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The role of NMDA receptor subunits in the regulation of synaptic plasticity in dorsomedial striatum in a model of paroxysmal dystonia

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I would like to dedicate my PhD research thesis to Dr Steven D. Kerr (Department of Pharmacology and Toxicology, Otago University, New Zealand) who has showed me the way to my science.

Acknowledgment

Ben Zoma (a Talmudic sage) asks: "Who is wise?"

He answers: "He who learns from every person".

(Pirke Avot 4:1)

Abstract

Dystonias are movement disorders whose pathomechanism is largely unknown. The dt^{sz} dystonic hamster mutant represents a model of primary paroxysmal dystonia, where alterations of striatal interneuron density and long term potentiation were described (Köhling et al., 2004, Gernert et al., 2000). In the present thesis, using corticostriatal slices, we explore in more detail whether long-term potentiation (LTP) and long-term depression (LTD) are shifted by a) behavioural stimulation or b) ontogenetic maturation using different stimulation protocols in the cortico-striatal synaptic pathway. The third aim of the thesis was c) to explore the role of NMDA receptors and their subunits in synaptic plasticity changes occurring with dystonia. Field extracellular recordings were conducted in dorsomedial striatum, and white matter was stimulated. Short and long term plasticity as well as input-output relationships were analysed. The main findings were: a. The occurrence of enhanced synaptic plasticity is not dependent on behavioural stimulation, while changes in excitability are. b. Ontogenetic maturation increases the dynamic range of synaptic plasticity under normal conditions, which is infringed in animals with dystonia, even though the symptoms have remitted. c. In dystonic tissue, LTP is dependent on NR2A, wheras in normal tissue, it depends on NR2B receptors. In conclusion, the functional shifts in NR2A vs. NR2B involvement in synaptic corticostriatal plasticity may be instrumental in the pathogenesis of dystonia in the dt^{sz} model.

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Abbreviations

1.	AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
2.	CA1	Cornu ammonis region 1
3.	CaMKII	Calcium Calmodulin dependent kinase two
4.	GABA	γ-aminobutyric acid
5.	GluR1	Glutamate receptor 1
6.	GPe	Globus pallidus external segment
7.	GPi	Globus pallidus internal segment
8.	HFS	High frequency stimulation
9.	D-AP5	D-2-amino-5-phosphonopentanoate
10.	D/NS	Dystonic non stimulated
11.	D/S	Dystonic stimulated
12.	D1 receptor	Dopamine 1 receptors
13.	D2 receptor	Dopamine 2 receptors
14.	dt^{sz}	Genetically dystonic hamster
15.	LFS	Low frequency stimulation
16.	LTP	Long- term potentiation
17.	LTD	Long-term depression
18.	NBQX	2,3-dioxo-6-nitro-7-sulfamoylbnzo(f)quinoxaline
19.	ND/NS	Non dystonic non stimulated
20.	ND/S	Non dystonic stimulated
21.	NMDA	N-methyl-D-aspartate
22.	NMDAR	N-methyl-D-aspartate receptor
23.	NR2A	N-methyl-D-aspartate receptor 2 subtype A
24.	NVP-AAAO77	(R)- [S)-1-(4-bromo-phenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl-phosphonic acid
25.	PKA	Protein kinase A
26.	PKC	Protein kinase C
27.	$_p$38 MAPK	$_p$38 mitogen-activated protein kinase
28.	Rap	Ras related protein

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Introduction

Striatum

Function

The basal ganglia are a major neural system which receives inputs from all cortical areas, and in turn affects the frontal cortex via its thalamic projections (Fig. 1) (Cavada and Goldman-Rakic, 1991; Faull et al., 1986). The striatum is the main input structure of the basal ganglia. It is divided into dorsal striatum which includes caudate and putamen, and the ventral striatum, which consists of the ventromedial parts of the caudate, putamen, olfactory tubercle, and nucleus accumbens which are further subdivided in medioventral shell and dorsolateral core. The neocortex projects mainly to the more dorsal parts of the striatum, while other regions such as amygdala and hippocampus project mainly to ventral parts of the striatum. While the dorsal striatum appears to be more important for voluntary motor functions, the ventral part of the striatum plays a major role in motivated and goal directed behaviours as well as the development and expression of addiction (Kelley, 1999). The caudate nucleus, putamen, nucleus accumbens and olfactory tubercle are collectively referred to as th*e striatum*. The term neo-striatum is sometime used to refer only to the caudate nucleus and putamen (Webster, 1979; Parent, 1986). The dorsal striatum is further divided into dorsolateral striatum and dorsomedial striatum. While visual and auditory cortical areas project to the dorsomedial *caudoputamen,* the motor areas and somatosensory areas of the cortex project to the dorsolateral part of the caudoputamen. Since the present PhD thesis is primarily concerned with the dorsomedial striatum, the reader should referred to Lopez-Fiqueroa et al., (1995); Yin and Knowlton, (2006); Voorn et al., (2004) for more details on dorsolateral striatum and Hyman, Malenka and Nestler, (2006), Hyman, (2006), and Nestler, (2002) for more details on ventral striatum. Physiological and anatomical studies have shown that different cortical areas project to distinct regions of the putamen and caudate. As a result, five different and parallel information processing circuits can be identified; a oculomotor circuit, a motor circuit, a dorsolateral prefrontal circuit, a anterior cingulated circuit, and a lateral orbitofrontal circuit (Alexander et al., 1986). The functional organisation of the corticostriatum is such that information initially processed in the cortex is transmitted to the striatum via the corticostriatal projection, integrated with the many other inputs to the basal ganglia (e.g. hippocampus, amygdala, intralaminar thalamic nuclei) which innervate the striatum, and then the information that was processed is transmitted to the output nuclei of the basal ganglia, the substantia nigra pars reticulata (SNr) and globus pallidus internal segment (GPi). These two output nuclei project to the ventral thalamus and then back to the cortex.

Little is known about the specific processing that takes place at each stage along the neocortico-striato-thalamic loop. Only a summary of the neocortico-striato-thalamic loop will be given here, but the reader is referred to Gerfen and Wilson, 1996; Smith et al., 1998; Utter and Basso, (2007) for more detail.

The cortico-basal ganglia-thalamocortical circuit

Albin and Delong (1989), (1990) proposed a unifying model of the functional organisation of the cortico-basal ganglia thalamocortical circuit. The transmission of cortical information through the basal ganglia occurs through two routes, the 'direct' and 'indirect' pathways (Figure 1) (Albin et al., 1989). In the *direct pathway* the information is transmitted directly from the striatum to the output nuclei, SNr and GPi. In the *indirect pathway,* corticostriatal information is transmitted indirectly to the output nuclei via external segment of the globus pallidus (GPe) which in turn projects to the subthalamic nucleus (STN) and then SNr and GPi. The information is then transmitted back to the cortex via the thalamus. Neurons in the direct pathway express dopamine D1 receptors, whereas striatal neurons of the indirect pathway bear dopamine D2 receptors (Obeso et al., 2000). Under resting conditions the output signal of the basal ganglia is inhibitory, and during the movement there is a loss of inhibition (DeLong, 1990; Albin, 1989). Projection neurons in the striatum are inhibitory GABAergic neurons. During associated behaviour i.e. when the system is activated by glutamatergic corticostriatal neurons, the activation of D1 dopamine receptors stimulates striatal output neurons via the direct pathway, which causes inhibition in SNr and GPi. This reduction in firing of the basal ganglia output leads to disinhibition of neurons in GPi and SNr, resulting in a net excitatory effect of GPi and SNr neurons. In contrast, activation of the indirect pathway leads to the opposite effect via D2 dopamine receptors, i.e. increased firing of output neurons and overall inhibition of basal ganglia activity. The balance between inhibition of output nuclei through the direct pathway and excitation through the indirect pathway is essential for normal motor function and it has been suggested that the indirect pathway acts to attenuate basal ganglia unwanted movement (Mink and Thach, 1993). A disruption in the balance between excitation and inhibition of the output nuclei via direct and indirect pathways is believed to underlie the motor dysfunction in certain diseases, including Parkinson disease's, Huntington's disease, paroxysmal dystonia (for more reading on cortico-basal ganglia-thalamocortical circuit the reader is refereed to (Haber, 2003; Utter and Basso, 2007 or Furuta and Kaneko, 2006).

Striatal Neurons

The striatum contains both projection neurons and few populations of interneurons (Kawaguchi et al., 1995). The principal type of neuron found in the striatum is a medium sized densely spiny neuron. These neurons comprise 95% of the total population of striatal neurons (Kemp and Powell, 1971a). Spiny neurons utilise GABA as their major neurotransmitter and are subdivided into two major subpopulations (Smith and Bolam, 1990; Smith et al., 1998). One subpopulation projects to GP and express D2 dopamine receptors and enkephalin. The second subpopulation projects to the output nuclei of the basal ganglia and express, in addition to GABA receptors, D1 dopamine receptors as well as substance P (Chang and Wilson, 1990). The remaining 5-10% of the neurons found in the striatum have medium to large perikarya with aspiny or spiny dendrites. They are believed to form several distinct classess of interneurons, for example cholinergic interneurons which comprise about 2% of all striatal neurons and appear to contact with dendritic spine and shafts of medium spiny neurons (Smith and Bolam, 1990; Bolam et al., 1984). Cholinergic interneurons are thought also to project directly to the substantia nigra or globus pallidus (Grofová, 1975). Corticostriatal terminals make synaptic contact with the heads of spines of spiny projection neurons which in turn give rise to the indirect and direct pathway (Hersch et al., 1995; Kincaid et al., 1998). The excitatory input from the cortex to spiny neurons is influenced by many other inputs to spiny neurons (Bolam and Bennett, 1995). The major synaptic target of the GABA interneurons are spiny output neurons (Bennet and Bolam, 1994) and this provide feed-forward inhibition of cortical information to spiny neurons (Plenz and Kitai, 1998; Jaeger et al., 1994). The precise role of this inhibition is currently unknown.

Neurochemistry of the Striatum

Like in other cortical regions, the medium sized spiny neurons of the striatum use the excitatory neurotransmitter glutamate (Garcia-Munoz et al., 1991) which in turn binds normally to D, L-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate acid (NMDA) receptors (Bernard and Bolam, 1998). Their activation leads to depolarization of the medium spiny neurons (Kita, 1996). The medium sized spiny neurons then uses GABA as neurotransmitter (Smith and Bolan, 1990). This suggests that the striatum is being activated by via corticostriatal synaptic pathway, whereas the GABAergic projections may mediate motor output. In addition, the corticostriatal synaptic pathway might

be influenced by other neurotransmitters including dopamine, acetylcholine, and opiates which may modulate striatal excitability which in turn interacts in a complex manner to influence learning and memory processes within the striatum. The precise mechanisms underlying the interactions between dopamine, acetylcholine and opiates are currently unknown (for extensive reading of neurochemistry and pharmacology of the major basal ganglia system please refer to David et al., 2005; Silkis et al., 2000; Joel and Weiner, 2000 and Conn et al., 2005).
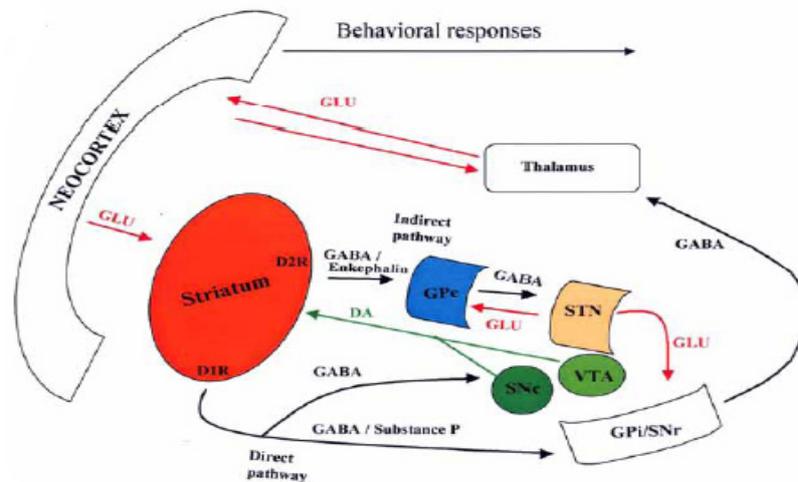
Figure 1. The circuitry of the striatum. Dorsal striatum receives dopaminergic inputs from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) and glutamatergic (GLU) afferents from the neocortex. GABAergic output from the striatum travels via the indirect pathway to the external segment of the globus pallidus (GPe) which in turn projects to the subthalamic nucleus (STN), resulting in a net excitation of the internal segment of the globus pallidus(GPi) and of the substantia nigra par reticulata (SNr). This effect is mediated by dopamine 2 receptors. Opposite inhibitory outputs from the striatum to the internal segment of the globus pallidus are generated via the direct pathway and the effect is mediated by dopamine 1 receptors. Internal segment of the globus pallidus and substantia nigra par reticulate then project inhibitory signals to the thalamus which releases excitatory output to the cortex (reproduced from Fasano and Brambilla, 2002 with permission).

Dystonia

General Background

Dystonia is a movement disorder characterised by twisted postures, muscle spasms, bizarre walking with bending, twisting of the torso, and development of sustained. Dystonia is the third most common movement disorder after essential tremor and Parkinson disease. The term dystonia was first described by Oppenheim in 1911, when he applied the name dystonia musculurum to a childhood-onset form of generalised dystonia (Oppenheim, 1911). The hallmark of dystonia is the cocontraction of agonist and antagonist muscles owing to a failure of normal reciprocal inhibition, with overflow or spread into other regions (Hallett, 1998; but see Malfait and Sanger, 2007). Dystonia in its various forms has emerged as a common neurological disorder (Marsden and Quinn, 1990). Dystonias are classified, depending on the etiology, on the age at onset and regional distribution of abnormal movements (Fahn et al., 1987). Dystonia can be further classified in terms of etiology; it may be arise sporadically (primary) or as a manifestation of a variety of definable neurological diseases and acquired brain lesions (secondary or symptomatic) (McGeer and McGeer, 1995; Calne and Lang, 1988). Dystonia is believed to be a disease of basal ganglia (Hallett, 1993), produce secondary dystonia lesions within the basal ganglia. However, the neurochemical and anatomical basis for dystonia is largely unknown at the present time.

Definition and Classification of Dystonia

Two main groups of dystonia can be classified by etiologically: primary (idiopathic) and secondary (symptomatic). Dystonia can be further classified according to the regional distribution of affected body parts. For example in focal dystonia, the abnormal movements affect a single body region. As more widespread form of focal dystonia, segmental dystonia two or more body segments affected. Cervical dystonia is the most common form of the focal dystonias. Dystonia can be also generalised or multifocal dystonia. Dystonia can vary by age at onset. Early onset dystonia (9 years old) usually affects an arm or leg. In late onset primary dystonia (20 years), neck or cranial muscles are commonly affected, and the legs less too (Fahn et al., 1987). Dystonia further include paroxysmal dyskinesias which are characterised by episodic sudden dystonic movement or posture (Fahn, 1994). The paroxysmal dyskinesias are further subdivided into paroxysmal kinesigenic dystonias and non-paroxysmal kinesigenic

(Fahn, 1994). Non-kinesigenic paroxysmal dystonia can be provoked by stress or alcohol, and dystonic attacks are longer (from to 4 hours up to 2 days), occur less frequently (no more than three times per day), whereas paroxysmal kinesigenic dystonia is usually short-lasting only short-lasting (only few seconds). This thesis is only concerned with the non-kinesigenic paroxysmal dystonia. For more reading on dystonia and on the treatment of dystonia the reader should referr to Geyer and Bressman, 2006; Richter and Löscher, 1997, Bhidayasiri and Tarsy, 2006.

Animal Model of Dystonia (Dystonic Hamsters)

There are number of experimental animal models that are exist for distonia. Since this thesis mainly concerned with dystonic hamsters, the reader is referred to Richter and Löscher, (1997) for an extensive overview. Dystonic hamsters (dt^{sz}) represent one of the best model of dystonia since it bears a close resemblance to generalized paroxysmal dystonia in humans. Usually it can be induced by stress, and dystonic attacks can last for hours, and vary in severity, progressing from head to the extremities (Yoon et al., 1976). The dystonic syndrome in dt^{sz} mutants shows an age-dependent time course. The severity of dystonia reaches a maximum at an age of about 32-42 days. Then, the severity slowly declines until complete remission of stress-inducible dystonic attack occur at an age about 10 weeks. The severity of dystonia can be rated by a score system: stage 1, flat body posture; stage 2, facial contortions, rearing with forelimbs crossing, disturbed gait with hyperextended forepaws; stage 3, hyperextended hindlimbs so that the animals appear to walk on triptoes; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; stage 6, a complete immobilization in a twisted, hunched posture with hind-and forelimbs tonically extended. The hamsters completely recover after about 30 minutes and onwards the immobilization is slowly declines.

Neuropathology of Dystonia

Primary (idiopathic) and secondary (symptomatic) dystonia are considered to be mainly the disorder of basal ganglia, with symptomatic dystonia being often associated with lesions within the basal ganglia, particularly within putamen, globus pallidus and the caudate nuclei (Hallet, 1993). Disruptions in the function of the basal ganglia circuit will lead to errors in

scaling of movement. These errors could lead to an either hypokinetic or hyperkinetic movement disorder. The hypokinetic disorder of movement develops as a result of excessive activity in GPi. Excessive activity of GPi occurs as a result of a decreased inhibitory output from GPe (indirect pathway) and an enhanced striatal projection to GPi (direct pathway) (Fig. 2). At the same time the excitatory projection from subthalamic nucleus (STN) to the GPi is also enhanced. Both the decrease of activity in the direct pathway and increase in activity in indirect pathway as well as an enhanced in activity from STN lead to an excessive increase in inhibitory output from the GPi, suppression of thalamocortical activity, and the development of hypokinetic disorder, like parkinsonism. In hyperkinetic disorders like in dystonia or drug-induced dyskinesias in Parkinson's disease, the opposite changes in mean discharge rate of GPi occurr (DeLong, 1990). It has been suggested that a loss of excitatory drive from the STN to GPi would lower inhibitory output from GPi to the thalamus which in turn disinhibits the motor thalamus and the cortex and results in excessive and involuntary movements. Indeed, several studied indicate that the firing rate of GPi is decreased in hypokinetic disorders (Filion et al., 1991; Gernet et al., 2000). Fillion et al., (1991) showed that all GPi neurons decreased their firing rate following apomorphine application. Interestingly, Gernet et al (2000) have demonstrated that striatal GABAergic interneuron density is reduced in dystonic hamsters. These results are consistent with the observation that a significantly decreased basal discharge rate of GPi was found in dystonic hamsters (Gernet et al., 1999a).
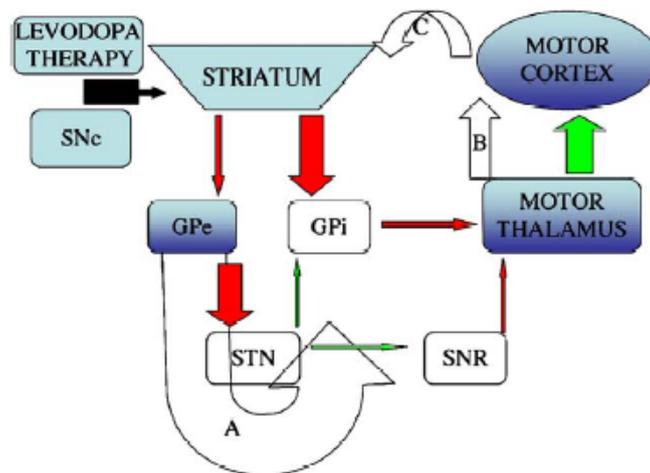
Figure 2. Pathophysiological theory of dystonia and drug-induced dyskinesias. It is suggested that an excessive decrease in GPi activity disinhibits the motor thalamus and the cortex, giving rise to abnormal increase in cortical drive and consequent excessive motor movements. (for more details see, Deogaonkar and Subramanian, 2005, reproduced from Deogaonkar and Subramanian, 2005 with permission).

