Influence of nucleus accumbens core or shell stimulation on early long-term potentiation in the dentate gyrus of freely moving rats

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To Benjamin Simon, my precious son

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Abstract

The nucleus accumbens (NAcc) is an integral part of the basal ganglia located within the ventral striatum. It is composed of two regions: core and shell which has been related to reward motivated behavior. It is positioned as an interface between the limbic and motor systems integrating signals arising from these structures, and to modulate limbic drive and motor planning. The NAcc is innervated by limbic structures and receives convergent excitatory afferents from the ventral hippocampus, basolateral amygdala (BLA) and medial prefrontal cortex. In addition, it receives dopaminergic input from the ventral tegmental area (VTA) which has been implicated in a number of functions related to neural reward processing. In the last years our laboratory has characterized the influences of several brain structures modulating synaptic plasticity in the dentate gyrus (DG) of the hippocampal formation. Synaptic plasticity characterized by changes in the efficacy of synaptic transmission at synapses, can contribute to storage of information within neural circuits. Two major forms of long-term changes in synaptic efficacy have been characterized: long-term potentiation (LTP) and long-term depression (LTD). These changes in synaptic strength can occur both on short-term and long-term basis depending on synaptic activity and the modulatory type of synapse. Characterizing the brain structure in question, it was electrically stimulated within a distinct time window prior to or after short-term plasticity induction in the DG. Under distinct circumstances, activation of modulatory brain structures can transform a protein synthesis-independent early long-term potentiation (early-LTP) to a late long-term potentiation (late-LTP) in the DG. Here, we stimulated the NAcc core or shell 15 minutes after induction of early-LTP in the DG via the perforant pathway (PP) stimulation. Summarizing, the stimulation of NAcc core or shell did not significantly modify the amplitude or the duration of DG early-LTP. Stimulation of the NAcc core 15 minutes prior to the induction of DG early-LTP via the PP completely prevented the induction of early-LTP of the field excitatory postsynaptic potential (f-EPSP) while the population spike amplitude (PSA) potentiated less than control and decayed very fast to baseline value. The stimulation of the NAcc shell before induction of DG early-LTP did neither modify significantly the amplitude nor the duration of DG early-LTP. In a set of control experiments, we investigated if stimulation of the NAcc core or shell alone, without tetanus to the PP, would have an effect on baseline values after stimulating the DG. The results for these control experiments indicated that NAcc core stimulation slightly but significantly depressed the PSA up to 8 h but not f-EPSP. In summary, NAcc stimulation after the induction of early-LTP seems to have no effect on the time course and late phases of the potentiation in the DG. However, NAcc stimulation before the induction of LTP had influences on the time course and the late phases of the potentiation.

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List of abbreviations

AD/DA	-	analog-digital/digital-analog
AMPA	-	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	-	2-way analysis of variance
AP	-	anterior-posterior
BLA	-	basolateral amygdala
Ca^{2+}	-	calcium ion
CA	-	cornu ammonis
CaM	-	calmodulin
CaMKII	-	calcium/calmodulin-dependent protein kinase II
cAMP	-	cyclic adenosine monophosphate
CRE	-	cAMP responsive element
CREB	-	cAMP response element-binding protein
DA	-	dopamine
DAG	-	diacylglycerol
DG	-	dentate gyrus
DNA	-	deoxyribonucleic acid
DV	-	dorso-ventral
Early-LTP	-	early long-term potentiation
EC	-	entorhinal cortex
EEG	-	electroencephalogram
ERKs	-	extracellular signal-regulated kinases
f-EPSP	-	field excitatory postsynaptic potential
GABA	-	gamma-(γ)-aminobutyric acid
HFS	-	high-frequency stimulation
H.M.	-	Henry Gustav Molaison
H_2O_2	-	hydrogenperoxide
I-O	-	input-output
i.p.	-	intraperitoneal
IPSPs	-	inhibitory postsynaptic potentials
IPIs	-	interpulse intervals
Late-LTP	-	late long-term potentiation
LHA/LPOA	-	lateral hypothalamic and lateral preoptic area

LHb	-	lateral habenula
LH	-	lateral hypothalamus
LS	-	lateral septum
LTP	-	long-term potentiation
MAPK	-	mitogen-activated protein kinase
MEK1	-	mitogen-activated extracellular kinase 1
Mg^{2+}	-	magnesium ion
ML	-	medio-lateral
MS	-	medial septum
MSNs	-	medium-size spiny neurons
mRNA	-	messenger ribonucleic acid
NAcc	-	nucleus accumbens
NMDA	-	N-methyl-D-aspartate
PAG/RF	-	periaqueductal gray
PaS	-	parasubiculum
PC	-	personal computer
PFC	-	prefrontal cortex
PKA	-	protein kinase A
PKC	-	protein kinase C
PKMζ	-	protein kinase Mzeta
PP	-	perforant pathway
PPD	-	paired-pulse depression
PPF	-	paired-pulse facilitation
PPI	-	paired-pulse index
PPTg/LDT	-	pedunculopontine and laterodorsal tegmentum
PP1	-	protein phosphatase 1
PRKs	-	PKC-related kinases
PRPs	-	plasticity-related proteins
PrS	-	presubiculum
PS	-	population spike
PSA	-	population spike amplitude
PSP	-	postsynaptic potential
PTP	-	posttetanic potentiation
RMTg	-	mesopontine rostromedial tegmental nucleus

RNA	-	ribonucleic acid
SN	-	substancia nigra
SEM	-	standard error of mean
STET	-	strong tetanus
STP	-	short-term potentiation
Sub	-	subiculum
SuM	-	nucleus supramammillaris
VP	-	ventral pallidum
VS	-	ventral striatum
VTA	-	ventral tegmental area
WTET	-	weak tetanus
6-OHDA	-	6-hydroxy-dopamine

1. Introduction

1.1. Learning and memory

One of the most essential and fascinating properties of the mammalian central nervous system is its ability for information processing and storage. Learning entails acquisition of new information and memory is the retention of, and ability to recall information (Squire 2004; Squire 2009). Neuronal basis of memory is often indirectly studied by monitoring the effects of brain damage on subsequent cognitive abilities or by monitoring neuronal activity in terms of hemodynamic, magnetic, or changes in electrical field. Cajal proposed at the beginning of 20th century that neuronal networks are not continuous cytoplasmatically (Jones 1994a; Jones 1994b), but communicate at distinct junctions with each other, which Sherrington named as 'synapses' (Sherrington 1906). External events in the brain may be represented as spatio-temporal patterns of activity within pre-existing neuronal circuits. Therefore, processes involved in learning and memory formation must occur within pre-existing neuronal circuits. The physical representation of a memory is referred to as the engram or memory trace (Dudai 1996; Dudai 2004).

It is now known that different types of information require the engagement of different neural systems. The two major, general subdivisions of memory are declarative (explicit) and non-declarative (implicit). Declarative memory is memory for facts and events, associated with awareness and intention to recall. Generally, it is rapidly acquired, flexible, and prone to distortion (Cohen and Squire 1980; Squire 2004; Squire 2009). Non-declarative memory includes priming, motor skill and

emotional memory. It is non-conscious, slowly acquired (except for priming), and inflexible (Squire 2004).

Declarative memories depend on structures in the medial temporal lobe, including the hippocampus and the entorhinal, parahippocampal, and perirhinal cortices (Squire et al. 1993). Lesions to these structures produce deficits in declarative memory tasks (Scoville and Milner 1957; Zola-Morgan et al. 1986; Squire et al. 1993; Squire and Alvarez 1995; Scoville and Milner 2000). Declarative memory can further be subdivided into episodic memory and semantic memory. Episodic memory involves recollections associated with a time and place and semantic memory is the recollection of facts without the environmental and temporal context. Patients with bilateral medial temporal lesions show both anterograde and retrograde amnesias (Scoville and Milner 1957; Scoville and Milner 2000). They can acquire neither new episodic memories nor retrieval of stored episodic memories shortly prior to the time of lesion. However, they can retrieve declarative memories learned in the more distant past. These suggest that the storage of such information may depend, at least temporarily, on intact and functional medial temporal lobes. A significant role of the hippocampus in declarative memory was identified following neuropsychological research involving a human patient who had undergone bilateral lesions of both the hippocampus and surrounding cortical structures (Scoville and Milner 1957). The removal of large sections of his temporal lobes including left of the hippocampus, “H.M.” (Henry Gustav Molaison best known as “H.M.”) was unable to form any new personal memories. But his tragic loss revolutionized the field of

neurobiology and made “H.M.” the most studied individual in the history of brain research.

Working memory is another type of memory associated with awareness involving the short-term retention of a perceptual representation. Working and declarative memory are separable because amnesic patients experience severe explicit memory deficits but normal working memory. Patients with parietal or frontal lobe lesions show poor working memory but normal explicit memory (Warrington and Weiskrantz 1971). The hippocampus is greatly considered to be critical for the initial storage of declarative memories. It receives extensive input from neocortical systems and feeds information back to those same systems (McClelland et al. 1995). The hippocampus has been suggested to provide a compressed trace for the temporary linking of component neocortical traces that must be activated together to read out the memory in its entirety. It plays a fundamental role in episodic memory, which will enable us to remember a pleasant dinner party years later. Unlike declarative memory, spatial memory appears to be confined to the hippocampus. This structure appears to have the ability to create a mental map of space with the help of “place cells” (O'Keefe and Dostrovsky 1971; Nakazawa et al. 2004; Rolls and Kesner 2006; Tonegawa and McHugh 2008).

1.2. Hippocampus

The hippocampus, named for its resemblance to the sea horse (*hippo* = horse, kampos = sea monster; Greek) is formed by two interlocking sheets of cortex. The Bolognese anatomist Guilio Cesare Aranzi (circa 1564) was the first to coin the

name “hippocampus” undoubtedly due to its resemblance to the sea horse. In a cross-section, it has a well defined laminar structure with layers visible, where rows of pyramidal cells are arranged. The different cell layers and sections are defined by the series of connections made.

Within the hippocampus, the information passes through three major distinct regions in succession. The hippocampus proper consists of regions with tightly packed pyramidal neurons which can further be divided into subregions mainly CA1, CA2 and CA3 (CA – *cornu ammonis* or Horn of Ammon) as well as the granular cells of the DG. The reference was to the ram’s horns of the Egyptian God Ammon, whose shape bears resemblance to these three regions together). The term, trisynaptic circuit or loop of the hippocampus was born out of the major three synaptic links in the hippocampal circuitry namely; entorhinal cortex (EC) to DG (synapse 1), DG to CA3 (synapse 2) and CA3 to CA1 (synapse 3) (Figure 1). Major information flows within the hippocampus uni-directionally via the adjacent EC axons known as the PP perforating through the subiculum. These axons form the first circuit connection with the granule cells of the DG. From the granule cells, the mossy fibers in turn project to form the circuit’s second connection with the dendrites of the pyramidal cells in the CA3 region. The axons of the pyramidal cells divide into two branches namely commissural fibers and Schaffer collateral. The information from the visual, auditory, and somatic associative cortices arrives first at the parahippocampal region of the cortex, and then passes through the EC to the hippocampus proper.

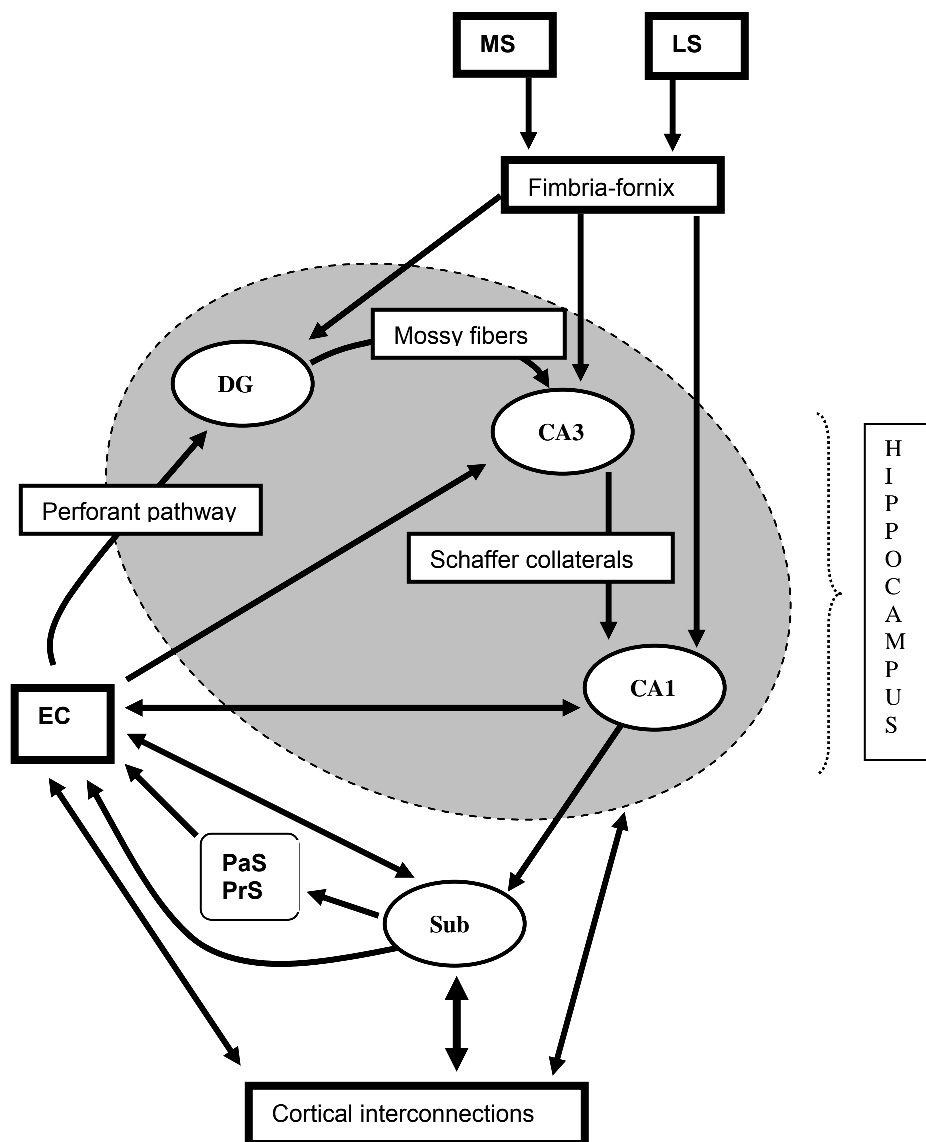


Figure 1: Schematic representation of major intrinsic connections of the mammalian hippocampal formation.

Abbreviations: EC – entorhinal cortex; DG – dentate gyrus; MS – medial septum; LS – lateral septum; Sub – subiculum; PaS – parasubiculum; PrS – presubiculum; CA1 and CA3 – Cornu ammonis 1 and 3 [Figure adapted from (Amaral and Witter 1989)].

The commissural fibers project to the contralateral hippocampus via the corpus callosum, while the Schaffer collateral pathways form the third connection in the circuit with the pyramidal cells in CA1 region (Figure 1).

The major excitatory neurotransmitter in the hippocampus is glutamate, whereas the major inhibitory neurotransmitter is gamma-(γ)-aminobutyric acid (GABA) (Dutar and Nicoll 1988). GABAergic synaptic inhibition strictly regulates the spatial and temporal extent of neuronal activity. The inhibition arises from feed-forward and feedback connections via inhibitory interneurons.

1.3. Long-term potentiation

Learning and memory entail ongoing adaptations of brain circuitry throughout its life time in response to the environment. Generally, they are thought to result from modifications in synaptic connectivity within the central nervous system (Hebb 1959; Konorski 1969). Changes in synaptic connectivity create new networks or circuits that are believed to represent newly acquired memories. Hebb in 1949 explained how networks of neurons might store information with a provocative theory that memories are represented by reverberating networks of neurons (Hebb 1949; Konorski 1948). Hebb later realized that a memory so represented cannot reverberate forever. Therefore, some modification in the network must occur to provide integrity to make the network a permanent trace and the trace could be reconstructed as a remembrance. Neurons communicate with each other mainly at the synapses and the activity of the network is most likely modified by changes in synaptic function. Hebb formalized this idea which is known as Hebb's Postulate:

“When an axon of cell A is near enough to excite cell B and repeatedly or 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 cells firing B, is increased.”

Hebb’s Postulate is very close to a more common definition of LTP. Bliss and Lomo first reported that brief tetanic stimulation of the PP in anesthetized rabbits increased the efficacy of synaptic transmission measured as changes of the f-EPSP recorded extracellularly in the DG. This prolonged increase in synaptic efficacy after brief high-frequency stimulation (HFS) of afferent fibers was named long-term potentiation (Bliss and Lomo 1973; Bliss and Gardner-Medwin 1973). LTP is currently considered as a cellular correlate of learning and memory (Bliss and Collingridge 1993). The best studied form of LTP as well as the one which can be induced in the intact healthy adult animal has been referred to as an ‘associative, N-methyl-D-aspartate (NMDA) -receptor dependent LTP’. The induction of LTP activates glutamatergic and neuromodulatory, heterosynaptic inputs required for its prolonged maintenance. This particular form of LTP requires the activation of various kinases as well as the synthesis of plasticity-related proteins (PRPs) (Reymann and Frey 2007; Frey and Frey 2008).

However, learning and memory are complex processes involving more than just local synaptic processes. Conventionally, LTP is a property of a single synapse or population of synapse in a restricted, artificial circuit activated by tetanization, which serves to investigate mechanisms of synaptic efficacy. Thus, study of LTP at the

synaptic or small network level can only contribute to the better basic understanding of the formation of elementary memory traces but not of memory taking part in an organism's behavior. Memory formation in the organism represents the property of several circuits including a multi-level, distributed storage system, which enables the adaptation of an individual organism to changes in its environment at least. Reviewed evidence shows that LTP shares a number of similar properties with memory consolidation such as requiring the synthesis of PRPs induced in an associative and heterosynaptic way (Frey and Morris 1997; Reymann and Frey 2007; Frey and Frey 2008).

1.3.1. Basic properties of LTP

Basic properties of LTP are input-specificity, associativity, cooperativity, and late-associativity (Bliss and Collingridge 1993; Bear and Malenka 1994; Frey and Morris 1998a; Malenka and Bear 2004; Frey and Frey 2008). LTP is *input-specific* in the sense that it is restricted to activated synapses rather than to all of the synapses on a given cell. If activation of one set of synapses triggered the activation of all other synapses, even inactive synapses being potentiated, it would be difficult to selectively enhance particular sets of inputs, as presumably required for learning and memory (Bliss and Collingridge 1993). The second basic property of LTP is *synaptic cooperativity*, i.e. LTP can be induced either by strong tetanic stimulation of a single pathway, or cooperatively via the weaker stimulation of many. Synaptic cooperativity is explained by the presence of a stimulus threshold that must be reached in order to induce LTP (Malenka and Bear 2004). Another essential

property of LTP is *associativity*. Weak stimulation of a pathway will not by itself trigger LTP. However, if a weakly pathway is activated at the same time when a neighboring pathway onto the same cell is strongly activated, both synaptic pathways undergo LTP. This selective enhancement of conjointly activated sets of synaptic inputs is often considered as a cellular analog of associative or classical conditioning. Generally, associativity is expected in any network of neurons that links one set of information with another. Lastly, *late-associativity* is a novel property of LTP. In contrast to normal associativity, it describes intersynaptic interventions within a time frame of a few minutes to a few hours (Frey and Morris 1997; Frey and Morris 1998a; Frey and Morris 1998b; Frey and Frey 2008). More clearly, a weak protein synthesis-independent early-LTP in one synaptic input can be transformed into a late, protein synthesis-dependent form, if a protein synthesis-dependent late-LTP is induced in a second synaptic input preceded or followed by the weak events in the first synaptic input (weak before strong or strong before weak) within a specific time frame (Frey and Morris 1997; Frey and Morris 1998a; Frey and Morris 1998b; Kauderer and Kandel 2000; Sajikumar and Frey 2004a; Frey and Frey 2008).

