line TgMXS*B6 Arc/Arg3.1 was not required for LTP maintenance - at least during five hours post-HFS. Instead, the consolidation processes stabilizing long-lasting LTP differed fundamentally in knock-out slices. Arc/Arg3.1 knock-out slices exhibited long-lasting LTP in the presence of protein synthesis inhibitors in contrast to wildtype slices in which LTP was diminished to baseline (Figure 4.21 B). This finding, that the absence of Arc/Arg3.1 at synaptic site rendered subsequent LTP induction insensitive to protein synthesis inhibition, indicates that the presence or absence of Arc/Arg3.1 implicates a critical shift in the molecular mechanisms underlying LTP consolidation. In Arc/Arg3.1 transgenic slices, in which local Arc/Arg3.1 translation in dendrites is prevented, no long-lasting LTP could be induced. This demonstrates that local Arc/Arg3.1 synthesis is critically required to transform transient into long-lasting LTP (Figure 4.13 and 4.21).

6 ABSTRACT

Learning and Memory are believed to be encoded by the activity of neuronal networks in the brain. These patterns of neuronal activity are shaped by modifications of synaptic strength and connectivity between individual neurons, a phenomenon termed synaptic plasticity. Specific forms of synaptic plasticity, which require *de-novo* protein synthesis are strongly involved in the formation of long-term memory. The immediate-early gene Arc/Arg3.1 was shown to play a pivotal role in the consolidation of memories and synaptic plasticity (Guzowski et al. 2000, Plath et al. 2006, Ploski et al. 2008). Arc/Arg3.1 is highly expressed following synaptic plasticity evoking stimuli (Link et al. 1995, Steward et al. 1998) which lead to transcription and translation of Arc/Arg3.1 at the soma but also to a very rapid translation at the activated synapses from dendritically localized Arc/Arg3.1 mRNA.

My data shows that the magnitude of transient LTP evoked by weak stimuli (1-HFS) was constrained by Arc/Arg3.1 protein at the synapses, but LTP maintenance was independent of Arc/Arg3.1.

Strong stimuli (3-HFS) evoked large and stable, protein synthesis dependent LTP in wildtype slices. The same stimuli evoked a similarly large and stable LTP in the Arc/Arg3.1 knock-out slices, however, this was no longer dependent on protein synthesis as it persisted in the presence of protein synthesis inhibitors for at least five hours. The latter results were surprising and novel because they show for the first time that some forms of long-lasting LTP might still exist in the absence of Arc/Arg3.1. However, the molecular mechanisms underlying this residual l-LTP are clearly different from l-LTP in wildtype synapses. Moreover, a novel role of Arc/Arg3.1 is revealed by these data, namely that of making l-LTP dependent on *de-novo* protein synthesis. Furthermore, the results obtained from transgenic slices demonstrated that locally and somatically translated Arc/Arg3.1 serve different functions in LTP. The local translation of Arc/Arg3.1 was found to be critically required to initiate LTP consolidation.

Because long term memory is entirely abolished in the Arc/Arg3.1 knock-out mice, while at least one form of long-lasting LTP still exists, it might be speculated that the role of Arc/Arg3.1 in memory is actually to couple synaptic plasticity to protein synthesis, independent of the persistence of plasticity.

7 ABBREVIATIONS

αCaMK2 ..... α-calcium-calmodulin-dependent kinase 2

AMPA receptor ..... α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid-type glutamate receptor

ANI ..... Anisomycin

Arc/Arg3.1 ..... Activity-regulated cytoskeleton-associated protein/ activity-regulated gene 3.1 protein

BDNF ..... Brain-derived neurotrophic factor

CA1 ..... Cornu ammonis 1

CA 3 ..... Cornu ammonis 3

CamKII ..... Ca^{2+}/Calmodulin-dependent protein kinase II

cAMP ..... Cyclic adenosine monophosphate

CRE ..... cAMP response element

CREB ..... cAMP response element-binding

CHX ..... Cycloheximide

con ..... Control pathway

eIF4E ..... Eukaryotic initiaion factor 4E

EPSC ..... Excitatory postsynaptic current

EPSP ..... Excitatory postsynaptic potential

ERK ..... Extracellular signal-regulated kinase

fEPSP ..... Field excitatory postsynaptic potential

FMRP ..... Fragile X mental retardation protein

FV ..... Fibre volley

GluR1, GluR2, GluR3 ..... Glutamate receptor type 1, type 2, type 3

HFS ..... High-frequency stimulation

IEG ..... Immediate early gene

IO-curve ..... Input-output curve

IRES ..... Internal ribosomal entry site

ISI ..... Inter-stimulus interval

KO ..... Arc/Arg3.1 knock-out mice

LTD ..... Long-term depression

LTP ..... Long-term potentiation

MAPK ..... Mitogen-activated protein kinase

MAP1B ..... Microtuble-associated protein 1B

mGluR ..... Metabotrophic glutamate receptor

n_c ..... Number of slices with control pathway

n_s ..... Number of slices with stimulated pathway

NMDA receptor ..... N-methyl D-aspartate-type glutamate receptor

ODN ..... Oligodeoxynucleotides
PKA ..... Protein Kinase A
PPF ..... Paired-Pulse Facilitation
PRP ..... Plasticity-related protein
PS, pop spike ..... Population spike
PSD ..... Postsynaptic density
SI ..... Stimulus intensity
stim ..... Stimulated pathway
Tg ..... Transgenic mice with the 3'UTR of the Arc/Arg3.1 encoding region is replaced with the 3'UTR of Zif268
WT ..... Arc/Arg3.1 wild-type mice

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JOHANNE KLÄSCHEN

PERSON

Familienstand:	ledig
Staatsangehörigkeit:	Deutsch
Geburtsdatum:	* 4.2.1988

SCHULAUSBILDUNG

1994 – 1998	Gustav-Peters Schule, Eutin-Fissau
1998 – 2006	Johann-Heinrich-Voss Gymnasium, Eutin
Juni 2006	Abitur (Note 1,6)

STUDIUM

2006 – 2013	Medizinstudium am Universitätsklinikum Eppendorf, Hamburg
2008	1. Staatsexamen (Note 1.5)
2013	2. Staatsexamen (Note 1.5)
	Gesamtnote 1,5
2010 – 2011	Laborarbeit für meine Dissertation am Institut für Molekulare und Zelluläre Kognition, Prof. Dr. Kuhl, ZMNH, UKE

Hamburg, 07. Dezember 2013

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