Synaptic Plasticity

A brief history of synaptic plasticity

Learning may be described as the mechanism by which new information about the world is acquired, and memory as the mechanism by which that knowledge is retained. Cajal (1913) originally hypothesized that information storage relies on the changes in the strength of synaptic connections between neurons that fire. In 1949, Hebb supported this hypothesis and proposed that if two neurons are active at the same time, the synaptic efficiency of the appropriate synapse will be strengthened (Hebb, 1949). In 1957, Scoville and Milner reported that bilateral medial temporal-lobe resection, including a structure called the hippocampus, causes a persistent impairment of recent memory (Scoville and Milner, 1957). They concluded that the hippocampus is critically involved in the retention of current experience. What was striking about their study is that other cognitive functions were preserved with the now famous patient H. M. H. M.' s language and reasoning abilities were unchanged and his performance on an IQ test was increased. Remote memories were intact. More recent anatomical studies of H. M., and other human patients with amnesic syndromes, as well as studies in animals , suggest that the memory deficits in H. M. arises most probably from damage to the hippocampus and cortical structures immediately surrounding the medial temporal lobe (Milner et al., 1998). In 1966, Anderson and Lomo reported that a single, short test shock, following an initial period of conditioning test shocks to the perforant path, elicited a potentiated response in the dentate gyrus. Lynch et al., (1983) reported that a tetanic stimulation of one pathway in the CA1 region of the hippocampus depresses the effectiveness of the other synapses. They called this phenomenon a heterosynaptic LTD which can be observed in the dentate gyrus and in the CA1 region of the hippocampus *in vitro*. Dudek and Bear (1992) reported that several hundred stimuli delivered at low frequency (1-3 Hz) produced a sustained depression of a modest, but significant amplitude. This phenomenon is known as homosynaptic long-term depression which is much harder to demonstrate in the dentate gyrus i*n vivo* than heterosynaptic LTD. The phenomena of LTP and LTD are not restricted to the synapses in the hippocampus, but found in many parts of the brain. Since this thesis is concerned with the dorsomedial striatal region, the reader is refereed to Jörntell and Hansel, (2006); Collingridge and Watkins, (1994), Anwyl, (2006), Lynch, (2004), Malenka and Bear, (2004), Richter-Levin, (2004), for more detail.

Collingridge et al., (1983) made the momentous discovery that the selective NMDA receptor antagonist DL-2-amino-5-phosphononalerate (APV) blocks the induction of LTP. In 1983, Lynch and his colleagues illustrated that intracellular injection of the calcium chelator N,N,N',N'-tetraacetic acid (EGTA) into pyramidal cells of the CA1 region of the hippocampus blocked the induction of LTP due to stimulation of the Schaffer collaterals. It became clear that the LTP phenomenon is an NMDA receptor dependent process. Low-frequency stimulation induced LTD usually too requires NMDA receptor activation (Dudek and Bear, 1992; Mulkey and Malenka, 1992). However, there are also reports of NMDA receptor independent LTP and LTD (discussed further below). More than 120 molecules have been implicated in LTP and LTD. The most important of them in corticostriatal synapses are NMDA and Dopamine receptors. The details of molecular and cellular mechanisms of synaptic plasticity in corticostriatal synapses will be revised in this introductory part of this thesis.

Synaptic plasticity in hippocampus

The ability to remember is profoundly the most significant and distinctive feature of our existence. We are largely defined by what we have learned and what we have remembered. Conversely, impairments in learning and memory can lead to devastating memory losses. Storage of memories in the brain almost certainly involves some form of synaptic modifications. The guiding principle for such modification was proposed by Donald Hebb in his book, The Organization of Behavior (Hebb, 1949). "When an axon of cell A is near enough to excite cell B or repeatedly or consistently takes part in firing it, some growth or metabolic changes takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased". Hebb postulates that if two neurons are active at the same time, the synaptic efficiency of the appropriate synapse will be strengthened. It took almost 25 years to discover a process by which strengthening of synaptic connections can be achieved, and the importance of one model that seemed to fit Hebb's idea.

This model is known as a long-term potentiation (LTP). LTP was originally induced in the hippocampus by stimulation of axons of the perforant path and potentiation of the postsynaptic potentials in the dentate gyrus (Bliss and Lomo,1973). The potentiation was found to be input-specific, in that stimulation of the medial path did not potentiate the lateral perforant path and vice versa. Since this thesis is concerned with LTP and LTD in the

corticostriatal synapses, the reader is referred to Collingridge and Watkins, (1994), Lynch, (2004), Ito, (2002), Jay, (2003), Malenka and Bear (2004), Richter-Levin et al., (2004), Collingridge et al., (2004). Bear and Abraham, (1996), Kemp and Bashir, (2001), Anwyl, (2006) for further details and discussion.

Briefly, LTP in the hippocampus has been widely studied since it is believed that the mechanisms involved in its induction, expression, and maintenance are fundamental to learning and memory (Bliss and Collingridge, 1993). The reasons for this are:

1. LTP is an enhancement of synaptic efficiency that can be induced by high frequency, or by low frequency stimulation (Bliss and Collingridge, 1993).

2. LTP can last for an extended period of time (from weeks to months *in vivo*) (Abraham et al., 2002).

3. It is most prominent in regions of the brain that are strongly implicated in learning and memory (e.g. neocortex and hippocampus).

4. LTP is specific to tetanized inputs: the non-tetanized inputs are not potentiated (MacNaugton, et al., 1978).

5. LTP has Hebbian-like properties, in that it requires conjoint pre-and post-synaptic activity for its generation (Bliss and Lomo, 1973).

6. There is the requirement for co-operativity amongst afferent fibres to induce LTP (MacNaugton et al., 1978).

7. Associativity amongst afferents can also be demonstrated, i.e. a tetanus too weak to elicit LTP will do so, if paired with a strong tetanus (MacNaugton et al., 1978).

8. Drug treatments that selectively block the induction of LTP also selectively impair learning and memory (Riedel et al., 2003).

Synaptic plasticity in neostriatum

The corticostriatal projection originates from all areas of the cerebral cortex (McGeorge and Faull, 1989) and releases glutamate into the striatum (Perschak and Cuenod, 1990). The two classical forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD) have been found at corticostriatal synapses on medium spiny neurons, both in *vitro* and in *vivo* (Fig. 3) (Partridge et al., 2000, Reynolds and Wickens, 2002). Since this thesis concerned with dorsomedial striatum, the reader is referred to Kelley et al., (2003) for more reading on synaptic plasticity in the ventral striatum. Recently, a complex form of LTP has

also been found in cholinergic interneurons after high frequency stimulation (for more reading on synaptic plasticity in cholinergic interneurons the reader is referred to Suzuki et al., 2001; Wang et al., 2006; Centonze et al., 1999). LTD was reported to be the major form of corticostriatal plasticity (Calabresi et al., 1992a). In the striatum, the induction of LTP and LTD is induced by the high frequency stimulation (HFS) of corticostriatal fibers. In the presence of physiological concentration of magnesium Mg^{2+} (1.2 mM) high frequency stimulation can induce LTD (Calabresi et al., 1992). Recently, Reynolds et al., (2000) have recently found that robust LTD is also expressed by corticostriatal synapses in *vivo*. However, the he anatomical location of the neurons within the striatum seems to influence the effect of corticostriatal plasticity. If the stimulation is conducted in the dorsolateral striatum which receives input primarily from sensorimotor cortex, it seem to show mainly depression of corticostriatal synapses, whereas neurons that are located in dorsomedial striatum indeed favour potentiation following HFS of corticostriatal synapses (Dang et al., 2006; Yin et al., 2006; Ronesi and Lovinger, 2005; Partridge et al., 2000). The reason for that it is currently unknown, but it may reflect the existence of D2-dopamine receptors (Joyce and Marshall, 1987). Lack of D2-dopamine receptors in dorsomedial striatum could favour the induction of potentiation in dorsomedial striatum. Another reason may rest on regional differences in glutamate and dopamine release presynaptically. Indeed, recently Smith et al., (2001) have found that short term plasticity and LTP is increased medially and the effect was dependent on the blockade of $GABA_A$ receptors as well as on elimination of dopaminergic input from nigrostriatal synapses (Smith et al., 2001). At low magnesium concentrations, HFS also in the lateral striatum produces NMDAR-dependent LTP which is blocked by AP-5, an NMDA receptor antagonist (Calabresi et al., 1992b). However, the presence or absence of Mg^{2+} and the anatomical location are the not the only switch between the induction of LTP and LTD. The age of the animal is also an important factor for the switch between the induction of LTP and LTD in corticostriatal synaptic pathway. Partridge et al., (2000) showed that the dorsolateral region of the striatum tends to express LTP from 12-14 days old animals, whereas LTD was found in slices from rats aged 15-34 days. Interestingly, these authors also found that synaptic plasticity is not changed in dorsomedial striatum, an NMDA-dependent LTP was found in both groups. Lastly, the location of the stimulating electrode used to activate corticostriatal afferents also influences the degree of synaptic plasticity in corticostriatal synaptic pathway. If the stimulating electrode is located near the white matter it can directly cause the release of certain neurotransmitters such as dopamine or GABA. This may induce an LTD in corticostriatal afferent. If the stimulating electrode is located above dorsomedial

striatum on the cortical side, LTP is easily induced. This minimal current spread into the striatum is believed to minimizes the release of large dopamine transients that bias toward LTD (Reynolds and Wickens, 2000, Wickens et al., 1996). In the next sections, we will examine several induction mechanisms of LTP and LTD at corticostriatal synapses.

Dopamine receptors and LTP in corticostriatal fibers

Dopamine is essential for induction of LTP in corticostriatal fibers. Localization studies have illustrated that D1-like (D1 and D5) receptors are located at the postsynaptic level, whereas D2-like receptors (D2, D3 and D4) receptors are located both at the presynaptic and the postsynaptic levels. D1-like receptors are mostly located on medium spiny output GABAergic neurons. D2-like receptors are located on dopaminergic neurons and on GABAergic output neurons (Bergson et al., 1995; Huang et al., 1992; Missale et al., 1998). At the receptor level, D1, D2, and D5 receptors are expressed both in the caudate-putamen and in nucleus accumbens. D3 receptors are mainly expressed in the nucleus accumbens and poorly expressed in caudate and putamen (Bouthenet et al., 1991; Diaz et al., 1994; Diaz et al., 1995). Both D1 and D2 receptors are found at high levels in the dorsal striatum. Blockade of dopamine D1-like receptors completely blocks the induction of NMDA-dependent LTP in corticostriatal synapses (Kerr and Wickens, 2001). Thus, NMDA receptor activation is not enough for LTP induction in free magnesium solution, and dopamine acting at D1-like receptors is a mandatory requirement. Recently, Centonze et al., (2003) have illustrated that the ablation of D1 receptors disrupts corticostriatal LTP, whereas pharmacological blockade of D5 receptors by SCH23390 prevents LTD in corticostriatal synapses. This suggests that D1 and D5 receptors differently regulate synaptic plasticity in corticostriatal fibers (for more information on dopamine-dependent plasticity, the reader is referred to (Reynolds and Wickens, 2002).

Dopamine receptors and LTD in corticostriatal fibers

Dopamine is also essential for the induction of the corticostriatal LTD. LTD in the corticostriatal synaptic pathway is non-NMDA dependent process (Calabresi et al., 1992b). In the striatum, the induction of LTD requires the activation of dopamine D1/D5 and D2 receptors. It is also requires the release of dopamine. LTD in the striatum is absent in mice lacking D2 receptors, whereas pharmacological blockade of D5 receptors prevents LTD in corticostriatal synapses (Calabresi et al., 1997; Centonze et al., 2003). Indeed, it has been recently shown that LTD induction in medium spiny neurons is dependent on activation of D2 dopamine receptors (Wang et al., 2006). It has been suggested that both D1 and D2 dopamine receptors cooperate to induce corticostriatal LTD, but with distinct cellular mechanisms. D1 dopamine receptors act via G-proteins, positively linked to the stimulation of adenylyl cyclase and lead to activation of protein kinase A (PKA) through cyclic adenosine 3'5' monophosphate (cAMP). Stimulation of D2-like dopamine receptors exerts opposite effects by inhibiting PKA (Vallone et al., 2000; Stoof and Kebabian, 1981). The exact cellular mechanism for D1/D2 cooperation in the induction of corticostriatal LTD and the locus of expression at the present time are not fully understood yet. Another molecule which is heavily implicated in corticostriatal LTD is dopamine-and cAMP-regulated phosphoprotein 32 kDA (DARPP-32). It is present in a high concentration in the striatum and it inhibits protein phosphatase-1 (PP-1) in phosphorylated form, (Greengard et al., 1999). Mice that are deficit of DARPP exhibit no LTD nor LTP (Calabresi et al., 2000b). This suggests that DARPP is a critical molecule for the induction of LTP and LTD in corticostriatal synapses. The induction of LTP and LTD by DARPP is mediated by two distinct pathways. LTP induction requires the activation of PKA, whereas the LTD induction seems to require the activation of nitric oxide via protein kinase G (PKG). For more information on corticostriatal LTD the reader is referred to Gubellini et al., (2004); Reynolds and Wickens, (2002); Pisani et al., (2005); Jay, (2003); Fasano and Brambilla, (2002); Anwyl, (2006); Centonze et al., (2001); Silkis, (2000), Calabresi et al., (1996).
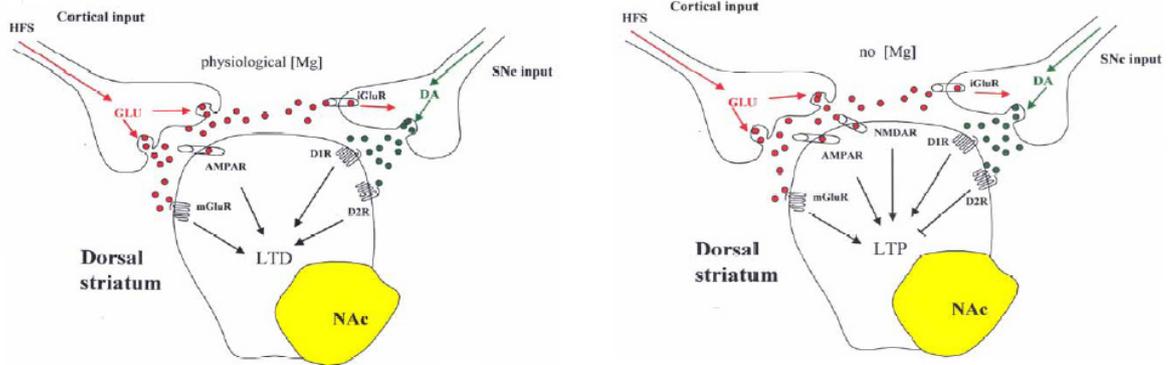
Figure 3. General mechanisms of synaptic plasticity in corticostriatal synapses. The general mechanisms of LTP and LTD in corticostriatal synaptic pathways. In dorsal striatum, in the presence of physiological magnesium, high frequency stimulation of the cortical input produces long term depression of the evoked responses. This process mediated by non-NMDA glutamate receptors (AMPA and mGluRs subfamilies), but also involves of Dopamine D1 and D2 dopamine receptors. In the absence of magnesium, high frequency stimulation of the cortical input produces long-term potentiation of the evoked responses. This phenomena relies on the activation of both NMDA and dopamine D1 dopamine receptors, but is inhibited by D2 dopamine receptors (Reproduced from Fasano and Brambilla, 2002 with permission).