1.3.2. Multiple phases of LTP

Brief high-frequency stimulation of the CA3-CA1 synapses can lead to LTP. LTP can be divided into several temporal phases characterized by different underlying mechanisms. Generally, it is divided into induction, expression and maintenance. The initial induction phase of LTP, i.e. so named ‘posttetanic potentiation’ (PTP) with a duration of several seconds to minutes is characterized by

presynaptic mechanisms, i.e. transient increase of neurotransmitter release. PTP is followed by a ‘short-term potentiation’ (STP) which lasts up to one hour. Postsynaptic events like activation of neurotransmitter receptors by local protein kinases (e.g. calcium/calmodulin-dependent kinase II (CaMKII)) (Dobrunz et al. 1997; Huang 1998) are responsible for the maintenance of that phase. STP can be followed by at least two further phases: early- and late-LTP (Matthies et al. 1990). Early-LTP is a transient form of LTP which lasts 2-4 h in both *in vitro* and *in vivo* while late-LTP lasts for 8-10 h in vitro and days or even months in freely moving animals (Figure 2) (Krug et al. 1984; Frey et al. 1988; Otani et al. 1989; Matthies et al. 1990).

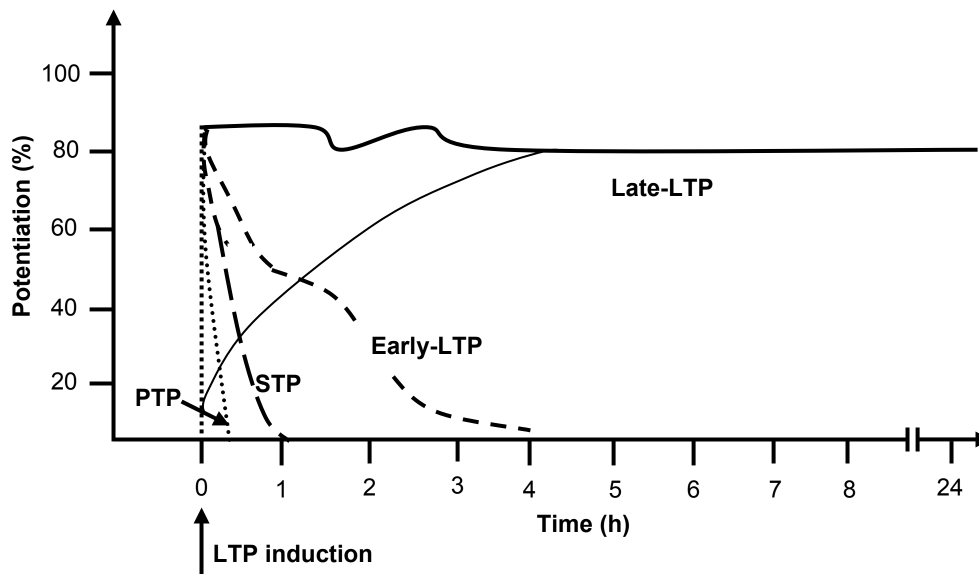


Figure 2: The multiple phases of LTP.

See text for detailed description [Figure adapted and modified from (Reymann and Frey 2007)]

The early-phase of LTP is transient and protein synthesis-independent, lasting about 2-4 h, induced by second messenger cascades, activated by calcium ion (Ca^{2+}) influx, and maintained by activated kinases like CaMKII and protein kinase C (PKC) (Malenka and Nicoll 1999; Soderling and Derkach 2000). Late-LTP begins gradually during the first 2-3 h and can last for 6-10 h in hippocampal slices *in vitro* and for days to months *in vivo* (Krug et al. 1984; Frey et al. 1988; Krug et al. 1989; Otani and Abraham 1989). The major difference between early-LTP and late-LTP is that late-LTP requires protein synthesis (Krug et al. 1984; Frey et al. 1988; Otani et al. 1989). Application of suppressors of ribonucleic acid (RNA) translation during LTP-induction resulted in an early-LTP while late-LTP was prevented (Krug et al. 1984; Frey et al. 1988; Otani et al. 1989; Frey et al. 1996; Mochida et al. 2001).

1.3.3. Cellular mechanisms of LTP

NMDA-receptor dependent LTP

The NMDA-receptor is a voltage-dependent glutamate receptor subtype. NMDA- receptors are composed of assemblies of NR1 and NR2 subunits, the later of which can be one of four separate gene products (NR2A-D) (Gomperts et al. 2000; Racca et al. 2000; Robert et al. 2000). The expressions of both subunits are required to form functional channels. The glutamate binding domain is formed at the junction of NR1 and NR2 subunits (Yamakura and Shimoji 1999). In addition to glutamate, the NMDA-receptor requires a co-agonist binding, glycine to allow the receptor to function. The glycine binding site is found on the NR1 subunit. The NR2B subunit also possesses a binding site for polyamines, regulatory molecules that modulate the functioning of the NMDA-receptor (Yamakura and Shimoji 1999). At

resting membrane potentials, NMDA-receptors are inactive. This is due to a voltage-dependent blockade of the channel pore by a magnesium ion (Mg^{2+}).

Induction of early-LTP in the DG of the hippocampus requires the activation of NMDA-receptors by presynaptically released glutamate when the postsynaptic membrane is sufficiently depolarized. Depolarization of the postsynaptic membrane relieves the voltage-dependent block of the NMDA-receptor by Mg^{2+}, which allows the entry of Ca^{2+} into the postsynaptic dendritic spines. The increase in postsynaptic Ca^{2+} concentration, the crucial trigger for LTP, activates complex intracellular signalling cascades that include several protein kinases, such as CaMKII (Lisman and Zhabotinsky 2001). Due to the sensitivity of NMDA-receptors to both presynaptic neurotransmitter release and postsynaptic depolarization, they act as Hebbian coincidence detectors (Collingridge 2003). Experimentally, NMDA-receptor activity can be triggered either by delivering high-frequency tetani to a critical number of presynaptic afferent fibers, or for instance, by pairing postsynaptic depolarization with presynaptic stimulation (Gustafsson et al. 1987). As the basic mechanism underlying the increase in synaptic strength during LTP, a change in α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) -receptor trafficking is discussed, that results in an increased number of AMPA-receptors in the postsynaptic membrane with no effect on NMDA-receptors (Malenka and Nicoll 1999).

The involvement of protein kinases in LTP

Protein kinases critically regulate synaptic plasticity in the mammalian hippocampus (Frey et al. 1993; Gass et al. 1998; Sweatt 1999; Rongo 2002; Sweatt 2004; Umemura et al. 2005; Schrader et al. 2009). Protein phosphorylation is mediated by protein kinases, and it is a key regulatory mechanism in neurons, enabling neuronal and modulating a plethora of important cellular processes, including neuronal development, growth, and plasticity (Walaas and Greengard 1991).

PKC consists of a family of ~15 different isoforms which plays an important role in neuronal signal transduction. Isoforms of all subclasses are prominently expressed in the rat hippocampus, as demonstrated by immunoblot with isozyme-specific antisera: conventional (Ca^{2+} /diacylglycerol (DAG) dependent), novel (Ca^{2+} -independent, DAG dependent) and atypical (Ca^{2+} / DAG-independent) (Nishizuka 1995). In addition, the zeta isoform is also found as the free, constitutively active catalytic domain, protein kinase Mzeta (PKMζ) (Ling et al. 2002; Hernandez et al. 2003). PKC inhibitors block different phases of hippocampal LTP (Reymann et al. 1988a; Reymann et al. 1988b). Activation of PKC is not essential for the initial phases of LTP, but is a necessary condition for a medium and a late, protein synthesis-dependent phase in this monosynaptic pathway, i.e. for the maintenance of synaptic LTP (Reymann et al. 1988a; Reymann et al. 1988b). Contrary, some results show that postsynaptic PKC is essentially involved in both the initial induction and the subsequent maintenance of LTP (Wang and Feng 1992).

PKC isoforms consist of an amino-terminal regulatory domain, containing an autoinhibitory pseudosubstrate sequence and second-messenger binding sites, and a carboxy-terminal catalytic domain (Nishizuka 1995; Ohno and Nishizuka 2002). PKC is normally held in an inactive basal state by interactions between these two domains. Second messengers activate PKC by binding to the regulatory domain and causing a conformational change that temporarily releases the autoinhibition. PKM, in contrast, consists of an independent PKC catalytic domain, which, lacking PKC's autoinhibitory regulatory domain, is autonomously active (Schwartz 1993). In brain, only a single isozyme, the atypical ζ, forms a stable PKM (Sacktor et al. 1993). In LTP, PKMζ increases by new protein synthesis through increased translation from a PKMζ messenger RNA (mRNA), producing the independent ζ catalytic domain (Hernandez et al. 2003). The persistent activity of PKMζ is both necessary and sufficient for maintaining LTP (Ling et al. 2002; Serrano et al. 2005; Sacktor 2008).

CaMKII, mitogen-activated protein kinase (MAPK), and adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase (PKA) are required for LTP induction as well as its prolonged maintenance (Frey et al. 1993; English and Sweatt 1997; Bortolotto and Collingridge 1998). Influx of Ca^{2+} stimulates calcium and calmodulin (Ca^{2+}/CaM) sensitive adenylyl cyclase, which synthetizes cAMP at least in juvenile tissue (Eliot et al. 1989). Cyclic AMP activates cAMP-dependent protein kinase A (PKA) or other cAMP-dependent processes. CaMKII and MAPK can promote the phosphorylation of each other, and MAPK is required for an increase in CaMKII levels produced by LTP-inducing stimulation (Giovannini et al. 2001). CaMKII undergoes autophosphorylation after the triggering of LTP (Fukunaga et al.

1995) and LTP induction was prevented in knockout mice lacking critical CaMKII subunits (Silva et al. 1992). PKA activation has been suggested to boost the activity of CaMKII indirectly by decreasing competing protein phosphatase activity (Lisman 1989; Blitzer et al. 1998). This presumably happens by phosphorylation of inhibitor 1, an endogenous inhibitor of protein phosphatase 1 (PP1). Recently, the MAPK cascade that activates extracellular signal-regulated kinases (ERKs) has been implicated in LTP as well as in some forms of learning and memory (Sweatt 2001; Sweatt 2004; Thomas and Huganir 2004).

A large number of chemical neurotransmitters, hormones, and other signalling substances use cAMP as an intracellular second messenger (Nguyen and Woo 2003; Abel and Nguyen 2008). The principal target for cAMP in mammalian cells is cAMP-dependent PKA, which is ubiquitously expressed and mediates intracellular signal transduction and intracellular signal transmission in invertebrates and vertebrates (Frey et al. 1993; Brandon et al. 1995; Nguyen and Kandel 1996; Abel and Nguyen 2008; Gelinas et al. 2008). Late-LTP requires cAMP-dependent PKA activity during LTP induction (Frey et al. 1993; Huang and Kandel 1994; Abel et al. 1997; Nguyen and Kandel 1997; Nguyen and Woo 2003). The application of PKA inhibitors attenuates LTP expression, apparently eliminating the ability of synapses to express LTP (Otmakhova et al. 2000). This result suggests that PKA activated by cAMP may gate the expression of late-LTP by direct, indirect or permissive activation of transcription factors.

The role of protein synthesis and transcription factors in LTP

Protein synthesis is assumed to be necessary for the cell to maintain synaptic changes over a long period of time, which require constant molecular turnover and eventually leads to synaptic growth. It has been further hypothesized that late-LTP requires the activation of transcription factors for sustaining prolonged periods of synaptic enhancement and finally making the synaptic change relatively permanent. Intraventricular application of anisomycin, a reversible translational inhibitor, prevents the long-term maintenance of LTP in the DG, an effect that parallels the block of long-term memory in several learning tasks (Krug et al. 1984; Otani and Abraham 1989). The application of anisomycin before, during, or immediately after tetanization produced a gradual decrease of potentiation after 4-6 h without affecting early-LTP. Application of anisomycin 1 h after tetanization had no effect.

Studies have shown that, the transcription factor cAMP-responsive element binding protein (CREB) differs in its activation following the induction of either short or long form of LTP (Matthies et al. 1997; Impey et al. 1998; Schulz et al. 1999). CREB is a member of the basic leucine zipper super family of transcription factors that modulate the transcription of genes by binding to a regulatory deoxyribonucleic acid (DNA) promoter known as cAMP responsive element (CRE) (Brindle and Montminy 1992; Mayr and Montminy 2001; Mayr et al. 2005). Nuclear CREB can be activated by several neural signalling pathways, including the cAMP and Ca^{2+} pathways which are known to be involved in memory and are activated or up-regulated by stimuli that induce LTP (Deisseroth et al. 1998; West et al. 2001). A variety of kinases induced by these pathways have been shown to activate CREB by

phosphorylating the Ser 133 site (Gonzalez and Montminy 1989; Sheng et al. 1991; Bito et al. 1996; Deisseroth and Tsien 2002; Ying et al. 2002). In contrast, CREB mutant mice showed normal LTP and intact learning in most hippocampus dependent tasks (Balschun et al. 2003).

Synaptic tagging

Gene expression and protein synthesis that mediate the long-term changes of LTP generally take place in the cell body, or for protein synthesis, in dendritic compartments, i.e. far away from the stimulated synapse. However, late-LTP is synapse-specific because other synapses that are not active at the time of LTP induction do not share in the potentiation induced in the tetanized pathway. Therefore, how pre-existing or newly synthetized PRPs interact with specific activated synapses, expressing LTP is the fundamental principle to the synapse-specificity believed to be critical for information processing and memory formation (Frey and Morris 1998a; Sajikumar and Frey 2004b; Frey and Frey 2008). Synaptic input-specificity can be explained in detail by the concept of 'synaptic tagging' (Frey and Morris 1997; Frey and Morris 1998a) which proposed that newly synthetized PRPs activated by heterosynaptic interactions (synergistic activation of both glutamatergic inputs and an additional neuromodulating neurotransmitter system during the induction of LTP triggers the synthesis of PRPs) bind to recently potentiated, glutamatergic 'tagged' synapses, thus maintaining LTP and input-specificity (Frey and Morris 1997; Frey and Morris 1998a; Frey and Morris 1998b; Frey and Frey 2008).

Interestingly, weak tetanic (WTET) stimulation which normally induces early-LTP could be transformed into late-LTP heterosynaptically, if an independent pathway is strongly stimulated within a distinct time window. The independently tetanized pathway can induce LTP with a variable persistence as a function of the prior history of activation of the neuron (Frey and Morris 1998b; Frey and Frey 2008). The tag is transiently active with an expected half-life of about 30 minutes in freely moving animals (Frey and Frey 2008). Also, it has been suggested that the PRPs are characterized by a specific, relatively short half-life of about 1-2 h (Frey and Morris 1998a; Sajikumar et al. 2005; Frey and Frey 2008). In an event where both processes, the synapse-specific tags as well as PRPs are available, then can the two interact and transform early- into late-LTP at the stimulated synapses (Frey and Morris 1998b; Frey and Frey 2008). The existences of tag- and PRP-dynamics therefore determine an effective, functionally important time window during which a normally transient form of functional plasticity can be transformed into long-lasting one. The tag does not necessarily has to be a single molecule, however, experimental data show that the tag must satisfy a number of criteria: (1) the tag is induced in a protein synthesis-independent manner, (2) the tag possesses a half-life of 30 min in vivo, (3) the tag is induced by both early-LTP and by late-LTP and the setting of the tag is protein synthesis independent, (4) the tag is formed in an input-specific and in a physically immobile manner, and (5) the tags interacts with the PRPs synthesized during late-LTP and facilitate synaptic tagging/capture process.

Late-LTP in the DG requires the associative activation of heterosynaptic inputs, for instance glutamatergic and noradrenergic or muscarinergic receptors in the DG

(Frey 2001). Therefore, it has been suggested that late-LTP requires concomitant activation of different neurotransmitter systems (Frey et al. 1989; Frey et al. 1990; Matthies et al. 1990; Frey et al. 1991; Frey and Morris 1998a; Sajikumar and Frey 2004a; Frey and Frey 2008).

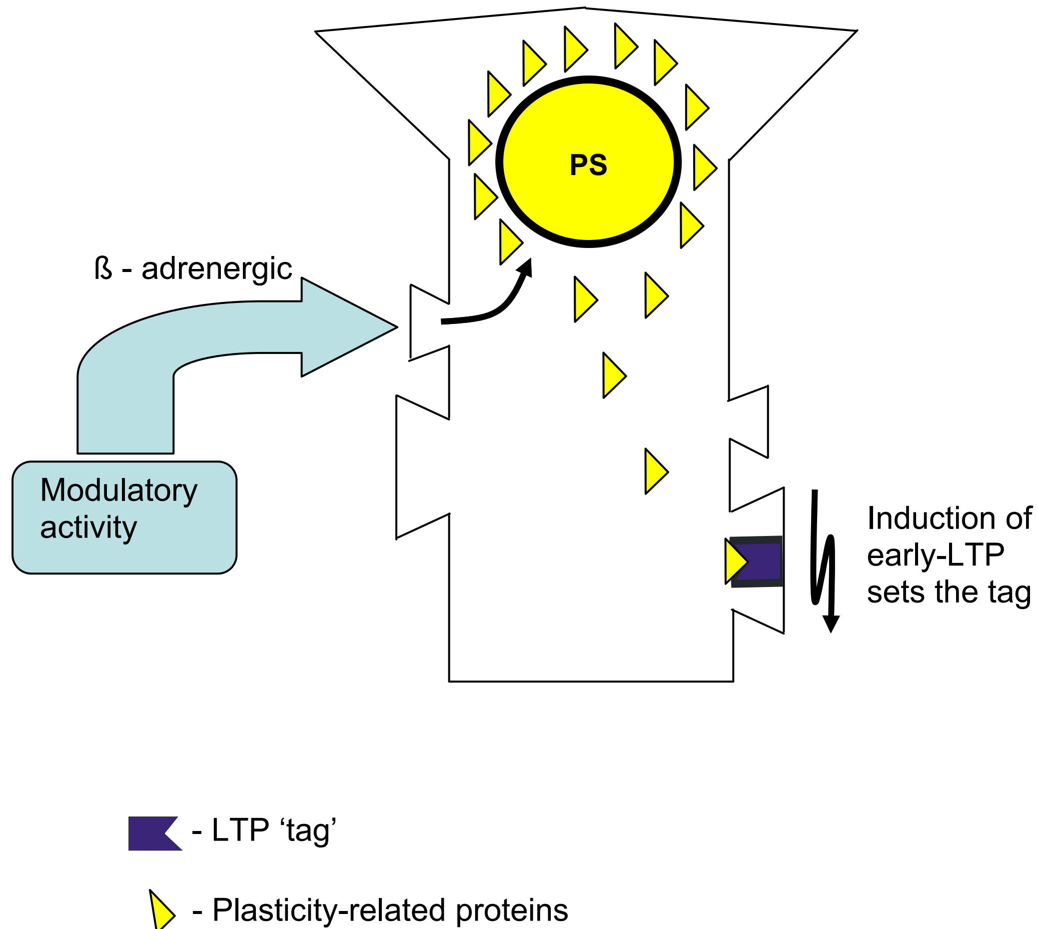


Figure 3: Synergistic activation of both glutamatergic and neuromodulatory inputs into DG. The schema describes "synaptic tagging" (Frey and Morris 1997): via a synaptic tag set by a weak tetanus (black zigzag arrow) that induces no protein synthesis (PS). A synapse can participate from PRPs induced by an independent modulatory input to the neuron. Thus, the early-LTP of the first input can be transformed into a late-LTP. The tag is only active during a limited time window. The activation of β-adrenergic receptor is known to stimulate the cAMP/PKA cascade (Stanton and Sarvey 1985; Watabe et al. 2000), one of the major pathways to induced protein synthesis-dependent late-LTP in the DG (Frey et al. 1993).

The typical LTP experiment, involving a brief period of HFS for LTP, where the activation of a population of glutamatergic synapses of an individual neuronal cell is likely to be accompanied by a dynamic activation of non-glutamatergic heterosynaptic inputs through neuromodulatory activities as shown in Figure 3 above. The tetanization used in LTP studies, involving simultaneous field activation of hundreds of fibers, activates more than one kind of neurotransmitter input, and it is that cooperative action of inputs that induces late-LTP. Therefore, it is suggested that a time-dependent convergence of two or more events is required for late-LTP (Frey and Frey 2008).