Mechanisms Underlying Long-Term Potentiation

NMDA receptor and Long-Term Potentiation

The key molecule that underlyies LTP, is the activation of the NMDA receptors (Elgersma and Silva, 1999; Collingridge et al., 1983). Collingridge et al., (1983) made the significant discovery that the selective NMDA receptor antagonist DL-2-amino-5-phosphononvalerate (APV) blocks the induction of LTP, but it has no effect on basal synaptic potentials following to stimulation of the Schaffer collaterals of the CA1 region of the hippocampus (Collingridge et al., 1983). Their conclusion was that NMDA receptors are involved in the induction of LTP. Subsequent studies have extended their finding (see Harris et al., 1984; Wigstrom et al., 1986a; Reymann et al., 1989). The question was to determine, which signal caused the activation of NMDA receptors to produce long-lasting enhancement of synaptic efficacy? Unlike the other glutamatergic receptors, the opening of NMDA receptor allows a considerable flow of Ca^{2+} ions into the postsynaptic cell (Lynch et al., 1983; Malenka et al., 1988). In addition, the NMDA receptor requires a large membrane depolarization in order to be functionally opened (Collingridge and Watkins, 1984). Lynch et al., (1983) showed that intracellular injection of the calcium chelator N, N, N',N'-tetraacetic acid (EGTA) into pyramidal cells of the CA1 region blocks the induction of LTP in the CA1 region of the hippocampus (Lynch et al., 1983). These results suggests, that the postsynaptic increase in calcium is mediated through the activation of the NMDA receptors. Due to the voltage dependent blockade of NMDA receptor by Mg^{2+} ions, the activation of NMDA receptor requires simultaneous presynaptic glutamate release and post-synaptic depolarization and therefore serves as a coincidence detector of correlated pre and postsynaptic activities (Nowak et al., 1984; Lynch et al., 2004). NMDA receptors as discussed in the previous chapter are multimeric proteins that exist in the central nervous system. NMDA and AMPA mediatd excitatory responses have been described in the striatum (Wilson, 1993; Calabresi et al., 1996). The AMPA and NMDA receptor subunits are primarily located at glutamatergic synapses and they do not express at GABAergic synapses in the striatum. NR1 and NR2A/B subunit coexist in a large proportion of glutamatergic synapses in the striatum (Amara et al., 2002; Ango et al., 2000). They are formed from two obligatory NR1 subunits, usually paired with two NR2A or two NR2B subunits (see Collingridge and Watkins, 1984). The question that puzzled many investigators was which subunit had a dominant role in LTP. Köhr et al., (2003) have shown that both NR2A and NR2B subunits of NMDARs activate signalling

pathways which lead to LTP. Hrabetova et al., (2000) have demonstrated that both subunits (NR2A and NR2B) are required for LTP. Recently Liu et al., (2004) using two NR2B specific antagonists together with NR2A specific antagonists, illustrated for the first time that the NR2A subunit is implicated in LTP in the CA1 region of the hippocampus. Liu et al., (2003) employed the NR2A selective antagonist (NVP-AAM077) to dissect subunit involvement of NR2A and NR2B in LTP and LTD in the CA1 region of the hippocampus. Morishita et al., (2006) have found that the activation of NR2B-containing NMDA receptors is not required for NMDA receptor-depedent LTD in the CA1 region of the hippocampus, whereas Fox et al., (2006) showed for the first time in *vivo* that both NR2A and NR2B can play roles in LTP and LTD in the CA1 region of the hippocampus. At the present time it is currently unknown which subunit does play role in the induction of LTP in corticostriatal pathway, but the recent study of Dang et al., (2006) has demonstrated that mice lacking NMDAR1 subunit in the striatum have no striatal LTP and impaired motor learning. This indicates that NR1 subunit is requires for NMDA-dependent LTP in corticostriatal synapses. An interesting question that arises is, how do different NMDA receptor subunits produce different form of plasticity? Although this remains a matter of debate, one possible explanation is that NR2A-containing and NR2B-containing NMDA EPSC's is have different kinetics (Cull-Candy et al., 2001) which provide different levels of intracellular calcium required for LTP and LTD (Lisman, 1989). In addition to distinct kinetics of calcium influx through NR2A and NR2B subunits, different NR2 subunits may couple to different postsynaptic signalling pathways (Sheng and Park, 2000), which could determine the direction of synaptic changes.

AMPA receptor phosphorylation and synaptic plasticity

Protein phosphorylation plays an important role in the regulation of neuronal function, as it does in almost every cell type (Greengard, 2001). Phosphorylation of ionotropic glutamate receptors has been shown to be a major event in their functional regulation and in the regulation of synaptic plasticity (Fig. 4) (Soderling et al., 2000). There is considerable evidence that indicates that protein kinases play a critical role in the generation of LTP. The question that arises is, what is the particular protein kinase involved in LTP? There are certain kinases, for example CaMKII, that have been proved to mediate direct signals which generate LTP, while others (e.g. PKA or PKC) may modulate its generation (Malenka et al., 1986). Ser831 and Ser845, which are located in the C-terminal domain of the GluR1, were shown to

be the major phosphorylation sites in the GluR1 subunit of AMPA receptor. During LTP, the CaMKII-phosphorylation site on the GluR1, Ser831, is phosphorylated (Barria et al., 1997, but see Hayashi et al., 2000). PKA which is another protein kinase, phosphorylates Ser845 (Roche et al., 1996). Phosphorylation of Ser831on the GluR1 subunit can increase conductance through the GluR1 subunit, and AMPA receptors show increased conductance during LTP (Benke et al., 1998). Phosphorylation of Ser845 of the GluR1 by PKA causes incorporation of the GluR1-containing AMPA receptors into the synapses, since mutations at Ser845, prevent delivery of the GluR1 to the synapses (Shi et al., 2001). Recent studies indicate that activity-driven phosphorylation of the GluR4 by PKA is necessary and sufficient for the delivery of these recombinant AMPA receptors to the synapses during development (Esteban et al., 2003, Zhu et al., 2000). Thus, a mechanism that mediates plasticity early in development (with GluR4) becomes a gate for plasticity (with GluR1) later in development.

AMPA receptor subunits and LTP

AMPA receptors are heterotetrameric complexes that are composed of various combinations of four subunits (GluR1-GluR4) (Hollmann and Heinemann, 1994). In the adult hippocampus, two major subtypes of AMPA receptor exist that contain either GluR1 and GluR2 or GluR2 and GluR3 subunits (Wenthold et al., 1996). GluR4 is mainly expressed early in development (Zhu et al., 2000). The question that arises is, what is the subunit of AMPA receptors which is implicated in LTP? Several findings indicate that GluR1 subunit of AMPA receptor is important during LTP (Lee et al., 2003; Andrasfalvy et al., 2003). GluR1 containing AMPA receptors are thought to be involved in the regulation of synaptic strength in many neurons including CA1 neurons. Lee et al., (2003) have shown that GluR1 phosphorylation site mutant mice show reduced LTP compared to wild-type littermates and lack NMDA receptor-dependent LTD. Andrasfalvy et al., (2003) showed that AMPA receptor currents of GluR1 mutant mice are severely reduced in amplitude. Interestingly, Meng et al., (2003) have demonstrated that LTP could be established and also dramatically enhanced in the absence of GluR2/3, indicating that GluR1 is sufficient for the expression of hippocampal LTP. These results are consistent with the observations that LTP is impaired in GluR1 knockout mice (Zamanillo et al., 1999). In contrast, a recent report illustrated an additional LTP which is independent of GluR1 phosphorylation at serine 831 and serine 845 and which is operative in mice under 3 weeks of age (Jensen et al., 2003). Jensen et al., (2003) have demonstrated that in very young mice, LTP is independent of the GluR1 subunit, and during maturation of

hippocampal connections between days 15 to days 42, the GluR1 independent form slowly decreases, while the GluR1 dependent form increases to become dominant in the adult brain. What is the subunit that is implicated in LTP in young animals? Kolleker et al., (2003) showed that in young animals, the GluR2 and not GluR1 subunit is heavily implicated in LTP. The GluR2 primary transcript gives rise to two C-terminal alternative splice variants, a "short" form and "long" form. Kolleker et al., (2003) further illustrated that the GluR2 long form and not the short form is implicated in LTP, since inhibition of GluR2 long form transport to the synapses during the induction of LTP resulted in a complete loss of LTP in mice lacking GluR1 subunit. These results suggest that LTP in immature animals is dependent on the GluR2 subunit of AMPA receptors, whereas in mature animals the GluR1 subunit is a critical subunit which is heavily implicated in enhancement of synaptic efficacy. Recent evidence suggested that the NMDA receptor dependent trafficking of postsynaptic AMPA receptors is a key element in plasticity (Lee et al., 1998; Lu et al., 2001; Shi et al., 1999). What is the molecular mechanism that links NMDA receptor activation to AMPA receptor trafficking? Two studies have shown the possible mechanistic link between NMDA receptor activation and AMPA receptor trafficking. Man et al., (2003) showed that a key molecule that links NMDA and AMPA receptors is CaMKII and the small Ras family GTPase Ras molecule (Man et al., 2003). Man et al., (2003) demonstrated that in cultured hippocampal neurons, the activation of NMDA receptors with glycine lead to a transient rise of $[Ca^{2+}]_I$ which in turn activates and recruites CaMKII and Ras. Ras then binds to, and activates AMPA-associated PI3K, which leads to long lasting facilitation of AMPA insertion. How exactly does the activation of PI3K lead to AMPA receptor insertion into the synapses? This remains to be established. Recently Krapivinsky et al., (2004) have demonstrated the role of the GTPase-activating protein SynGAP in the signalling transduction cascade between NMDA and AMPA receptors in cultured hippocampal neurons. Krapivinsky et al., (2004) proposed that in dormant neurons, SynGAP is phosphorylated by CaMKII. SynGAP and CaMKII are coupled via direct binding to PDZ domains of the multi-PDZ domain protein MUPP1. During stimulation of NMDA mediated Ca^{2+}, or disruption of the SynGAP-MUPP1, SynGAP is dephosphorylated, which lead to dissociation of CaMKII from the SynGAP-MUPP1 complex. This dephosphorylation inactivates indirectly another molecule, which is known as p38 MAPK via the inactivation of another small GTPase molecule known as Rap. The inactivation of p38MAPK causes incorporation of AMPA receptors into the synapse. These results suggest that small Ras family GTPases Ras and Rap are crucial for the maintenance and the expression of LTP. Recently, Palmer et al, (2005) showed for the first

time that hippocalcin which is a calcium sensor regulate AMPA receptor endocytosis by directing AP2 to AMPA receptors to enable their regulation through internalization in response to appropriate synaptic activity. Palmer et al., (2005) further illustrated that hippocalcin, act as a sensor which couples NMDA-dependent activation to regulate endocytosis of AMPA receptors during LTD. Thus, this result suggest that hippocalcin is critical molecule for the induction of LTD. Studies of AMPA receptor trafficking in synaptic plasticity have focused on the idea that an alternation in AMPA number is one of the mechanisms for LTP and LTD expression. Although LTD of AMPA receptor dependent LTD has been extensively studied, little is know about the mechanism of NMDA receptor dependent LTD, although recently, number of groups have shown the mechanism underlying the downregulation of NMDA receptor dependent LTD (Morishita et al., 2005; Montgomery et al., 2005). Montgomery et al., (2005) have illustrated that NMDA receptor dependent LTD involve primarily by dynamin-mediated endocytosis of NMDA receptors, since NMDA receptor-mediated LTD is blocked by endocytosis inhibitors, whereas Morishita et al., (2005) have reported that AMPA receptor dependent LTD is not affected by endocytosis inhibition. Montgomery et al., 2005; Morishita et al., 2005). Morishita et al., (2005) have shown that the NMDA receptor-mediated LTD is dependent on protein phosphotase 1 (PP1), since inhibitors of PP1 activity impair LTD of NMDA receptors. Morishita et al., (2005) further illustrated that NMDA receptor-mediated LTD is also requires actin depolymerisation, since phalloidin, which inhibits actin depolymerisation and stabilizes actin, block LTD of NMDA receptor, whereas LTD of AMPA receptor were unchanged (Morishita et al., 2005). These results suggest that the mechanisms of AMPA receptor and NMDA receptor mediated LTD appear to be different. The role of AMPA and NMDA receptors in trafficking during LTP and LTD is more complex and extensive, but the reader is refereed to Derkach et al., (2007), Collingridge et al., (2004), or Groc and Choquet, (2006) for more detail and for the latest development on this field of science.

The Role of NMDA receptors in Learning and Memory

There is considerable evidence that the dorsal striatum plays role in learning and memory. (Adams, Kesner and Ragozzino, 2001; Yin and Knowleton, 2004; Graybiel, 1995). Electrophysiological recordings in the dorsolateral striatum indicate that changes in neuronal firing occur during the acquisition and execution of stimulus-response tasks (Jog et al., 1999).

Several studies have indicated that NMDA receptors play a critical role in learning and memory in the corticostriatal fibers. NMDA receptor blockade in the dorsomedial striatum impaired spatial learning in an eight-arm radial maze (Smith-Roe et al., 1999). Palencia et al., (2005) have illustrated that infusions of the NMDA antagonist AP-5 into the dorsolateral striatum selectively impairs acquisition of an egocentric response discrimination. Recently Schenberg et al., (2006) found that in rats during contextual fear conditioning task NR2A subunit expression was significantly higher in the caudate-putamen compare to the control animals. This suggests that NR2A subunit does play very important role in learning and memory in the corticostriatal synapses. Atallah et al., (2006) have shown that ventral and dorsal striatum play different roles in an instrumental conditioning task. The author have illustrated that the dorsal striatum is responsible for performance but not learning, whereas the ventral striatum responsible for both learning and performance (Atallah et al., 2006). NMDA receptors do play a critical as well as episodic memory in humans and other primates (Scoville and Milner, 1957). Early support for the link between the NMDA activity, and spatial learning and memory came from Morris and colleagues. Morris et al., (1989) have shown that blockade of the NMDA receptors by the NMDA receptor antagonist AP5 impairs spatial learning and memory. This result indicated that NMDA receptors in the CA1 region of the hippocampus are crucial for spatial learning and memory. An alternative approach to study the role of the NMDA receptor in learning and memory, is to develop genetically engineered mice, in which deletion or over-expression of individual NMDA receptors in the hippocampus has allowed a focus on individual subregions of hippocampus (see Nakazawa et al. 2004, (BOX 2). For example, Tsien et al., (1996) created a mouse strain in which the NR1 gene was postnatally knocked out mainly in the CA1 region of the hippocampus. CA1-NR1-knockout mice showed severely impaired spatial learning and memory (Tsien et al., 1996). Recently, Nakazawa et al., (2003) used a conditional knockout mouse strain in which the deletion of the gene encoding the NR1 was targeted and restricted to the CA3 pyramidal cells of adult mice. Nakazawa and colleagues trained CA3-NR1 knockout mice in a delayed matching to place version of the water maze task. In this task, mice were trained to search for a hidden platform that was moved to a new location on each day of training. While all animals were significantly faster on trial one, only control mice were significantly faster on trial two. Mutant mice, however, showed only weak improvement and needed more trials to learn where the platform was hidden. Interestingly, learning was retarded only when the platform appeared in a new place. These results suggest that the NMDA receptors in the CA3 region play a crucial role in the immediate encoding. Indeed, several studies have demonstrated that

the NMDA receptors in the CA3 region are necessary for rapid learning, whereas NMDA receptors in the CA1 region are necessary for learning of a longer timescale (Lee and Kesner, 2002; Lee et al., 2004). In contrast to these results, overexpression of the NR2B subunit in forebrain structures enhanced synaptic plasticity in the hippocampus and spatial learning and memory (Tang et al., 1999).

The role of glutamate receptor subunits in learning and memory is more extensive and complex, but the reader is referred to Riedel et al., (2003), Graybiel (1998), Ragozzino et al., (2002), Haber et al., (2000), Palensia and Ragozzino (2006), Devan and White (1999) or Nakazawa et al., (2004) for more detail.
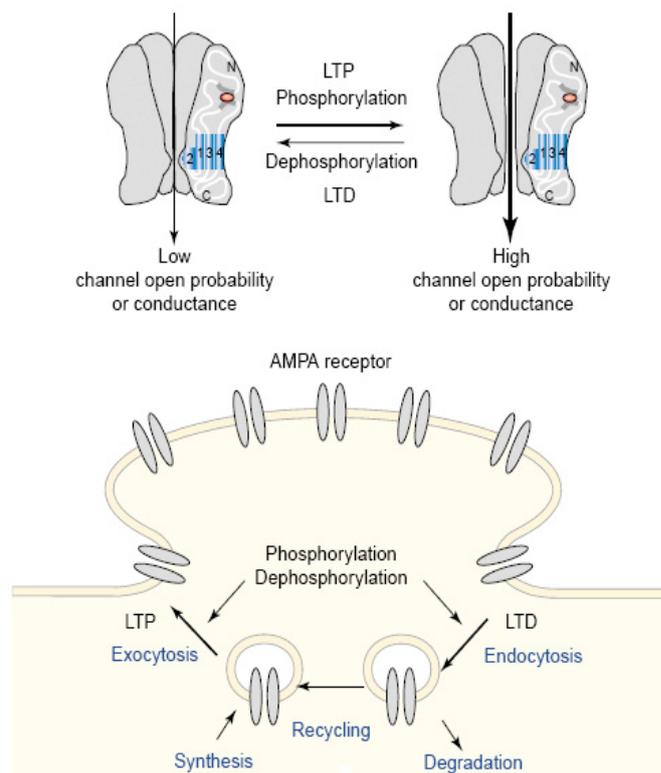
Figure 4. Regulation of AMPA receptors during synaptic plasticity. During LTP AMPA receptors are phosphorylated by several kinases and inserted into the synapses. During LTD AMPA receptor level decreases via dephosphorylation by phosphatases (Reproduced from Song and Huganir, 2002 with permission).

Pathophysiology of Dystonia

Cellular and Molecular Mechanisms in Dystonic Hamster

Dystonia is a common movement disorder that is thought to result from basal ganglia dysfunction. The pathophysiology of dystonias (primarily paroxysmal dystonia) are still remain elusive (Richter and Löscher, 1998) although there are several neurochemical and electrophysiological evidence exists in the manifestation of paroxysmal dystonia in dystonic hamsters. For example, Hamann et al., (2004) have shown that dystonia in hamster can lead to changes in the level of dopamine. This extracellular levels of dopamine were significantly enhanced within the striatum in the manifestation of primary paroxysmal dystonia (Hamann et al., 2004). Nobrega et al., (2002) have illustrated that dystonia significantly enhances ^{3}[H] AMPA receptor binding in the CA1 region of the hippocampus, in the ventral caudate putamen and in the dorsomedial part of the striatum (Nobrega et al., 2002). Nobrega et al., (1997) have also demonstrated that NMDA receptor binding was enhanced during dystonic attacks in dystonic mutant hamsters in several regions, including the striatum and ventrolateral thalamic nucleus, which is thought to be associated with altered basal ganglia output (Nobrega et al., 1997). These results suggest that overactivity of glutamatergic system may be involved in the manifestation of paroxysmal dystonia in the dystonic hamsters. Indeed, several studies have shown that NMDA receptor antagonists exert beneficial effects in certain experimental models of movement disorders, such as dystonia and parkinsonism (Kulkarni and Verma, 1991; Richter et al., 1991). Interestingly, a recent study has illustrated that the NR2A subunit of the NMDA receptor is increased in dyskinetic rats which may well suggest that NR2A subunit may also be upregulated in dystonias (Gardoni et al., 2006). Indeed, recently Richter et al, (2003) have found that the selective NR2B antagonist Ro-256981 fails to improve the dystonic syndrome in mutant hamsters which suggests that overstimulation of NR2A-containing NMDA receptors may be involved in the pathogenesis of dystonic episodes in mutant hamsters. At the present time, the role of NR2A as well as NR2B subunits in synaptic plasticity in corticostriatal synapses is unknown.