1.4. Reinforcement of early-LTP and the requirement of neuromodulatory brain structures

In addition to the above described heterosynaptic requirement, it has been demonstrated that hippocampal early-LTP can be transformed into late-LTP by the influence of the “motivational status” of the animals by applying electrical stimulus coupled with high motivational value (Seidenbecher et al. 1995; Frey et al. 2001). Direct electrical stimulation of specific associative brain modulatory systems within a distinct time window can transform a protein synthesis-independent early-LTP to a protein synthesis-dependent late-LTP in the rat DG (Frey et al. 2001; Frey and Frey 2008) and such process is known as “structural reinforcement”. Moreover, the restricted time window is within the range of 30 minutes before or after the induction of early-LTP in the DG via the PP. The hypothesis is that early-LTP induction in the DG sets a tag and marks the activated synapses in a specific way, which enables

them to capture PRPs to convert early- into late-LTP. Activation of the modulatory inputs involves the production and diffuse distribution of PRPs that are captured and utilized only at those synapses possessing a tag (see Figure 3). Until now, studies have shown that the BLA, the medial septum (MS), the locus coeruleus and the nucleus supramammillaris (SuM) can have a modulatory effect on DG early-LTP (Frey et al. 2001; Frey et al. 2003; Bergado et al. 2007; Frey and Frey 2008).

1.5. Nucleus accumbens as a candidate neuromodulatory structure for early-LTP – reinforcement in the DG

The NAcc is an integral part of the basal ganglia located within the ventral striatal complex. The basal ganglia is considered to be one of the oldest phylogenetic structures in the brain and are found in the forebrain of all amniotic vertebrates (Marin et al. 1998; Smeets et al. 2000; Zahm 2000; Wise 2004; Sesack and Grace 2010). The concept of 'striatal complex' was introduced in the mid- 1970s composed of the NAcc, the caudate nucleus, the olfactory tubercle, and the putamen (Heimer and Wilson 1975). The origin of the concept had it basis from the extrinsic connections within these brain regions and the striking similarities in chemo- and cytoarchitecture in the rat. The emerging idea at the time from the classical publication from Heimer and Wilson classified the NAcc and the medium-celled parts of the olfactory tubercle as the ventral striatum (VS). Like the other parts of the striatal complex, the NAcc receives extensive excitatory afferents from the cerebral cortex and thalamus. It projects to the ventral pallidum (VP), which innervates the

mediodorsal and other thalamic division (Zahm and Brog 1992; O'Donnell et al. 1997; Sesack and Grace 2010).

There exists a functional segregation between dorsal and ventral parts of the striatum. However, it is worth emphasizing that the uttermost division of the striatum into dorsal and ventral should not subvert the idea that they are related closely and the differentiation between the two regions is not clear cut as the subdivisions might impose (Voorn et al. 2004). In mammals, the NAcc is presently accepted to be an integral part of the VS complex together with the olfactory tubercle. The dorsal part of the striatum consists of the caudate nucleus and putamen which has been associated with processes of task-oriented motor sequences from the cortex and habit learning (Graybiel 1995; Wise 2004; Barnes et al. 2005; Grace et al. 2007; Sesack and Grace 2010). The NAcc has been implicated to be more involved in working memory, reward motivated behaviors and development of addiction (Alexander et al. 1986; McBride et al. 1999; Robinson and Berridge 2000; Lovinger et al. 2003; Lewis et al. 2004). The afferents from the allocortex preserved the association of the NAcc with the limbic part of the brain (Heimer and Wilson 1975).

Anatomical connection and functional properties of NAcc

Multiple limbic associated structures provide the excitatory cortical innervations to the NAcc (Figure 4). These structures include medial and lateral divisions of the prefrontal cortex (PFC), EC and ventral subiculum of the hippocampus, and BLA (Brog et al. 1993; Totterdell and Meredith 1997; Reynolds and Zahm 2005; Sesack and Grace 2010). NAcc is a rostral region of the brain, which is similar in its

subcortical connections to the rest of the striatum. However, the NAcc differs in the inputs it receives from other brain regions such as the hippocampus, VTA, amygdala, lateral hypothalamus (LH) and sublenticular substancia innominata (Meredith et al. 1992).

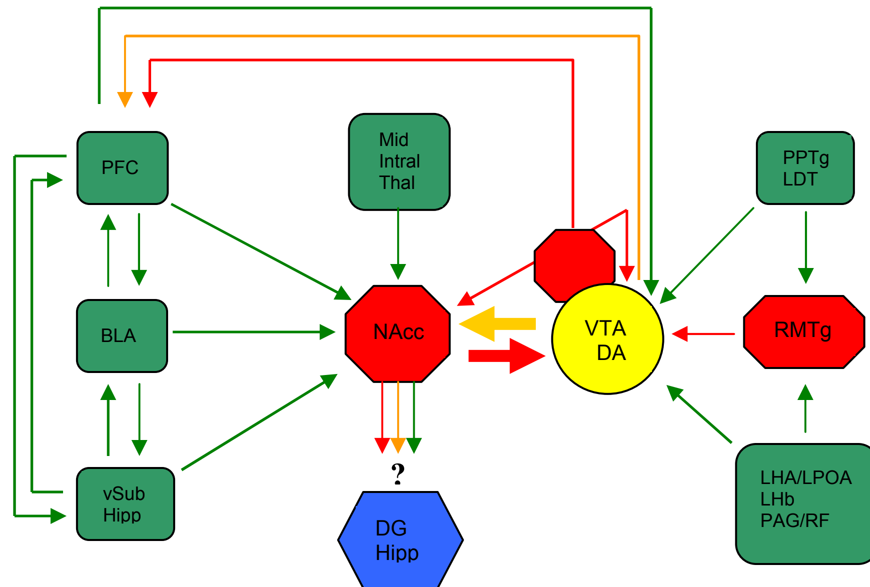


Figure 4: Principal efferent pathways from the NAcc.

The figure illustrates principal afferents linking brain regions to the NAcc and VTA. Red indicates inhibitory structures and pathways, green excitatory connections, yellow the modulatory influence of dopamine (DA), and blue much is not known about any direct projection from the NAcc (?). Abbreviation: LHA/LPOA – lateral hypothalamic and lateral preoptic areas; LHb – lateral habenula; PAG/RF – periaqueductal gray; RMTg – mesopontine rostromedial tegmental nucleus; PPTg/LDT – pedunculopontine and laterodorsal tegmentum [Figure modified from (Sesack and Grace 2010)].

In general, the NAcc is the target for most afferents arising from the limbic structures (including the PFC) whereas the other parts of the striatum receive inputs from sensory- and motor-related cortical areas (O'Donnell and Grace 1993). Thalamic afferents to the VS arise from midline and intralaminar nuclei (Figure 4) (Smith et al.

2004). Some thalamic neurons innervating the NAcc send collateral projections to the PFC (Otake and Nakamura 1998).

The predominant class of neurons in the striatum and the NAcc is the medium-size spiny neurons (MSNs) projection. These MSNs projection receives inputs from several brain regions and in turn projects to midbrain structures such as the substantia nigra (SN) and globus pallidus (Chang and Kitai 1985; Pennartz and Kitai 1991). Approximately 90% of the cells in the NAcc are typical MSNs (Meredith et al. 1992) believed to be GABAergic neurons. The remaining cells are local circuit neurons including cholinergic neurons which play a vital role in striatal function (Kawaguchi et al. 1995; Wang et al. 2006).

Yang and Mogenson showed a functional relationship between excitatory inputs to the NAcc from the hippocampus and mesolimbic dopaminergic inputs from the VTA (Yang and Mogenson 1984). In this study, the subiculum of the hippocampus was stimulated and the recordings were made from the NAcc cells. They showed that the stimulation resulted in excitation of the NAcc cells. However, the NAcc neurons excitation was attenuated when trains of conditioning pulses were delivered to the VTA 100 ms prior to stimulation of the hippocampus (Yang and Mogenson 1984). In order to establish that the attenuation observed occur as a result of dopaminergic action, they showed that both pre-treatment of the VTA with 6-OHDA (6-hydroxy-dopamine) and the application of iontophoretically DA mimicked reduced the attenuation (Yang and Mogenson 1984).

The hippocampal projection originates primarily in the subiculum and CA1 region (Swanson and Cowan 1975), has been found to be distributed along the entire length of the medial NAcc (Kelley and Domesick 1982). Meanwhile, the NAcc core receives projections from the dorsal subiculum whereas the NAcc shell receives its main input from the ventral subiculum (Groenewegen et al. 1987; Brog et al. 1993). Likewise, the NAcc has also been demonstrated to receive inputs from insular, perirhinal, entorhinal and piriform regions (Berendse et al. 1992).

Unlike the CA1 and subiculum, the DG does not project to the NAcc or vice versa. However, it is possible some indirect physiological mechanisms that could underlie communication between the NAcc and the hippocampus. Heimer et al. described a direct anatomical connection between the NAcc and LH (Heimer et al. 1991). A further direct connection originating in the posterior parts of the LH to the DG granule cells in the hippocampus has also been described by Wayner et al. (Wayner et al. 1997). This NAcc – LH – DG granule cells circuitry could be an indirect possible pathway through which the NAcc could influence synaptic plasticity events in the DG of the hippocampus. Also, the studies from Heimer et al. revealed an extended relation of the NAcc selective innervation of the amygdala (Heimer et al. 1991; Heimer et al. 1997). A functional link between the amygdala and hippocampal LTP has been demonstrated (Ikegaya et al. 1994; Ikegaya et al. 1995a; Ikegaya et al. 1995b; Kamiya and Ozawa 1998; Akirav and Richter-Levin 1999a; Akirav and Richter-Levin 1999b) but there are no known direct efferents linking the amygdala with the DG (Chen et al. 1999). An extensive study from our laboratory has shown a modulatory influence of the BLA on DG early-LTP (Frey et al. 2001)

which seems to involve noradrenergic and cholinergic afferent innervation whereas lesion of the amygdala attenuates DG early- and late-LTP (Ikegaya et al. 1994). This suggests that the locus coeruleus and the MS participate in these processes as the main sources of noradrenergic and cholinergic innervation to the DG (Vizi and Kiss 1998; Frey et al. 2003; Bergado et al. 2007) .

In addition, the NAcc indirectly connects to the hippocampus via the ventral pallidum and VTA. The NAcc inhibitory efferents projects to the VTA via ventral pallidum and the VTA project dopaminergic efferent to the hippocampal formation (Zahm and Heimer 1990; Zahm 2000; Sesack and Grace 2010). However, the dopaminergic contribution to DG LTP (Kusuki et al. 1997) is not clear as in the CA1 region (Kulla and Manahan-Vaughan 2000) with dopaminergic projection from the VTA to the CA1 region directly (Gasbarri et al. 1994). Therefore, any influence of dopaminergic projection from VTA to the DG might be through an indirect action or processes. Lisman and Grace recently suggest that the hippocampus and VTA form a functional loop designed to detect novelty and this novelty signal is use to control the entry of behaviorally significant information into the hippocampus store of long-term memory (Lisman and Grace 2005). According to their proposal, the hippocampus – VTA functional loop has two arms: down arc and upward arm. The down arc of this loop carries novelty signals from the hippocampus to the VTA while the upward arm carries novelty signal from the VTA to the hippocampus. The upward arm of the loop involve the release of DA which enhances LTP and the role of DA in CA1 LTP has been clearly demonstrated, but not at the cortical synapses onto the DG granule cells as described above (Lisman and Grace 2005).

Compartmentalization of the NAcc

The NAcc is divided into two major regions at higher level of anatomical differentiation. The division consists of: the core is the central region directly beneath and continuous with the dorsal striatum and surrounding the rostral limb of the anterior commissure, and the shell occupies the most ventral and medial portion of the NAcc (Zahm and Brog 1992; Meredith et al. 1993; Zahm and Heimer 1993; Jongen-Relo et al. 1994; Groenewegen et al. 1999). Core and shell subregions of the NAcc can be differentiated based on several criteria. For example the differences exist based on several criteria such as the differences of efferent and afferent connections, immunohistochemistry, levels of peptides, binding of various receptor ligands, density of cholinergic neurons and in the membrane properties (Meredith et al. 1992; Meredith et al. 1993; Jongen-Relo et al. 1994; Meredith et al. 1996; Usuda et al. 1998; Meredith 1999; Sesack and Grace 2010). In core neurons, for instance, the resting potential is more negative, and the input resistance lower than in shell neurons (Pennartz et al. 1992), suggesting that shell neurons will be more excitable than core neurons (Meredith et al. 1992). Neither core nor shell neurons of the NAcc have been observed to be spontaneously active at resting membrane potentials, though depolarization by current injection in either type can generate both single spikes and trains of action potentials (O'Donnell and Grace 1993).

1.6. Aims of the dissertation

On account of these, considering the manner in which the NAcc is involved in motivation and reward coupled with the strong projections it receives from the BLA,

we hypothesized that the NAcc might differentially modulate the hippocampal DG early-LTP. Taking into account that BLA projects strongly to the NAcc and feedback projections to the BLA, its stimulation might activates heterosynaptic afferents, releasing neurotransmitter substances capable of activating metabolic cascades leading to regulation of translation, and therefore, the synthesis of PRPs. This hypothesis is partly one of our departmental broader working hypotheses aiming at the characterization of various brain regions that might modulate hippocampal plasticity as described previously. This dissertation was therefore set out to further electrophysiologically characterize the two subregions of the NAcc in its differential modulatory effect on the DG early-LTP. Furthermore, according to our working hypothesis, we wanted to study if and how NAcc stimulation can interfere with tagging processes in the DG. We hypothesized that early-LTP can set a transient tag and NAcc stimulation could interfere with the synthesis of PRPs. The detailed experimental outline entails:

- the induction of DG early-LTP with the animals bearing implanted electrode in the NAcc,
- effects of the NAcc stimulation on basal synaptic transmission,
- NAcc stimulation before or after DG early-LTP induction within 15 minute time window.

Recent findings by others (Lopez et al. 2008) suggested NAcc role for DG – synaptic plasticity in anesthetized rats. Here, we expand these studies in freely moving rats.

2. Materials and methods

All experimental procedures were performed according to the guidelines and with permission from the ethical committee of animal experimentation, the regional council of Saxony-Anhalt that was in accordance with the European Communities Council Directives (86/609/EEC). The principles of laboratory animal care were strictly followed. Every possible effort was made to minimize the number of animals used and their suffering.

2.1. Laboratory animals

The animals were male Wistar (Wistar - Schoenwalde) rats (8 weeks old at the time of surgery) inbred from the breeding colony of the Leibniz Institute for Neurobiology, Magdeburg. The animals were housed in a plastic translucent standard breeding cage (55 x 35 x 18) cm in groups of five per cage weighing between 270 g and 320 g before surgery. After surgery, the animals were housed in individual cages (40 x 25 x 18) cm. They were allowed at least 10 days of post-recovery period before the experiments commenced. The base of the cage was covered with commercial bedding material (ssniff, R/M-H, Soest). The animals were kept in our departmental animal facility under standard housing conditions with temperature (22 ± 2°C) and humidity (55 ± 5%) controlled conditions under 12 h light-dark cycle with light on at 6:00 a.m. The animals had free access to food pellets (ssniff, R/M-H, Soest) and water *ad libitum*. All experimental procedures were carried out in the light phase of the cycle. The bedding material and water were changed weekly for the animals in our departmental animal facility.

2.2. Electrode Implantation

For simultaneously recording of both – the f-EPSP and the PSA in the DG – a special “double” recording –electrode was designed. This “double” –recording was made out of two single lacquer insulated stainless steel wires (diameter per wire: 125 µm) which were straightened close together to ensure the recording from one and the same cell population. Because both wires of the recording electrode were fixed together for better handling, a compromise had to be made: the tips of both recording electrodes were cut at a constant distance according to the anatomical state of the DG. The distance of about 400-420 µm between the tips were optimal for the rat strain used (Wistar - Schoenwalde) to guarantee the simultaneous recording of the f-EPSP and PSA (Frey and Frey 2009). Stimulation and recording electrodes were both referenced and grounded to stainless steel screws (1.4 mm Ø, Schließblockschrauben, OPTOTEC, Rathenow, Germany) soldered to silver-coated copper wires (0.4 mm Ø, Conrad, Hirschau, Germany). Similarly, the two lacquer isolated stainless steel wires (diameter per wire: 125 µm) used as bipolar stimulation electrode was 1.0 mm apart when straightened together and its length was cut to 4-5 mm for implantation. Two teflon isolated tungsten wire (diameter per wire: 200 µm), 0.5 mm apart was used as bipolar stimulation electrode for the NAcc stimulation. Its length was cut to of 8.0 mm for implantation.

2.2.1. Anesthesia and surgical preparation

Prior to surgery, the animals were anesthetized with an initial intraperitoneal (i.p.) injection of Nembutal (sodium pentobarbital, Sigma-Aldrich Chemie GmbH,

Munich, Germany) at 40 mg/kg. The anesthetized animals were mounted in a stereotaxic frame (TSE Systems GmbH, Bad Homburg, Germany) and fixed with the help of a rat adaptor and lateral ear bars. After the animal fixation and stabilization within the stereotaxic frame, the animals' forehead and neck were shaved. The scalp was incised to expose the skull by cutting part of the skin and removing periosteum. For cleaning and dehydration, the skull bone was swabbed three to four times with 3% of hydrogenperoxide (H_2O_2) and abraded with a sharp bone-spoon. During the electrode implantation process, foot and tail withdrawal as well as eyes reflexes were checked to assess the depth of anesthesia. If required, an additional dose of Nembutal (0.5 ml i.p.) was administered.

For stereotaxic coordinates, bregma was marked and subsequently referred to as the anterio-posterior (AP) and medio-lateral (ML) zero point. The skull was aligned on a horizontal plane without any angle or slope. A point 9.0 mm behind bregma on the midline was marked known as lambda (as shown in Figure 5). If the distance between the bregma and lambda was not equal to 9.0 mm then a factor was deduced to determine the AP between the bregma and lambda for each coordinates. The AP coordinates for the drill holes were marked stereotactically onto the saggittal margin. Thereafter, the ML positions were calculated and marked unilaterally onto the right parietal bone. The stereotaxic coordinates for stimulations and recording sites were derived from the atlas of Paxinos and Watson (Paxinos and Watson 1998). The brain was exposed through small burr holes on the skull by the marked coordinates with a trepan (Trend WD-56 EM, W&H Deutschland GmbH, Oberbayern, Germany).