Electrophysiological studies have also suggested a link between pathophysiological aspects of dystonia to movement disorders. Gernert et al., (2000) provided an evidence that the mean discharge rate of neurons in GPi is dramatically reduced in dystonic hamsters (Gernert et al., 2000). Gernert et al., (2000) recently have also demonstrated that the number and density of

parvalbumin-immunoreactive GABAergic interneuron in all striatal subregions were markedly reduced in mutant hamsters (Gernert et al., 2000). The loss of inhibitory interneurons in dystonic hamsters and the reduction of the mean discharge rate of GPi activity are the essential factors for striatal overactivity in dystonias. The most compelling evidence for striatal overactivation came from the study of Köhling et al., (2004) who has illustrated that LTP and the paired pulse facilitation were larger in dystonic hamsters than controls. Köhling et al., (2004) have also shown that the population spike amplitude (excitability) was enhanced in dystonic mutant hamsters compared to control and that the effect was likely a presynaptic in nature (Köhling et al., 2004). This study suggests that the corticostriatal synaptic pathway is overexcited in mutant hamsters. Another evidence to suggest that striatal projection neurones may be more excitable in dystonic hamsters came from the study of Siep et al., (2002) who has demonstrated that fast sodium currents are less depressed by sodium channels blocker lamotrigine, suggesting that changes in sodium channel function may be involved in the model of paroxysmal dystonia (Siep et al., 2002; Ptacek, 1997). Another channel that might be affected in primary paroxysmal dystonia is voltage-gated K^+ channels. Recently, Richter et al., (2006) have demonstrated that the openers of K^+ channels, retigabine and flupirtine possess antidystonic effects, whereas the K^+ channel, blocker XE-991 aggravate the dystonic syndrome. Thus potassium channels openers may be effective in various model of dystonias (Richter et al., 2006).

In summary, dystonias are common movement disorders characterised by involuntary, sustained contractions of opposing muscles, frequently causing twisting movements or abnormal postures. It is believed that dystonia is a disorder that is thought to result from basal ganglia dysfunction. The principal function of the striatum is thought to be important for motor learning and memory and understanding the dynamics of corticostriatal synaptic information processing in health and in certain diseases is one of the main challenges in the basal ganglia field. Since dystonic symptoms in dt^{sz} hamsters are (1) paroxysmal, and bridged by clinically normal periods, since (2) dystonic symptoms cease altogether with maturation and since (3) preliminary findings suggest an involvement of NMDA-receptors in the hyperexcitability associated with dystonia in these animals, in this thesis three specific questions were addressed:

A. Is an acute dystonic attack particularly reflected in synaptic plasticity / excitability changes in vitro, and, conversely, is there a difference to findings during symptom-free intervals.

B. Do the in-vitro changes in excitability / synaptic plasticity of tissue from dt^{sz} hamsters cease during maturation, i.e. with full clinical recovery?

C. Is the plasticity change observed in vitro dependent on NMDA receptors, and which subunits are involved?

Materials and Methods

Material and Methods

Introduction

In all cases, experiments were conducted randomly based on the age of animals. Young hamsters (30 to 42 days old) were divided into non dystonic non stimulated (ND/NS), non dystonic stimulated (ND/S), dystonic non stimulated (D/NS), and dystonic stimulated (D/S). The old hamsters (100 to 120 days old) comprised remission state were divided into old non dystonic non stimulated (ND/NS), non dystonic stimulated (ND/S), dystonic non stimulated (D/NS), and dystonic and stimulated (D/S). The dystonic syndrome in dt^{sz} mutants shows an age-dependent time course (e.g. Richter and Löscher, 1998). The severity of dystonia reaches a maximum at an age of about 32-42 days. Thus, the investigation of electrophysiological changes in young hamsters were carried out at age of (32-42 days) in prepubertal animals. The severity of dystonia slowly declines until complete remission of stress-inducible dystonic attacks occurs at an age of about 10 weeks. Thus, the investigation of electrophysiological changes in old hamsters were carried out at age of (90-120 days). Non dystonic stimulated group was stimulated in the same manner as the mutant hamsters during the experiments as well as before electrophysiological experiments; that is, these animals were together with mutant hamsters also tested after weaning at the age of 21 days by triple stimulation procedure three times per week. The stimulation procedure took place only on Monday, Wednesday and Friday mornings. The difference between dystonic stimulated and dystonic non stimulated was that the experiments with dystonic stimulated mutants were conducted on the same day, whereas with dystonic non stimulated mutants the experiments were conducted 24 hours after the induction of dystonic attack in mutant hamsters.

Induction of dystonic attacks and severity score of dystonia

The present experiments were carried out in dt^{sz} mutant Syrian golden hamsters at ages of 32-42 days and in 90-120 days. The experiments were done in compliance with the German Animal Welfare Act and were approved by the responsible governmental agency in Rostock and Berlin. The hamsters were obtained from Angelika Richter group(generous gift) from the Institute of Pharmacology and Toxicology, School of Veterinary Medicine, Freie Universitität Berlin, Berlin, Germany. All hamsters were kept under the same controlled and constant environmental conditions. In mutant hamsters, dystonic attacks can be reproducibly induced

by stress such as handling and tactile stimuli. After stressful stimuli by i.p. injection of 0.9% of NaCl, dt^{sz} hamsters develop a sequence of abnormal movements and postures. The severity of dystonia can be rated by the following score system (Richter and Löscher, 1998): stage 1, flat body posture; stage 2, facial contortions, rearing with forelimbs crossing, disturbed gait with hyperextended forepaws; stage 3, hyperextended hindlimbs so that the animals appear to walk on triptoes; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; stage 6, immobilization in a twisted hunched posture with hind-and forelimbs tonically extended forward. The individual maximum stage of dystonia is usually reached within 2-3 hours after the hamsters were placed in the new cage. Theafter, the hamsters completely recover. Immediately, after stage 6, the animals were taken for electrophysiological procedure. Only hamsters with the stage 6 were included in dystonic group.

Slice preparation

Slice preparation of hamster brains were prepared following procedures to those described by Köhling et a., (2004). Only angulated slices at 40$^{°}$ were used for these experiments to preserve corticostriatal synaptic network. The study was conducted on randomized design. Briefly, brains were quickly removed and chilled in ice-cold artificial cerebrospinal fluid (ACSF) containing in (mM): NaCI 125, NaHCO3 26, KCI 4, NaHPO$_4$ 1.25, CaCl$_2$, 2, MgCl$_2$ 1.3 and glucose 10. The brain was trimmed on the dorsal side at an angle of approximately 40$^{°}$ from the horizontal and glued to a vibratome based on that side (Integraslice 7550 mm, Campden Instruments Ltd). From this slanted tissue block, 500-μm slices were made which contained the motor cortex and entire striatum, with connections between these two regions still intact (Fig. 5). Immediately after the slicing, two slices were transferred to the interface-type recording chamber and were incubated at room temperature for at least 1 hour and then for another hour at 32-33 $^{°}$ C. The rest of the slices were transferred to an incubation submersion-type bath filled with the same ACSF as it stated above. The recording was only conducted on dorsomedial side of the striatum (Figure 5) and was only performed after at least 2.5-3 hours of incubation of the slices.

Electrophysiological Recording

Field excitatory post synaptic potentials were obtained from the dorsomedial striatum of the hamsters. As a recording site, the dorsomedial part of the corticostriatum was chosen, as it receives most of the motor-cortical input (Nobrega et al., 2002). A single silver coated tungsten wire recording electrode was used for this study. Stimulation was conducted at the white matter of the cortex with monosynaptically evoked field responses at 0.25 Hz using 50 μm diameter of bipolar platinum wires. For input-output characterisation of evoked field potential, stimulus intensity was stepwise increased until reaching saturating responses. For paired-pulses paradigms, and likewise for long-term plasticity experiments, stimulus strength was set at 50% of saturating intensity. Paired-pulse paradigm was conducted at 40 ms delay and the ration between the second and the first amplitude of the fEPSPs was calculated. Following 10 to 15 minutes of stable baseline, long-term potentiation (LTP) and long-term depression (LTD) were induced with several paradigms:

1. Paradigm A: 100 Hz for 3 seconds, 3 times, 20 seconds apart

2. Paradigm B: 100 Hz for 1 second, 3 times, 20 seconds apart

3. Paradigm C: 10 Hz for 300 seconds (once)

4. Paradigm D: 1 Hz for 600 seconds (once)

High-frequency stimulation were given at the same stimulus intensity. We choose paradigm A since we found that this paradigm produces a robust LTP in the dorsomedial striatum. Paradigms B and C were taken since a number of reports showed that these two paradigms produce reliable LTD in corticostriatal slices in rats (Ronesi and Lovinger, 2005, Fino et al., 2005). Paradigm B was established to investigate responses in an intermediate setting. All the experiments were conducted with 1.3 mM of $MgCl_2$ (except in a normal healthy animals, where 1 mM of Mg^{2+} was used). Signals were processed and digitised with a Power1401 A/D converter using Signal 2.03 software (CED, Cambridge, UK). Data were expressed as mean ± SEM% of the baseline fEPSP slope. Data were analysed using Signal 2.03 software (CED, Cambridge, UK). Values are given as means ± SEM. Statistical analysis was done with the

aid of SigmaStat software (SPSS; Chicago, IL, USA) performing Two-Way Anova and post-hoc test was done with Holm-Sidak method or One-Way ANOVA with the post hoc test was conducted with Fisher LSD method, and differences were considered significant when P<0.05. The initial slopes of the fEPSPs were measured and expressed as a percentage change from the baseline level, calculated from an average of the last 10 minutes of the baseline recording period. The degree of LTP for each experiment was measured as the average of the last 10 minutes of the post-HFS or postdrug recording period, whereas the group means were expressed as the percentage ± SEM change. A star based system was used: * p<0.05; ** p<0.01. The 'n' values reported refer to the number of slices.

Drug Application

All pharmacological compounds were included in the extracellular standard solution at the concentration indicated and were given at least 30 minutes before the baseline recording. To test for the contribution of NMDA receptors in the generation of synaptically evoked potentials, D (-)-2-amino-5-phosphonopentanoic acid (APV, 50 μm) was bath-applied for a minimum of 20-30 minutes. For the elucidation of different roles of NMDA receptor subunits in synaptic plasticity the NR2A selective antagonist (R)-[S]-1-(4-bromo-phenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl-phosphonic acid (NVP-AAM077, Novartis, 50 nM) and the NR2B selective antagonist (αR, ßS)-α-(4-hydroxyphenyl)-ß-methyl-4-(phenylmethyl)-1-piperidinepropanol (Ro25-6981, Novartis) (5 μM, or 10 μM) were used. NR2A selective antagonist was a gracious gift from Novartis.
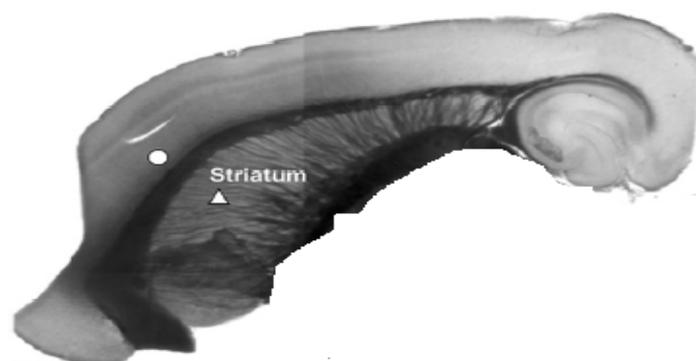
Figure 5. The recording and stimulation of the corticostriatal synapses. The stimulating was conducted in the white matter, whereas the recording was done in the dorsomedial site of the corticostriatal synaptic pathway (Reproduced from Smeal et al., 2007 with permission).

Results

RESULTS

A. Synaptic plasticity and network excitability after dystonic attacks and in symptom-free intervals

In a previous paper (Köhling et al., 2004), cortico-striatal long-term synaptic plasiticity was shown to be increased significantly in dt^{sz} hamsters. These experiments, however, had been carried out on animals for which it remained unclear whether they had suffered a recent dystonic attack or were in a symptom-free interval. Since dystonia in these animals is a paroxysmal one, it is likely that activity-dependent states determine network function. To discern the effect of acute attacks on synaptic function, and conversely to differentiate these from symptom-free intervals, experiments were carried out on animals shortly after a dystonic attack had been provoked by triple-stimulation paradigm (D/S) and corresponding controls (ND/S), as well as in tissue from animals in the symptom free phase (D/NS and ND/NS).

1. Long-term potentiation in corticostriatal slices in young dystonic hamsters

To determine whether the appearance of a dystonic episode has any influence on the expression of long-term synaptic plasticity in cortico-striatal preparations, experiments were conducted using a stimulation paradigm known to robustly induce LTP (paradigm A, Köhling et al., 2004). Further, to determine whether any changes would apply to the whole spectrum of induction paradigms, all groups were also tested in paradigms B, C, and D, as detailed in the Methods section.

As shown in Fig. 6, with paradigm A (100 Hz, 3s, 3x), robust long-term potentiation (LTP) only appeared in dystonic tissues, but not in control, confirming in essence the findings of Köhling et al. (2004). Thus, mean relative slopes in the different groups were: 0.97 ± 0.064 (ND/NS), 1.08 ± 0.05 (ND/S), 1.26 ± 0.05 (D/S), and 1.39 ± 0.08 (D/NS) (Fig. 6C). Importantly, post hoc analysis of Two-Way ANOVA revealed that the deciding factor determining the expression of LTP was the presence of dystonia in general, whereas stimulation of the animals (i.e. the acute expression of a dystonic attack) did not influence synaptic plasticity changes (Fig. 6C). More specifically, the D/NS group was significantly different from both ND/NS and ND/S groups (both p=0.001). Further, the D/S group also was

significantly different from ND/NS and ND/S (p = 0.007 and p=0.044, respectively). No further significant differences emerged, particularly also not between S and NS groups (Fig. 6).

The next question which was addressed was whether the significant differences found in LTP induction using paradigm A would prevail also in other induction paradigms, and whether indeed with other induction paradigms, dystonic and non-dystonic tissues would differ in their expression of LTP or LTD.

As Figs. 7-9 demonstrate, stimulation paradigms B to D were not effective in disclosing differences between dystonic and non-dystonic tissue. Thus, stimulation paradigms B (100 Hz, 1s, 3x, Fig. 7) and C (10 Hz, 300s; Fig. 8) had no effect on long-term plasticity in any of the experimental groups, whereas in paradigm D (1 Hz, 600s; Fig. 9), a tendency towards development of LTD could be observed, albeit non-significantly so.

Regarding findings in stimulation paradigm B (100 Hz, 1s, 3x), mean relative slopes in the different groups were: 0.99 ± 0.10 (ND/NS), 1.02 ± 0.08 (ND/S), 1.02 ± 0.09 (D/S), and 1.02 ± 0.09 (D/NS) (Fig. 7C). Similarly, in stimulation paradigm C (10 Hz, 300s), mean relative slopes in the different groups were: 0.98 ± 0.14 (ND/NS), 1.02 ± 0.19 (ND/S), 1.02 ± 0.06 (D/S), and 1.01 ± 0.06 (D/NS) (Fig. 8C). A qualitative alteration could be observed in experiments using stimulation paradigm D, which in some publications was shown to induce LTD in rats (cf. Ronesi and Lovinger et al. 2005). However, in our hands, and in a different species, no significant LTD could be seen, although a tendency towards depression became apparent: mean relative slopes in these experiments in the different groups were: 0.89 ± 0.10 (ND/NS), 0.91 ± 0.11 (ND/S), 0.95 ± 0.11 (D/S), and 1.07 ± 0.09 (D/NS) (Fig. 9C).

2. Input-output relationships of afferent stimulation in young dystonic hamsters

Since in the initial paper describing altered LTP in dystonic tissue, also differences in input-output characteristics were described pointing to alterations of network excitability, in the present thesis the input-output relationships were established as well by inducing fEPSP at increasing stimulation intensities up to saturating responses prior to induction of LTP. As demonstrated in Fig. 10A, both the presence of dystonia and pre-treatment of the animals with stimulation in the triple-stimulation test lead to a significant increase in the input-output

relationship over a wide range of stimulation intensities. While ND/NS preparations were thus significantly different from all groups from stimulation intensities of 20 V and above, no differences emerged among the other groups ($p < 0.007$; two-way ANOVA; Fig. 10A), suggesting stimulation itself to raise excitability in young naïve, i.e. non-dystonic, animals.

3. Paired-pulse facilitation before and after long-term synaptic changes

Since excitability changes per se do not seem to correlate with the difference of LTP-expression in dystonic vs. non-dystonic tissues, the next question addressed was whether pre-synaptic changes might be responsible, and whether this would be differentially so, in D and ND preparations. Paired pulse plasticity is thought to reflect activity-dependent changes of transmitter release from presynaptic terminals (Zucker, 1999). Paired pulse facilitation (PPF), in this context (e.g. paried pulse ratio – PPR – >1) is thought to result from residual Ca^{2+} in the synaptic bouton, which upon the second stimulus releases more transmitter in otherwise low-release probability synapses (Zucker, 1999). Hence, paired-pulse facilitation usually reflects presynaptic changes. To test for these, short term plasticity was tested using double stimulation at short intervals (40 ms), which can reveal alterations in the slope of the second response relative to the first. If there is an increase in this ratio, release probability would likely be increased; with a decease, the opposite, i.e. a decrease in synaptic release probability would become evident. As can be seen in Fig. 11, both before and after LTP induction (using paradigm A), PPR was >1 in all cases, indicating an additional recruitment of released vesicles upon the second stimulus (due to, e.g. residual Ca^{2+} in the synaptic bouton). However, no significant differences could be seen (two-way ANOVA), neither among groups nor between conditions pre- and post-LTP. Specifically, the PPR for the different conditions and groups were: 1.06 ± 0.13 and 1.16 ± 0.08 (ND/NS, pre and post), 1.26 ± 0.09 and 1.24 ± 0.1 (ND/S, pre and post), 1.23 ± 0.11 and 1.22 ± 0.09 (D/NS, pre and post) and 1.27 ± 0.13 and 1.17 ± 0.08 (D/S, pre and post).

B. Synaptic plasticity and network excitability in dystonic hamsters in remission state

The dystonic syndrome in dt[sz] mutants shows an age-dependent time course (see Richter and Löscher, 1998). The severity of dystonia reaches a maximum at an age of 32-42 days; at 70 days, the animals are considered to be in remission state, i.e. no spontaneous or provoked dystonic attacks occur from this age on. The remission in these older animals raises the

question whether synaptic plasticity changes, as observed in the young groups with active dystonia can also be observed in the remission, post-dystonic age group. A recent study by Hamann et al., (2007) illustrating a recovery from an initial deficit of striatal PV^+ GABAergic interneurons in the remission group suggest functional changes in the basal ganglia may also subside. Thus, our second aim was to see whether synaptic plasticity would also preserved in old hamsters (post-dystonic mutant hamsters) at an age of 90-120 days.

1. Long-term potentiation in corticostriatal slices in old dystonic hamsters in remission state

Since no literature data exist on synaptic plasticity in old hamsters, we initially performed a series of experiments to determine whether LTP would induced with high frequency stimulations in stimulation paradigm A (100 Hz, 3s, 3x) in physiological magnesium (1.3 mM). Our hypothesis in this case was that the enhancement of LTP as observed in young, actively dystonic hamsters would be compromised in post-dystonic hamsters. To further assess whether stressing the animals with the triple-stimulator technique would have an impact, also in these experiments, apart from ND/NS and D/NS, also ND/S and D/S groups were tested.