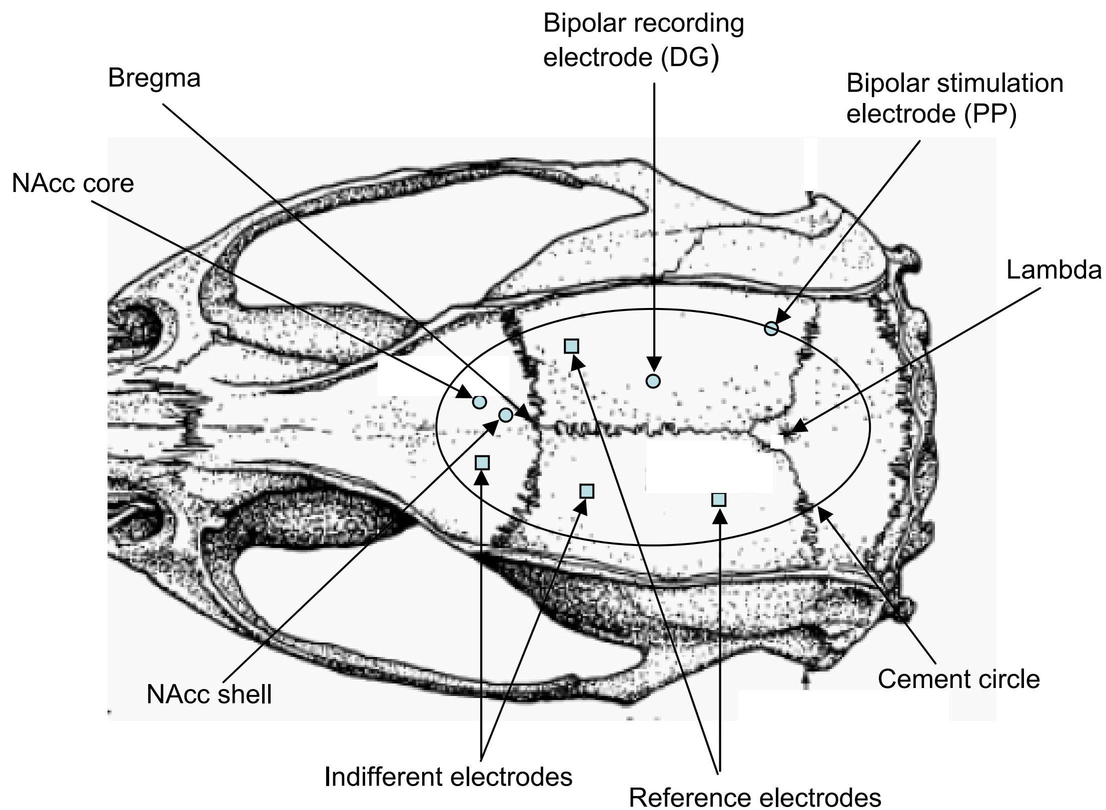


Figure 5: Trephine hole sites.

Electrode implantation locations of drillings are provided for adequate coordinates. The square boxes are locations for the ground and indifferent screws while the circles are the locations for the NAcc core or shell, stimulation and recording –electrodes. The bregma and lambda points are marked as depicted on the skull. [Figure adapted and modified from: (Paxinos and Watson 1998)].

The drill heads of about 1.3 mm in diameter (Size 12, Bohrköpfe, Hager and Meisinger GmbH, Neuss, Germany) was used to drill the holes. Four miniscrews were attached at the skull in the left frontal bone (1 miniscrew), left parietal bone (2 miniscrew) and near the right parieto-frontal junction (1 miniscrew), which served as ground and indifferent electrodes (as shown in Figure 5). For NAcc preparation, animals were additionally implanted unilaterally on the right hemisphere with bipolar stimulating electrode. The dura was pierced through with the bipolar stimulation

electrode and lowered into the NAcc slowly over a period of 5 minutes to the required depth. After the NAcc bipolar stimulating electrode placement was completed, it was sealed and fixed together with the frontal miniscrew to the skull with dental acrylic (Paladur, Heraeus Kulzer GmbH, Hanau, Germany).

2.2.2. Electrophysiologically-guided electrode insertion

For PP–DG preparation, animals were implanted unilaterally on the right hemisphere. The bipolar stimulating electrode was implanted into the medial angular bundle to stimulate the medial PP. Also, the “double” -recording electrode was then implanted into the granule cell layer in the DG. The PP stimulating coordinates were 7.5 mm AP and 4.1 mm ML relative to bregma with ca. 2.9 - 3.1 mm dorso-ventral (DV) from the dura surface. Whereas DG recording coordinates were 4.0 mm AP and 2.3 mm ML relative to bregma with ca. 2.4 - 2.6 mm DV from the dura surface. The stimulating electrode implanted within the core or shell subregion of the NAcc was aimed at the following coordinates: for the core regions of the NAcc (AP: +2.2 mm; ML: +1.2 mm; DV: -6.5 mm from the dura surface) and for the shell region of the NAcc (AP: +1.6 mm; ML: +0.8 mm; DV: -6.4 mm from the dura surface).

The “double” -recording electrode was fixed on the left stereotaxic micromanipulator (advanced 3-dimensional precision model, TSE Systems GmbH, Bad Homburg, Germany). Thereafter, the “double” -recording electrode was placed in position of the drilled hole. The DV zero point was set to the point where the electrode touched the dura surface. The bipolar stimulating electrode was also fixed

on the right stereotaxic micromanipulator. The DV zero point was calculated in the same manner like the “double” -recording electrodes. The electrodes were then connected to an electrophysiological set up. The dura was pierced through with both electrodes and were carefully lowered into the brain (beneath the drilled holes) to a starting position of 1.5 mm and 2.0 mm below zero for recording and stimulation – electrodes respectively.

The f-EPSP slope and PSA were evoked manually by applying single biphasic constant current square wave test pulses of 0.1 ms per half-wave duration at the stimulus intensity of 400 μA using a stimulation isolator (2100 Isolated Pulse Stimulator, A-M System, Sequim, USA) The evoked potentials were recorded and amplified differentially (Differential Amplifier, INH, Science Products GmbH, Hochheim, Germany) with gain of x100 and filtered at 0.1 Hz – 5 kHz bandpass. The acquired signals with the aid of Intracell software were further sampled at the rate of 10 kHz through analog-digital/digital-analog (AD/DA) converter (CED 1401-plus, micro CED, Cambridge Electronic Design, Cambridge, UK) and saved to an online computer. Intracell software is scientific physiological data acquisition software developed at the Department of Neurophysiology (Intracell, Leibniz Institute for Neurobiology, Magdeburg, Germany). A further connection to an oscilloscope (Oszilloskop Gould 1602, CALDI GmbH, Ratingen, Germany) allowed the variable visualization of the recorded signals manually triggered by the stimulation unit.

After lowering the electrodes, the brain was allowed to recover for about 30 to 45 minutes. The final depth positioning of all electrodes was done visually under

electrophysiological control and was set to optimize the response from the implanted pathway. This was done by taking recordings of evoked field potentials by simultaneously recording the f-EPSP slope and the PSA via the implanted electrodes by means of online stimulation and recording. The f-EPSP slope and PSA responses during the lowering of the electrodes were monitored through the use of stimulating unit which triggered the oscilloscope and also viewed on the computer monitor with Intracell software. The evoked potentials were recorded via the implanted electrodes throughout the surgery.

The procedure of exact electrode positioning was conducted as follows: the longer tip of the bipolar recording electrode start position was located in the outer dendritic layers within the middle third of the stratum moleculare. A test stimulus evoked a negative-going f-EPSP slope. Lowering of the bipolar recording electrode stepwise increased the resulting f-EPSP slope and revealed a positive-going population spike (PS) around the axon hillock while the shorter tip registered the f-EPSP slope within the middle third of the stratum moleculare. Once the PS amplitude and the f-EPSP slope approached maximum, the “double” -recording electrode was lowered stepwise slowly and fixed at maximum. The longer tip was adjusted into the hilus of the DG to record the maximum PSA whereas the shorter tip into the middle third of the stratum moleculare to record the f-EPSP as described by (Frey and Frey 2009). No changes were made for 30-45 minutes to allow a stable level to be reached. To ensure that the responses remained maximal, evoked field potentials were frequently checked and the positioning of the electrodes readjusted if they were not. When stable evoked field potentials were obtained, the electrodes

were permanently fixed. Electrode placement verification was ascertained by a distinctive f-EPSP slope and PSA responses associated with the correct placement (as shown in Figure 6). The correct electrodes depth was typically 2.5 mm below the dura surface for the “double” -recording electrode and 3.0 mm below the dura surface for the bipolar stimulating electrode.

During the surgery process, liquid and blood was removed and gelaspon (Bausch & Lomb Chauvin Ankerpharm, Berlin, Germany) was used to cover the holes around the implanted electrodes. The entire assembly, electrodes and miniscrews, were fixed to the skull with dental cement and the electrodes were disconnected from the electrophysiological set up. Thereafter, gently removing the electrodes from the manipulators, the sockets were fixed and sealed with dental cement. To avoid wound infection, an antibiotic powder (Chlorhexidin Puder, Riemer Arzneimittel AG, Greifswald, Germany) was applied. The animals were individually caged and allowed to recover for at least 10 days after surgery before the experiments commenced.

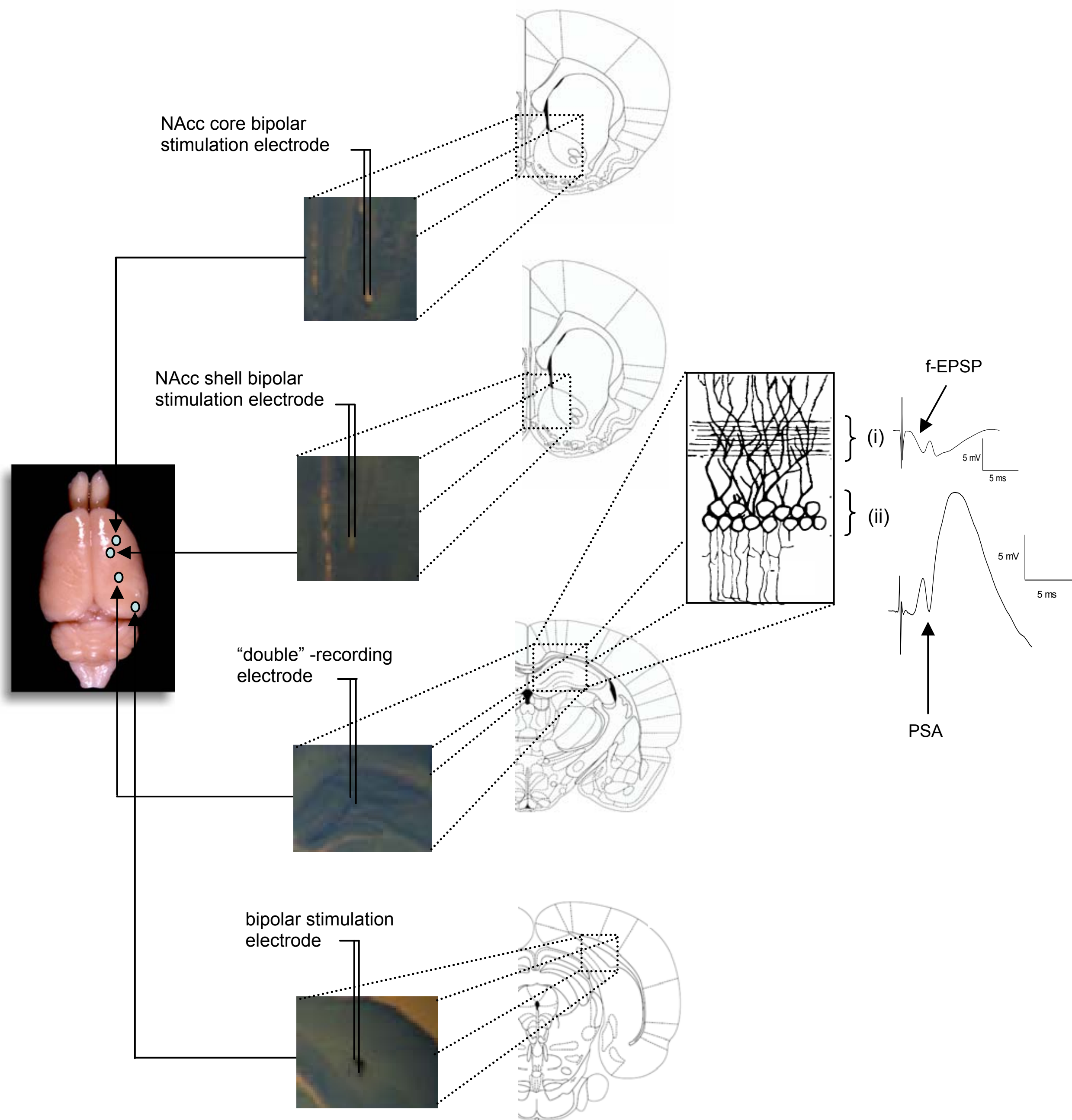


Figure 6: Implantation sites of the electrodes.

Left panel: Shows a photograph of a rat brain from the above. The electrodes insertion sites are visibly marked with small circles. Middle left panel: The corresponding coronal section in a Nissl staining showing the exact electrodes positioning in depth. Middle right panel: For an overview the schematic drawings adapted from (Paxinos and Watson 2003) showing the corresponding AP coordinates. Right panel: Shows the granule cell layers in a rectangular box with the analog trace signal (f-EPSP(i) from the dendrites and PSA(ii) from the soma): [The granule cell layers figure was adapted from (Bliss and Lomo 1973)].

2.3. Electrophysiological experiments

All the electrophysiological experimental recordings were performed after the animals were placed into the recording chambers (40 x 40 x 40) cm for habituation a day before the beginning of the experiments. During the initial habituation, the animals were exposed to the recording boxes for at least 4 h. Thereafter, they were connected to a swivel connector via a ribbon cable and then left undisturbed for 45–60 minutes to allow them to recover from any anxiety due to handling before constructing the input–output (I-O) curve. Throughout the experiment, the animals were allowed to move freely with *ad libitum* access to food and water. However, the influence of hippocampal electroencephalogram (EEG) on field potentials are strongly linked to the behavior of the animal at the time the stimulus is delivered (Winson and Abzug 1978; Cao and Leung 1991; Green et al. 1993). Adapting the animals to the recording chamber prior to testing initiation minimized this confounding effect by administering stimuli only during specific and stable behavior states.

The implanted electrodes on the head-stage of the animals were connected to a swivel connector (LEMO Elektronik GmbH, München, Germany) via a ribbon cable to the stimulation unit and amplifier. The ribbon cable allowed the animals to move freely in the recording box (as shown in Figure 7). Evoked responses were generated by applying single biphasic constant current square wave pulses of 0.1 ms per half-wave duration and manually specified stimulation intensity of 50–800 µA using a stimulation isolator.

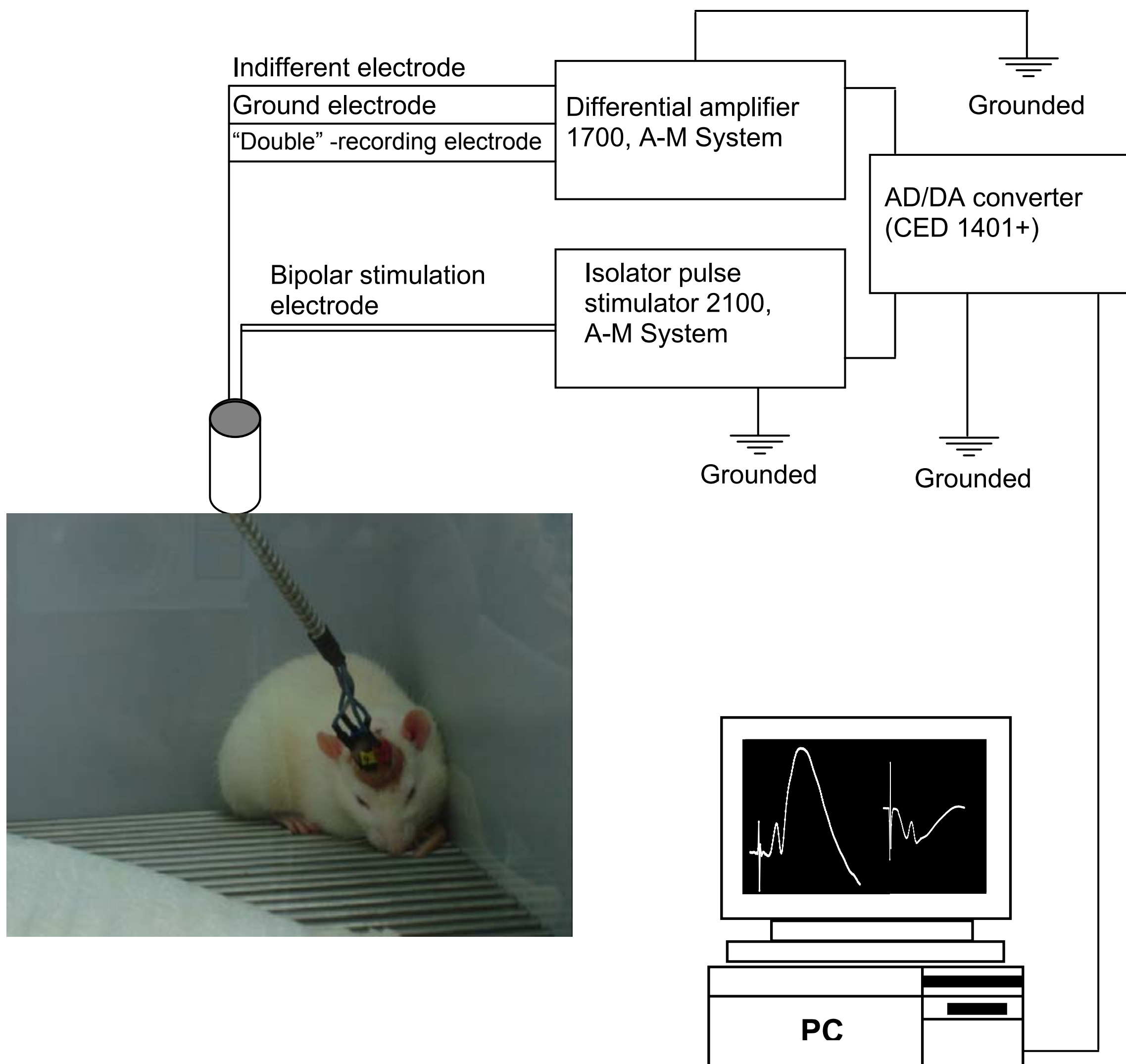


Figure 7: Electrophysiological recording set-up with connected freely moving animal.
The implanted bipolar stimulating and “double” -recording electrodes in PP-DG were connected to the set-up via a ribbon cable and swivel connector. Stimuli were applied with the aid of a personal computer (PC) supported intracell software via the stimulation isolator into the PP. The applied stimulus generated field potentials in the granule cell layer of the DG. The evoked field potentials were amplified differentially by an amplifier and digitized by AD converter and analyzed online with PC.

The signals from the recording electrodes were amplified (Differential AC Amplifier, Model 1700, A-M System, Sequim, USA) with gain (x100), filtered (0.1 Hz – 5 kHz

bandpass) and digitized at 10 kHz sampling rate through AD/DA converter. Waveforms, displayed and analyzed on a computer with custom made data acquisition software called Intracell, were saved to the hard disk. The hippocampal EEG signal was monitored throughout the course of each experiment with an oscilloscope using the same electrode for recording the f-EPSP slope.

2.3.1. Analysis of field evoked potentials at the DG

Extracellular field potential recordings represent the summed responses from a number of neurons in the vicinity of the recording electrode. As the name implies, field potentials reflect the summated response of an entire population of neurons which includes both excitatory and inhibitory influences. However, the orderly orientation of the dendritic fields to the cell body of the granules cell layers presents a unique situation in which field potentials recorded *in vivo* offer valuable information about the activity from the dendrites to the cell bodies (Andersen et al. 1966; Lomo 1971). The responses were evoked by stimulating at low frequency test pulse. For each recording time point, five evoked responses were averaged for both f-EPSP slope and PSA. The PP fibers stimulation generates a dipole of current flowing from the synaptic region (negative-going sink) to the cell body layer (positive-going source); in the case for an f-EPSP. An extracellular “double” -recording electrode in the DG containing synapses, records a small voltage difference as a negative potential at and above the region of incoming PP fibers.

The heterogeneous f-EPSP slope was measured as the slope at the steepest point of the potential between the two markers provided in Figure 8(ii) taking into

consideration that the real amplitude of the corresponding f-EPSP is overlaid by the development of the field for the PS as shown in Figure 8(ii). The f-EPSP slope is considered as a ratio of the f-EPSP amplitude and f-EPSP latency with unit mV/ms. The f-EPSP slope represents depolarization at the postsynaptic membrane, indicating that synaptic transmission has occurred. The amplitude of the PS was measured from the first positive peak deflection of the evoked potential to the peak of the preceding negative potential in mV. The PSA reflects the synchronous discharge of action potentials by the granule cell population. At higher stimulus intensity, more neurons fires synchronously and the amplitude gets larger, the peak of the negative potential reflecting the PS becomes narrow.