As demonstrated in Fig. 12, in post-dystonic hamster tissues, irrespective of prior stressing of animals, LTP is expressed equally to the young groups. Thus, the relative slopes after stimulation paradigm A were 1.29 ± 0.18 (D/S) and 1.32 ± 0.23 (D/NS). These values were not different among the two groups, nor in comparison to young groups (p>0.05; Fig. 12 vs. Fig. 6). Interestingly, in the ND groups, differences did become apparent (two-way ANOVA): In the ND/NS group, maturation of the tissue appeared to significantly facilitate the emergence of LTP; relative slopes here were 1.31 ± 0.13 (as compared to 0.97 ± 0.06 in the young ND/NS group; p=0.018; Figs. 12C and 6C, respectively). More importantly still, in the ND/S old group, handling and stress using the triple-stimulator technique prior to the experiment did significantly influence long-term synaptic plasticity in vitro: In this group, the relative slope after stimulation paradigm A was 0.83 ± 0.07 (Fig. 12C), and thus significantly reduced against all other old remission groups, as well as compared to the young ND/S group (p=0.029; Fig 12C vs. Fig. 6C).

To test whether differences in synaptic plasticity would emerge also using other stimulation paradigms, experiments were conducted with stimulation paradigms B (100Hz, 1s, 3x), C (10 Hz, 300 s), and D (1 Hz, 600 s). Since the initial experiments were conducted on NS animal groups (Köhling et al., 2004), we first conducted these experiments on these groups, i.e. D/NS and ND/NS. In these investigations, none of the other stimulation paradigms was able to significantly induce either LTP or LTD (Fig. 13). Thus, the relative slopes in the different groups were as follows. Paradigm B: 0.98 ± 0.10 (ND/NS) and 1.18 ± 11.5 (D/NS); paradigm C: 0.97 ± 0.12 (ND/NS) and 0.99 ± 0.07 (D/NS), and paradigm D: 0.97 ± 0.08 (ND/NS) and 1.16 ± 0.16 (D/NS). Since no LTP or LTD was elicited, and since no differences arose either among the groups or versus the corresponding young groups with active dystonia (and respective controls, ANOVA), no further experiments were done with the stimulation paradigms B through D.

2. Input-output relationships of afferent stimulation in old dystonic hamsters in remission state.

Similar to the experiments in the young hamster groups, also in the old groups analyses on network excitability, i.e. on the input-output characteristics were conducted. As shown in Fig. 10B, the input-output relationships of the old groups were similar to the one of the young groups at first sight. A closer analysis, however, reveals that a significant divergence (two-way ANOVA) can be observed in the D/S group, were excitability is significantly lower than all other groups within the stimulation range of 25 to 60 V, suggesting, in turn, that stimulation itself decreases excitability in dystonic old animals in the remission state.

3. Paired-pulse facilitation before and after long-term synaptic changes in old dystonic hamsters in the remission state

Since excitability changes again (as in young animals, even though in an opposite fashion) seem to be influenced by activity of the animals prior to the experiment, i.e. handling and stress induced by the triple stimulator technique, at least in the dystonic remission group, experiments were conducted to determine the PPR in the different groups, both prior to and after application of stimulus paradigm A. Similar to the young groups, no significant differences among the groups, nor between treatments could be observed (two-way ANOVA), except for the D/S group prior to LTP induction (Fig. 14). Specifically, the PPR for the

different conditions and groups were: 1.17 ± 0.03 and 1.19 ± 0.05 (ND/NS, pre and post), 1.16 ± 0.15 and 1.18 ± 0.12 (ND/S, pre and post), 1.21 ± 0.11 and 1.31 ± 0.08 (D/NS, pre and post) and 0.92 ± 0.07 and 1.05 ± 0.18 (D/S, pre and post). Testing with two-way ANOVA, a significant reduction of PPR could be observed in the D/S groups prior to LTP as compared to the corresponding D/NS group (p=0.044), as well as its corresponding group (D/S) in young animals (1.27 ± 0.13; p=0.03; cf. Fig. 11), suggesting stimulation and maturation to play a role here.

C. Role of NMDA-receptors in dystonia associated synaptic plasticity changes

Coming back to the main question of mechanisms underlying the induction of dystonia, and more specifically, the interaction between synaptic plasticity changes at the cortico-striatal synapse and the appearance of dystonic syndromes, with the following experiments we concentrated on elucidating the role of glutamate receptors in LTP generation in young dystonic hamsters. Among the glutamate receptors, NMDA receptors (NMDARs) play a crucial role for synaptic plasticity and for learning and memory (Bliss and Colligridge, 1993).

1. Blockade of NMDA receptors

In order to determine the contribution of NMDA receptors in the enhancement of LTP in D/NS and D/S mutants, we used D-AP5 to block NMDA receptors during the entire duration of LTP-induction experiments. In these studies, only the paradigm resulting in robust LTP, paradigm A, was tested. Using 50 µM D-AP5, the induction of LTP was totally blocked both in D/S and D/NS groups. Thus, compared to intact NMDA transmission, in the D/NS group, relative slopes decreased significantly from 1.39 ± 0.08 to 1.05 ± 0.11 (ANOVA, P=0.039, Fig. 15). Likewise, in the D/S groups, a decrease from 1.26 ± 0.05 to 0.91 ± 0.12 was observed (p=0.009, ANOVA, Fig. 15). These results show that the effect was postsynaptic in nature, and secondly, we can conclude that the enhancement of synaptic plasticity which was seen in dystonic groups was totally dependent on NMDA receptor activation.

2. Role of NMDA-receptor subunits

NMDARs are composed of a combination of NR1 and NR2 subunits. NMDARs are heteromers composed of two essential NR1 subunits and two or three NR2 subunits (Behe et

al., 1995, Laube et al., 1998). There are four NR2 subunits (A-D), but the NR2A and NR2B subunits predominate in the forebrain. Several papers suggest that NR2A and NR2B subunit-containing NMDARs have a separable role in synaptic plasticity, with NR2A subtypes regulating LTP and NR2B subtypes regulating LTD, at least in the hippocampus (Liu et al., 2004, Massey et al., 2004). Recent data, again in the hippocampus, contradict the initial findings that NR2A and NR2B play distinct roles in regulating synaptic plasticity and instead suggest that both subunits are capable of inducing LTP and LTD (Fox et a., 2006, Berberich et al., 2005, Weitlauf et al., 2005). For the cortico-striatal synapse, and more so for dystonic conditions, no data are available, raising the question whether also in our conditions, NR2A and NR2B receptors will serve differential roles. To test this, a selective NR2B antagonist, RO 25-6981 as well as a selective NR2A antagonist, NVP-AAM077, was used. Using induction paradigm A, under control conditions LTP was robustly induced (see above; A1), both in D/NS as well as D/S groups (Figs. 16 and 17). Blocking NR2A receptors in the D/NS group (Fig. 16A), LTP is lost (mean slope reduced to 1.03 ± 0.08, p=0.023, ANOVA). with this paradigm (139.62 ± 8.69% above the baseline, n=15). Blocking NR2B receptors in turn, a reduction, but no significant (ANOVA) block of LTP could be observed, even in non-selective concentrations of 5 and 10 µM. Specifically, the relatives slopes were 1.18 ±0.05 at 5 µM RO-25-6981, and 1.09 ± 0.07 with 10 µM of the blocker (Fig. 16 B). Since these dosages (at least at 10 µM) were non selective, and yet no significant block was achieved, the NR2A receptor appears to be crucial for LTP in D/NS preparations. The same holds true for D/S preparations (Fig. 17). Again, NR2A block abolished LTP (slope reduction to 0.96 ±0.12, p=0.006, ANOVA, Fig. 17A), while NR2B block, even at maximal concentrations, had no effect (relative slopes 1.36 ± 0.14 and 1.35 ± 0.23 with 5 and 10 µM concentrations of the blocker, respectively; ns, ANOVA, Fig. 17B).

3. The role of NMDA receptor subunits in the induction of LTP in normal corticostriatal slices from healthy animals

Since the previous experiments showed that NR2A subunits are crucially involved in LTP generation of dystonic hamsters, the question arises whether they play the same role also in synaptic plasticity of normal, non-dystonic tissue. The precise role of NMDA receptor subunits and the physiological functions of these receptor subunits in corticostriatal synaptic pathway have remained enigmatic and unknown. The induction of LTP in vitro of corticostriatal in brain slices of normal, non-dystonic animals usually requires specific

experimental conditions; as evidenced by the present experiments, LTP could not be induced under normal recording conditions (Fig. 6). Most commonly, LTP in healthy tissue is induced with high frequency stimulation (HFS) of corticostriatal afferents while the slice is bathed in a solution with low Mg^{2+} concentration, or nominally Mg^{2+}-free solution. This relieves striatal NMDA receptors from physiological blockade at resting potentials (Kita, 1996). Therefore, we induced LTP in corticostriatal slices with only 1 mM of Mg^{2+} (instead 1.3 mM as in the previous experiments), using paradigm A (100 Hz, 3s, 3x). As shown in Fig. 18, this induced consistent LTP in corticostriatal slices ($p=0.008$, 1.6 ± 0.15 after the induction of LTP versus 1.0 ± 0.08 before, ANOVA). As expected in the presence of D-AP5 at 50 μM, LTP was blocked ($p=0.018$, slope reduction to 1.09 ± 0.09, ANOVA, 18A). Contrary to dystonic tissue, however, LTP was not blocked with NR2A receptor block, in this case, even a non-significant increase in mean fEPSP slope to 1.97 ± 0.28 occurred (Fig. 18B). NR2B receptors, in turn, in this ND/NS groups do play a role in LTP generation: Blocking NR2B receptors with selective concentrations of either 1 or 5 μM Ro256981, fEPSP was reduced to 1.18 ± 0.13 and 0.95 ± 0.09, respectively, the latter of which was significant ($p=0.003$, ANOVA, Fig. 18C). Thus, LTP in the corticostriatal synaptic pathway in healthy tissue appears to be purely dependent on NR2B subunits, in contrast to the dystonic mutants plasticity where LTP is predominantly dependent on the activation of NR2A subunits.
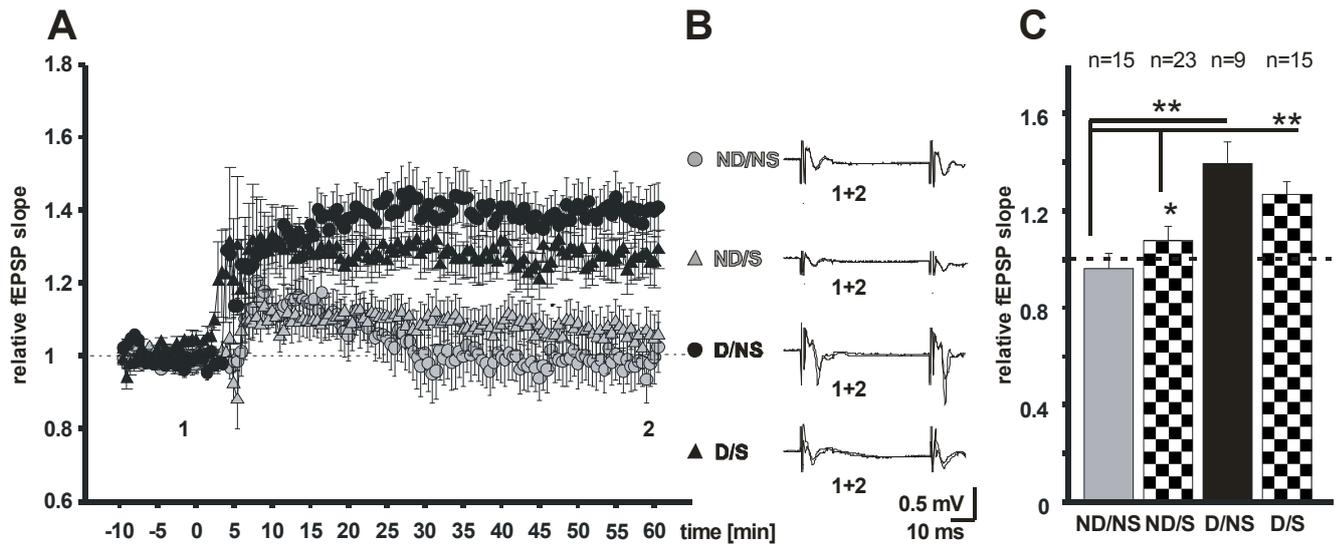
Figure 6. Comparison of Long-Term Potentiation in young non dystonic non stimulated (ND/NS), young non dystonic and stimulated (ND/S), young dystonic non stimulated (D/NS), and in young dystonic stimulated (D/S) hamsters in dorsomedial corticostriatal synaptic pathway with 100 Hz for 3 seconds, 3 times, 20 seconds apart. Long-Term Potentiation was significantly different between D/NS from both ND/NS and ND/S (P=0.001). Further, the D/S group also was significantly different from ND/NS and ND/S (p = 0.007 and p=0.044, respectively). Each point bar represents the mean relative ± SEM for n= 9 to 23 separate observations. **A**. Dot plot of relative fEPSP slopes before (1) and after (2) LTP induction paradigm delivered at timepoint 0. **B**. Examples of superimpose traces of different experimental groups of timepoints (1) and (2), i.e. before and after LTP-induction paradigm A. **C**. Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups.
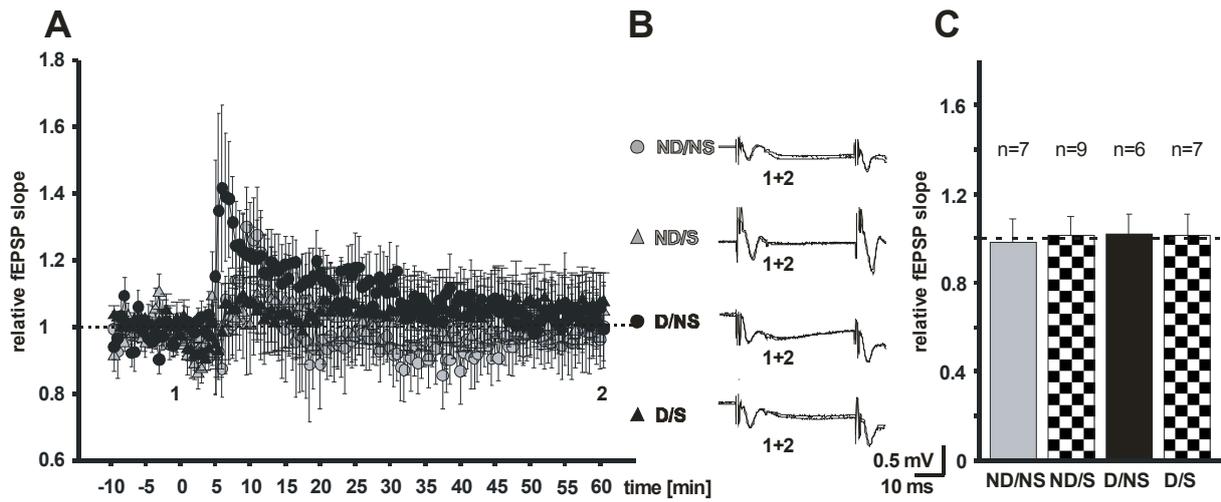
Figure 7. Comparison of Long-Term Potentiation in young non disease non stimulated (ND/NS), young non dystonic and stimulated (ND/S), young dystonic non stimulated (D/NS), and in young dystonic stimulated (D/S) hamsters in dorsomedial corticostriatal synaptic pathway with stimulation paradigm of 100 Hz for 1 second, 3 times, 20 seconds apart. Long-Term Potentiation was not significantly different between young ND/NS, ND/S, D/NS, and D/S ($P>0.05$). Each point bar represents the mean relative ± SEM for n= 6 to 9 separate observations. **A**. Dot plot of relative fEPSP slopes before (1) and after (2) LTP induction paradigm delivered at timepoint 0. **B**. Examples of superimpose traces of different experimental groups of timepoints (1) and (2), i.e. before and after LTP-induction paradigm B. **C**. Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups.
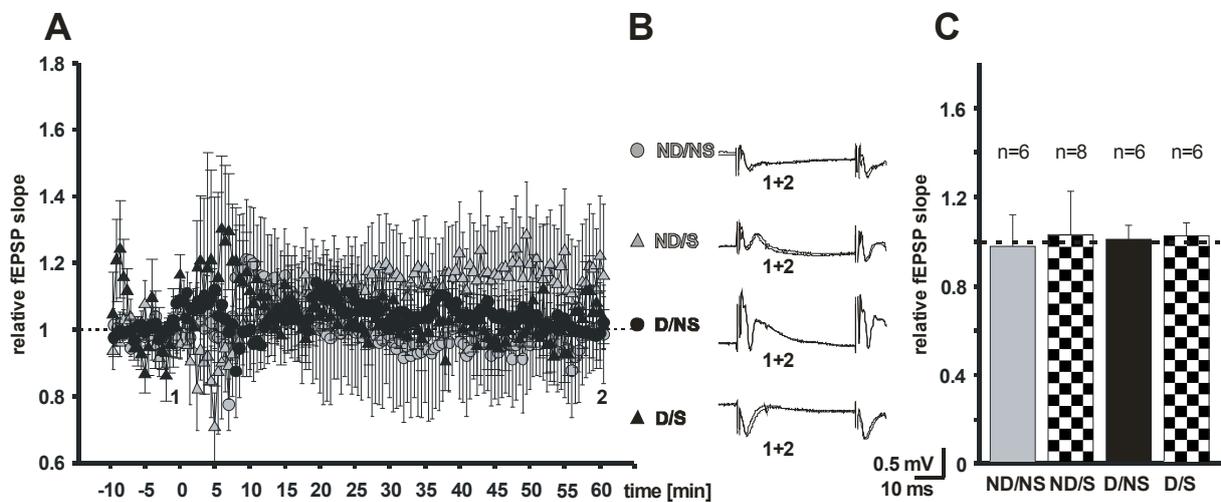
Figure 8. Comparison of Long-Term Depression in young non dystonic non stimulated (ND/NS), young non dystonic and stimulated (ND/S), young dystonic non stimulated (D/NS), and in young dystonic stimulated (D/S) hamsters in dorsomedial corticostriatal synaptic pathway with 10 Hz for 300 seconds. Long-Term Potentiation was not significantly different between young ND/NS, ND/S, D/NS, and D/S ($P > 0.05$). Each point bar represents the mean relative ± SEM for n= 6 to 9 separate observations. **A**. Dot plot of relative fEPSP slopes before (1) and after (2) LTP induction paradigm delivered at timepoint 0. **B**. Examples of superimpose traces of different experimental groups of timepoints (1) and (2), i.e. before and after LTP-induction paradigm C. **C**. Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups.
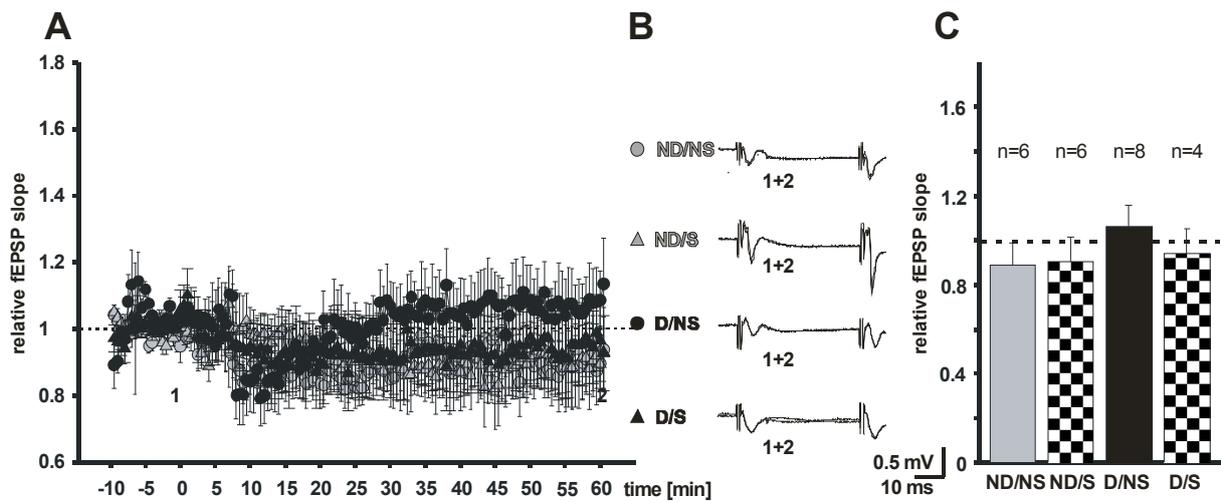
Figure 9. Comparison of Long-Term Depression in young non dystonic non stimulated (ND/NS), young non dystonic and stimulated (ND/S), young dystonic non stimulated (D/NS), and in young dystonic stimulated (D/S) hamsters in dorsomedial corticostriatal synaptic pathway with 1 Hz for 600 seconds. Long-Term Potentiation was not significantly different between young ND/NS, ND/S, D/NS, and D/S (P>0.05). Each point bar represents the mean relative ± SEM for n= 6 to 9 separate observations. **A**. Dot plot of relative fEPSP slopes before (1) and after (2) LTP induction paradigm delivered at timepoint 0. **B**. Examples of superimpose traces of different experimental groups of timepoints (1) and (2), i.e. before and after LTP-induction paradigm D. **C**. Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups.

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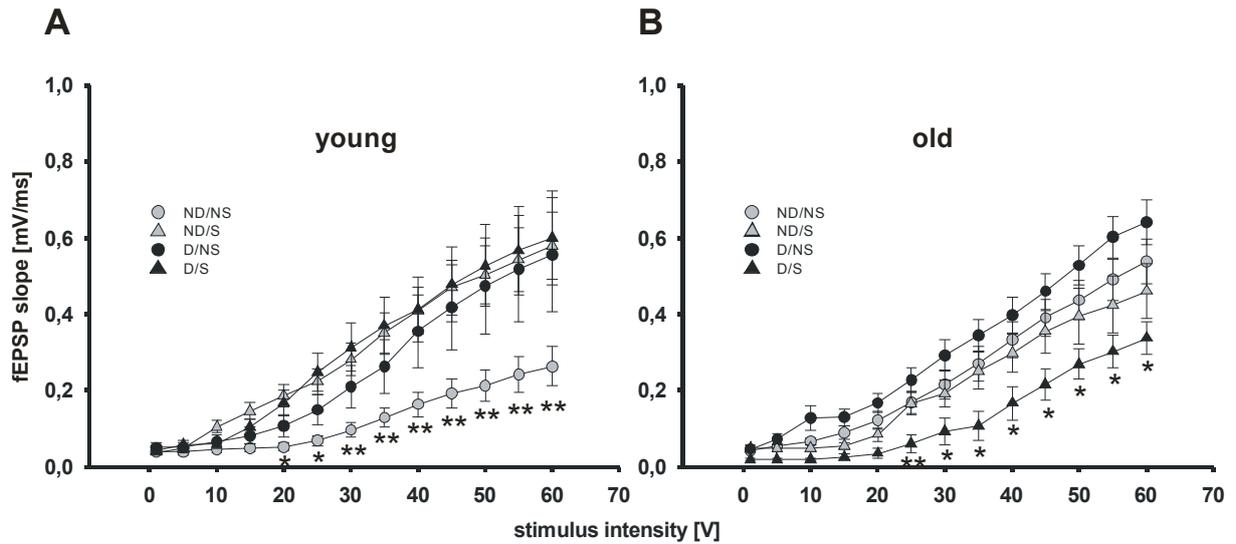
Figure 10. Overall slope of the field of excitatory post synaptic potential in young non dystonic non stimulated (ND/NS), young non dystonic and stimulated (ND/S), young dystonic non stimulated (D/NS), and in young dystonic stimulated (D/S) hamsters, as well as in old non dystonic non stimulated (ND/NS), old non dystonic and stimulated (ND/S), old dystonic non stimulated (D/NS), and in old dystonic stimulated (D/S) hamsters. **A.** Input/Output curves were significantly different between ND/NS, and ND/S, D/NS, and from D/S with the stimulation range of 20 to 60 volts (P<0.007, n= 22 to 43 separate observations). **B.** Input/Output curves were significantly different between D/S, and ND/NS, ND/S, and from D/NS with the stimulation range of 25 to 60 volts (P<0.05, n= 20-22 for each separate observations).
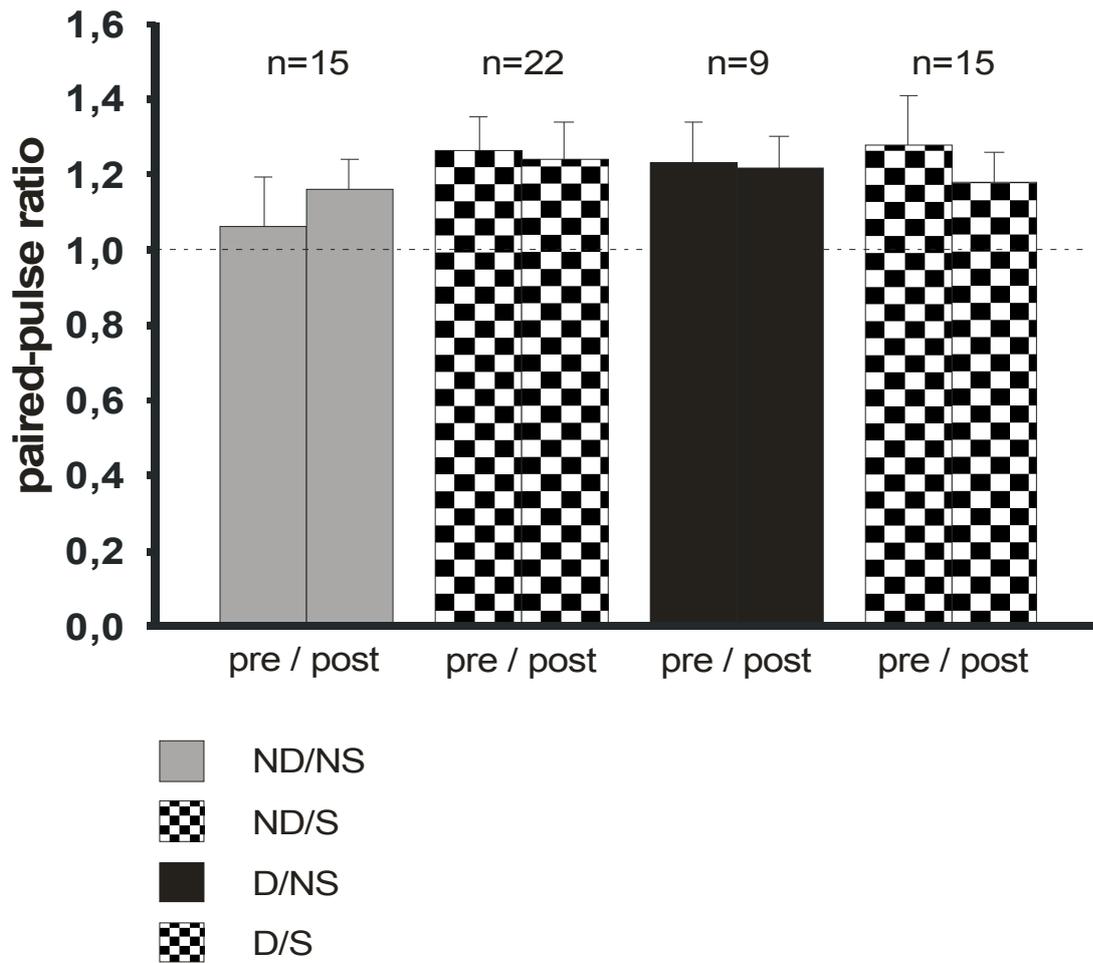
Figure 11. Comparison of paired-pulse facilitation in young non dystonic non stimulated (ND/NS), young non dystonic and stimulated (ND/S), young dystonic non stimulated (D/NS), and young dystonic and stimulated (D/S) hamsters before and after high frequency stimulation (HFS) with stimulation paradigm of 100 Hz for 3 seconds, 3 times, 20 seconds apart. Bar chart shows non significant difference in Paired-Pulse Facilitation before and after HFS between young ND/NS, ND/S, D/NS, and D/NS (P>0.05). Each point bar represents the mean relative ± SEM for n = 9 to 22 for each separate observations.
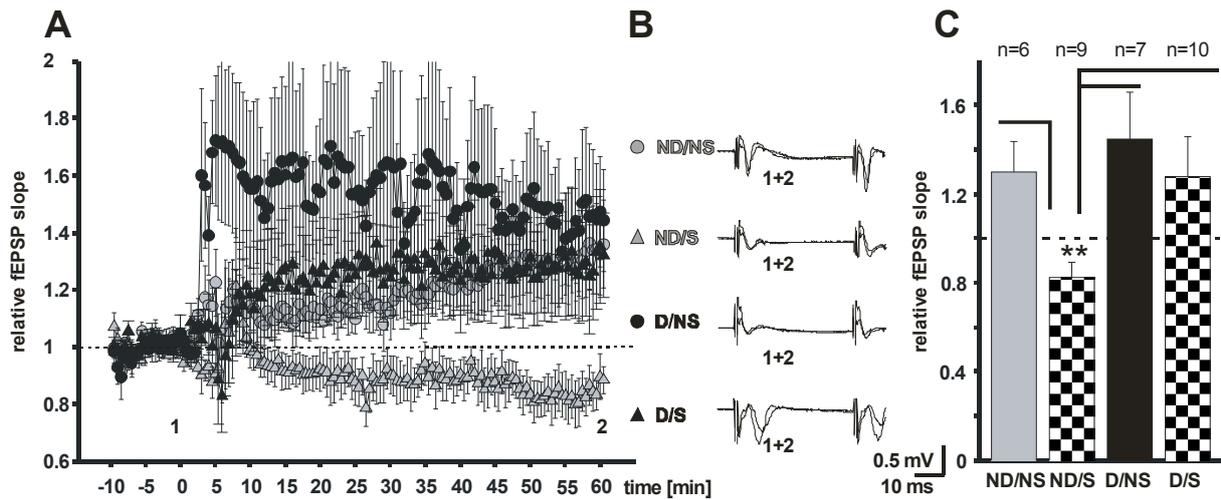
Figure 12. Comparison of Long-Term Potentiation in old non dystonic non stimulated (ND/NS), old non dystonic stimulated (ND/S), old dystonic non stimulated (D/NS), and in old dystonic and stimulated (D/S) hamsters in dorsomedial corticostriatal synaptic pathway with stimulation paradigm of with 100 Hz for 3 seconds, 3 times, 20 seconds apart. Long-Term Potentiation was significant different between ND/S from ND/NS, D/NS, as well as from D/S (P=0.001). Each point bar represents the mean relative ± SEM for n = 6 to 9 separate observations. **A**. Dot plot of relative fEPSP slopes before (1) and after (2) LTP induction paradigm delivered at timepoint 0. **B**. Examples of superimpose traces of different experimental groups of timepoints (1) and (2), i.e. before and after LTP-induction paradigm A. **C**. Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups.
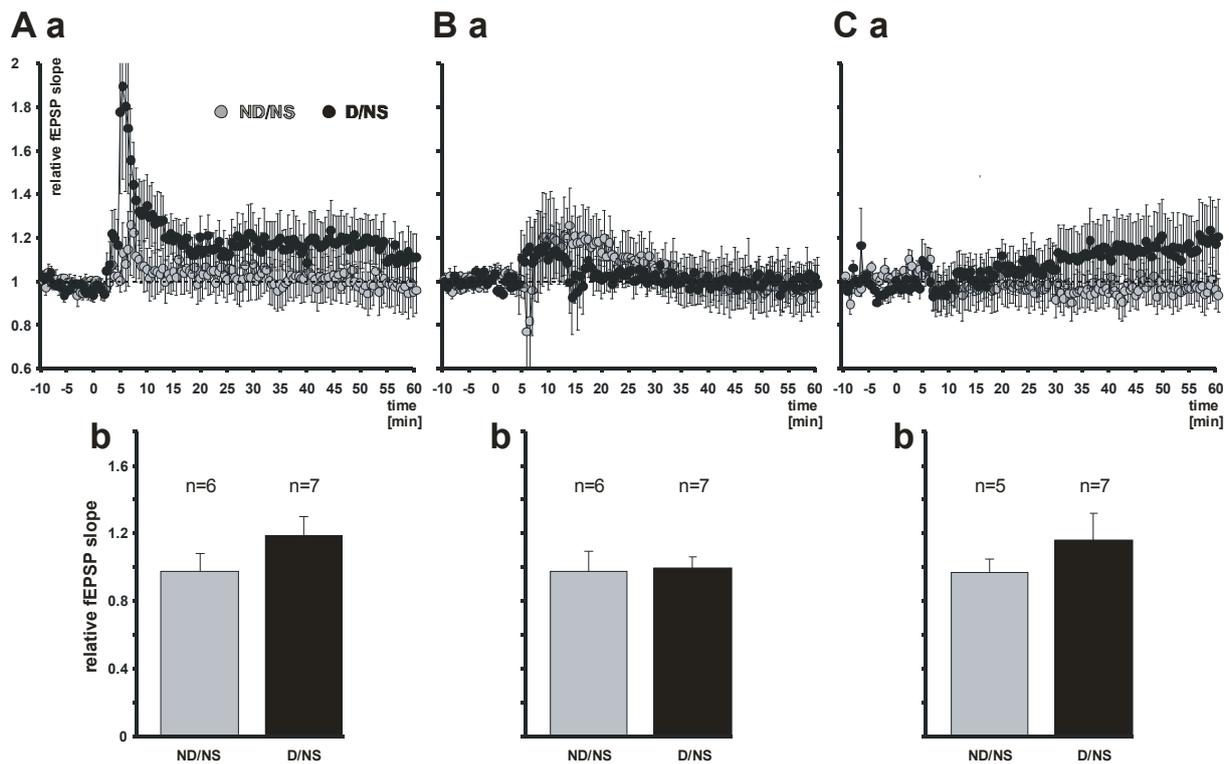
Figure 13. Comparison of Long-Term Potentiation and Long-Term Depression in corticostriatal synaptic pathway with stimulation paradigm of 100 Hz for 1 second, 3 times, 20 seconds apart between old non dystonic non stimulated (ND/NS) and old dystonic non stimulated old hamsters(D/NS), with stimulation paradigm of 10 Hz for 300 seconds between old non dystonic non stimulated (ND/NS) and old dystonic non stimulated hamsters (D/NS), and with stimulation paradigm of 1 Hz for 600 seconds between old non dystonic non stimulated (ND/NS) and old dystonic non stimulated (D/NS) hamsters. Long-Term Potentiation was not significant different between ND/NS, and D/NS hamsters with stimulation paradigm of 100 Hz for 1 second, 3 times, 20 seconds apart (P>0.05, Figures **A**a and **A**b). Each point bar represents the mean relative ± SEM for n = 6 to 7 separate observations. **A**a. Dot plot of relative fEPSP slopes before (1) and after (2) LTP induction paradigm delivered at timepoint 0. **Ab.** Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups. Long-Term Depression was not significant different between ND/NS, and D/NS hamsters with stimulation paradigm of 10 Hz for 300 seconds (P>0.05, Figures **B**a and **B**b). Each point represents the mean relative ± SEM for n = 6 to 7 separate observations. **B**a. Dot plot of relative fEPSP slopes before (1) and after (2) LTP induction paradigm delivered at timepoint 0. **Bb.** Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups. Long-Term Depression was not significant different between ND/NS, and D/NS with stimulation paradigm of 1 Hz for 600 seconds (P>0.05, Figure **C**a and **C**b). Each point represents the mean relative ± SEM for n = 5 to 7 separate observations. **C**a. Dot plot of relative fEPSP slopes before (1) and after (2) LTP induction paradigm delivered at timepoint 0. **Cb.** Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups.
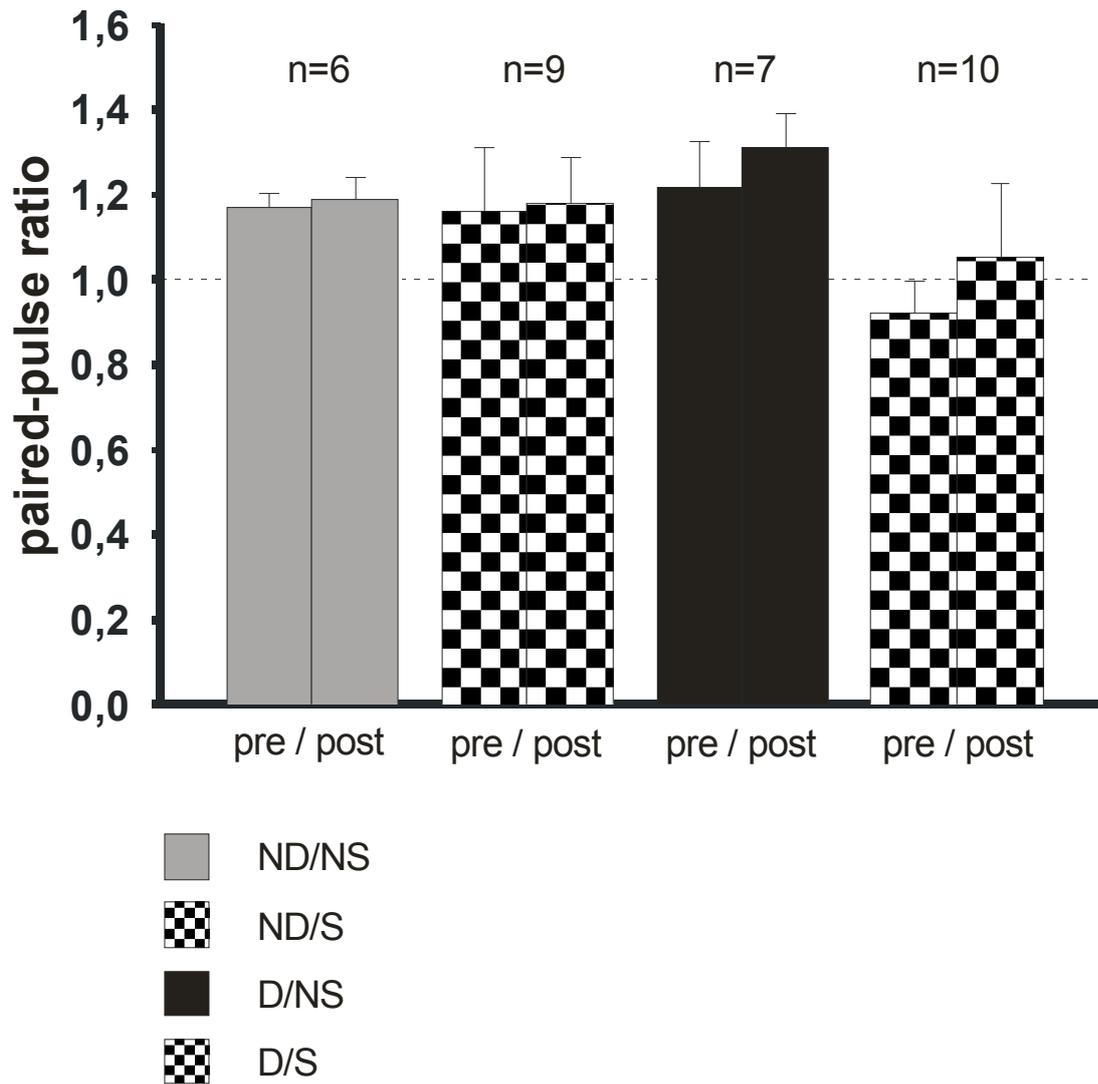
Figure 14. Comparison of paired-pulse facilitation in old non dystonic non stimulated (ND/NS), old non dystonic and stimulated (ND/S), old dystonic non stimulated (D/NS), and old dystonic and stimulated (D/S) hamsters before and after high frequency stimulation (HFS) with stimulation paradigm of 100 Hz for 3 seconds, 3 times, 20 seconds apart. Bar chart shows non significant difference in Paired-Pulse Facilitation before and after HFS between old ND/NS, ND/S, D/NS, and D/NS (P>0.05). Each point bar represents the mean relative ± SEM for n = 9 to 22 for each separate observations.
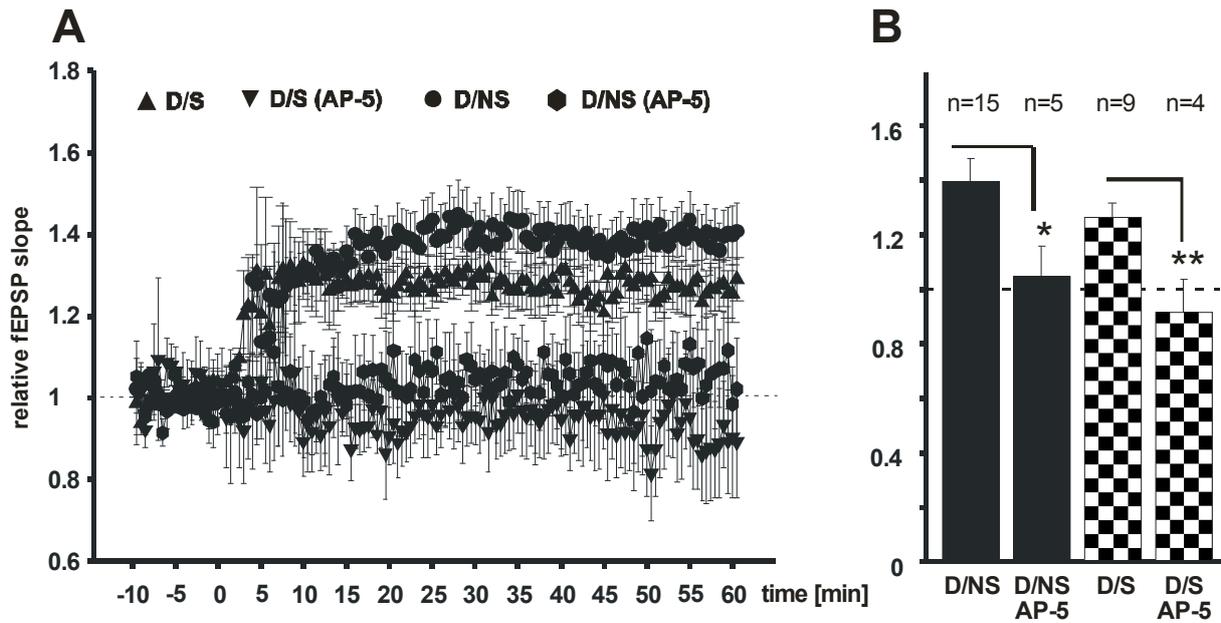
Figure 15. Long-Term Potentiation is fully blocked in the presence of D-AP5 (50 μM) in a young dystonic non stimulated mutants as well as in young dystonic stimulated mutants. **A.** Dot plot shows the relative field EPSP slope (mean relative ± SEM) of LTP measurement in D/NS, and in D/NS in the presence of D-AP5 (50 μM) as well as in D/S, and in D/S in the presence of D-AP5 (50 μM) at stimulus intensity of 100 Hz for 3 seconds, 3 times, 20 seconds apart. LTP in D/NS in presence of D-AP5 was fully blocked (P=0.039, n= 5 to 15 for each separate observations). Likewise, LTP in D/S was also fully blocked with the application of D-AP5 (p=0.009, n= 4 to 9 for each separate observations). **B.** Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups.
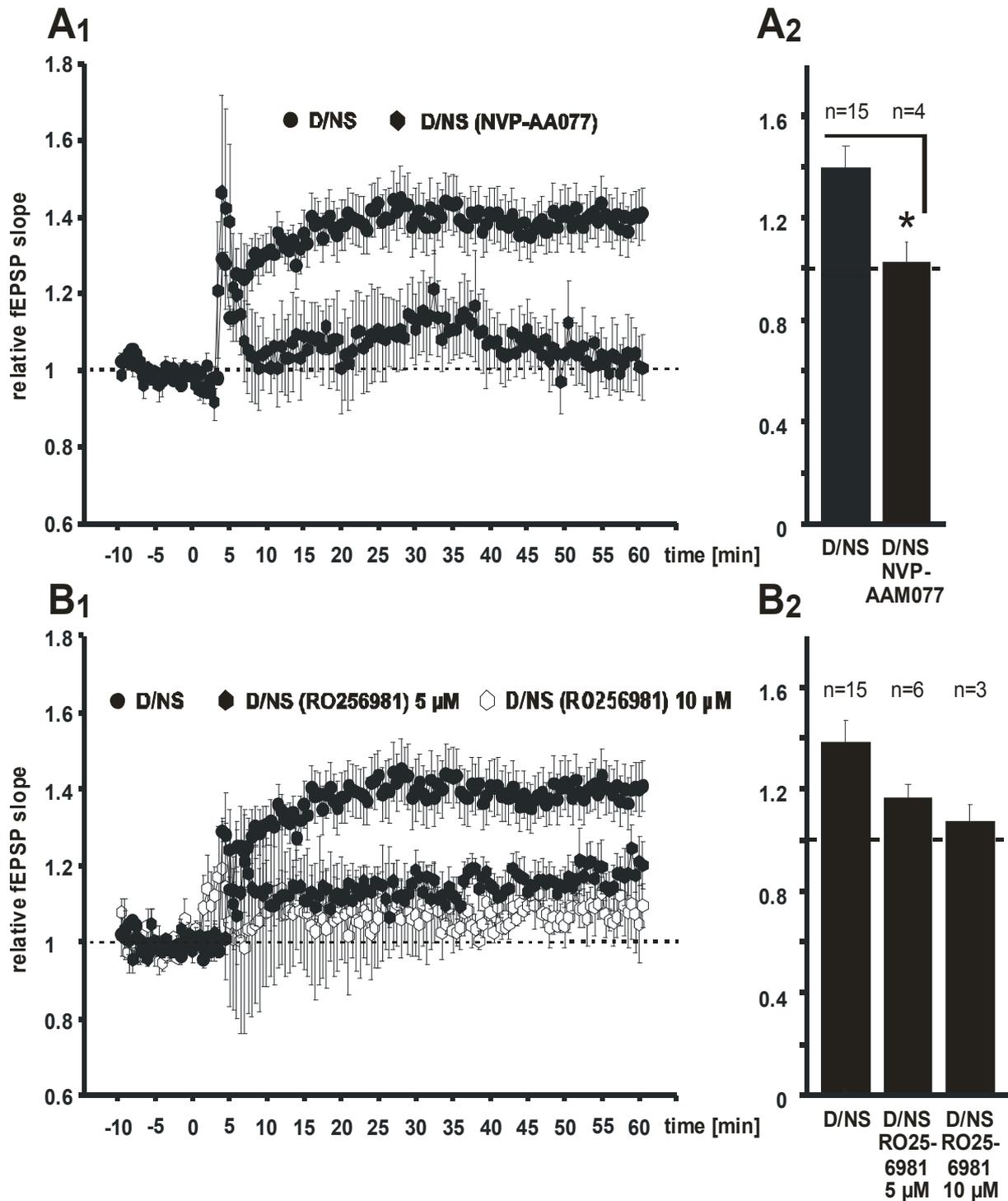
Figure 16. Long-Term Potentiation is only partially blocked in the presence of selective NR2B antagonist, Ro 25-6981, and complete blocked under in the presence of selective NR2A antagonist NVP-AAM077 in young dystonic non stimulated mutants (D/NS). **A$_1$.** Dot plot shows the relative field EPSP slope (mean relative ± SEM) of LTP measurement in D/NS, and in D/NS mutants in the presence of selective NR2A antagonist (50 nM) at stimulus intensity of 100 Hz for 3 seconds, 3 times, 20 seconds apart. LTP in D/NS was blocked with the application of NVP-AAM077 (50 nM) (P=0.023, n = 4-15 for each separate observations). **A$_2$.** Bar chart shows of mean ± SEM of relative fEPSP slopes