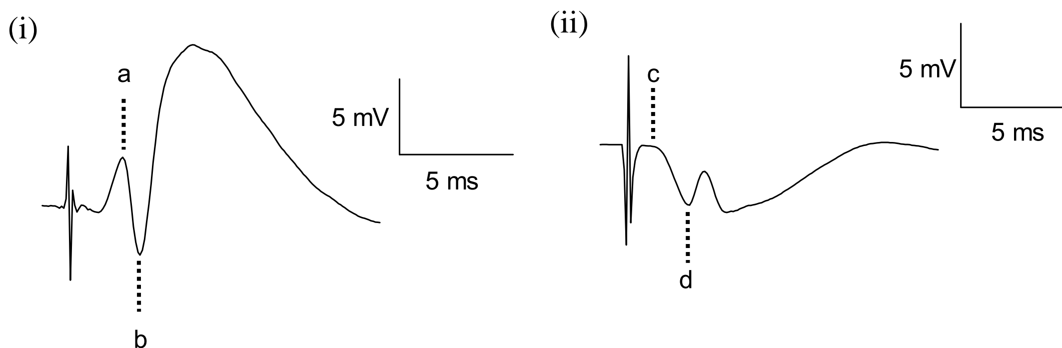


Figure 8: Analysis of evoked field potentials in DG.

The figure shows the evoked response of PSA and f-EPSP in the granule cell layer to the stimulation of PP in the DG. The points *a* and *b* mark the peaks in between which the PS amplitude was measured. The f-EPSP slope was measured at the steepest point between the mark points *c* and *d*.

2.3.2. Input - output curve construction

An I-O function is the relationship between the amount of current applied to the afferent fiber bundle (input current) and the resulting response (output voltage). The absolute voltage of the field potential for a given stimulus intensity varies from animal to animal *in vivo*. In order to obtain a more complete profile of the excitability of the cell population, a range of stimulus intensities were administered, extending from just above threshold for evoking an f-EPSP slope to that which evokes a maximal PS. The I–O curves were generated for each animal by applying varying stimulation current intensities (50–800 μA) to the PP to establish the test intensity used in subsequent experiments. The current pulses were generated by a constant current isolation unit at low frequency. The stimulus intensity was increased stepwise at constant time intervals from below threshold to saturation by so doing, the maximum PSA and f-EPSP slope was determined. Maximum amplitude of the PS and f-EPSP slope was normalized and expressed as 100%. The stimulation intensities required to evoke DG field potentials of about 60% of the maximum slope function of the f-EPSP and 40% of the maximum amplitude of the PS were calculated. The 40% of the maximal PSA was used to tetanize the PP irrespective of the “double” -recording electrode techniques used. All further potentials were evoked with the calculated stimulus intensity. The animals were disconnected from the swivel and stayed in the recording box overnight to continue experiments the next day.

2.3.3. Initial baseline

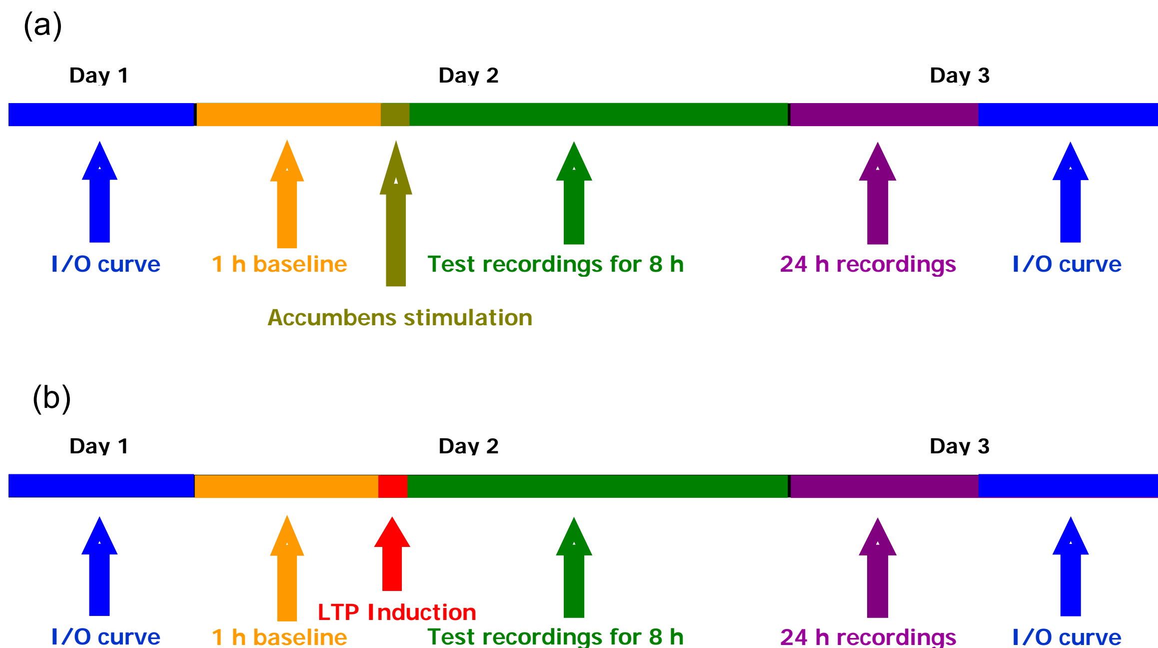
On the day of the experiment, at least one hour stable baseline was recorded. After connecting the animals to the swivel via a ribbon cable, a 30 minutes time interval was allowed for the animals to recover from handling. Apart from the insertion of ribbon cable at the start of the experiment, disturbance of the animals were kept to an absolute minimum. Ambient conditions were kept constant to prevent environmental influences on behavioral state that might affect the LTP expressed and the recording process as a whole. The calculated values for the f-EPSP slope (60%) and PSA (40%) were evoked and the stimulus intensity confirmed as criteria for baseline. At a change in the calculated values, the stimulation isolation unit was adjusted accordingly. This test section was followed by a non-recording period of 30 minutes before the start of the baseline. An hour stable baseline was recorded with test-pulses given every 5 minutes for both f-EPSP slope and PSA alternatively. The responses were evoked by stimulating at low frequency test-pulses (0.1 Hz, 0.1 ms stimulus duration). For each recording time point, five evoked responses were averaged

2.3.4. Experimental design

The average of five single test stimuli (10 s interpulse interval) was stored alternatively every 10 minutes for the f-EPSP slope and PSA. The induction of an early-LTP by application of WTET (see below) to the PP in combination with prior (15 minutes before) stimulation of the NAcc core or shell to prove whether this might influence early-LTP induction or its time course. To ascertain whether NAcc core or

shell stimulation could modify early-LTP in the DG when activated after early-LTP induction, the alternative experimental pattern was also tested, i.e. early-LTP induction followed (15 minutes after) by the stimulation of the NAcc core or shell. The experimental design is shown below in Figure 9.

The initial test record after the tetanus was 5 minutes and then every 15 minutes five test stimuli were applied at low frequency of 0.1 Hz. The mean values of the PSA and the slope of the f-EPSP were recorded consecutively. In control experiments, either of the NAcc core or shell regions were stimulated after stable baseline was recorded. These experiments were prerequisite to test the possible drift in response to electrical stimulation of both regions of the NAcc on basal synaptic transmission. The time course was followed up for 8 h and a final test recording was followed up to 24 h on the next day.



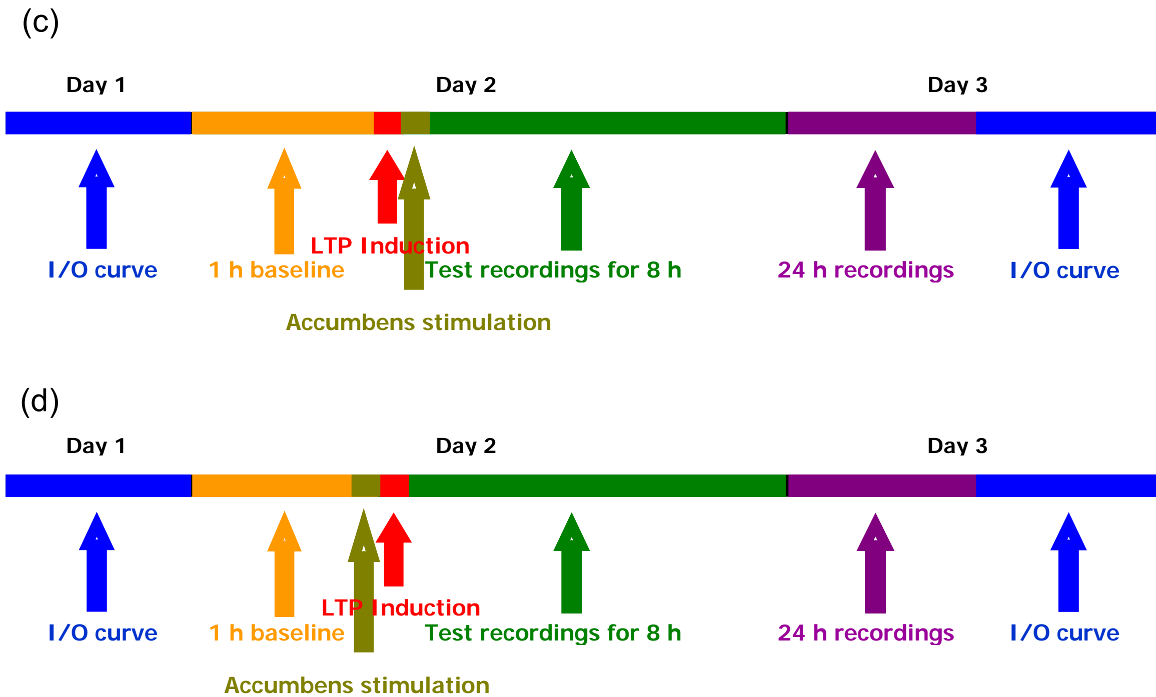


Figure 9: Experimental design.

(a): The effects of NAcc core or shell stimulation on basal synaptic transmission. (b): Early-LTP induction in animals bearing electrodes in the NAcc core or shell. (c): The NAcc core or shell was stimulated 15 minutes after early-LTP induction. (d): The effect of stimulating NAcc core or shell 15 minutes prior to early-LTP induction.

Stimulus paradigms

After initial baseline recording, a stimulation protocol was applied to induce early-LTP with WTET in the DG. A stimulus intensity of 40% of the maximum PSA was used for all the tetani without regards to the two separate intensities used for the test recordings of the f-EPSP slope (60% of the maximal slope function) and the PSA (40% of the maximal PSA).

Weak tetanization

A WTET protocol was applied to induce transient early-LTP at the PP-DG synapses. It consists of 3 burst (interburst intervals of 10 s) of 15 impulses at a frequency of 200 Hz with 0.2 ms pulse duration per half-wave for each stimulus.

Nucleus accumbens stimulation

NAcc core or shell was stimulated with “weak” or “strong” tetanic stimulation as used at the PP. The strong tetanization protocol consists of 4 trains of 20 burst (interburst interval 10 s) of 15 impulses at a frequency of 200 Hz with 0.2 ms pulse duration per half-wave for each stimulus. It can not be ascertained whether the stimulation paradigm used in stimulating the NAcc was weak or strong. A series of tests was conducted to establish a level of NAcc stimulation which would be suitable to activate the NAcc without causing detectable stress to the animal. The NAcc tetanus consists of 3 burst of 15 impulses at a frequency of 0.1 Hz with 0.1 ms pulse duration per half-wave and 10 s interburst interval with a stimulus intensity of 400 μA.

2.4. Histological analysis

Upon completion of the experiments, the placements of the electrodes were examined histologically. The animals were deeply anesthetized with 2-Bromo-2-chloro-1, 1, 1-trifluoroethane (Sigma–Aldrich Chemie GmbH, Munich, Germany) and thereafter decapitated. The brains were carefully removed out of the cranium, snap-frozen at approximately –30°C in isopentane and stored at –70°C. Using a freezing microtome, coronal section of 40 μm thick for the NAcc and 30 μm thick for the PP

as well as the DG were cut using a cryostat (Microm HM 560, Microm International GmbH, Walldorf, Germany).

The slices were air-dried on a heating plate (37°C) and stained. After 7 days of equilibration the tissue was completely dry for staining. The slides were stirred for 25 minutes in 10% of Formalin, tab water and 96% ethanol, then washed in distilled water and stained in Toluidine. Slices were washed in distilled water and dehydrated in an ascending percentage of ethanol series and degreased in Xylene. Bubble-free embedding was done with glass slides for microscopy (Carl Roth GmbH, Karlsruhe, Germany) with Histomount (Thermo Shandon, Pittsburgh PA, USA). For histological examination, a stereo microscope without filter in the translucent mode was used (Leica Z16 APO, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Digital images were made with digital camera if necessary. Only animals with correct electrode placement (i.e. within the structures of interest) were considered for final analysis.

2.5. Statistical analysis

Six averaged measurements per animal were obtained during the initial baseline recordings. At each recording time point, 5 sweeps were recorded for both f-EPSP and PSA and the values averaged. The mean values of the average sweeps were obtained and expressed as a percentage. Each averaged f-EPSP and PSA value was then plotted as the percentage of their respective mean values. Data of animals which has undergone the same treatment were pooled together, and values

of every time point were shown as the mean percentage ± the standard error of mean (SEM).

Repeated measures two-way analysis of variance (ANOVA) using group (to consider the different sites of stimulation) and time (repeated measures) were performed, after checking for normality using the Kolmogorov-Smirnov test (Lilliefors correction) although the *F*-test is remarkably robust to deviations from normality (Lindman 1974). Additionally, in baseline stability control experiments, a paired samples t-test was used to check for deviation from initial baseline level over time in individual groups. In all tests a $P<0.05$ was considered statistically significant. PC-based software was used for off-line statistical analysis (Microsoft Excel, GraphPad Prism).

3. Results

3.1. NAcc stimulation and its effect on basal synaptic transmission in DG

The results of control experiments were carried out to establish the effects of stimulating the NAcc core or shell on basal synaptic transmission at the PP-DG synapses are shown in Figure 10A, B, C, D. Stimulation of NAcc core or shell on basal synaptic transmission groups (open squares for NAcc core and open triangles for NAcc shell) are compared to control stimulation groups (filled circles) for both f-EPSP in Figure 10A, C and PSA in Figure 10B, D. The stimulation of the NAcc shell had no effect on basal f-EPSP and PSA as shown in Figure 10A, B. The individual analysis for each group compared with their own baseline (*t*-test for paired samples) showed no significant differences at any time point. This is confirmed by the ANOVA showing no significant influence of time on the f-EPSPS (Figure 10A) ($F_{(15,\ 270)}$ = 1.015, ns 2-way ANOVA repeated measures) and PSA (Figure 10B) ($F_{(15,\ 270)}$ = 0.5005, ns) as well as no differences among groups for f-EPSP (Figure 10A) ($F_{(1,\ 270)}$ = 0.02804, ns) and PSA (Figure 10B) ($F_{(1,\ 270)}$ = 0.2415, ns). Stimulation of the NAcc core induced a reduction of the granule cell output measured as the PSA in response to PP stimulation as shown in Figure 10D but not in the f-EPSP as shown in Figure 10C. The statistical analysis using ANOVA, (i.e. comparing the two groups and all recording points) showed no significant differences in time on the f-EPSP (Figure 10C) ($F_{(15,\ 270)}$ = 2.051, ns) and PSA (Figure 10D) ($F_{(15,\ 300)}$ = 2.098, ns) or among groups for f-EPSP (Figure 10C) ($F_{(1,\ 270)}$ = 0.08584, ns) and PSA (Figure 10D) ($F_{(1,\ 300)}$ = 2.471, ns). However, comparing the NAcc core results with their own

baseline values using a paired samples *t*-test, significant differences were observed from 2 to 8 h after stimulation, suggesting a slight depressive action of NAcc core stimulation on basal PS function at the DG.

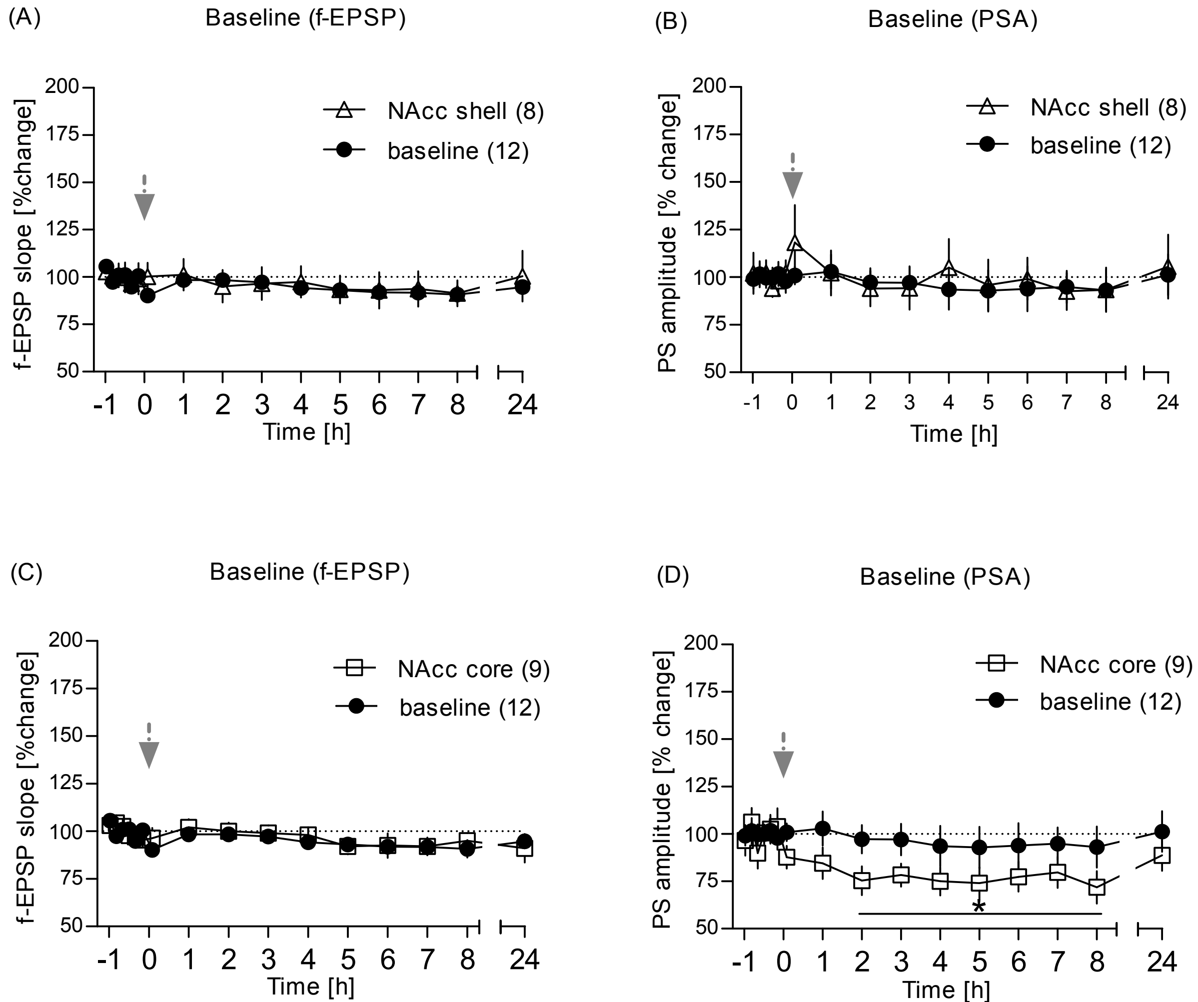


Figure 10: Influence of NAcc core or shell stimulation on basal synaptic transmission in DG. The graphs show the effects of the stimulation of the NAcc core (grey arrow) (open squares) or shell (grey arrow) (open triangles) on basal synaptic transmission at the PP-DG synapses (grey arrow) compared to non-stimulated animals (baseline) (filled circles). The ANOVA showed no difference among groups, both regarding the f-EPSP (A, C) or the PSA (B, D), but in the case of the PSA, a *t*-test for paired samples showed a significant reduction in the PSA after the NAcc core stimulation compared to its own baseline values, from 2 to 8 h, as indicated by the asterisk above the line. Values are expressed as mean ± SEM in all graphs.

The co-stimulation of both, NAcc core or shell and the PP produced no modification in the I-O curves within a range of 50 to 800 μA (Figure 11) in comparison to PP stimulation alone.

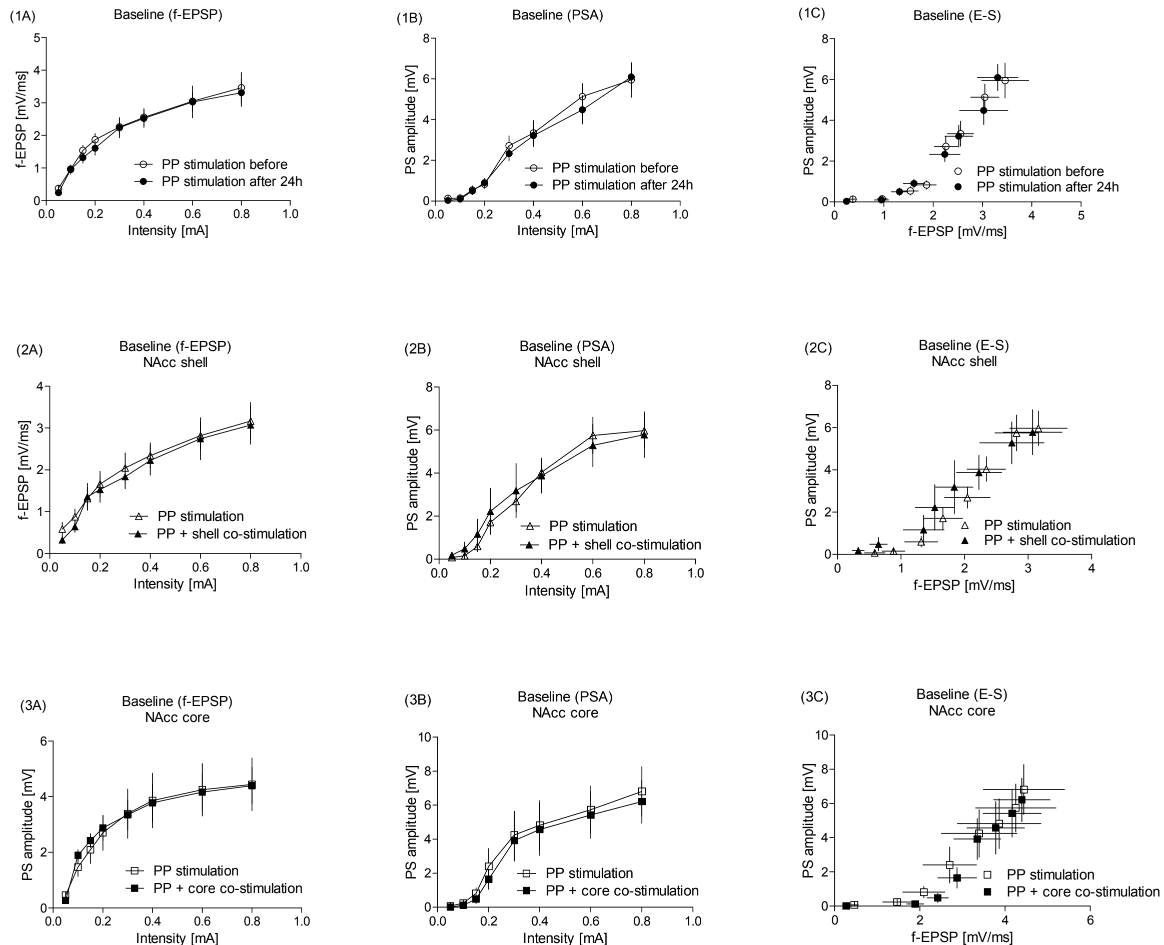


Figure 11: I-O curves and E-S relations for NAcc stimulation on baseline in DG.

Co-activation of the NAcc core or shell and the PP does not change input-output relationships in the DG. (1A, B, C) show the effects of control basal synaptic transmission when only the PP was stimulated before and after the experiment. (2A, B) show the effects of co-stimulating the NAcc shell on the f-EPSP and PSA input-output relationships (filled triangles), respectively; and compared with those obtained when only the PP was stimulated (open triangles). (2C) show the E-S relationship where E-S relation connotes EPSP-Spike relation. (3A, B, C) show the same for the NAcc core. Mean ± SEM are represented in all graphs.

3.2. Control early-LTP in the DG

In order to study the influence of NAcc stimulation on early-LTP with certainty, there was the need to carry out these control experiments. Control early-LTP experiments were performed with animals with only bipolar stimulation and “double” -recording electrodes in the PP and DG respectively. The second group of control experiments was carried out with animals bearing bipolar stimulation electrodes implanted into the NAcc core or shell. These control experiments were a necessity to determine with certainty that there is no own effect of the implanted bipolar stimulation electrode in either the NAcc core or shell region. However, the animals that exhibit potentiation at the application of WTET proceed to the next phase of the experiments, whereby either region of the NAcc was stimulated prior to or after WTET was applied.

Control early-LTP group (filled circles, Figure 12) is compared to control early-LTP with animals bearing electrodes in either region of the NAcc (open squares for NAcc core and open triangles for NAcc shell, Figure 12). The results show that the induction of early-LTP by application of WTET at the PP-DG synapses was the same in both control groups statistically as shown in Figure 12 (for f-EPSP and PSA). A two-way repeated measures ANOVA, the group and time factors showed a significant time effect on the f-EPSP (Figure 12A) ($F_{(9,\ 189)}$ = 22.25, * P<0.05) and PSA (Figure 12B) ($F_{(9,\ 207)}$ = 27.52, * P<0.05), but no influence of group factor on the f-EPSP (Figure 12A) ($F_{(1,\ 189)}$ = 0.5325, P>0.05) and PSA (Figure 12B) ($F_{(1,\ 207)}$ = 3.388, P>0.05) or interaction between factors on the f-EPSP (Figure 12A) ($F_{(9,\ 189)}$ =

2.947, P>0.05) and PSA (Figure 12B) ($F_{(9, 207)}$ = 1.432, P>0.05) for control early-LTP in the NAcc shell group.

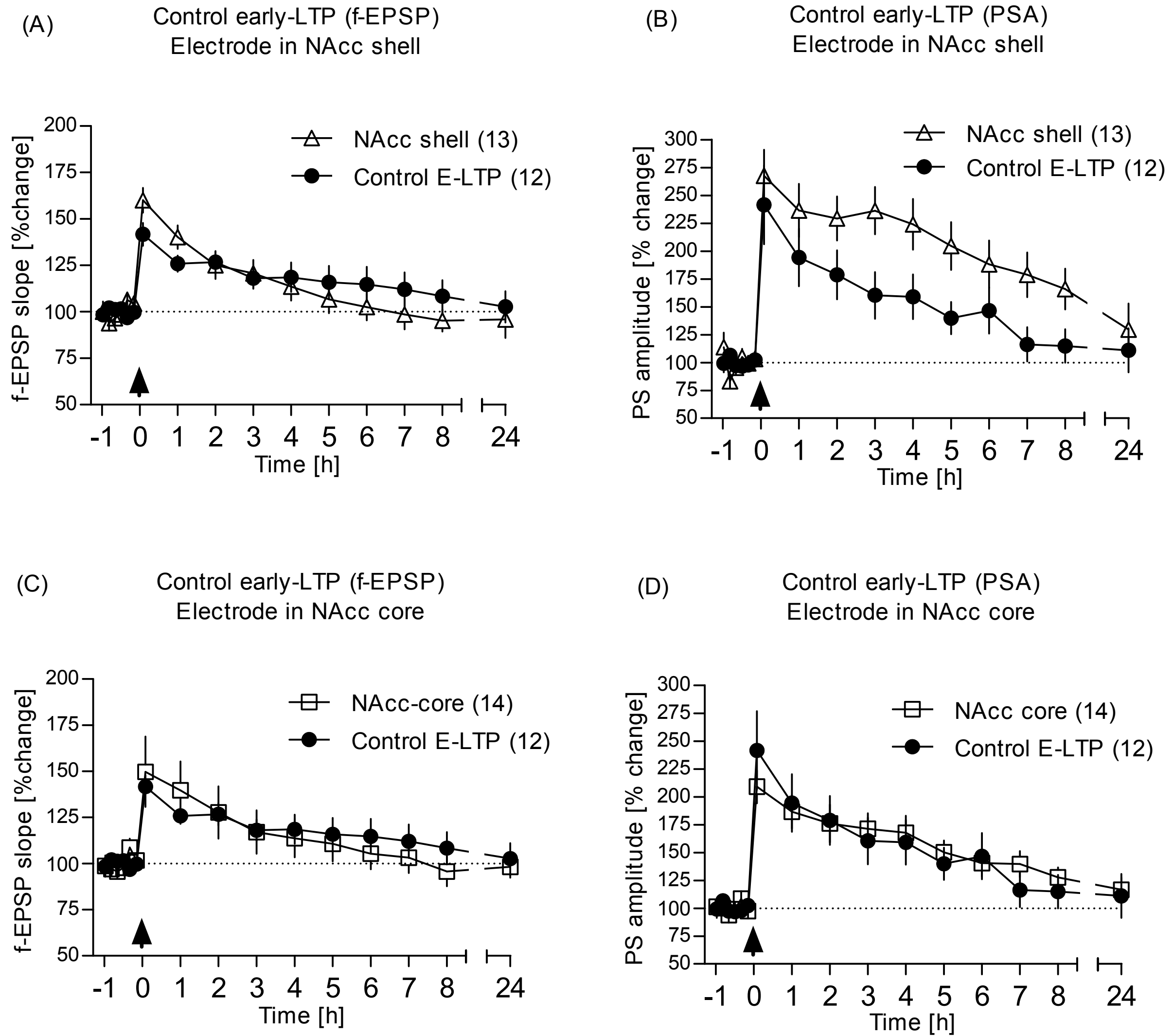


Figure 12: Control early-LTP groups in DG.

Control early-LTP groups after PP tetanization (filled circles) compared to control early-LTP with animals bearing electrode in the NAcc shell (open triangles) does not show statistical differences among groups (repeated measures two-way ANOVA) for the both f-EPSP (A) and PSA (B). C and D show the same effect for f-EPSP and PSA respectively in the NAcc core group (open squares). Thus, the control early-LTP without animals bearing electrode in the either the NAcc core or shell region was used as a control group for the rest of the analysis. The black arrow indicates early-LTP induction at time zero.

Similarly, the control early- LTP in the NAcc core group shows the same effect as shown in Figure 12C, D. The group and time factors showed a significant time effect on the f-EPSP (Figure 12C) ($F_{(9, 216)}$ = 15.71, * P<0.05) and PSA (Figure 12D) ($F_{(9, 216)}$ = 33.55, * P<0.05), but no influence of group factor on the f-EPSP (Figure 12C) ($F_{(1, 216)}$ = 0.03324, P>0.05) and PSA (Figure 12D) ($F_{(1, 216)}$ = 0.01050, P>0.05) or interaction between factors on the f-EPSP (Figure 12C) ($F_{(9, 216)}$ = 1.293, P>0.05) and PSA (Figure 12D) ($F_{(9, 216)}$ = 1.706, P>0.05).

The significant difference in time was due to the fact that the initial early-LTP potentiation for both f-EPSP ((160.1 ± 6.70) with animals bearing electrodes in NAcc shell and (141.8 ± 6.13) control early-LTP group) as well as PSA ((267.6 ± 23.30) with animals bearing electrodes in the NAcc shell and (241.6 ± 35.29) control early-LTP group) after 5 minutes but decayed to the baseline value as shown in Figure 12A, B. Similarly, the same effect applies to the NAcc core group in Figure 12C, D. Initial potentiation of the early-LTP for animals bearing electrodes in the NAcc core group was for f-EPSP (149.8 ± 19.08) and PSA (208.9 ± 14.78) and decayed to the baseline value. Co-stimulation of the PP by the application of WTET produced no modification in the I-O curves within a range of 50 to 800 μA (Figure 13) in comparison to PP stimulation alone.

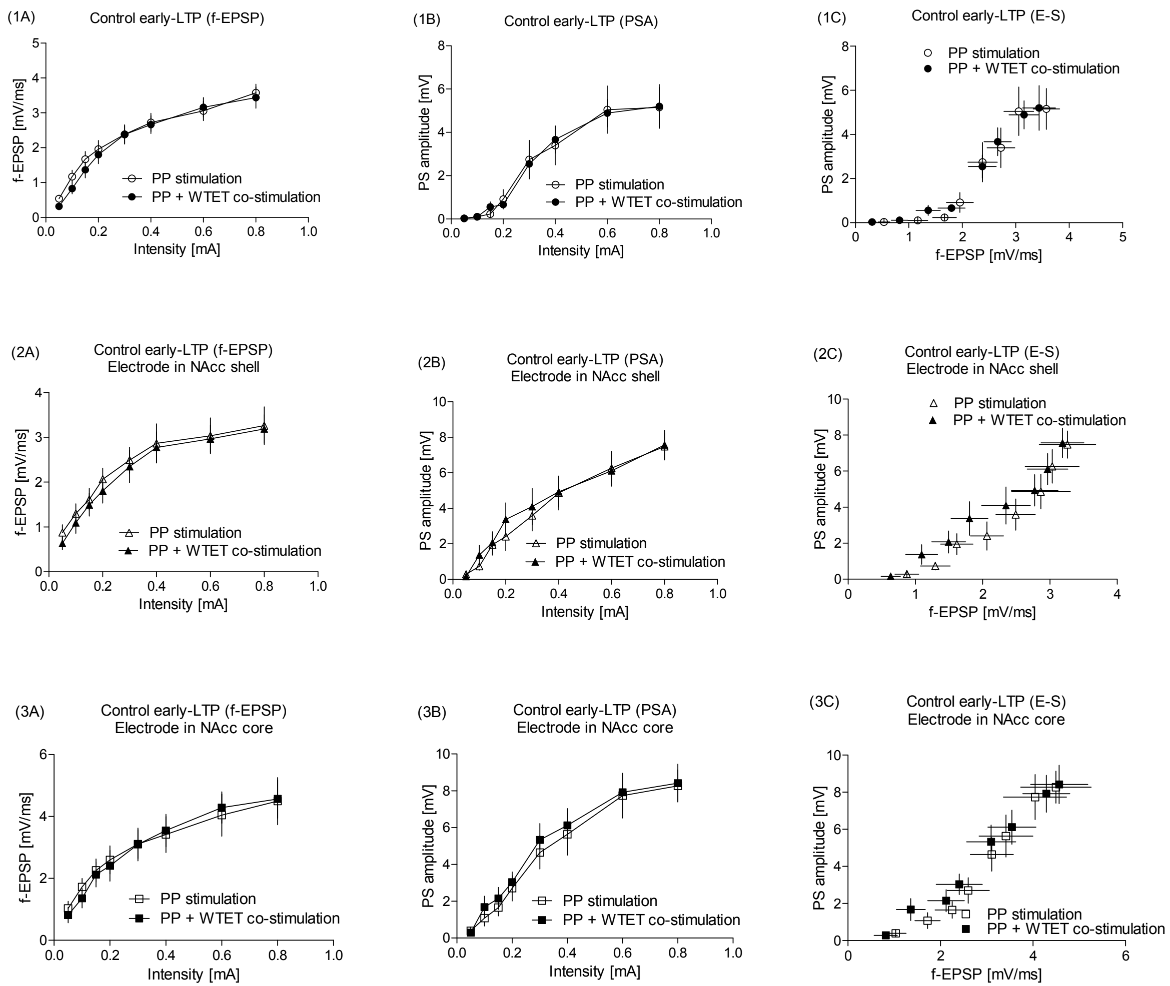


Figure 13: I-O curve and E-S relation for control early-LTP in DG.

Application of WTET to the PP does not change input-output relationships in the DG. (1A, B) show the effects of early-LTP induction at the PP on the f-EPSP and PSA input-output relationships (filled circles), respectively; and compared with those obtained when only the PP was stimulated (open circles) with the E-S relationship (1C). (2A, B, C) and (3A, B, C) show the same effect for the NAcc shell and core groups respectively.

3.3. DG early-LTP and NAcc stimulation

3.3.1. NAcc stimulation after WTET in DG

In this set, the NAcc core or shell was stimulated 15 minutes after the induction of early-LTP in the DG. The stimulation of the NAcc core or shell 15 minutes after the induction of early-LTP in the DG showed no short- or long-term effect on the time course of early-LTP. The NAcc shell stimulation 15 minutes after early-LTP induction (open triangles, Figure 14A, B) was compared to control early-LTP (filled circles, Figure 14A, B). Two-way repeated measures ANOVA with group and time as factors show no difference among the groups on the f-EPSP (Figure 14A) ($F_{(1,\ 198)}$ = 0.2445, ns) and PSA (Figure 14B) ($F_{(1,\ 198)}$ = 0.04662, ns) or interaction between factors on the f-EPSP (Figure 14A) ($F_{(9,\ 198)}$ = 0.5164, P>0.05) and PSA (Figure 14B) ($F_{(9,\ 198)}$ = 0.6544, P>0.05) but time influence on the f-EPSP (Figure 14A) ($F_{(9,\ 198)}$ = 15.79, * P<0.05) and PSA (Figure 14A) ($F_{(9,\ 198)}$ = 35.63, * P<0.05). The initial potentiation 5 minutes after WTET application was for f-EPSP (148.1 ± 6.42) (Figure 14A) and PSA (223.1 ± 10.28) (Figure 14B), and decayed to baseline value. Similarly, the NAcc core stimulation 15 minutes after early-LTP induction group (open squares, Figure 14C, D) was compared to control early-LTP (filled circles, Figure 14C, D) with no effect among the group on the f-EPSP (Figure 14C) ($F_{(1,\ 207)}$ = 0.1208; ns) and PSA (Figure 14D) ($F_{(1,\ 207)}$ = 0.05399; ns) or interaction between factors on the f-EPSP (Figure 14C) ($F_{(9,\ 207)}$ = 0.3081, P>0.05) and PSA (Figure 14D) ($F_{(9,\ 207)}$ = 1.008, P>0.05) but time influence on the f-EPSP (Figure 14C) ($F_{(9,\ 207)}$ = 13.26, * P<0.05) and PSA (Figure 14D) ($F_{(9,\ 207)}$ = 36.86, * P<0.05). The initial potentiation 5 minutes after WTET application was for f-

EPSP (144.2 ± 5.16) and PSA (218.5 ± 15.94) and both decayed to baseline value after 6 h.