of different experimental groups. **B**$_1$ Dot plot shows the relative field EPSP slope (mean relative ± SEM) of LTP measurement in D/NS, and in D/NS mutants in the presence of selective NR2B antagonist (5 µM and 10 µM) at stimulus intensity of 100 Hz for 3 seconds, 3 times, 20 seconds apart. LTP in D/NS was not blocked with the application of RO 25-6981 (5 µM) ($P > 0.05$, n = 6-15 for each separate observations), and only partially blocked with the application of RO 25-6981 (10 µM) ($P > 0.05$, n = 3-15 for each separate observations). **B**$_2$. Bar chart shows of mean ± SEM of relative fEPSP slopes of different experimental groups.
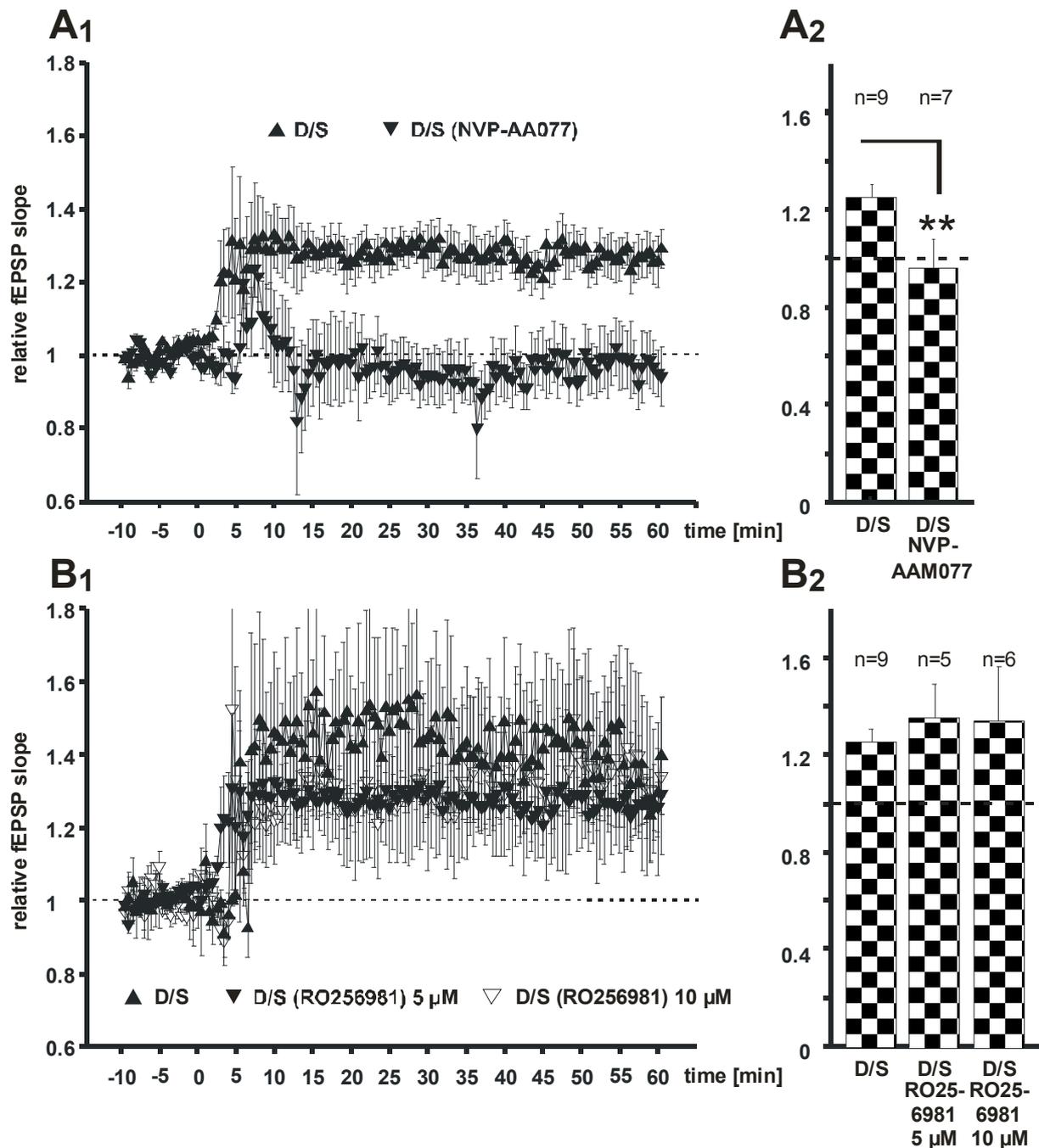
Figure 17. Long-Term Potentiation is only partially blocked in the presence of selective NR2B antagonist, Ro 25-6981, and complete blocked under in the presence of selective NR2A antagonist NVP-AAM077 in young dystonic stimulated mutants (D/S). **A₁.** Dot plot shows the relative field EPSP slope (mean relative ± SEM) of LTP measurement in D/S, and in D/S mutants in the presence of selective NR2A antagonist (50 nM) at stimulus intensity of 100 Hz for 3 seconds, 3 times, 20 seconds apart. LTP in D/S was blocked with the application of NVP-AAM077 (50 nM) (P=0.06, n = 7-9 for each separate observations). **A₂.** Bar chart shows of mean ± SEM of relative fEPSP slopes of different experimental groups. **B₁.** Dot plot shows the relative field EPSP slope (mean relative ± SEM) of LTP measurement in D/S, and in D/S mutants in the presence of selective NR2B antagonist (5 µM and 10

µM) at stimulus intensity of 100 Hz for 3 seconds, 3 times, 20 seconds apart. LTP in D/S was not blocked with the application of RO 25-6981 (5 µM) ($P > 0.05$, n = 5-9 for each separate observations), and only partially blocked with the application of RO 25-6981 (10 µM) ($P > 0.05$, n = 6-9 for each separate observations). **B$_2$.** Bar chart shows of mean ± SEM of relative fEPSP slopes of different experimental groups.
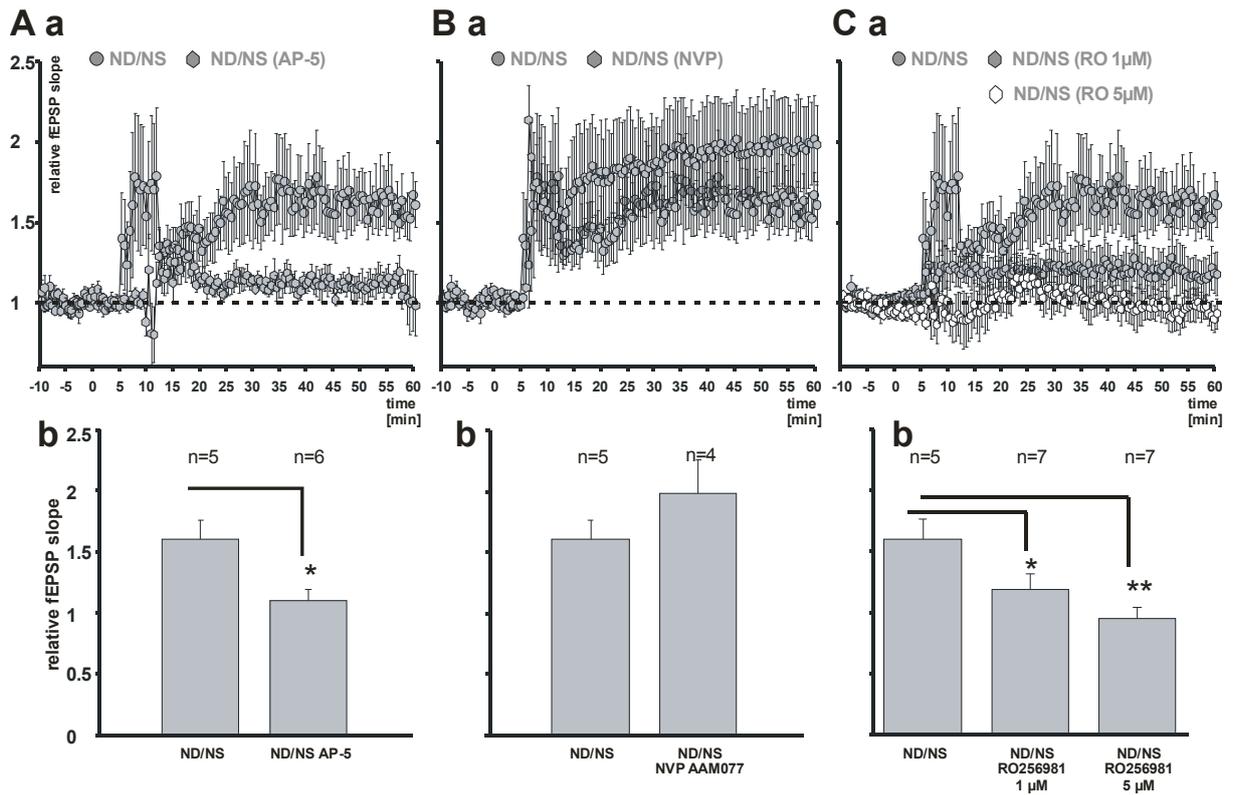
Figure 18. Long Term Potentiation is NMDA dependent, NR2B dependent, but independent of NR2A subunit in normal healthy animals with the stimulation of 100 Hz for 3 seconds, 3 times, 20 seconds apart. **Aa.** Dot plot of relative field EPSP slope (mean relative ± SEM) of LTP measurement in healthy animals, and in healthy animals in the presence of D-AP5. LTP in corticostriatal slices was blocked with the application of D-AP5 (50 µM) (P=0.018, n= 5-6 for each separate observations). **Ab.** Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups, **Ba.** Dot plot of relative field of EPSP slope (mean relative ± SEM) of LTP measurement in healthy animals, and in healthy animals in the presence of 50 nM of selective NR2A antagonist NVP-AAM077. LTP was not blocked in healthy animals in the presence of NVP-AAM077 850 nM) (P>0.05, n= 4-5 for each separate observations). **Bb.** Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups. **Ca.** Dot plot of relative field EPSP slope (mean relative ± SEM) of LTP measurement in healthy animals, and in health animals in the presence of RO 25-6981 (1 µM) as well as in the presence of RO 25-6981 (5 µM). LTP in healthy animals was blocked with the application of RO 25-6981 (1 µM) (P=0.019, n= 5-7 for each separate observations), and in the presence of 5 µM of RO 25-6981 (P=0.003, n= 5-7 for each separate observations). **Cb.** Bar chart of mean ± SEM of relative fEPSP slopes of different experimental groups.

Discussion

Discussion

In the present thesis, three main questions on the mechanisms of paroxysmal dystonia in the genetic dt^{sz}-hamster mutant were addressed:

A. Is an acute dystonic attack particularly reflected in synaptic plasticity / excitability changes in vitro, and, conversely, is there a difference to findings during symptom-free intervals.

B. Do the in-vitro changes in excitability / synaptic plasticity of tissue from dt^{sz} hamsters cease during maturation, i.e. with full clinical recovery?

C. Is the plasticity change observed in vitro dependent on NMDA receptors, and which subunits are involved?

The main findings pertaining to these questions were:

A. Synaptic plasticity changes in dystonic hamsters do not depend acutely on previous dystonic attacks; rather, the presence or absence of the dystonic phenotype per se determines whether LTP is expressed or not. Along with this, also synaptic excitability is increased.

B. With full clinical recovery, LTP appears both in normal tissue and in slices from dystonic hamsters in the remission state. At first sight, this indicates that both groups, parallel to clinical normalisation, also show similar characteristics in expressing synaptic plasticity. This, however, holds true only partly, since only in healthy control tissue, behaviourally dependent (stress induced) LTD could be unmasked, suggesting that activity dependent synaptic plasticity in dt^{sz} hamsters is infringed and altered also in remission state.

C. The increase of synaptic plasticity in dystonic hamsters is due to NMDA-receptor mediated mechanisms, and more specifically, to a functionally increased involvement of NR2A subunits. Conversely, synaptic plasticity in (young) normal tissue, emerging under conditions of partial Mg^{2+}-block-release of NMDA receptors, is carried by NR2B receptor subunits.

Long-term plasticity in young dystonic mutants

Two forms of long term synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD) are widely expressed at excitatory as well as at inhibitory synapses throughout the brain and have been described at corticostriatal synaptic pathway (Partridge et al., 2000, Reynolds and Wickens, 2002). Köhling et al., (2004) have shown an enhancement

of LTP in young dystonic hamsters with the stimulus paradigm using 100 Hz stimuli for 3s, repeated thrice at 20s intervals, at induction stimulation intensity of 200% saturating current intensity, our first aim was to replicate these data using a similar induction stimulation series (paradigm A), with the hypothesis that LTP-induction in dystonic tissue might depend on the emergence of paroxysmal dystonic attacks, i.e. hypothesising that long-term plasticity would be activity dependent in a metaplastic way. In the experiments, a) the data of Köhling et al. (referring to D/NS and ND/NS groups) could be replicated, showing that also in this experimental series, LTP was increased in dystonic tissue, even at physiological Mg^{2+} levels (Partridge et al., 2000). However, the hypothesis stipulating LTP expression to be activity dependent could not be verified; LTP was expressed to a similar degree in both D/NS and D/S groups and the only determining factor was the presence of dystonic phenotype, rather than exogenous activity/stress. Synaptic excitability, in turn, was increased in all groups relative to naïve, i.e. ND/NS tissue. In this context, Nobrega et al., (1997) illustrated that NMDA receptor binding is enhanced during dystonic attacks in dystonic mutant hamsters, whereas in dorsolateral part of the corticostriatum the density of NMDA receptor are unaltered (Nobrega et al., 1997). Such an an increase of NMDA-receptor density might account for an increase in excitability in D/NS and D/S groups, but it does not so in the ND/S group. Forced activity (triple stimulator technique), however, may be speculated to have a similar effect, since excitability was equally enhanced in ND/S groups: This opens two possible interpretations: First, presumed overactivity in the striatum (Gernert et al, 2000), either induced by external manipulation or by the presence of dystonic phenotype, leads to an increase in excitability. Second, since this increase in excitability also occurred in ND/S tissue, the mechanisms of increased synaptic plasticity (LTP expression) versus increased excitability (shift of I/O curve) are not identical. What could be the reason for this enhancement of synaptic plasticity in dystonic mutants? There are number of reasons that might contribute for the enhancement of LTP in dystonic hamsters. One obvious reason for this effect might be the loss of inhibitory interneurons in dt[sz] hamsters in the striatum and decrease single unit activity in the output structure of the basal ganglia, the entopeduncular nucleus (EPN) (the rodent's homolog of the globus pallidus interna (GPi) in primates), onto which striate output neurones project (Gernert et al., 1999, 2000). It is well known that inhibitory interneurons are important for the inhibition of GABAergic spiny neurons which project to the globus pallidus and to the basal ganglia output nuclei (Bennet and Bolam, 1994). Thus, the deficit of GABAergic level in the striatum, may lead to the lowered activity of EPN which in turn may causes an increased thalamic activity to facilitate motor pattern generators in the cerebral cortex and could

therefore contribute to the enhancement of LTP and to the occurrence of dystonic attacks. This notwithstanding, the differences among the different groups in young tissue were lost using other induction paradigms. Specifically, paradigms B and C did not elicit any long-term plasticity, and paradigm D resulted in the expression of minimal, but not significant, depression, and this again to a similar degree in all groups. All in all, this points to a low dynamic range of synaptic plasticity in young, immature corticostriatal synapses, which is increased to a certain degree by the dystonic phanotype. As the paired-pulse experiments reveal, this alteration is unlikely to be presynaptic. This contrast previous findings, in that the enhancement of LTP was hinted to be at least partially dependent on presynaptic changes (Köhling et al. 2004). One difference between this study and the cited one is that different stimulation paradigms were employed due to technical reasons (current vs. voltage outputs of the stimulators). The other possible reasons for the differences are the differences of intestimulus intervals that were employed. In the current study, a shorter (40 ms) interval was chosen to more specifically address the question of pre- versus postsynaptic changes. In principle, paired-pulse responses can be both governed by presynaptic depletion kinetics of the vesicle pool and recurrent inhibition. The latter is thought to be dominated by excitatory drive at higher stimulation intensities (Ling and Benardo, 1995), and more importantly, to occur at slower kinetics (approx. 60 ms for a bisynaptic loop; Brunel and Wang, 2003). Thus, even though contributions of fast feedforward inhibition cannot be excluded (Turner, 1990), at a shorter, 40 ms interval, presynaptic mechanisms will be unveiled better than at longer intervals. Further, Akopian and Walsh (2002) have illustrated that NMDA as well as AMPA-mediated transmission at the cortico-striatal synapse can undergo differential paired-pulse changes depending on stimulus intensity.

Functional changes during maturation

The second main finding of the thesis is that with maturation, LTP is expression appears to be the physiological response to high-frequency activation of the striatum. Importantly, this seems to hold true irrespective of a history of dystonia; tissue from animals in the remission phase (D/NS and D/S groups) appears to be normalised in this respect. What could be the reason for this? Recently, Hamann et al., (2007) have shown that the deficit of striatal PV+ GABAergic interneurons has completely disappeared in older hamsters in the dorsal and posterior parts of the striatum (Hamann et al., 2007) which may reflect an inability to sustain LTP in dystonic mutants relative to the control ones. Previous findings of an age-dependent

normalization of striatal activity have also been reported by Gernert et al (2002) who illustrated a complete normalization of the discharge pattern of GPi after remission of dystonia in dt^{sz} hamsters. Thus, the present work on corticostriatal plasticity in mutant hamsters and with the work of Hamann et al., (2007) and Gernert et al, (2002, 2000) suggests that alterations of inhibitory interneurons could be an important factor in the manifestation of paroxysmal dystonia in dt^{sz} mutant and the enhacement of LTP in corticostriatal pathway in dystonic hamsters, and conversely, the apparent normalization in older tissue. These considerations notwithstanding, a full functional remission in dystonic tissue can be debated on the grounds of the experiments of this thesis, where it could be demonstrated that behavioural stimulation of healthy hamsters prior to in-vitro high-frequency stimulation preconditioned the tissue to express LTD, rather than LTP. This suggests that the dynamic range of plasticity responses in normal tissue is much wider than in dystonic one, or indeed in young tissue, ranging from LTD to LTP depending on behavioural preconditioning, and that this dynamic range is narrowed down by a history of dystonia, even if dystonic attacks do not arise any more. Could presynaptic mechanism acoount for this? As evidenced by the analysis of paired pulse responses, this seems unlikely, since there is no difference among any of the ND/NS, ND/S and D/NS groups, with the PPR being > 1 in all of them. Thus, both in mature and young tissue, expression of LTP in the paradigms used is not presynaptically determined. Yet, presynaptic mechanisms may play a role in the alterations of synaptic excitability, i.e. the downregulation of I/O curves within the D/S group. In this group, the PPR was significantly reduced to values < 1 both in relation to the age-matched D/NS group and to the corresponding young D/S group. This may suggest behavioural stimulation, at least in mature animals, can might lead to presynaptic changes, which do not have an impact on LTP expression, but on excitability.

The role of NMDA receptors in LTP expression in dt^{sz} mutants

Since we find that LTP in dystonic mutant represents a postsynaptic effect, we investigated the role of NMDA receptor and the role of NMDA receptor subtypes in the induction of LTP in dt^{sz} mutant. We found that LTP in dystonic hamsters was primarily mediated by NMDA receptors. In the striatum, LTP is considered a cellular model of learning and memory. In the striatum, LTP is preferentially induced in the absence of external magnesium ions (Calabresi et a., 1992a), although other groups report LTP to arise also under increased Mg^{2+} concentrations, provided the dorsomedial part of the striatum is investigated (Partridge et al.,

2000), the region which was in fact focused on in this thesis. Here, it was demonstrated that under these conditions, LTP is expressed in dystonic, but not in normal tissue. To elicit LTP in normal tissue, indeed the Mg^{2+}-concentration had to be lowered to 1.0 mM, replicating findings in rats reported by other groups also in hamsters. In either case, LTP was dependent on NMDA-receptors, since it was blocked by D-AP5. A number of studies have shown that with the strong stimulation intensity of corticostriatal afferents, NMDA-dependent components of EPSP can be observed in striatal neurons (Akopian and Walsh, 2002; Cherubuni et al., 1988). The contribution of NMDA receptor-mediated transmission might be also dependent on the anatomical location. Thus, Smith et al. (2001) have illustrated that in dorsomedial striatum (the region addressed also in this thesis) displays strong short and long term plasticity changes dependent on the activation NMDA receptors even in the presence of magnesium ions. At odds with this, Calabresi et al., (1996) have demonstrated that a major component of the excitatory synaptic potential recorded from striatal neurones was primarily mediated by AMPA receptors and several authors have shown a failure or only a weak reduction of evoked synaptic potentials by NMDA receptor antagonists (Vilagi et a., 1995; Herrling et al., 1985). Again, the reason for this discrepancy very likely lies in regional differences of receptor expression, with dorsomedial regions expressing NMDA receptors, and dorsolateral ones not. Interestingly, a previous report shows that systemic application of competitive and non competitive NMDA receptor antagonists attenuates dystonic attacks in dt^{sz} hamsters (Richter et al., 1991). An antidystonic effect with a non-competitive NMDA receptor antagonist has also been reported in patients with dystonia (Fredow and Löscher, 1991). At the biochemical level, Nobrega et al., (1997) have shown that NMDA receptor binding was enhanced during dystonic attacks in dystonic mutant hamsters in several brain regions, including in the ventrolateral thalamic nucleus, which may be associated with altered basal ganglia output. Thus, these results suggest that the involvement of excessive glutamatergic transmission might contribute to the manifestation of paroxysmal dystonia in the dt^{sz} mutant. Interestingly, a recent report of Sander et al., (2007), have illustrated that intrastriatal injection of NMDA receptor competitive antagonists (D-AP5) failed to affect the severity of dystonic attacks in the dt^{sz} hamstes. This result suggests that with systemic administration of NMDA receptor antagonist, the antidystonic effects might be mediated by extrastriatal brain regions, too. The same author shows that intrastriatal injection of the AMPA competitive antagonist NBQX exerts antidystonic effects (Sander et al., 2007). In addition to that, autoradiographic studies showed decreased AMPA receptor binding in the dorsomedial as well as in dorsolateral part of the striatum, which may suggest a compensatory

reaction to the enhancement of NMDA receptor binding in ventrolateral thalamic nucleus (Nobrega et al, 2002).

The role of NMDA receptor subunits in long-term plasticity in corticostriatal synapses of dt^{sz} mutants and normal hamster

Since we found that LTP in dystonic mutant was primarily mediated by NMDA receptors, the last step of this project was to determine which of the NMDA receptor subunits NR2A or NR2B were involved in the enhancement of synaptic plasticity in dorsomedial striatum in dystonic mutant. In addition to that, we also conducted a separate experiments to determine the role of different NMDA receptor subunits (NR2A and NR2B) in the regulation of synaptic plasticity in a tissue of normal hamsters. For this, we used a selective NR2B antagonist, Ro25-6981 and the selective NR2A antagonists, NVP-AAM077. Our results unequivocally show that in dystonic mutants, LTP predominantly is driven by NR2A receptors, whereas in normal tissue, NR2B receptors are responsible. A note on the different concentrations of the drugs used may be appropriate here: Since the NR2A receptor antagonist is considered to be slightly less selective, in the Experiments on dystonic groups, we used rather large (and at 10 µM unspecific) concentrations of the NR2B antagonist. The fact that even under these high concentrations of the NR2B antagonist, LTP was not significantly suppressed underscores that notion that it is NR2A which is solely responsible for synaptic plasticity in the corticostriatal synapse of dystonic hamsters. In normal tissue, in turn, NR2A antagonist application had virtually no effect, and consequently, here lower, specifically acting concentrations of the NR2B blocker (1 and 5 µM) were already effective, underlining its importance in normal tissue. The results suggest that an overexpression of NR2A subunits may be involved in the enhancement of LTP in dt^{sz} mutant. Interestingly, Gardoni et al., (2006) have demonstrated that NR2A subunits are increased in dyskinetic rats in a symptomatic model of L-DOPA- induced dyskinesia. The same author found that the level of NR2B subunits shows a significant reduction in dyskinetic rats (Gardoni et al., 2006). Recent studies in primates have also suggested that upregulation of NR2A subunit in synaptosomal membranes may be an important factor in L-DOPA-induced dyskinesias (Hallet et al., 2005). Thus, the biochemical as well as our pharmacological approaches revealed that NR2A subunit does upregulate, at least functionally, during dystonic attacks and it might be a crucial factor in the manifestation of paroxysmal dystonia in the dt^{sz} mutant. In this context, the precise function and the role of NR1 receptor subunits in the regulation of corticostriatal synaptic

pathway is completely unknown, although recently Dang et al, (2006) have illustrated that the deletion of NR1 subunit of NMDA receptors from the striatum, abolished striatal LTP in dorsomedial part of the corticostriatum and impaired motor learning. One must bear in mind, however, that by the deletion NR1 subunit, NMDA function is completely eliminated (Dang et al. 2006).

What might be the mechanism of the induction of LTP in dorsomedial part of corticostriatum? There is evidence that parvalbumin-containing GABAergic interneurons in the striatum express mainly NMDA receptors with NR2B subunits (Landwehrmeyer et al., 1995). Since LTP in dorsomedial striatum is primarily dependent on the activation of NR2B receptors in normal tissue, this would suggest an involvement of interneurons in this process. As mentioned above, during dystonia the level of parvalbumin-containing GABAergic interneurons is decreased in the striatum in young dt^{sz} hamsters which in turn will downregulate NR2B subunit density. Since LTP in dystonic hamsters is mainly dependent on NR2A subunits, we suggest that during dystonia there is a switch from NR2B to NR2A subunits, and perhaps indeed from interneuron mediated to directly-mediated LTP, which may provide a compensatory mechanism for the induction of synaptic plasticity in dystonic mutant.

We hypothesize that this switch may be important for regulating the threshold for the induction of synaptic plasticity and the magnitude of the expressed plasticity which might be important for homeostatic plasticity. In light of these considerations, understanding the role of NMDA receptor in the regulation of synaptic plasticity in dorsomedial striatum in dystonic mutant might result in novel therapeutic strategies to prevent abnormal movement which is associated with abnormalities in basal ganglia.

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