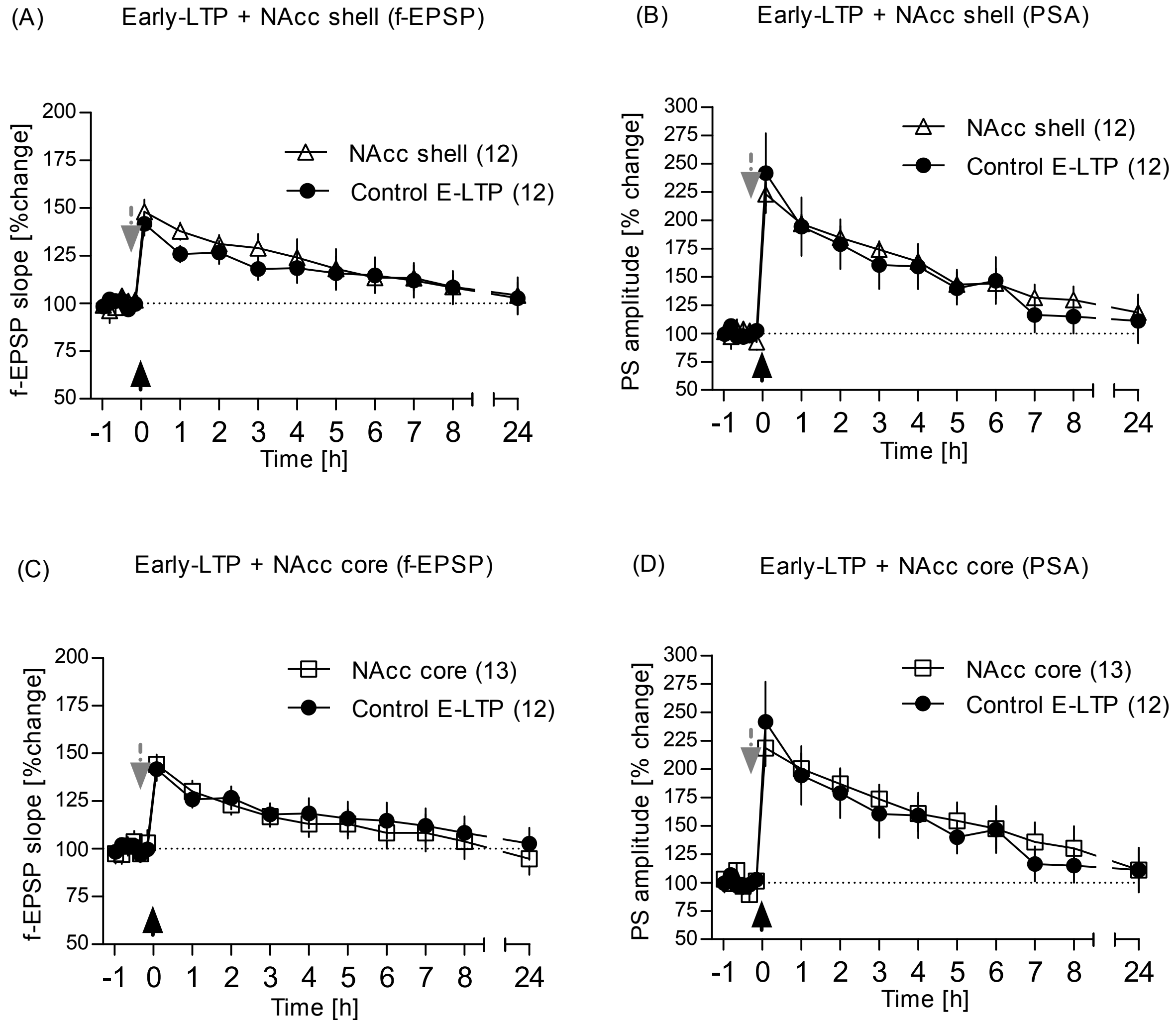


Figure 14: NAcc core or shell stimulation 15 minutes after WTET application in DG.

The graphs show the effect of stimulating the NAcc core (grey arrow) (open squares) or shell (grey arrow) (open triangles) 15 minutes after induction of early-LTP in the DG (black arrow) compared with control early-LTP (filled circles) for f-EPSP (A, C) and PSA (B, B). 2-way repeated measures ANOVA with group and tine as factors show no difference among the groups or interaction between factors for both f-EPSP and PSA.

The co-activation of NAcc core or shell and the PP produced no changes in the I-O curves within a range of 50 to 800 μA (Figure 15) in comparison to PP stimulation alone.

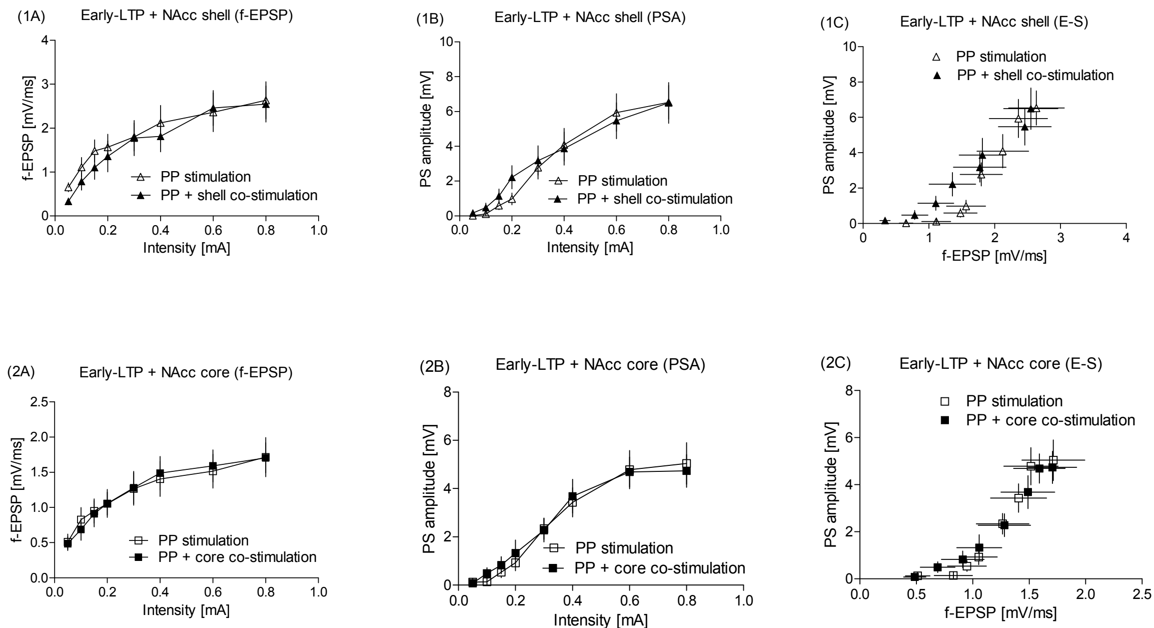


Figure 15: I-O curves and E-S relation for NAcc stimulation after WTET application to the DG.

The co-stimulation of the NAcc core or shell and the PP does not produce any changes in the input-output relationships in the DG. (1A, B) show the effects of co-stimulating the NAcc shell on the f-EPSP and PSA input-output relationships (filled triangles), respectively; compared with those obtained when the PP was stimulated alone (open triangles). (1C) show the E-S relationship. (2A, B, C) show the same for the NAcc core.

3.3.2. NAcc stimulation before WTET in DG

The NAcc core or shell was stimulated prior to WTET application in the DG (Figure 16). The stimulation of the NAcc core or shell 15 minutes before the induction of early-LTP in the DG, the NAcc core stimulation modified the initial level

of potentiation at the f-EPSP and PSA (Figure 16C, D). In comparing the NAcc stimulation prior to early-LTP induction group (open squares for NAcc core and open triangles for NAcc shell, Figure 16) to control early-LTP (filled circles, Figure 16), the NAcc shell (Figure A, B) shows no influence of the group factor on the f-EPSP (Figure 16A) ($F_{(1,\ 189)}$ = 0.01659; ns) and PSA (Figure 16B) ($F_{(1,\ 189)}$ = 0.4252; ns) or interaction between factors on the f-EPSP (Figure 16A) ($F_{(9,\ 189)}$ = 2.778, P>0.05) and PSA (Figure 16B) ($F_{(9,\ 189)}$ = 1.093, P>0.05) but time effect on the f-EPSP (Figure 16A) ($F_{(9,\ 189)}$ = 17.83, * P<0.05) and PSA (Figure 16B) ($F_{(9,\ 189)}$ = 41.61, * P<0.05). The initial potentiation 5 minutes after WTET application was (167.2 ± 11.76) and (274.3 ± 23.20) for f-EPSP (Figure 16A) and PSA (Figure 16B) respectively.

On the contrary, the stimulation of the NAcc core before early-LTP induction significantly influenced the initial potentiation and the time course of the early-LTP (Figure 16C, D). The stimulation of the NAcc core modified significantly the outcome of the PP tetanization of the factor group on the f-EPSP (Figure 16C) ($F_{(1,\ 216)}$ = 6.812, * P<0.05) and the post hoc Tukey test confirmed significant difference among the groups. A similar pattern appeared with respect to the PSA potentiation (Figure 16D). Stimulation of the NAcc core (factor group) influenced significantly the outcome of tetanization to the PP ($F_{(1,\ 216)}$ = 2.978, * P<0.05), the Tukey test confirmed a significant difference among the groups. Initial potentiation 5 minutes after WTET application was (106.5 ± 2.68) for f-EPSP (Figure 16C) and (167.9 ± 8.04) for PSA (Figure 16D) and decayed very fast to the baseline value. The potentiation of both: the f-EPSP and the PSA seemed to be favored by the stimulation of the NAcc shell, and are clearly reduced by the stimulation of the NAcc

core. The time course of early-LTP, shown in Figure 16C, D, suggests that the effect modified mainly the induction of early-LTP but not its late phases.

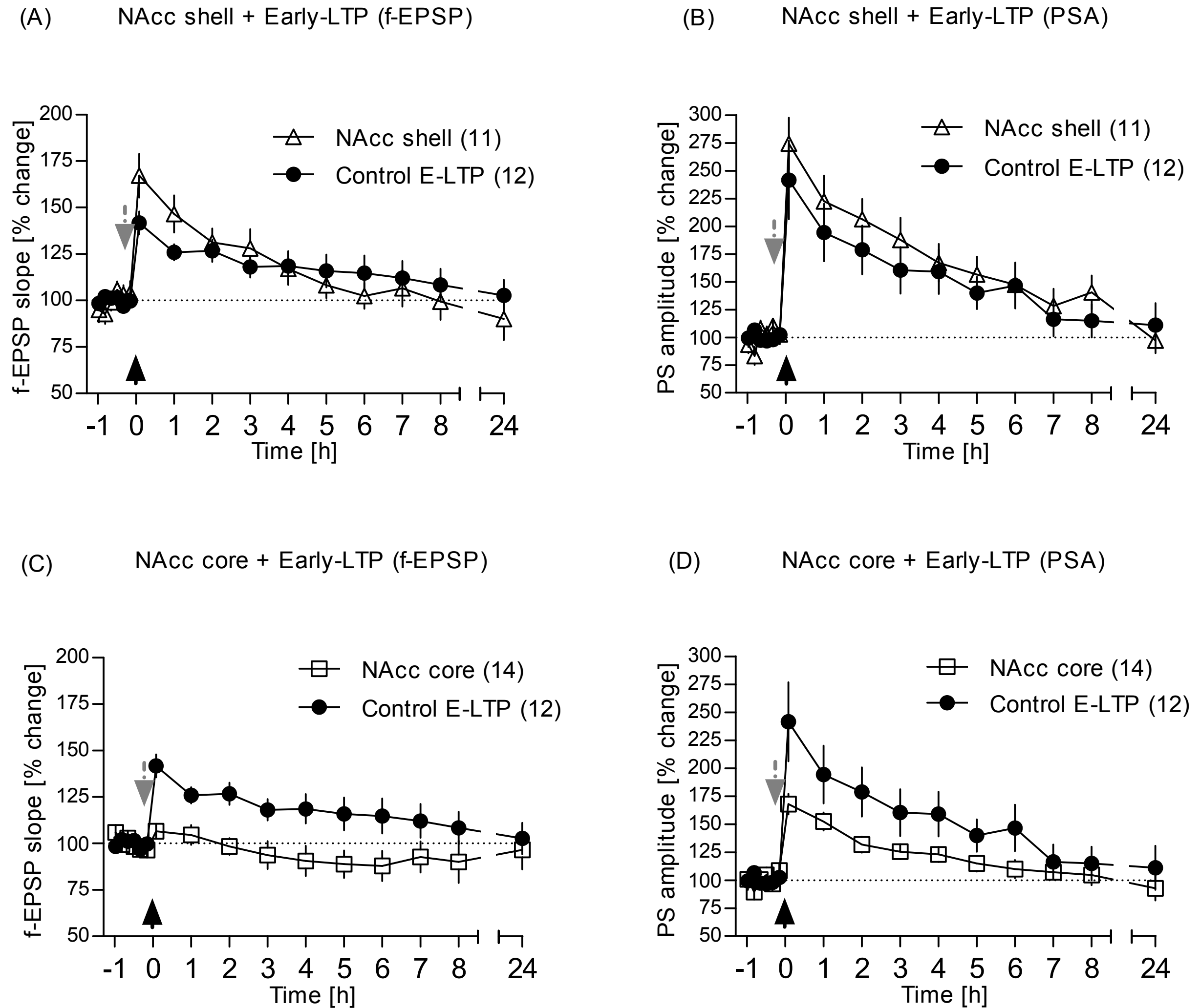


Figure 16: NAcc core or shell stimulation before WTET application in DG.

The graphs show the effects of stimulating the NAcc core or shell 15 minutes (grey arrow) before induction of early-LTP in the DG (black arrow). (A, B) show no significant difference between NAcc shell stimulated group (open triangles) and the control early-LTP group (filled circles). (C, D) show the influence of NAcc core stimulation before early-LTP induction in the DG on the f-EPSP and PSA respectively (open squares). The ANOVA showed significant group effects (* P<0.05) and the post hoc Tukey test showed that the NAcc core stimulated group differs significantly from the control early-LTP group in the f-EPSP (C) and PSA (D).

The co-stimulation of both, NAcc core or shell and the PP produced no changes in the I-O curves within a range of 50 to 800 μA (Figure 17) in comparison to PP stimulation alone.

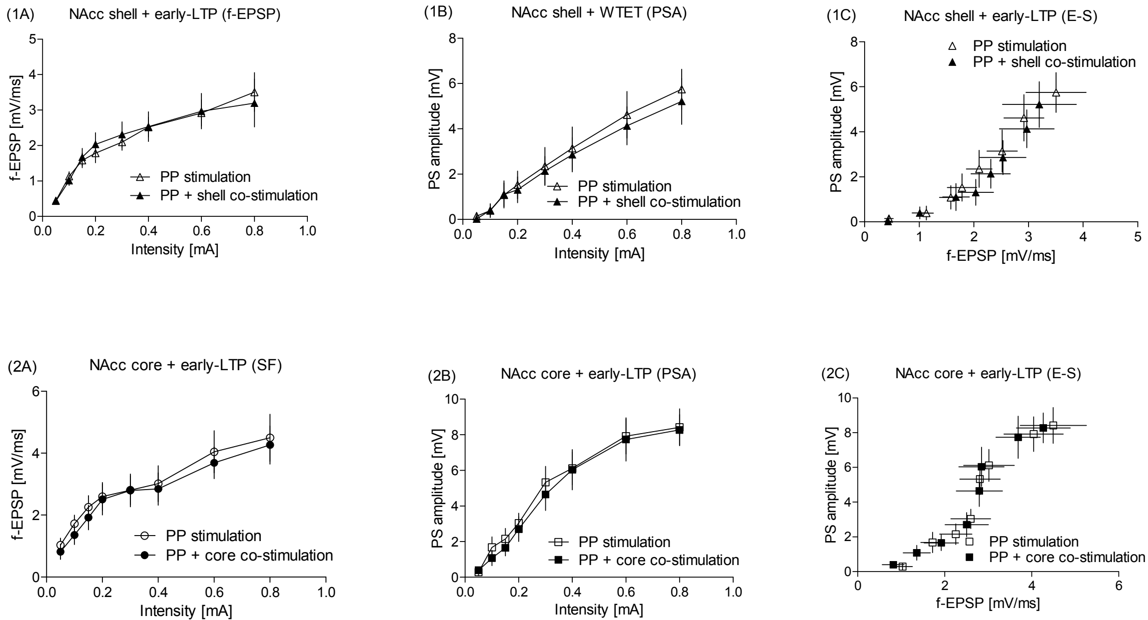


Figure 17: I-O curves and E-S relation for NAcc stimulation before WTET was applied to DG.

The co-stimulation of the NAcc core or shell and the PP does not change the input-output relationships in the DG. (1A, B) show the effects of co-stimulating the NAcc shell on the f-EPSP and PSA input-output relationships, respectively (open triangles); compared with those obtained when only the PP was stimulated (filled triangles). (1C) show the E-S relationship. (2A, B, C) show the same for the NAcc core.

4. Discussion

The major finding of this study provides evidence for a differential modulatory effect of NAcc core or shell stimulation on early-LTP induction in the DG in freely moving animals. These results suggest that the observed effects of NAcc core or shell stimulation on DG early-LTP depend on indirect influences of synaptic plasticity process in intermediate substructures and not on direct changes of plasticity of the granular cells in the DG. We have shown an opposite effect of the NAcc core and shell activation on the induction of LTP. Stimulation of the NAcc core showed a strong inhibitory effect on the f-EPSP and the PSA components measured. On the contrary, stimulation of the NAcc shell facilitated the initial potentiation of the f-EPSP and the PSA even though the facilitation effect was not that pronounced in freely moving animals in comparison to anesthetized animals (Lopez et al. 2008). This obvious difference in the facilitation effect between the freely moving and anesthetized animals might probably be a result of counterbalance by complementary facilitating influences acting in the freely moving animals than under narcosis. Additionally, and in line with the inhibition exerted on DG early-LTP, the stimulation of the NAcc core caused, by itself, a prolonged reduction of the PSA.

The changes which are often observed in I-O function of the f-EPSP slope and the PSA has led to the common practice of expressing the I-O function as a relationship between these two variables. Even though the intercept of this function according to experimental treatment may fluctuate, the slope of the I-O curve between the waveform parameters remains relatively constant. This suggests that

the cellular coupling mechanism of f-EPSP to action potential generation does not change. A selective effect on the f-EPSP slope suggests basically a dendritic locus of action, whiles changes in the PSA in the absence of the f-EPSP slope changes primarily indicates somatic focus. I-O curve of f-EPSP/PSA with leftward shift can reflect a greater synchrony in the cell firing for the same level of synaptic activation, while a rightward shift with this function can reflect a diminish synchronous cell firing. The most likely synaptic events that could bring about the directional shift in the I-O curve may include a change in neurotransmitter release, synaptic efficacy, cell excitability, cell loss, a change in postsynaptic sensitivity or number of non-NMDA glutamate receptors, or a change in inhibition. Future studies should verify if influences of local inhibition contribute to changes observed in the time course of potentials after NAcc stimulation in the DG. For that, for instance homosynaptic and heterosynaptic paired pulse stimulation protocols will be used.

Differential effect of NAcc core and shell

The differential effect of the NAcc core and shell activation has been documented using behavioral models (Gal et al. 1997; Jongen-Relo et al. 2003; Blaiss and Janak 2009; Kelsey et al. 2009; Pedroza-Llinas et al. 2009) as well as hippocampal LTP (Lopez et al. 2008) which was confirmed in the present experiments. Opposite roles for these territories are difficult to interpret in terms of the nature of the projecting cells, or the transmitter they release in their axon terminals because both NAcc regions projection neurons are essentially identical: medium size spiny neurons as in the rest of the striatal complex, from which the NAcc is the most ventral component. A recent report, based on a computational

model, proposed the existence of differences in several biophysical properties of spiny neurons within the NAcc (Steephen and Manchanda 2009) but in conclusion, all these neurons were GABAergic and inhibitory at their target.

The most likely explanation for opposite NAcc core-shell effects may depend on the different efferent projections of both regions. The NAcc shell has a strong projection to the lateral hypothalamus and has been implied in the regulation of feeding behavior, while the NAcc core projects mainly to the VP and is likely related to the reinforcement of motor patterns (Kelley 2004). Considering that LH inhibits LTP induction at the DG (Wayner et al. 1997), a facilitatory role of activating the NAcc shell can be predicted, though the functional significance of this effect seemed to be minor in awake animals comparing to our present results with those obtained under narcosis (Lopez et al. 2008). On the contrary, stimulation of the NAcc core, showed a strong inhibitory effect on early-LTP induction at the DG, both in awake or anesthetized animals. A strong inhibition is compatible with a GABAergic projection, but no direct projection from the NAcc core to the DG has been described yet. The main target of the NAcc core, the VP is also GABAergic, but again a direction connection to the DG remains to be proven.

The NAcc is involved in neural plasticity, especially reward-motivated processes, ranging from addictions (Kelley et al. 1982; Di et al. 2004) to memory (Floresco et al. 1996; Levita et al. 2002; Fenu and Di 2003; Dalley et al. 2005; Hernandez et al. 2005; LaLumiere et al. 2005; Ferretti et al. 2007; Ramirez-Lugo et al. 2007). However, only a few studies have addressed the distinction between the

NAcc core and shell subregions (Li and Fleming 2003; Jongen-Relo et al. 2003; Klein et al. 2004). Since LTP is considered as a cellular correlate of learning and memory, our results lead to the prediction that the NAcc shell positively modulates memory acquisition, at least in hippocampus-dependent memory tasks. In agreement with our NAcc shell stimulation prior to DG early-LTP induction results, Gal et al (Gal et al. 1997) have shown that rats with electrolytic lesions in the NAcc shell had deficits in reference and working memories during the acquisition phase of a radial maze task. However, there are also reports showing no effects of NAcc shell manipulations on acquisition in hippocampus-dependent tasks (Floresco et al. 1996). Regarding the NAcc core, the prediction would be of a reduced memory acquisition. Reports on the NAcc core contribution to acquisition in hippocampus-dependent tasks are indicative of positive modulation (Smith-Roe et al. 1999) or no effect (Jongen-Relo et al. 2003).

Excitability differences have been observed between the NAcc core and shell neurons (Pennartz et al. 1992). The neurons in the NAcc core have negative mean resting potential and the mean input resistance is lower than that of the shell neurons. This means that, the NAcc shell projection neurons will be more excitable than that of the core neurons because of their passive membrane properties (Pennartz et al. 1992; Meredith et al. 1993). These results reflect the outcome of the present study, where stimulation of the NAcc core or shell before induction of DG early-LTP exhibit different modulatory effects except their output influences were indirect in the DG. The ambiguities over the pathway through which they modulate neuronal activities in the DG are still yet to be determined. Our results lead to the

prediction that the NAcc shell projection neurons are more excitable than the NAcc core projection neurons.

Similarly, Zahm et al. proposed an alternative route through which the NAcc shell subregion can indirectly modulate the activity of the NAcc core subregion in the rat brain (Zahm and Brog 1992; Zahm 1999). In their distinct description of the anatomical connections of the NAcc core and shell subregion, the shell project predominantly to the ventromedial ventral pallidum, this then project to the mediodorsal thalamus. The mediodorsal thalamus projection, then project back to the dorsal PFC which selectively project to the NAcc core subregion. However, through this indirect pathway, the NAcc shell subregion could potentially affect the activity of the NAcc core region output (Zahm and Brog 1992; Zahm 1999).

Networks mediating the NAcc influences on DG early-LTP

The network mediating the core influence on DG early-LTP remains therefore still to be clarified, but the effect of NAcc core stimulation reducing the baseline amplitude of the PS amplitude is also compatible with a GABAergic inhibitory effect. GABA receptors of type A, can reduce the excitability of its postsynaptic targets by a hyperpolarizing or shunting effect of opening chloride channels (Hille 1992; Kandel and Siegelbaum 2000; Olsen and Betz 2006). Tetanic stimulation of the PP (glutamatergic afferents) under this condition may fail to induce LTP, in correspondence with our results.

The long-term inhibition of the DG early-LTP suggests the involvement of other mechanisms beyond changes in membrane potential or membrane resistance. The hippocampal contains $GABA_B$ (B1a and B2) receptors coupled to protein G-second messenger systems that can exert long-term inhibitory effects on membrane potentials, transmitter release and act as negative regulator of the adenylate cyclase-PKA system (Couve et al. 2000) and thus, could appear as a likely mediator for this prolonged effect of stimulating the NAcc core on DG early-LTP. $GABA_B$ receptor inhibition is mainly mediated due to activation of K^+ channels initiating an outward K^+ conductance with a delayed onset but it could also be due to Ca^{2+} channels blockade resulting to inhibition of transmitter release if $GABA_B$ receptors are presynaptically located (Davies 2003). GABAergic projection originating in the EC runs in the PP to synapses directly on DG granule cells has been observed (Germroth et al. 1989; Fifkova et al. 1992) but it remain to be determine how stimulation of the NAcc core might activate this pathway to inhibit DG early-LTP induction.

In a recent review, Lisman and Grace (Lisman and Grace 2005) proposed the existence of a functional hippocampal-VTA loop involving the hippocampus, the subiculum, the NAcc, the VP and the VTA based on a functional studies (Floresco et al. 2001a; Floresco et al. 2001b; Floresco and Grace 2003; Goto and Grace 2005). They hypothesized that the loop has two arms namely downward arc and upward arm as described earlier in the introduction. The downward arc of the loop activation begins when the hippocampus detects newly arrived information that is not already stored in its long-term memory. But they suspect that an ultimate function of the

hippocampal-VTA loop relates to the need to protect previously stored information. Because synaptic modification is set in motion by neuronal activity, there is the potential that activation of this network under any condition may overwrite stored information (Lisman and Grace 2005). The downward arc of the proposed loop confirms our results of stimulating the NAcc core or shell after DG early-LTP induction with no modulatory influences, where NAcc core or shell stimulation 15 minutes after DG early-LTP induction has no modulatory effects on already established or ongoing plasticity processes. On the other hand, according to our results of prior stimulation of the NAcc 15 minutes before DG early-LTP induction, the upward arm of the loop view may apply to the NAcc shell, but not to the NAcc core. The induction of DG early-LTP 15 minutes after the NAcc core or shell stimulation, the NAcc core stimulation impaired DG early-LTP induction while the NAcc shell enhances it. More functional studies distinguishing the NAcc core and shell are required to clarify this aspect.

Additionally, the NAcc shell/LH/PP – granular cells' circuitry might possibly mediate the facilitating effect of NAcc shell stimulation on DG early-LTP induction (Lopez et al. 2008). Stratford and Kelley have shown that intra-shell muscimol administration markedly activates Fos expression throughout the LH (Stratford and Kelley 1999) and Heimer et al also have described a direct anatomical connection between the NAcc shell and the lateral hypothalamus (Heimer et al. 1991). Taking into account that the electrical stimulation of the LH inhibits LTP induction in the medial PP-DG synapses (Wayner et al. 1997). All these anatomical connection from the NAcc shell to LH and from the LH to DG is direct, therefore, NAcc shell

stimulation could release an inhibition on the LH (dis-inhibition) leading to an excitation of the efferent structure, DG (Taha and Fields 2006), thereby facilitating the effect of NAcc shell stimulation on DG early-LTP, since most of the projecting neurons from the NAcc are GABAergic (Meredith et al. 1993). An alternative but not exclusive possibility would involve the VTA, since this mesencephalic nucleus receives projections from the NAcc shell, both directly (Heimer et al. 1991; Usuda et al. 1998) and indirectly over the VP (Zahm 1989; Groenewegen et al. 1993). The VTA projects dopaminergic efferents to the hippocampal formation. Nevertheless, the contribution of DA to LTP in the DG (Kusuki et al. 1997) is not as clear as in the CA1 (Kulla and Manahan-Vaughan 2000), which might be a consequence of the fact that the major projections from the VTA are directed to CA1 (Gasbarri et al. 1994). Therefore, if the VTA is involved in processes described here, it might be an indirect action.

Even though, some fibers of the NAcc core project to the LH and the VTA, they are not as pronounced as those arising from the NAcc shell (Heimer et al. 1991; Usuda et al. 1998). The path through which the NAcc core inhibits DG early-LTP induction at the PP-DG synapses seems less evident since NAcc core efferents are mainly projected to the classical basal ganglia output structures (Heimer et al. 1991; Zahm and Brog 1992). Such pathways involve inhibitory action on pallidal neurons affecting the function of the subthalamic and thalamic neurons (Nakano 2000), from which a connection to the hippocampus are difficult to trace. However, there are evidence of a contribution of structures like the subthalamic nucleus, and the pedunculopontine nucleus. These structures receive a considerable direct projection

from the NAcc core (Usuda et al. 1998) and contribute to cognition and motivation (Dellu et al. 1991; Temel et al. 2005). All together, this evidence seems to indicate that their function goes beyond motility, and indirectly supports the effects described above. In addition, the pedunculopontine nucleus is able to modulate hippocampal theta rhythm (Kinney et al. 1998; Nowacka et al. 2002), a feature that further supports our tentative hypothesis.

No role of the NAcc in DG - synaptic tagging

It has been shown that the synergistic action of NMDA receptor activation and modulatory inputs leads to late-LTP in the DG (Swanson-Park et al. 1999; Frey et al. 2001). β-adrenergic receptors could modulate LTP via stimulation of the cAMP/protein kinase A cascade (Stanton and Sarvey 1987), which is of paramount importance for late-LTP (Frey et al. 1993; Quevedo et al. 1999). Furthermore, interactions between β-adrenergic and other types of receptors, such as muscarinic or serotonergic receptors (Wang et al. 1999; Watabe et al. 2000), could be also important for LTP modulation. It has been shown that an early-LTP induced by the application of WTET in the DG of freely moving animals, can be transformed into a late-LTP by the application of behavioral stimuli with a high motivational value within a distinct time window before or after DG early-LTP induction (Seidenbecher et al. 1997; Straube et al. 2003). This effect seems to depend on the ability of such stimuli to activate modulatory brain regions projecting to the DG, resulting in the synthesis of plasticity-related proteins required for late-LTP to occur. This has been well documented by the fact that the application of anisomycin, a protein synthesis

inhibitor, prevented behaviorally-induced LTP-reinforcement (Bergado et al. 2003; Straube et al. 2003).

The induction of LTP sets a tag at the activated synapses which allows the identification and insertion of the proteins required for a long lasting enhancement of synaptic efficacy. Affective modulation of memory is mediated by limbic structures such as the amygdala (McIntyre et al. 2003; Richter-Levin 2004), and it can be described as a universal mechanism, not restricted to a particular form of plasticity process or brain locus (Bergado et al. 2007). Taken into consideration, the fact that the NAcc receives a strong input from the BLA, we initially proposed that activation of the NAcc might activate heterosynaptic afferents, releasing neurotransmitters substances able to activate metabolic cascades leading to regulation of transcription, and therefore, the synthesis of plasticity proteins. The lack of reinforcement with the NAcc stimulation but rather inhibition, strongly suggest that it is not reinforcement candidate of the DG early-LTP.

Modulatory effect of DAergic activation

In a recent publication, we have shown that blockade of dopaminergic D1/D5-receptors in the NAcc resulted in a delayed-onset depression of baseline recordings in the DG as well as the prevention of any LTP-induction (Kudolo et al. 2010). There is a suggestive similarity between the results after NAcc core stimulation and those after injection of the D1/D5 receptor blocker, with the only exception of the f-EPSP baseline, which resulted depressed by SCH23390, but not by NAcc core stimulation. These coincidence is however difficult to interpret. DA D1 receptors are located pre-

and post-synaptically in the NAcc (Wong et al. 1999). On the other hand, SCH23390 exerts an inhibitory influence on the firing rate of NAcc neurons (Nicola and Deadwyler 2000) probably because it reduces the glutamate release (Kalivas and Duffy 1997) raising the apparent contradiction that stimulating the NAcc produced the same result as inhibiting it. Finally, it has to be remembered that though the injection was performed in the NAcc core, the small size of the injected molecules does not allow disclosing that some diffusion might have occurred to neighbouring regions like the NAcc shell or the caudate nucleus. Although these data do not allow an explanatory conclusion, they point to a complex interaction among different brain structures. As there is no direct evidence to establish a dopaminergic connection between NAcc and DG, the effects must involve indirect mechanisms, such as glutamatergic and/or GABAergic processes (see above).

Future studies will address the output in the DG early-LTP if DA in the NAcc is inhibited or facilitated by the application of DA antagonist or agonists which will partly help our understanding on the probable role of DA in NAcc and in DG LTP. In addition to DA, the studies will seek to explore the mechanisms through which the NAcc modulate neuronal activities in the DG and the neurotransmitters systems. Also, further functional studies may be required to clarify and distinguish the NAcc core and shell modulatory influences on DG synaptic plasticity. Further studies on the role of the amygdala and VTA as a gating system for information flow from the NAcc into the DG will help our understanding to the complex interplay of these brain structures

5. Conclusion

In summary, the principal findings of this dissertation are as follows:

- The results show that the induction and maintenance phase of plasticity processes in a specific brain region i.e. the DG of the hippocampus may depend on the direct and indirect inputs from other brain structures.

- NAcc activity can influence the induction of plasticity processes in other brain regions like the DG but has no effect on already established or on-going plasticity processes in that region.

- Stimulation of the NAcc core had a slight and statistically significant depressing effect on the control baseline while the NAcc shell had no effect on this baseline.

- Electrical activation of the NAcc core or shell has a differential modulatory effect on plastic phenomena in the DG.

- NAcc stimulation before or after DG early-LTP induction does not mimic the effect of the BLA as shown before by others. NAcc is not a suitable candidate brain structure to reinforce DG early- into late-LTP.

- Since LTP is considered to be a cellular model for learning and memory, the results indicate, that NAcc core and shell function can differentially modulate information processing in the dentate gyrus of the hippocampus.

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Appendices

I Zusammenfassung der Dissertation

Der Nucleus Accumbens ist ein im ventralen Striatum lokalisiertes Areal und zählt zu den Basalganglien. Er besteht aus zwei Teilen, dem Kern und der Hülle. Funktionell werden beide Kerne mit motiviertem Verhalten assoziiert. Der Nucleus Accumbens liegt zwischen dem limbischen System und dem Motorsystem. Er kombiniert und moduliert Eingänge aus diesen Strukturen, wie limbische Affekt-motivierte Handlungen und die motorische Planung. Innerviert wird der Nucleus Accumbens von limbischen Strukturen. Seine exzitatorischen Afferenzen kommen aus dem ventralen Hippocampus, der basolateralen Amygdala und dem medialen präfrontalen Kortex. Zusätzlich erhält er dopaminerge Inputs aus dem ventralen Tegmentum, welches mit Belohnungsprozessen assoziiert wird. Unser Labor hat in den letzten Jahren den Einfluss unterschiedlicher Gehirnstrukturen auf die synaptische Plastizität im Gyrus Dentatus charakterisiert, der/ein Teil der hippocampalen Formation ist. Unter synaptischer Plastizität versteht man eine Änderung der Effektivität in der Impulsübertragung an Synapsen, welche zum Speichern von Informationen in neuralen Netzwerken beitragen kann. Synaptische Plastizität kann sowohl von kurzfristiger als auch langanhaltender Dauer sein. Dies hängt sowohl von der synaptischen Aktivierung als auch von dem modulierenden heterosynaptischen Input ab. In der hier vorliegenden Untersuchung wurde das entsprechende Gehirnareal (Kern und Hülle des Nucleus Accumbens) in einer definierten Zeit vor oder nach der Induktion einer Langzeitpotenzierung im Gyrus dentatus elektrisch stimuliert. Bei der Aktivierung von heterosynaptisch

modulierenden Gehirnstrukturen ist es möglich eine frühe Proteinbiosynthese-unabhängige Langzeitpotenzierung (early-LTP), in eine späte Proteinbiosynthese-abhängige Langzeitpotenzierung (late-LTP) im Gyrus dentatus umzuwandeln. Wir stimulierten den Nucleus Accumbens Kern oder die Nucleus Accumbens Hülle durch den Tractus Perforans 15 Minuten vor oder nach der Induktion von early-LTP. Durch die Stimulation des Kerns oder der Hülle des Nucleus Accumbens nach early-LTP wurden weder Amplitude noch Dauer der early-LTP im Gyrus Dentatus signifikant verändert. Bei einem weiteren Experiment wurden Kern oder Hülle des Nucleus Accumbens 15 Minuten vor der Induktion von early-LTP im Gyrus Dentatus durch den Tractus Perforans stimuliert. Dies verhinderte die Induktion von early-LTP, bezogen auf das exzitatorische postsynaptische Feldpotential (f-EPSP). Dahingegen wurde die Populations-Spike-Amplitude (PSA) weniger als bei Kontrollen potenziert und fiel schnell auf Basalniveau ab. Die Stimulierung der Hülle modifizierte weder Amplitude noch Dauer der early-LTP mit statistischer Signifikanz. In Kontrollexperimenten wurde die Auswirkung der Stimulierung von entweder nur dem Kern oder der Hülle des Nucleus accumbens auf die Basalwerte im Gyrus Dentatus untersucht (ohne Aktivierung durch den Tractus Perforans). Die Ergebnisse dieser Kontrolluntersuchungen zeigten, dass die Stimulation einen geringfügigen, aber statistisch signifikanten, hemmenden Effekt auf die PSA bis zu 8 Stunden nach der Stimulation hatte. Keinen Effekt hatte die Stimulation im Rahmen dieser Kontrollexperimente jedoch auf das f-EPSP. Histologische Analysen wurden nach den Experimenten durchgeführt, um zu überprüfen, ob die Elektroden bei den Versuchstieren korrekt platziert wurden. Versuchstiere, bei denen dies nicht der Fall

war, wurden nicht in die Analyse mit einbezogen. Zusammenfassend lässt sich festhalten, dass die Stimulation des Nucleus Accumbens nach der Induktion von early-LTP im Gyrus Dentatus keinen Effekt auf den Zeitverlauf oder die späte Phase der Potenzierung hatte. Allerdings gab es einen Effekt auf den Zeitverlauf und die späte Phase der Potenzierung, wenn man den Nucleus Accumbens *vor* der Induktion von early-LTP im Gyrus Dentatus stimulierte.

II Curriculum vitae

John J.K. Kudolo

Personal information

Date of birth: 22-04-1977
Nationality: Ghanaian
Place of birth: Adidome, Ghana
Marital status: Married with a son

Education

Mar. 2007 – Nov. 2010:	**Biology/Neurobiology**, PhD. Otto-von-Guericke University of Magdeburg, Germany.
PhD. Dissertation:	“Influence of nucleus accumbens core or shell stimulation on early-LTP in the dentate gyrus of freely moving animals”
2004 – 2006:	**Biomedical Engineering**, MSc. University of Lübeck/ University of Applied Sciences, Lübeck, Germany
Master thesis:	“Stimulation of nerve fibers with uniform field”
2005 – 2006:	**Biomedical Eng. & Informatics**, MSc. Guest student, Aalborg University, Denmark
1997 – 2001:	**Physics** & Geology, BSc. University of Ghana (UG), Legon
1992 – 1996:	**Secondary school education** (Pre-university education), Ghana

III Scientific publications

1. **Kudolo J**, Tabassum H, Frey S, López J, Hassan H, Frey JU, Bergado JA (2010): Electrical and pharmacological manipulations of the nucleus accumbens core impair synaptic plasticity in the dentate gyrus of the rat. Neuroscience, 14; 68 (3):723-31.

Abstract & conference proceedings

1. **John Kudolo**, Thomas Scherf, Julietta U. Frey, Sabine Frey (2010): Influence of electrical stimulation of the ventral tegmental area on field potentiation in hippocampal CA1 of freely moving rats: synaptic tagging/capturing mechanisms in vivo, 550.3.2010, Neuroscience Meeting Planner. San Diego California. Society for Neuroscience 2010

2. **John Kudolo**, Jorge A. Bergado, Hadir Hassan, Julietta U. Frey (2009): Influence of nucleus accumbens core or shell stimulation on early long-term potentiation in the dentate gyrus of freely moving rats, 319.2.2009 Neuroscience Meeting Planner. Chicago IL: Society for Neuroscience, 2009. Online.

3. **John Kudolo**, Jorge A. Bergado, Julietta U. Frey (2009): Influence of nucleus accumbens core or shell stimulation early long-term potentiation in the dentate gyrus of freely moving rats. Proceedings of Göttingen Meeting of the German Neuroscience Society

IV Selbständigkeitserklärung

Erklärung

Hiermit erkläre ich, John J.K. Kudolo, dass ich die von mir eingereichte Dissertation mit dem Thema

“Influence of nucleus accumbens core or shell stimulation on early long-term potentiation in the dentate gyrus of freely moving animals”

selbständig verfaßt, nicht schon als Dissertation verwendet habe und die benutzten Hilfsmittel und Quellen vollständig angegeben wurden.

Weiterhin erkläre ich, dass ich weder diese noch eine andere Arbeit zur Erlangung des akademischen Grades doctor rerum naturalium (Dr. rer. nat.) an anderen Einrichtungen eingereicht habe

Magdeburg, 09.11.2010 Unterschrift: