

**BEHAVIOURAL ANALYSIS OF TRANSGENIC MICE  
OVEREXPRESSING GAMMA-SYNUCLEIN**

**Hatem Salem**

**Cardiff University**

**2011**

## **Acknowledgment**

I am grateful to Vladimir Buchman, my supervisor and Steve Dunnett, my second supervisor for their guidance and support.

Many thanks to Simon Brooks, Natalia Ninkina and Abdelmojeeb Al-wandy who have helped and supported me in my study. And thanks for all people who have helped me in so many ways along my study.

Special thanks to Libyan peoples bureau in London.

## **Abstract**

The alpha-synucleinopathies is a diverse group of diseases characterised by malfunctioning of alpha-synuclein protein. Parkinson's disease is the most common of all alpha-synucleinopathies.

Intracellular inclusions, like Lewy bodies and certain other abnormal structures are the pathological hallmarks of this group of neurodegenerative diseases. The main protein component of these structures is alpha-synuclein.

Alpha-synuclein is a member of the synuclein family, which consists of 3 closely related proteins, alpha-, beta- and gamma-synucleins. Alpha-synuclein is more extensively studied because of its direct involvement in human diseases, while our knowledge about two other family members is very limited. Neither beta-synuclein, nor gamma-synuclein is a component of Lewy bodies and other pathological structures typical for neurodegenerative diseases.

However, there are several reports of accumulation of beta-synuclein and particularly gamma-synuclein in atypical pathological structure in brains of patients suffering from neurodegenerative diseases and mice carrying a deletion of UCHL1 gene.

Therefore, it has been suggested that malfunction of gamma-synuclein could lead to neuronal pathology similar to pathology caused by malfunction of alpha-synuclein.

To question this hypothesis, a transgenic mouse line expressing a high level of mouse gamma-synuclein under control of pan-neuronal Thy-1 promoter has been produced.

A battery of various behavioural tests has been selected to test gamma-synuclein transgenic (heterozygous and homozygous) mice and their wild type littermates at different ages.

These tests included acoustic startle test, gait test, locomotor activity count test, rotarod test, and beam walking test. the Results of the locomotor activity test were unconvincing but the other four tests revealed that gamma-synuclein transgenic mice develop age-dependent motor dysfunction, the onset of which correlated with the genotype. These findings support the hypothesis that gamma-synuclein may play a role in pathogenesis of neurodegenerative diseases.

**Declaration by candidate**

I hereby declare that this thesis is my own work, and it has not been submitted to any other university for examination either in the united kingdom or overseas. Also, I declare that other sources of information have been acknowledged.

## Table of Contents

CHAPTER 1- INTRODUCTION .....	7
1.1- THE SYNUCLEIN FAMILY .....	7
1.1.1 ALPHA SYNUCLEIN .....	8
1.1.2 BETA- SYNUCLEIN .....	11
1.1.3 GAMMA-SYNUCLEIN .....	13
1.2- FUNCTION OF SYNUCLEINS IN THE NERVOUS SYSTEM .....	15
1.3 SYNUCLEINOPATHIES .....	18
1.3.1 PARKINSON'S DISEASE .....	19
1.3.2 AETIOLOGY OF PARKINSON DISEASE .....	19
1.3.3 CLINICAL SIGNS AND HISTOPATHOLOGICAL FEATURES OF PARKINSON'S .....	20
1.3.4 GENERAL PRINCIPAL OF PARKINSON'S DISEASE TREATMENT .....	21
1.4 BEHAVIOURAL TESTING OF ANIMALS MODELLING NEURODEGENERATIVE .....	23
1.4.1 BEAM WALKING TEST .....	24
1.4.2 Rota-rod test .....	24
1.4.3 GAIT TEST (FOOT PRINT) .....	25
1.4.4 LOCOMOTOR ACTIVITY COUNT .....	25
1.4.5 ACOUSTIC STARTLE STIMULUS AND PREPULSE INHIBITION .....	26
1-5 GENERATING OF TRANSGENIC MICE OVEREXPRESSING GAMMA SYNUCLEIN .....	27
CHAPTER 2- MATERIALS AND METHODS .....	29
2.1 EXPERIMENTAL ANIMALS .....	29
2.2 ACOUSTIC STARTLE TEST .....	29
2.3 GAIT TEST (FOOTPRINTING) .....	32
2.4 BEAM WALKING TEST .....	33
2.5 ROTA-ROD TEST .....	35
2.6 MOTOR ACTIVITY TEST .....	37
2.7. DNA PURIFICATION .....	38
2.7.1 EXPERIMENTAL GROUPS AND GENOTYPING OF GAMMA-SYNUCLEIN .....	39
2.8. STATISTICAL ANALYSIS .....	41
CHAPTER 3-RESULTS .....	42
3.1 PREPARATION OF EXPERIMENTAL ANIMAL GROUPS .....	42
3.2 PREPULSE INHIBITION OF ACOUSTIC STARTLE RESPONSE .....	43
3.3 GAIT TEST .....	47
3.4 LOCOMOTOR ACTIVITY COUNT TEST .....	52
3.5 ROTA-ROD TEST .....	59
3.6 BEAM WALKING TEST .....	62
CHAPTER 4- DISCUSSION .....	66
REFERENCES .....	73

## **Chapter 1- Introduction**

### **1.1- The synuclein family**

Synucleins were discovered in 1988 by Maroteaux, while he was working on the pacific electric ray. Using an antiserum against cholinergic vesicles and the immunostaining technique a novel protein was identified and localised to the presynaptic terminals and the nuclear membrane of the electric organ neurons, therefore this protein was named synuclein (Maroteaux et al., 1988). Few years later, three isoforms of synuclein were found in the rat brain and designated SYN1, SYN2, and SYN3 (Maroteaux and Scheller, 1991). Subsequently, synucleins were identified in the human cerebral cortex, and the encoding cDNA was cloned and sequenced. Two abundant isoforms of 140 and 134 amino acids were named alpha-, and beta-synucleins respectively (Jakes et al., 1994). More recently, another protein was identified as a protein highly expressed in breast cancer tissue (Ji et al., 1997) or in mouse peripheral nervous system (Buchman et al., 1998). Because of its similarity to alpha- and beta-synuclein it was named gamma-synuclein (Lavedan, 1998). Using immunocytochemical and subcellular fractionation approaches it has been proved that synucleins are indeed abundant at presynaptic terminals of rat (Maroteaux and Scheller, 1991), song bird (George et al., 1995), and human brain (Jakes et al., 1994; Irizarry et al., 1996). The three human synucleins share 44% overall identity, however beta-synuclein has 78% identity with alpha-synuclein, while, gamma-synuclein shares 60% identity with alpha-synuclein (Goedert, 2001).

Human alpha-, beta-, and gamma-synuclein genes (SNCA, SNCB, and SNCG) are mapped to chromosomes 4q21.3-q22, 5q35, and 10q23, respectively (Campion et al.,

1995; Shibasaki et al., 1995; Lavedan, 1998; Ninkina et al., 1998; Spillantini et al., 1995).

### 1.1.1 Alpha synuclein

Alpha-synuclein is a heat stable protein that is abundant in the brain; by some estimates this protein makes 1% of the total protein in the soluble cytosolic brain fraction (Iwai et al., 1995). Alpha-synuclein consists of 140 amino acids in human and rodents and the amino acid sequence identity between human and mouse or rat alpha-synuclein is 95% (Maroteaux and Scheller, 1991) (Figure 1).

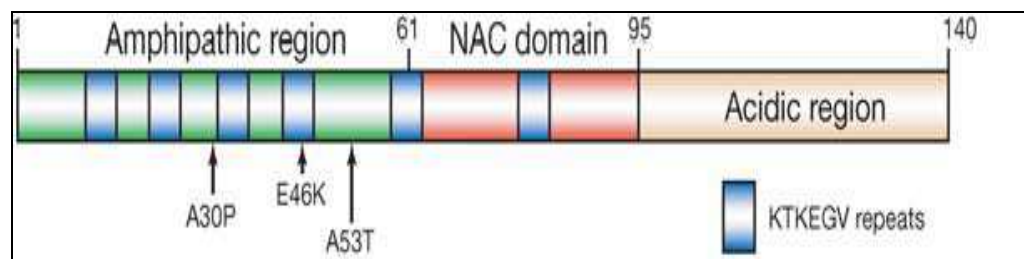
ALPHA-SYNUCLEIN		
Rat	MDVFMKGLSKAKEGVVAAAEEKTKQGVAEAAAGKTKEGVLYVGSKTKEGVVH	50
Mouse	MDVFMKGLSKAKEGVVAAAEEKTKQGVAEAAAGKTKEGVLYVGSKTKEGVVH	50
Human	MDVFMKGLSKAKEGVVAAAEEKTKQGVAEAAAGKTKEGVLYVGSKTKEGVVH	50
Rat	G V T T V A E K T K E Q V T N V G G A V V T G V T A V A Q K T V E G A G N I A A A T G F V K K D Q M	100
Mouse	G V T T V A E K T K E Q V T N V G G A V V T G V T A V A Q K T V E G A G N I A A A T G F V K K D Q M	100
Human	G V <span style="border: 1px solid black;">A</span> T V A E K T K E Q V T N V G G A V V T G V T A V A Q K T V E G A G <span style="border: 1px solid black;">S</span> I A A A T G F V K K D Q <span style="border: 1px solid black;">L</span>	100
Rat	G K G E E G Y P Q E G I L E D M P V D P S S E A Y E M P S E E G Y Q D Y E P E A	140
Mouse	G K G E E G Y P Q E G I L E D M P V D P <span style="border: 1px solid black;">G</span> S E A Y E M P S E E G Y Q D Y E P E A	140
Human	G K <span style="border: 1px solid black;">N</span> E E G <span style="border: 1px solid black;">A</span> P Q E G I L E D M P V D <span style="border: 1px solid black;">F</span> <span style="border: 1px solid black;">D</span> <span style="border: 1px solid black;">N</span> E A Y E M P S E E G Y Q D Y E P E A	140

**Figure 1.** Amino acid sequences of rat, mouse, and human alpha-synucleins. Amino acids inside boxes are different between at least two of these three species.

Structurally, alpha-synuclein protein has three modular protein domains (Ueda et al., 1993) (Figure 2). The first one is a highly conserved amino terminal lipid binding domain (residues 1-61) that is capable of forming alpha-helix. Residues 61 to 95 represent the second, internal hydrophobic (NAC) domain, which is the building block of synuclein aggregates - the GAV motif (residues 66-74) within the NAC domain has been demonstrated to be responsible for fibrilization of alpha-synuclein



(Du et al., 2003). The third structural domain is a carboxyl terminal acidic tail (residues 95-140).



**Figure 2.** This scheme adapted from Moore et al., (2005) shows the three main regions of alpha-synuclein protein and the location of the three points of mutation (A53, A30P, and E46K).

The primary structure of alpha-synuclein has seven imperfect 11-residue repeat sequences capable of forming five amphipathic helices on the amino-terminal half (Davidson et al., 1998; Jao et al., 2004). Alpha-synuclein helices 1-4 were predicted to associate with lipid vesicles (Biere et al., 2000; Perrin et al., 2000), whereas, helix 5 might be responsible for protein-protein interactions (Davidson et al., 1998).

Three pathogenic missense point mutations in human alpha-synuclein gene have been identified in association with the early onset hereditary form of Parkinson's disease. In 1997 Polymeropoulos and colleagues described a mutation in Greek and Italian families that resulted in substitution of alanine to threonine in the position 53 (A53T) of alpha-synuclein. This alteration may lead to the disruption of the alpha-helical structure, and expansion of the beta-sheet structure, which increases protein propensity to aggregate (Polymeropoulos et al., 1997). In 1998 Kruger and colleagues reported another alpha-synuclein gene mutation in a German family with an early onset hereditary form of Parkinson's disease. This mutation results in changing of alanine to proline in position 30 (A30P) (Kruger et al., 1998). The third mutation was found in 2004 by Zarranz and colleagues in a Spanish family with a hereditary form

of Parkinson's disease characterised by atrophy in substantia nigra, lack of Alzheimer's pathology, and multiple alpha-synuclein and ubiquitin immunopositive Lewy bodies in cortical and subcortical areas. This mutation leads to substitution of glutamic acid to lysine in position 46 of human alpha-synuclein (E46K) (Zarranz et al., 2004). Moreover, overexpression of alpha-synuclein or Parkinson's disease associated alpha-synuclein mutants, in mouse, fly, worm, and even yeast suggest that excess accumulation of alpha-synuclein leads to cellular toxicity (Moore et al., 2005; Lee and Trojanowski, 2006).

Alpha-synuclein gene duplication and triplication have been also associated with autosomal dominant familial forms of Parkinson's disease (Singleton et al., 2003; Farrer et al., 2004; Forman et al., 2005; Savitt et al., 2006). Patients with alpha-synuclein gene triplication show earlier onset of the disease with rapid cognitive decline, more severe non-motor symptoms, more widespread neurodegeneration and faster disease progression than patients with duplication of the gene, which suggests that gene dosage affects the pathogenesis of Parkinson's disease (Singleton et al., 2003; Farrer et al., 2004).

It is commonly accepted the pathological effect of alpha-synuclein is connected with its ability to aggregate and form filaments and fibrils. Alpha-synuclein aggregates into cross  $\beta$ -amyloid type structure spontaneously, and the NAC region seems to play an important role in forming the aggregates (Giasson et al., 2001). The full-length recombinant wild type alpha-synuclein has the ability to self-aggregate and form fibrils (Hashimoto et al., 1998; Giasson et al., 1999). This process depends on the protein concentration, time, temperature, pH, and the presence of certain molecules and ions, particularly heavy metal ions (Ostrerova-Golts et al., 2000; Uversky et al., 2001; Uversky et al., 2002; Golts et al., 2002). Atomic force microscopy and electron

microscopy demonstrated that filaments formed by recombinant protein in vitro have diameter of 8-10 nm (Conway et al., 1998, 2002; Giasson et al., 1999; Narhi et al., 1999). These filaments have a straight appearance (Conway et al., 1998, 2002; Serpell et al., 2000) and are similar to those extracted from brain tissues of patients with Parkinson's disease, Lewy body dementia (Conway et al., 1998; Spillantini et al., 1997) or multiple system atrophy (Arima et al., 1998) although the diameter of the latter type is larger.

All three known missense mutations (A53T, A30P and E46K) potentiate the aggregation of recombinant alpha synuclein (Conway et al., 2000; El Agnaf et al., 1998; Woong et al., 2004).

### **1.1.2 Beta- synuclein**

Beta-synuclein, the second member of the synuclein family, was first identified by Jakes and colleagues using a monoclonal antibody, which was raised against paired helical filaments of Alzheimer's disease brains (Jakes et al., 1994). It was independently isolated from bovine brain and named phosphneuroprotein-14 (Tobe et al., 1992; Galvin et al., 1999). The human beta-synuclein consists of 134 amino acids and shares 78% identity with human alpha-synuclein, but lacks 11 hydrophobic amino acids located in the central region of alpha-synuclein molecule (Goedert, 2001). The absence of this fragment, which is thought to play an important role in alpha-synuclein aggregation (Uversky et al., 2002), might be the main reason why beta-synuclein shows very low propensity to aggregate.

Mouse and rat beta-synucleins have identical amino acid sequences and share 97.8% identity with human beta-synuclein (Lavedan, 1998) (Figure 3).

In the rat brain beta-synuclein immunoreactivity has been detected in the cerebral cortex, granular cell layer of the olfactory bulb, hippocampus, striatum, cerebral

cortex, caudate putamen, thalamic reticular nuclei, and brain stem (Nakajo et al., 1994; Mori et al., 2002; Li et al., 2002). Lower levels of expression have been found in the locus coeruleus and in the perikarya of the neurons in the substantia nigra pars compacta (Li et al., 2002) and in non-neuronal tissues (Nakajo et al., 1996). In neurons beta-synuclein is found predominantly in the presynaptic nerve terminals where it co-localizes with synaptophysin (Jakes et al., 1994; Nakajo et al., 1994; Murphy et al., 2000).

The human beta-synuclein gene has been mapped to human chromosome 5q35 (Giasson et al., 2000; Clayton and George, 1999) and mouse beta-synuclein - to mouse chromosome 13 (Sopher et al., 2001). Two mutations of the gene encoding beta-synuclein that lead to amino acid substitutions (V70M and P123H) in highly conserved region of the protein have been associated with predisposition to dementia with Lewy bodies (Ohtake et al., 2004). However, in agreement with its low propensity to aggregate, beta-synuclein-positive inclusions have not been detected in the brain of patients with these mutations or in brains of patients with idiopathic forms of Parkinson's disease or dementia with Lewy bodies.

BETA- SYNUCLEINS		
Rat	MDVFMKGLSMAKEGVVAAAETKQGVTEAAETKEGVLYVGSKTKEGVVQ	50
Mouse	MDVFMKGLSMAKEGVVAAAETKQGVTEAAETKEGVLYVGSKTKEGVVQ	50
Human	MDVFMKGLSMAKEGVVAAAETKQGVTEAAETKEGVLYVGSKTREGVVQ	50
Rat	GVASVAEKTKEQASHLGGAVFSGAGNIAAATGLVKKEEFPTDLKPEEVAQ	100
Mouse	GVASVAEKTKEQASHLGGAVFSGAGNIAAATGLVKKEEFPTDLKPEEVAQ	100
Human	GVASVAEKTKEQASHLGGAVFSGAGNIAAATGLVKREEEFPTDLKPEEVAQ	100
Rat	EAAEEPLIEPLMEPEGESYEDSPQEEYQEYEPEA	134
Mouse	EAAEEPLIEPLMEPEGESYEDSPQEEYQEYEPEA	134
Human	EAAEEPLIEPLMEPEGESYEDPPQEEYQEYEPEA	134

**Figure 3.** Amino acid sequences of rat, mouse, and human beta-synucleins. Amino acids inside boxes are different between the human and rodent proteins.

### 1.1.3 Gamma-synuclein

Human gamma-synuclein is composed of 127 amino acids and shares 60%, and 56% similarity with human alpha- and beta-synuclein, respectively. It also shares 87.7% and 83.8% identity with mouse and rat gamma-synuclein, respectively (Figure 3). Gamma-synuclein is highly expressed in the embryonic and adult peripheral nervous system and spinal cord (Buchman et al., 1998). In the adult brain gamma-synuclein expression was detected in the substantia nigra, hippocampus, thalamus, caudate nucleus and amygdala but at lower levels than in the peripheral nervous system. Gamma-synuclein is also expressed in selected non-neuronal tissues, namely in skin epidermis (Ninkina et al., 1999) and white fat depots (Oort et al., 2008; Frandsen et al., 2009). In breast and ovarian malignant tumours and tumour cell lines very high levels of gamma-synuclein expression was detected (Ji et al., 1997; Ninkina et al., 1998), while in the epithelial cells of the normal mammary gland and ovary the level of gamma-synuclein expression was hardly detectable (Lavedan, 1998; Bruening et al., 2000).

The human gamma-synuclein gene has five exons, (Ninkina et al., 1998; Lavedan, 1998) and has been mapped to chromosome 10q23 (Ninkina et al., 1998; Giasson et al., 2000; Clayton and George, 1999; Ji et al., 1997).

Gamma-synuclein propensity to aggregate is much lower than of alpha-synuclein but substantially higher than of beta-synuclein. Recombinant gamma-synuclein forms amyloid fibrils but even in higher concentration solution the kinetics of their formation is ~20 fold slower than for recombinant alpha-synuclein (Uvesky et al., 2002).

Although, gamma-synuclein is not a component of the pathological inclusions typical for Parkinson's disease and dementia with Lewy bodies (Spillantini et al., 1997;

Iwatsubo, 2003), high levels of gamma-synuclein have been detected in the brains of patients with sporadic cases of these diseases (Rockenstein et al., 2001). Moreover, gamma-synucleins have been detected in axonal spheroid-like lesions in the hippocampal dentate molecular layer of some Parkinson's disease and dementia with Lewy bodies patients, but were not detected in control brains tissues (Galvin et al., 1999).

In Gad mice with inactivated ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) gene, a model of gracile axonal dystrophy, Wang and his colleagues found gamma-synuclein immunoreactive spheroids in the gracile nucleus from 3 weeks of age. Accordingly they suggest that gamma-synuclein contributes in the pathogenesis of axonal swelling in gad mice (Wang et al., 2004).

High levels of gamma-synuclein have been observed in patients with glaucoma (Surgucheva, et al., 2002) and in the retina of patients with Alzheimer's disease (Surgucheva et al., 2001).

GAMMA- SYNUCLEINS		
Rat	MDVF <sup>K</sup> KGFSIA <sup>R</sup> EGVVGAVEKT <sup>T</sup> QGVTEAAEKTKEGVMY	39
Mouse	MDVF <sup>K</sup> KGFSIA <sup>K</sup> EGVVGAVEKTKQGVTEAAEKTKEGVMY	39
Human	MDVF <sup>K</sup> KGFSIA <sup>K</sup> EGVVGAVEKTKQGVTEAAEKTKEGVMY	39
Rat	VGAKTK <sup>G</sup> ER <sup>G</sup> TSVTSVAEKTKEQANAVSEAVVSSVNTVAT <sup>E</sup> TV <sup>E</sup> EEAENIV	89
Mouse	VG <sup>T</sup> TKTK <sup>E</sup> ENVVTSVTSVAEKTKEQANAVSEAVVSSVNTVA <sup>N</sup> KTVEEAENIV	89
Human	VGAKTKENVVTSVTSVAEKTKEQANAVSEAVVSSVNTVATKTVEEAENI <sup>A</sup>	89
Rat	VTTGVVRKEDLEPPAQDQEA <sup>-</sup> - <sup>-</sup> KEQEE <sup>-</sup> GEEAKSGGD	123
Mouse	VTTGVVRKEDLEPPAQDQEA <sup>-</sup> - <sup>-</sup> KEQEE <sup>-</sup> <sup>N</sup> EEAKSG <sup>E</sup> ED	123
Human	VT <sup>S</sup> GVVRKEDI <sup>R</sup> P <sup>S</sup> A <sup>P</sup> Q <sup>E</sup> GVASK <sup>E</sup> K <sup>E</sup> EE <sup>V</sup> AEEA <sup>Q</sup> SGGD	127

**Figure 4.** Amino acid sequences of rat, mouse, and human gamma-synucleins. Amino acids inside boxes are different between at least two of these three species.

## **1.2- Function of synucleins in the nervous system**

The normal function of alpha-synuclein is still not fully understood and it is possible that this protein might have many different functions or might be involved in many intracellular processes as a chaperon-like protein (Ostrerova et al., 1999; Chandra et al., 2005). Indeed, numerous studies demonstrated effects of this protein on activity of various enzymes and intracellular systems. For example, alpha-synuclein is able to selectively inhibit phospholipase D2 by direct interaction with this enzyme at the membrane surface (Jenco et al., 1998). Alpha-synuclein has also been implicated in regulation of dopamine production (Perez et al., 2002 ; Liu et al., 2008). In the dopamine synthesis pathway tyrosine is converted to L-3, 4-dihydroxyphenylalanine (L-DOPA) by the rate-limiting enzyme tyrosine hydroxylase (TH). Overexpression of alpha-synuclein in cells decrease levels of TH mRNA and protein (Baptista et al., 2003; Yu et al., 2004), by reducing activity of the TH promoter (Gao et al., 2007). Also, alpha synuclein binds to TH and prevents its phosphorylation as well as inhibits its activity by promoting the activity of protein phosphatase 2A (PP2A) (Perez et al., 2002; Peng et al., 2005). In contrast, increased phosphorylation of TH and elevation of its activity has been shown in cell culture models following suppression of alpha-synuclein expression (Liu et al., 2008). Tehranian and his colleagues have found that alpha-synuclein interacts with another enzyme involved in dopamine synthesis, L-aromatic amino acid decarboxylase (AADC), and demonstrated that overexpression of alpha-synuclein reduced serine phosphorylation and decreased AADC activity in dopaminergic cell models, possibly via altering PP2A activity (Tehranian et al., 2006).

In 1999 Kholodilov and colleagues found that in a rodent model alpha-synuclein has a protective role in neuronal cell injury (Kholodilov et al., 1999). Moreover,

overexpression of wild type alpha-synuclein protects neuronal cells from oxidative stress, while overexpression of mutated alpha-synuclein (A53T or A30P) increases susceptibility of these cells to the oxidative stress damage (Lee et al., 2001). Increased expression of alpha-synuclein also rescues neurological phenotype that leads to developmental retardation and premature death of mice lacking a presynaptic chaperone protein CSP-alpha (Chandra et al., 2005). It may also play a part in neuronal function by interacting with other proteins and regulating membrane biogenesis (Rochet et al., 2004; Lee et al., 2002; Danzer et al., 2007).

Because alpha- and beta-synuclein are predominantly located in neuronal pre-synaptic terminals it has been suggested that the normal function of synucleins is related to this localisation. Sharon and colleagues suggested that alpha-synuclein may have a role in transportation of fatty acids between the aqueous and membrane phospholipid components of presynaptic terminals (Sharon et al., 2001). It also appears that alpha-synuclein plays a role in maintenance and transport of synaptic vesicles (Marteaux et al., 1998; Jenco et al., 1998). However, alpha-synuclein is not important in synaptogenesis as it is expressed after synapse formation (Murphy et al., 2000), and no abnormalities detected in synaptogenesis in mice lacking alpha-synuclein (Abeliovich et al., 2000). Recently, the role of alpha-synuclein in redistribution of secretory vesicle pools and neurotransmitter release in presynaptic terminals has been shown in cell culture and transgenic mouse models (Nemani et al., 2010).

In the zebra finch (songbird) a high level of alpha-synuclein (synelfin) was observed in synaptic terminals of neurons involved in song learning only during learning period, and after that the quantity of synuclein decreased significantly, this support an idea that alpha-synucleins play an important role in neuronal plasticity (George et al., 1995).



Very recent experimental data clearly demonstrated that alpha-synucleins via interaction with synaptobrevin/VAMP2 promotes the SNARE complex assembly at the presynaptic membrane (Burré et al., 2010) and therefore, is important for the regulation of neurotransmitter release.

Surprisingly, complete inactivation of the alpha-synuclein gene in knockout mice (Abeliovich et al., 2000; Cabin et al., 2002; Dauer et al., 2002; Schluter et al., 2003) leads to very limited changes of animal physiology and no clinical signs of pathology develop even in aged mice (Al-Wandi et al., 2010). This might be caused by functional substitution of missed alpha-synuclein by two other closely related members of the synuclein family. A similar absence of significant changes was observed in knockout mice lacking beta-synuclein or gamma-synuclein or even two members of the synuclein family (Ninkina et al., 2003; Robertson et al., 2004; Chandra et al., 2004). Inactivation of all three synuclein genes caused substantial changes in neurotransmission and neurodegeneration in ageing knockout mice (Burré et al., 2010; Greten-Harrison et al., 2010 and V.L.Buchman, personal communication), which confirms the idea of functional redundancy within the synuclein family.

The normal function of beta-synuclein in the nervous system has been substantially less studied. Like alpha-synuclein, beta-synuclein interacts with and inhibits phospholipase D2 (Jenco et al., 1998). It also has p53-dependent anti-apoptotic activity and inhibits caspase-3 activity (da Costa et al., 2003).

It has been suggested that because of its negligible propensity to aggregate and the ability to inhibit alpha-synuclein aggregation in vitro (Uversky et al., 2002; Park and Lansbury, 2003; Yamin et al., 2005) as well as accumulation in cell bodies of neurons that do not develop alpha-synuclein pathology in synucleinopathy patients (Mori et

al., 2003), beta-synuclein might reduce neuronal pathology caused by alpha-synuclein aggregation. Several studies in cell culture models and mice co-expressing both synucleins confirmed that beta-synuclein indeed could ameliorate alpha-synuclein pathology (Hashimoto et al., 2001; Hashimoto et al., 2004a; Fan et al., 2006). The mechanism of this effect is not clear and several mechanisms have been proposed including direct interaction with alpha-synuclein (Hashimoto et al., 2001, 2004a), regulation of alpha-synuclein expression (Fan et al., 2006), reduction of proteasomal inhibition by aggregated alpha-synuclein (Snyder et al., 2005) and activation of Akt protein kinase (Hashimoto et al., 2004b). It has also been demonstrated that beta-synuclein or beta-synuclein-derived peptides have neuroprotective effect in animal models of Alzheimer's disease (Windisch et al., 2004).

Information about the neuronal function of gamma-synuclein is even scarcer. Strong inhibition of ubiquitin-independent proteolytic activity of the 20S proteasome by gamma-synuclein has been demonstrated (Snyder et al., 2005). In cultured non-neuronal cells overexpressed gamma-synuclein inhibits function of co-expressed serotonin transporter (Wersinger and Sidhu, 2009). In cultured primary neurons overexpression of gamma-synuclein leads to significant loss of neurofilament staining, suggesting that this protein affects neurofilament network integrity (Buchman et al., 1998).

### **1.3 Synucleinopathies**

This term has been given to a group of degenerative diseases characterized by the presence in neurons or/and glial cells of characteristic inclusions, whose main constituent is aggregated and fibrillated alpha-synuclein (Spillantini et al., 1997).

This group includes Parkinson's disease, Dementia with Lewy bodies (Spillantini et

al., 1997; Baba et al., 1998), multiple system atrophy (Arima et al., 1998; Tu et al., 1998; Wakabayashi et al., 1998; Gai et al., 1999; Fujiwara et al., 2002), neurodegeneration with iron accumulation type 1 (Arawaka et al., 1998; Saito et al., 2000) and Lewy bodies variant of Alzheimer's disease. The most common of these disorders is Parkinson's disease.

### **1.3.1 Parkinson's disease**

Parkinson's disease symptoms were first time systematically described in 1817 by James Parkinson, although the disease was known for many years, initially as paralysis agitans. Only in 1862 Jean Martin Charcot has given Parkinson's disease its current name. It is also known as primary Parkinsonism (Parkinsonism is a group of disorders that have similar symptoms) or idiopathic Parkinson's disease (idiopathic means a disease which cause still unknown). Parkinson's disease is the most common movement disorder, and it has been considered second common and socially important neurodegenerative disorder after Alzheimer's disease. In developed countries 1-2 % of general population over the age of 65 years are affected.

### **1.3.2 Aetiology of Parkinson disease**

About 90% of Parkinson's disease cases are sporadic (idiopathic), where the causative agent is unknown, but 10% is considered to be familial, caused by modifications of genetic information (Pallone et al., 2007). Among all the risk factors that were suggested to be involved in Parkinson's disease development and progression, only advanced age has been proven as a critical one. However, some epidemiological and

experimental studies suggest that environmental factors, such as herbicides and metal ions may play a role in the pathogenesis of Parkinson's disease (Andre et al., 2005).

For long time Parkinson's disease was considered as a purely non-hereditary disease. But since the PARK1 locus was discovered in 1996 (Polymeropoulos et al., 1996), several more loci have been identified that are linked to Parkinson's disease. These loci have been designated PARK 1-13, and mapped to chromosomes 4q21, 6q25.2-q27, 2p13, 4q21, 4p14, 1p35-p36, 1p36, 12q12, 1p36, 1p32, 2q36-q37, X-chromosome, and 2p13 respectively.

For both PARK1 and PARK4 that mapped to 4q21 genetic defects were identified in the same, alpha-synuclein, gene, which became the first evidence of its role in the pathogenesis of Parkinson's disease (Polymeropoulos et al., 1997; Kruger et al., 1998; Spillantini et al., 1998). Alpha-synuclein mutations associated with the disease were described above. Some of PARK loci and corresponding gene mutations are associated with autosomal dominant (for instance alpha-synuclein and LRRK2 mutations) and some – with autosomal recessive (for instance parkin, DJ-1 and PINK1 mutations) forms of familial Parkinson's disease.

### **1.3.3 Clinical signs and histopathological features of Parkinson's disease**

Parkinson's disease is symptomatically characterized by resting tremor, muscles rigidity, bradykinesia (slowness of movement), and postural instability (impaired balance). These signs usually develop gradually and worsen with time. Moreover, in addition to these motor features the disease is characterised by certain non-motor symptoms. Surveys have demonstrated that about 90% of Parkinson's disease patients suffering from at least one non-motor symptom, and about 10% display 5 such

symptoms (Miyasaki, 2006). These non-motor symptoms include neuropsychiatric, sleep disturbances, autonomic and/or sensory dysfunction, impulse control disorders, fatigue and weight loss.

Major motor symptoms of Parkinson's disease occurs as a result of neurons in the substantia nigra pars compacta die or become impaired. These neurons produce dopamine and use it for transmitting neuronal signals. Major histopathological features of Parkinson's disease, which have been described by Lewy in 1912, are eosinophilic, round cytoplasmic inclusions, later named Lewy bodies, and dystrophic neurites also known as Lewy neurites (Lewy, 1912). In Parkinson's disease patients Lewy bodies are mainly but not exclusively found in dopamine neurons of the substantia nigra (Tretiakoff, 1919). The loss of dopamine neurons, another hallmarks of Parkinson disease, is not uniform, it has been reported that the most pronounced nerve cell loss occur in the caudal and ventral parts of the substantia nigra (Hassler, 1936).

#### **1.3.4 General principal of Parkinson's disease treatment**

There is no drug or other type of treatment to effectively cure Parkinson's disease and all currently used therapeutic interventions are able to control only disease symptoms and only temporarily. The medications used for Parkinson's disease are divided into three categories, the aim of the first and most important is to increase the level of dopamine in the brain or enhance dopamine signalling. The second type of the medications works on other neurotransmitter systems that reduce some of the symptoms of the disease. The targets of the third category are various non-motor symptoms of the disease.

Levodopa (the generic name of drugs with L-DOPA as an active compound) treatment increases dopamine content by carboxylation of L-DOPA, which converts it to dopamine. This is the most efficacious treatment for Parkinson's disease available now, but it is not recommended at the early stage of Parkinson's disease to avoid early development of motor fluctuation and dyskinesia (Olanow et al., 2009). COMT inhibitors are often used to improve levodopa action by reducing L-DOPA and dopamine catabolism. Another drug commonly used in combination with levodopa and COMT inhibitors are inhibitors of aromatic-L-amino-acid decarboxylase (DOPA decarboxylase), like carbidopa or benserazide, that also increase L-DOPA half-life. Monoamine oxidase type B inhibitors that slow down dopamine catabolism are used in early, mild Parkinson's disease. They are effective in reducing disability, believe to slow neuronal degeneration and have less adverse effects than levodopa or dopamine agonists (Caslake et al., 2009; Olanow et al., 2009). Various dopamine agonists activate dopamine receptors and may be effective in preventing dyskinesia.

Examples of drugs that affect non-dopaminergic neurotransmission are amantadine, a glutamate antagonist that indirectly promotes dopamine release and various anticholinergic drugs. Both type of drugs are commonly used at early stages of the disease progression and often in combinations with each other and other drugs.

Managing of non-motor symptoms of Parkinson's disease, autonomic dysfunction, sleep disorders, psychological and cognitive problems, and sensory abnormalities, is very important because they are often causes of morbidity. Various specific drugs and their combinations are used for treating each of these symptoms.

Surgical technique was used a lot before the levadopa was introduced. In the last few decades after improving the surgical techniques, it has become used again in some advanced cases, when the drug treatments proved to be inefficient.

These techniques included thalamotomy and thalamic stimulation, which are effective for reducing parkinsonian tremor (Kelly et al., 1987; Narabayshi et al., 1982). In addition, pallidotomy and pallidal stimulation technique helped in reducing and alleviate dyskinesias (Baron et al., 2000; Fine et al., 2000).

Some studies suggest that deep brain stimulation technique may help in reducing dyskinesia and relieve tremor (Benabid et al., 2009).

Regenerative approaches are seen as a future of the Parkinson's disease therapy. So far, transplanting neural cells into corpus striatum to produce dopamine were used in several clinical trials but it does not work well. Recently transplanting of fetal nigral cells has been found to improve symptoms in few patients ( Olanow et al. 1996).

#### **1.4 Behavioural testing of animals modelling neurodegenerative diseases**

Numerous and various behavioural tests are used to analyse behavioural changes in animals that model human neurodegenerative diseases. Each test was designed to reveal changes in particular functional system, however in the majority of cases this aim has not been achieved because many neural systems are involved in the execution of even simple animal behaviour. Nevertheless, by using combination of several behavioural tests researchers sometimes manage to demonstrate dysfunction of a particular neural system.

To achieve that a few steps should be taken in consideration before conducting any behavioural experiment. First, the number of animals in each experimental group should be sufficient for obtaining statistically significant data. Usually the minimal number required is 10 to 12 mice. If the effect of the genotype is studied, the age of

experimental animals in each group should be in the same range and, except of special cases, not lower than 70 days (Crawley and Paylor, 1997). Second, the animal's general health should be screened before starting any experiment for presence of any physical or emotional problems.

In this study five behavioural tests have been used to measure motor performance of three different genotype groups of animals, which are wild type, heterozygous and homozygous transgenic mice overexpressing gamma-synuclein.

#### **1.4.1 Beam walking test**

This test has been designed to assess the motor coordination and balance capabilities of mice by measuring their ability to traverse a narrow beam.

In addition, there are two types of beams used in this test one of them is square and the other is round.

There are three different diameters of square beam 28mm, 12mm, and 5mm, which used respectively for training and used according to animals age and weight. The round beams of three different diameters, 28mm, 17mm and 11mm, are usually used (Carter et al., 1999). The round beams were used in this study.

#### **1.4.2 Rotarod test**

The devise for this test was designed in 1957 by Dunham and Miya, and it was further developed in 1968 by Jones and Roberts (Jones and Roberts, 1968). The rotating rod of the apparatus has a soft rubber cover to prevent the animals from holding the rotating rod and rotating with it rather than running on top, or falling from it. This test is used to measure the motor coordination, balance, and ataxia. Moreover there are two variants of this test:



Constant mode: mice running for 180 seconds on constant speed of the rod rotation (i.e. 24 rpm).

Accelerating mode: mice running for 300 second with gradual acceleration of the rod rotation (i.e. 4-40 rpm).

### **1.4.3 Gait test (Foot print)**

This test shows the pattern of mouse walking and detect if there is any gait abnormalities, therefore it is a measure of the animal coordination. The gait test is designed to be conducting in a dark room and the starting point of the devise should provide with a light source. There are three main parameters that have to be measured in this test to analyze the foot print patterns of the mice (Carter et al., 1999).

a-The first parameter is the stride length, which include four measurements:

- 1- Right hind stride.
- 2- Right front stride.
- 3- Left hind stride.
- 4- Left front stride.

b-The second parameter is the front and hind base width, front base is the distance between right and left fore limbs in each step, while the hind base is the distance between the right and left hind limbs in each step.

c-The front and hind limb overlap, which is the distance between the two centres of front and hind paws in each step.

### **1.4.4 Locomotor activity count**

This test uses metal cages where the animals placed for testing their locomotor activity. Test cages have two infrared photo beams sources and sensors, and beams

breaks by mouse recorded by the software on the computer connected to these cages.

And there are three parameters measured in this system:

- 1- Perseveration, where one beam is broken repeatedly.
- 2- Alternation, where the two beams are broken in sequence.
- 3- Total activity.

#### **1.4.5 Acoustic startle stimulus and prepulse inhibition**

Startle reflex is a motor response to a sudden environmental stimulus, which consists of rapid contraction of head, neck, trunk, and legs muscles. Measurement of acoustic startle responses can provide general information regarding sensorimotor processing, however prepulse inhibition provide more important information than startle reflex.

Prepulse inhibition is acoustic stimulus prior to the startle stimulus, and the intensity of prepulse inhibition is lower than startle stimulus, and it attenuates the response to the startle stimulus (Ison et al., 1997).`

The device used to measure startle response consists of a wooden sound-proof box that isolates the animal from the surrounding environment. This cubical box provided with acoustic startle sound source, speakers producing white noise, a small fan for ventilation and also provides some level of background noise, and the light source. Within the wooden box is a plastic tube where an animal is placed for testing. This tube has two movable plastic doors to make it easy to place the animal inside this

tube. A computer with specialised software is connected to the apparatus for collecting the data for analysis

## **1-5 Generating of transgenic mice overexpressing gamma synuclein**

Mice expressing high levels of wild-type mouse gamma-synuclein were generated by using Thy-1 neuron-specific expression cassette, identical to the cassette that was previously used to generate lines expressing high levels of alpha-synuclein (van der Putten et al., 2000; Zhou et al., 2008) and beta-synuclein (Hashimoto et al., 2001) expression.

Whereas, a fragment of mouse gamma-synuclein c DNA including 34 bp of 5'-UTR and 64 bp of 3'-UTR was PCR amplified using pD53 plasmid DNA (Buchman, et al. 1998) as a template and oligonucleotides with XhoI linkers to facilitate subsequent cloning into XhoI site of Thy-1 promoter plasmid 323-p TSC21k (Van der Putten et al., 2000). The fragment for microinjecting mouse oocytes was isolated by digestion of the resulting plasmid DNA with NotI. Transgenic founders were produced on C57BI/6 genetic background. Mice positive for transgene expression were crossed with C57BI/6 wild-type mice.

The colony of transgenic mice was maintained by backcrossing hemizygous Thy1mgamma-synuclein mice with C57BL/6 mice.

During their first year of life hemizygous animals cannot be distinguished from their wild type littermates. Later on, these mice developed a clasping reflex, abnormal posture and gait, and other signs of motor impairments. Thy1mgamma-synuclein homozygous animals were generated to increase the pathology, and this resulted in doubling of the transgene expression. In the dorsal root ganglia of 12 month old mice

transgene expression levels were seven times higher than the level in endogenous gamma-synuclein mRNA. Although, homozygous animals expressed high level of gamma-synuclein mRNA throughout their nervous system they appeared normal during the first few months of life.

## **Chapter 2- Materials and methods**

### **2.1 Experimental animals**

Male mice of three genotypes, wild type and gamma-synuclein transgenic hemizygote (Het) or homozygote (TG/TG), were used for behavioural testing that was carried out at four age points (6, 9, 12, 18 months old). These animals were housed either in a separate cage for each animal, or up to five littermates in the same cage. Animals had free access to food and water, and the animal holding room had a 12 h light/dark cycle.

All experiments were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986) and local ethical review. Accordingly, animals which reveal paralysis of their limbs and loss of righting reflex were sacrificed to prevent further suffering.

### **2.2 Acoustic startle test**

#### **Startle apparatus**

Equipment manufactured by San Diego Instruments was purchased via Sandown Scientific, Surrey, United Kingdom.

The system consists of a soundproof chamber, which contains a plexiglass cylinder (5 cm in diameter by 12.8 cm long) mounted on a rigid plastic platform. The cylinder has two movable doors, which make the operation of placing the animals and taking them out of the cylinder more easy. The chamber contains a fan for ventilation and providing some level of background noise, and a loudspeaker 28 cm above the

cylinder, which is the acoustic sound source capable to produce full spectrum white noise that is computer controlled for duration and decibel level (Figure 5).



**Figure 5.** The startle apparatus used in this study. The picture shows two chambers connected to the computer, which save the data as Excel files.

### **Procedure**

Prior to the onset of the trials mice were habituated in the chamber for 5 minutes under 70 dB background white noise. Each trial was preceded by a variable 20-30 second interval, used to prevent the mice from predicting the stimulus presentation. Two startle intensities were used, 105 and 120 dBs of 50ms duration.

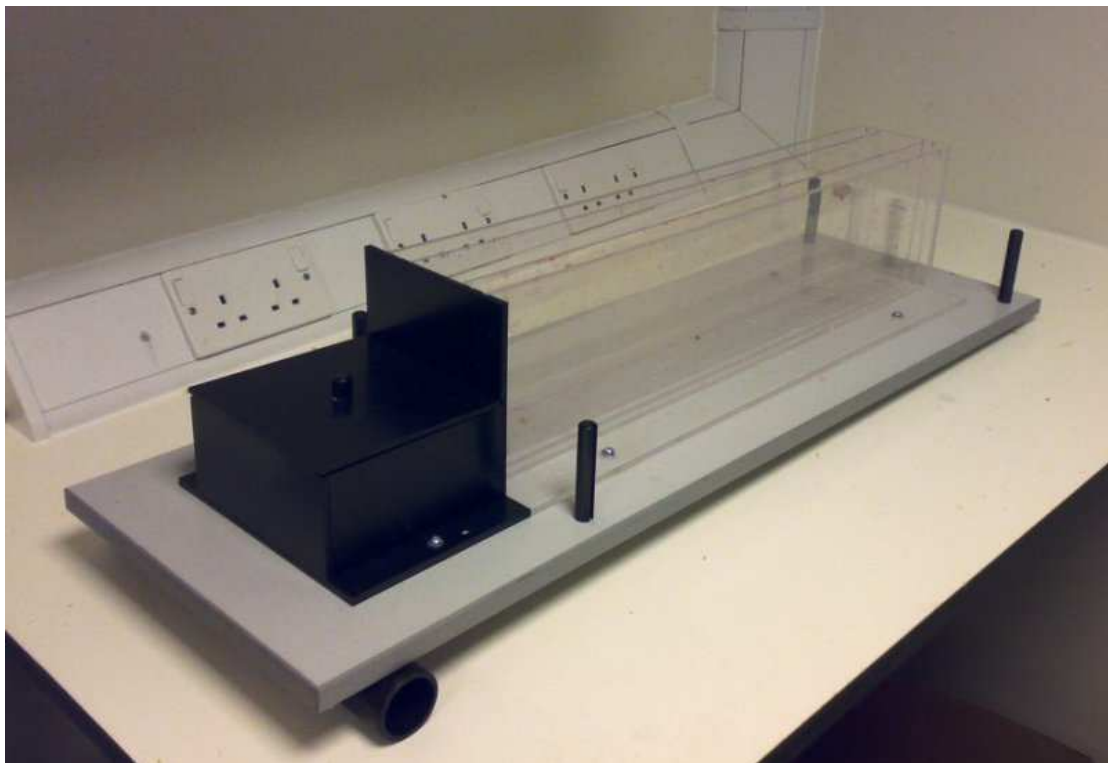
For the PPI trials, pre-pulse duration was 20ms with a gap between the pre-pulse and the startle stimuli of 20ms. For each of the primary startle stimuli, trials with differing PP intensities were used: 0, 2, 4, 8, and 16dBs above background (70db), with an additional no-stimulus control trial, whereby neither pre-pulse or startle stimulus was

used. 11 trials types were used, each replicated 12 times in each session, hence each session consisted of 132 trials in total. The data was collected and stored on a PC. For each test values were collected for the peak startle response corrected for baseline movements prior to analyses.

## **2.3 Gait test (footprinting)**

### **Gait test device**

The test device consists of a corridor runway (60cm length x10cm height) with adjustable width (5 or 10cm), lined with white paper. At the end of the device there is a black goal box (15x15cm), baited with food to encourage the mice to run down the runway (Figure 6). A light source (about 60 W) was placed above the starting point of the tunnel to stimulate animals to run toward the goal box.



**Figure 6.** Gait test device that used in this study and it includes wooden base, Perspex corridor and black acrylic goal box.



## **Procedure**

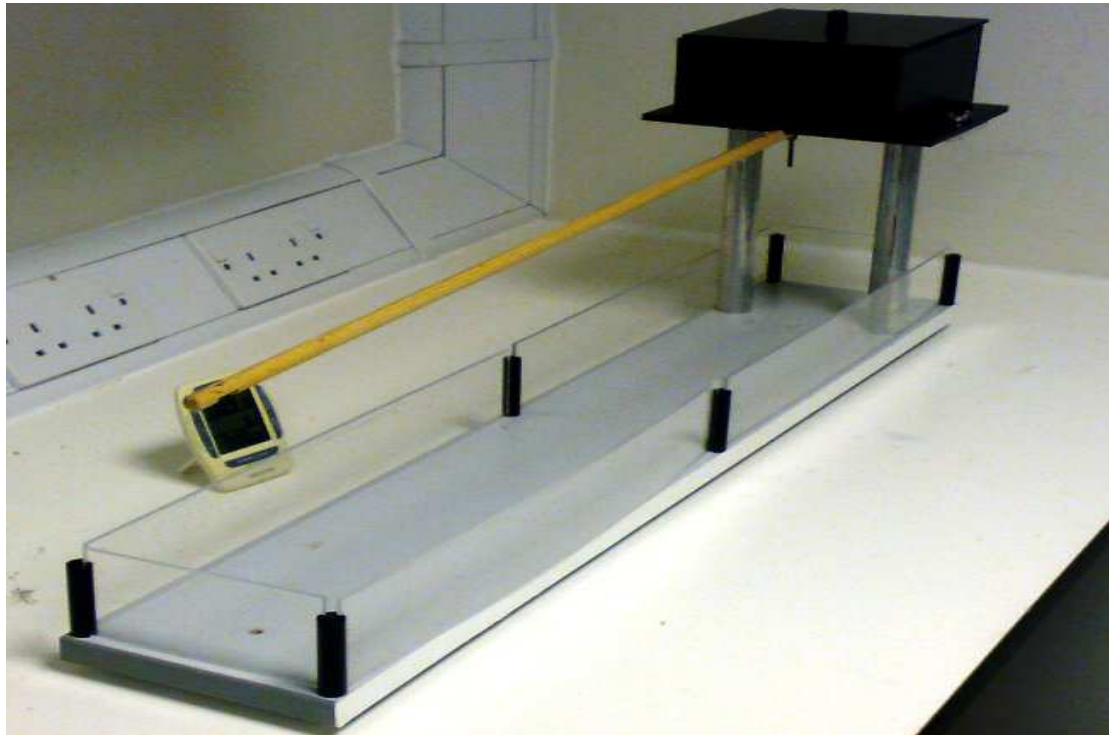
Animals were trained for 5 consecutive days (one 5 minute session per day). The training and experiments were conducted in a darkened room. The fore and hind paws were brushed with blue and red non-toxic paints respectively and the mice placed at the starting point from where they ran down the runway to the goal box.

From the footprints collected, eight parameters were measured (right hind stride, left hind stride, right front stride, left front stride, hind base, left base, right overlap, and left overlap). For each mouse measurements were taken for three full strides from the mid-run period, when mice were neither accelerating nor decelerating. The average then assessed for each parameter and used in statistical data analysis. Stride length was assessed by measuring the distance between the centre of front and hind paws of right and left side. Base widths were assessed by measuring the distance between the right and left front paws, and right and left hind paws. Overlap is the distance between the hind and fore paws centres. If the centre of the front footprint falls on the centre of the hind footprint the overlap value was recorded as zero.

## **2.4 Beam walking test**

### **Beam test device**

The horizontal beam test device consists of a 62 cm long wooden strip of either 11 mm or 17 mm diameters, used according to the age and size of animals, attached to a raised goal box (15x15 cm) (Figure 7). Animals from 4 months to 12 months old were tested on 11 mm beam, whilst the 17 mm beam was used to test coordination at 18 months old due to the large size of wild type and some heterozygous mice.



**Figure 7.** Beam test device consists of a raised horizontal wooden beam and goal box.

## **Procedure**

For training mice were placed near to the goal box, allowed to enter, then promptly removed, placed on the beam and the operation was repeated, each time increasing the distance between the animal and the goal box. For the last attempt the mouse was placed facing away from the goal box, enforcing the animal to turn 180° and cross along the beam to reach the goal box.

On the day of testing, animals were transferred from the housing room to the testing room inside their home cages. The test was conducting by placing the mouse facing away from goal box on the free end of the strip and at the same time the timer switched on. The amount of time taken by the mouse to turn 180° to face the goal box was recorded, followed by the time taken to cross the strip to reach the goal box. As

some animals cannot complete this test, other parameters were recorded (freezing time, time to fall). If the animal was not able to move and stay as it is for more than 3 minutes then freeze was recorded. If the mouse fell from the beam, fall time was recorded.

## **2.5 Rota-rod test**

### **Rota-rod apparatus**

The apparatus (UGO BASILE COMERIO (VA) - ITALY) basically consists of a 3 cm drum, divided by six flanges into five test areas, so five animals can be recorded at the same time. Each test area has a synchronized counter, which is stopped by the animal falling on a trip switch.

The apparatus is provided by a geared motor that provides three modes of operation (Acceleration, Lock, Reset) controlled by a switch on the front panel (Figure 8).

### **Procedure**

The animals were trained a day before the day of testing and in each training run five animals were placed on the rotation rod for 240 seconds, with the apparatus on a constant speed of 24 rpm. Each mouse had three training runs within a 90 minute interval.

This test includes two methods, constant mode and accelerating mode, and allows constant mode conducting first.

### **Constant mode**

The apparatus is set to run at a speed of 24 rpm. Animals were placed on the drum one by one and trip switches set. Each mouse had three trials of 240 seconds and the time each mouse spent on the drum recorded.

### **Accelerating mode**

Animals were placed on the drum then the speed allowed increasing constantly from 4-40 rpm per minute, over a 5 minutes period. The period of time each mouse spent on the rotating rod was recorded. Each mouse was tested three times with 90 minutes interval.



**Figure 8.** Mice rota-rod test apparatus (UGO BASILE COMERIO (VA) - ITALY) used to analyse the mice motor coordination and balance performance.

## **2.6 Motor activity test**

### **The apparatus**

Eight metal cages (38x25x24 cm) were used in parallel to measure the general motor activity. Activity was recorded by two infrared photo beam emitters and sensors that detected beam breaks caused by movement. Sensors were positioned 13cm apart along the length of the cage, with one 12.5cm from the door (front wall) and the other 12.5cm from the rear wall. The sensors were positioned 1cm above the floor surface (Figure 9). Three basic parameters were measured with this system, perseverative movement, where one beam was broken repeatedly, locomotor activity where both beams were broken in sequence, and total activity. The cages were connected to an Acorn Electron microcomputer and printer that stored and processed the data. The program controlling the computer was written using the Arachnid (Paul Frey Ltd, Cambridge, UK) computer programming software.

### **Procedure**

Individual mice were placed in a cage with access to food and water and the recording software activated. The session length can be up to 24 hours, but in this study 1 hour session length was used, divided into twelve 5 minute blocks.



**Figure 9.** Motor activity apparatus comprising of cages and infrared detectors connected to a computer to record data.

## **2.7. DNA Purification**

DNA purification was conducted using the NucleoSpin Tissue Kit according to the manufacturer's (Macherey-Nagel) instructions.

Tissue samples were obtained by cutting about 25 mg of tissue from the ear (ear biopsy).

180  $\mu$ l of T1 buffer and 25  $\mu$ l of Proteinase K solution were added to the tissue sample, vortexed to mix and incubated at 56°C for 1-3 hours or overnight until complete lysis is obtained. 200  $\mu$ l of B3 buffer was added to the lysate and mixed vigorously, then 210  $\mu$ l of ethanol (96-100%) was added and mixed.

For each sample, one NucleoSpin Tissue column was placed into a 2 ml collecting tube. The sample was loaded to the column and centrifuged for 1 minute at 11000-13000 rpm. The content of the collecting tube was discarded and the column returned into the collecting tube.

The silica membrane of the tube was washed twice, firstly, by adding 500 µl BW buffer and centrifuging for 1 minute at 11000 rpm and secondly by adding 600 µl of B5 buffer and centrifuging at 11000 rpm. The content of the collecting tube was discarded and the column returned into the collecting tube.

The column was centrifuged once again for 1 minute to dry the silica membrane.

The NucleoSpin Tissue column was placed into a 1.5 ml microcentrifuge tube and 100 µl distilled water or prewarmed elution buffer BE was added to elute DNA, and centrifuge for 1 minute at 11000 rpm.

Eluted purified DNA was used for PCR analysis immediately or kept at 4°C.

### **2.7.1 Experimental groups and genotyping of gamma-synuclein transgenic mice**

To prepare groups of experimental animals we bred hemizygous males and females from the colony of gamma-synuclein transgenic mice produced in collaboration with Dr. Herman van der Putten. These mice have additional copies of mouse gamma synuclein gene under control of neurospecific Thy-1 promoter. As the position of transgene integration in the genome of gamma-synuclein transgenic mice is not known, it was not possible to use PCR technique for discrimination between hemi- and homozygous transgenic mice. PCR technique was used to identify only the presence of additional copies of gamma-synuclein gene. However, two step process

allowed us to reveal whether there are two alleles (TG/TG) or one allele (Het) in the genome of each mouse:

### **Step 1. Genotyping by using PCR technique for the presence of gamma synuclein gene**

For PCR analysis 5 µl of purified DNA was mixed with 44 µl distilled water, 5 µl 10XPCR buffer, 0.4 µl 25 mM dNTP mix, 0.25 µl 100 µM Forward primer (HP45 ThyI f2: 5'-acaccctaaagcatacagtcagacc-3'), 0.25 µl 100 µM Reverse primer (HP84 m Gsn 5'-ggccttctagtcttctccactcttg-3) and 0.25 µl Taq polymerase 25 cycles of amplification for 30 sec at 95°C/ 40 sec at 62°C/40 sec at 72°C were carried out.

Reaction products were analysed on 1% agarose gel stained with ethidium bromide . A 1 kb band is detected when DNA from either homo- or hemizygous animals was used for amplification but DNA from wild type animals gives no amplification product.

### **Step 2. Discriminating between hemi- and homozygous transgenic mice by backcrossing**

#### **Genotyping of males**

At the age of 7-8 weeks each TG positive male mouse was placed into a separate cage and mated with two CD1 females.

Females were checked regularly for pregnancy and those obviously pregnant were separated to a separate cage to produce a litter. Pups were tested for the presence of transgene by PCR analysis of DNA samples from ear biopsies as described for Step 1. Cross-contamination of samples was avoided by cleaning the forceps and scissor each time.



### **Genotyping of females**

TG positive females ( $\geq 8$  weeks old) were mated with a wild type C57BL/6 male. Females were checked regularly, those obviously pregnant were separated to a single cage, and when the litter was born, tissue biopsies were collected and PCR analysis of DNA samples carried out.

### **Analysis of genotyping results**

If all pups in a litter were positive, their parent was suggested to be homozygous (TG/TG). For a conclusive determination of homozygosity, at least two litters and 10 pups in total have to be genotyped. If some pups in litters were negative, their parent was considered to be hemizygous (Het).

## **2.8. Statistical analysis**

The statistical software used to analyse data of this study has been developed by statsoft (Statistica 1997).

Statistical analyses were conducted by using ANOVA (one way or two -way ANOVA which was applied to the data as required), with a statistical significance of  $P < 0.05$ .

Beam walking data was subjected to non-parametric Mann-Whitney test.

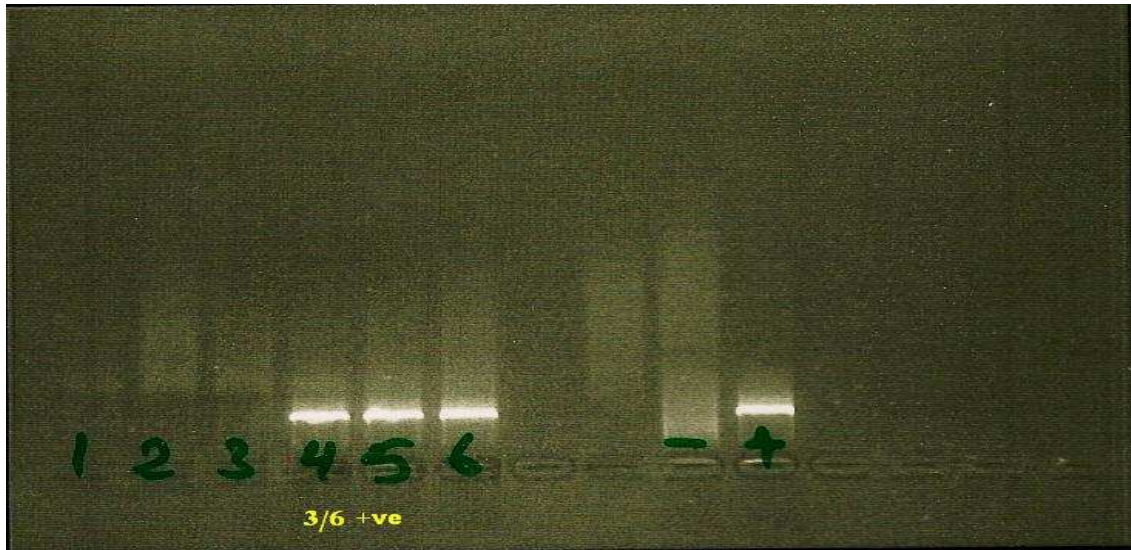
## **Chapter 3-Results**

### **3.1 Preparation of experimental animal groups**

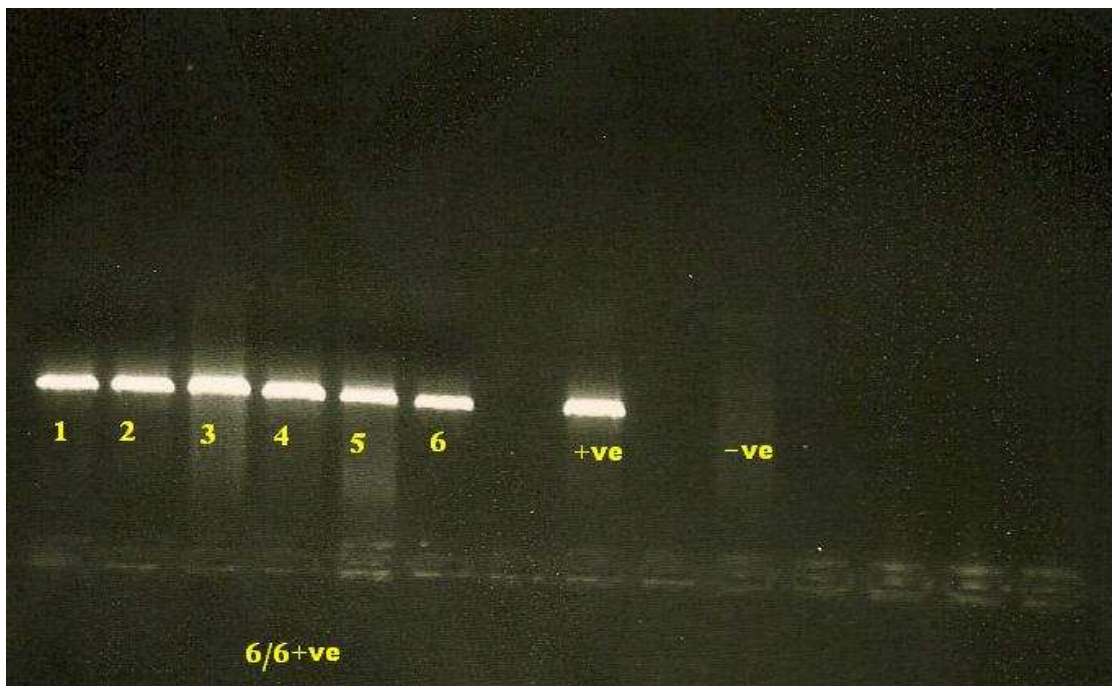
Heterozygous gamma-synuclein transgenic mice obtained by backcrossing with C57BL/6 wild type mice were intercrossed to obtain groups of wild type, hemizygous and homozygous transgenic mice. Transgenic and wild type animals were discriminated by the presence or absence of 1 kb product of PCR reaction after amplification of DNA extracted from the mouse ear biopsy (see Chapter 2.7.1).

For confident discrimination between homo- and hemizygous animals they were backcrossed with wild type mice and the offspring was analysed for the presence of the transgenic allele as described in Materials and Methods, Chapter 2.7.2. The presence of wild type animals in the offspring (an example shown in Fig.10a) suggested that the parent carried only one transgenic allele (i.e. is hemizygous). Only if all offspring animals in at least two litters (total number >12) carried the transgenic allele (see Fig.10b) was the parent declared homozygous.

Experimental groups for behavioural studies were formed exclusively from male animals.



**Figure 10a.** This figure shows only three of six samples are positive which mean that this animal is hemizygous.

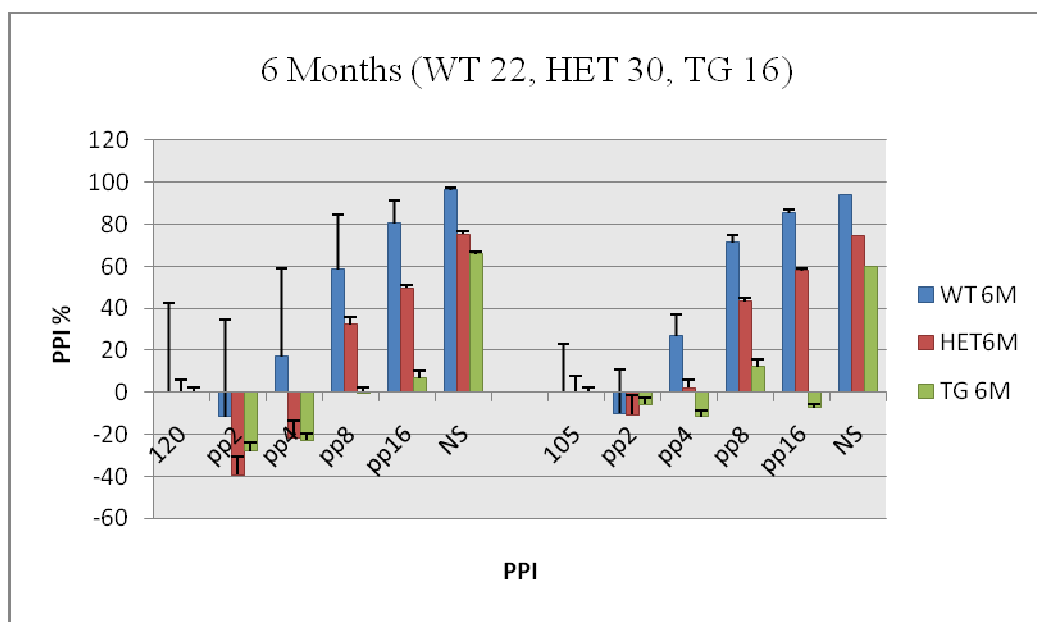


**Figure 10b.** All the samples are positive which mean that this animal is homozygous.

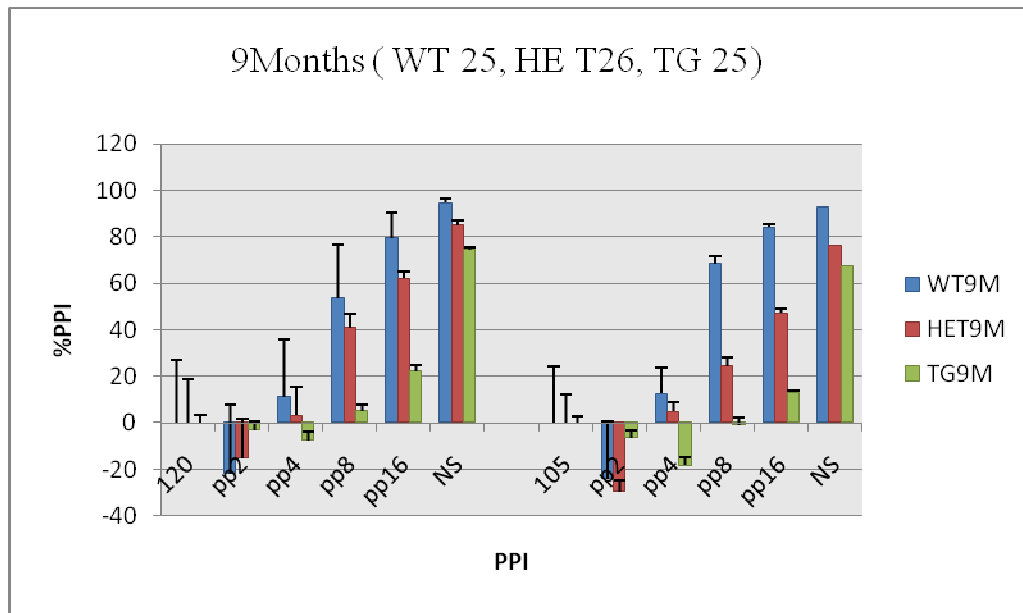
### 3.2 Prepulse inhibition of acoustic startle response

The standard design of experiments to measure prepulse inhibition was used and all experimental data were analysed accordingly. The statistical analysis of acoustic

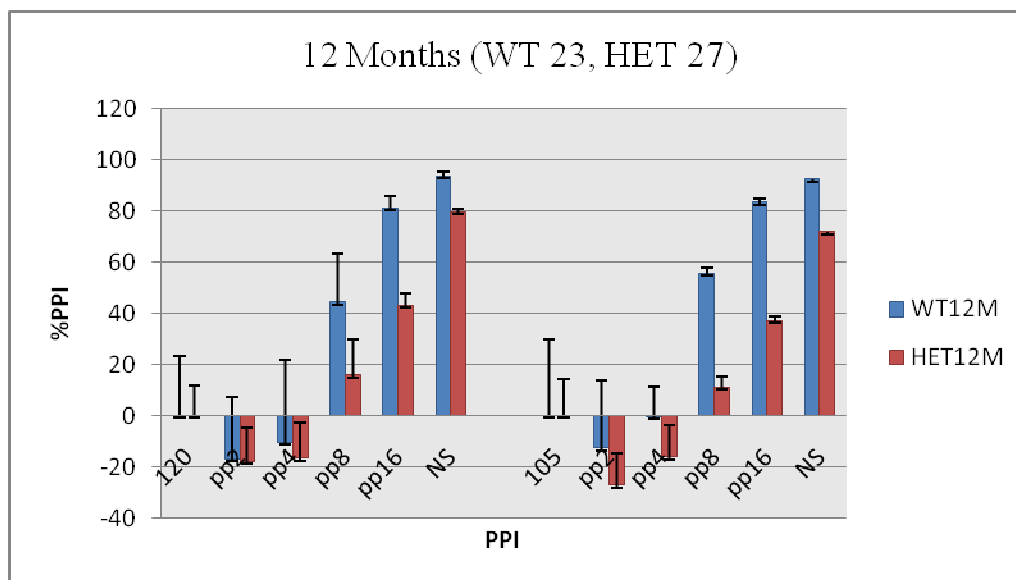
startle test data revealed that hemizygous and homozygous animals displayed significant differences compared with wild type mice as shown in figures (11a, b, c, d). A 2 way ANOVA test of PPI to 120 and 105 dB startle stimuli showed a significant effect between wild type and hemizygous mice, as well as between wild type and homozygous mice ( $P < 0.001$ ). But some sets of data for transgenic mice were found strange and unreliable and the possible reason will be discussed in the discussion chapter.



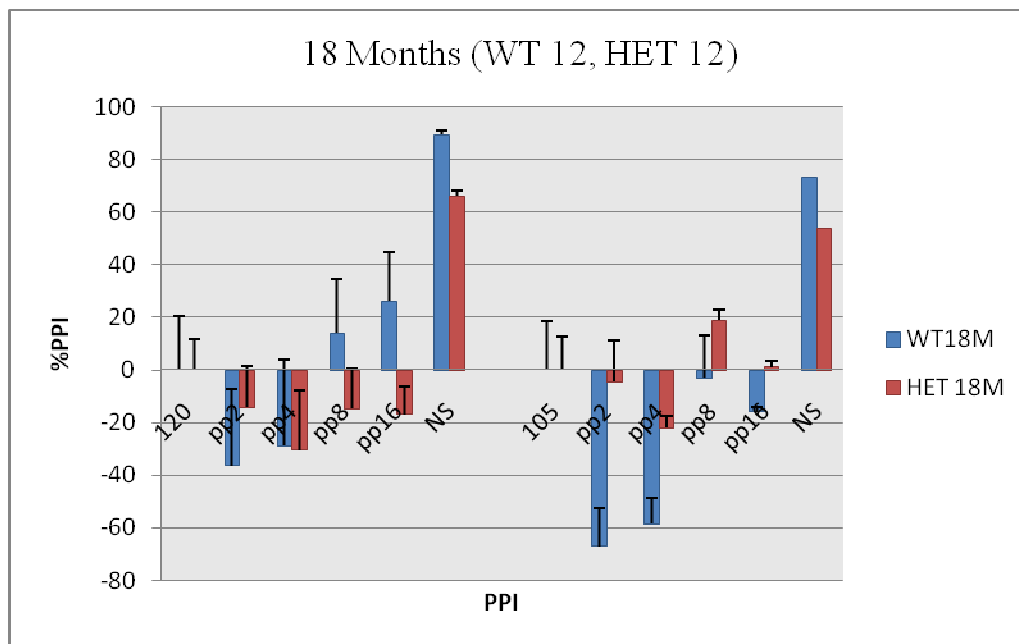
**Figure 11a.** Prepulse inhibition of acoustic startle response in hemizygous (HET) and homozygous (TG) compared to wild type (WT) mice at 6 months of age. Both hemizygous and homozygous show impaired prepulse inhibition (n= 22 WT, 30 HET and 16 TG).



**Figure 11b.** Prepulse inhibition of acoustic startle response in hemizygous (HET) and homozygous (TG) compared to wild type (WT) mice at 9 months of age. Both hemizygous and homozygous mice show progressive decline comparing to animals at 6 months of age (n=25 WT, 26 HET and 25 TG mice).



**Figure 11c.** Prepulse inhibition of acoustic startle response in hemizygous (HET) compared to wild type (WT) mice at 12 months of age (n=23 WT, 27 HET mice).



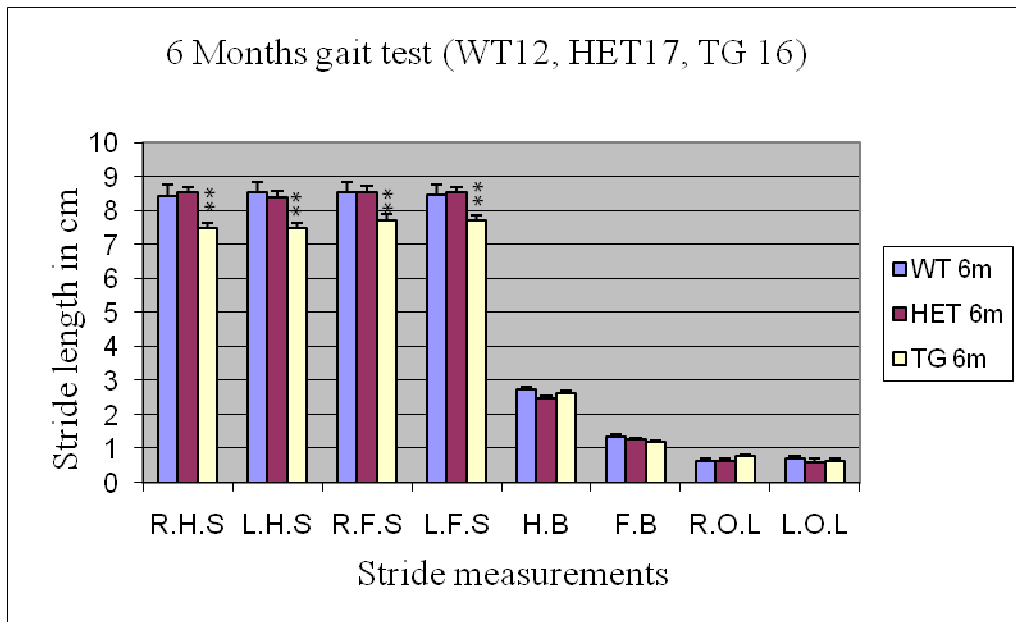
**Figure 11d.** Prepulse inhibition of acoustic startle response in hemizygous (HET) compared to wild type (WT) mice at 18 months of age (n=12 WT, 12 HET mice).

### 3.3 Gait test

Footprint patterns of 6-, 9-, 12- and 18-month old male mice were analysed to find if and when gamma-synuclein transgenic mice develop any gait abnormalities. No significant difference in stride length of wild type and hemizygous littermates has been found for 6, 9 and 12 months age groups (Fig. 12a-c). However statistical analysis of the stride length of 18-month old wild type and hemizygous mice revealed significant differences (genotype:  $F(1,110)=7.86$ ,  $P<0.01$ ), (Fig. 12d).

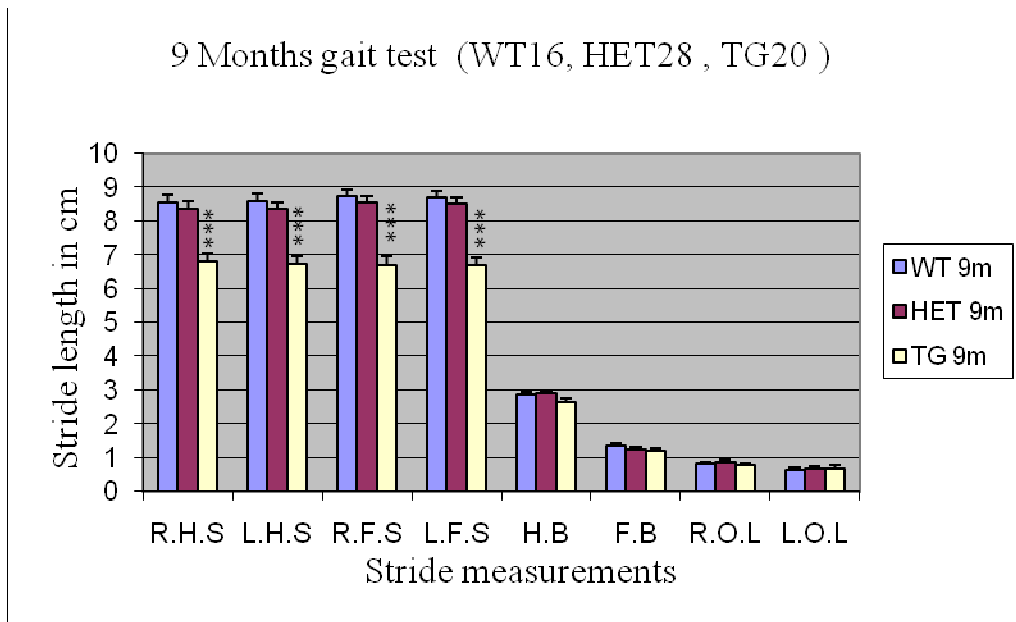
No differences have been found for hind and front paws base width (genotype:  $F(1,42)=0.10$  ;  $P=0.7$ ) or overlaps measurements (genotype:  $F(1,42)=3.7$  ;  $P=0.598$ ), for all ages of wild type and hemizygous gamma-synuclein transgenic mice (Fig. 12a-d).

However, a statistically significant difference between stride length of wild type and homozygous transgenic mice was found for both studied ages (genotype:  $F(1,110)=116.16$ ;  $P<0.001$ ) (Fig. 12a, b). However, at these ages neither hind and front paws base width, nor overlaps measurements were different between wild type and homozygous transgenic mice (genotype:  $F(1,54)=0.372$ ;  $P=0.39$ ) and (genotype:  $F(1,54)=0.0$ ;  $P=0.517$ ), respectively (Fig. 12a, b).

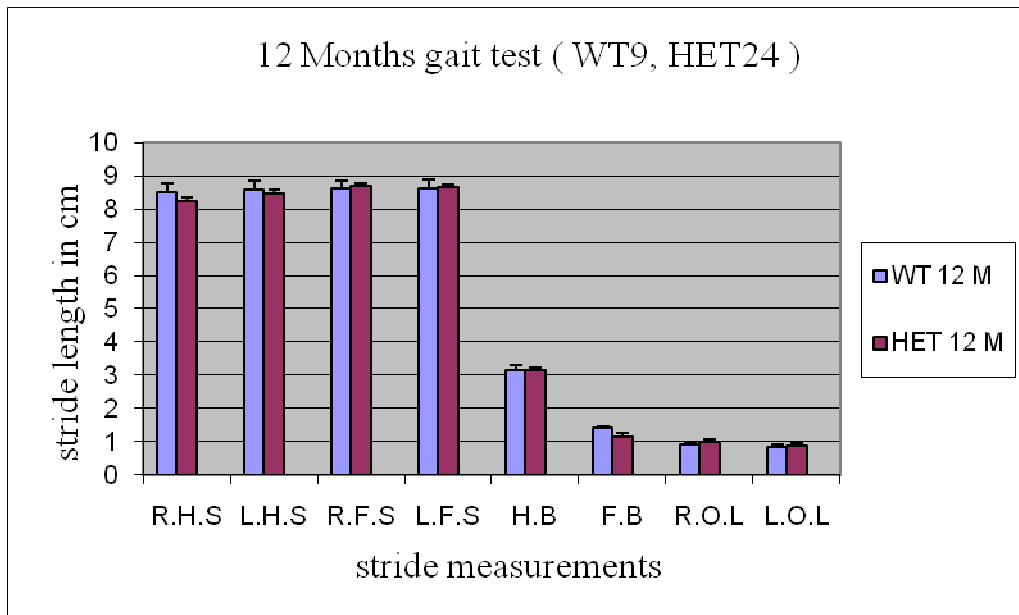


**Figure 12a.** The bar chart shows results (mean  $\pm$  SEM) of the analysis of various parameters of 6-month old animal stride (R.H.S - right hind stride; L.H.S - left hind stride; R.F.S - right front stride; L.H.S - left front stride; H.B. - hind base; F.B. – front base; R.O.L. - right front and hind limb overlap; L.O.L. - left front and hind limb overlap). Groups of wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice were tested. Values statistically different from the wild type value for the same parameter are designated (\*\*P<0.01, ANOVA test) .

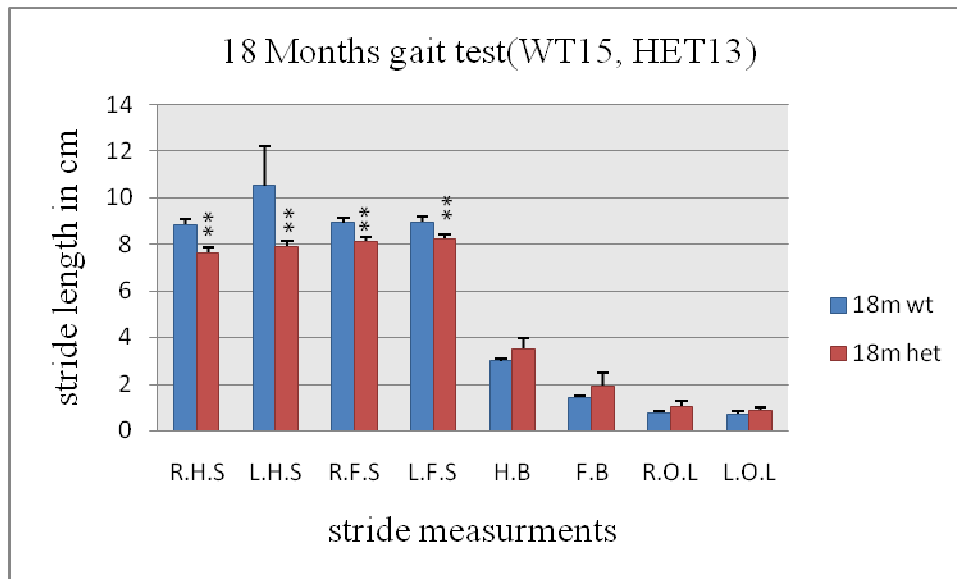




**Figure 12b.** The bar chart shows results (mean  $\pm$  SEM) of the analysis of various parameters of 9-month old animal stride (R.H.S - right hind stride; L.H.S - left hind stride; R.F.S - right front stride; L.H.S - left front stride; H.B. - hind base; F.B. – front base; R.O.L. - right front and hind limb overlap; L.O.L. - left front and hind limb overlap). Groups of wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice were tested. Values statistically different from the wild type value for the same parameter are designated (\*\*\*) $P < 0.001$ , ANOVA test).



**Figure 12c.** The bar chart shows results (mean  $\pm$  SEM) of the analysis of various parameters of 12-month old animal stride (R.H.S - right hind stride; L.H.S - left hind stride; R.F.S - right front stride; L.H.S - left front stride; H.B. - hind base; F.B. – front base; R.O.L. - right front and hind limb overlap; L.O.L. - left front and hind limb overlap). Groups of wild type (WT) and hemizygous (HET) transgenic mice were tested. No statistically significant differences were found.

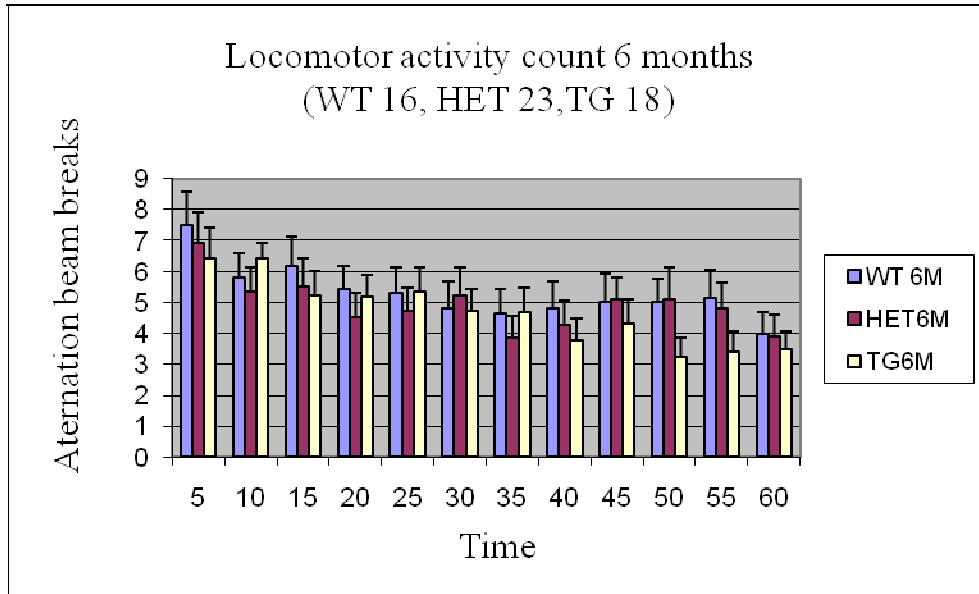


**Figure 12d.** The bar chart shows results (mean  $\pm$  SEM) of the analysis of various parameters of 18-month old animal stride (R.H.S - right hind stride; L.H.S - left hind stride; R.F.S - right front stride; L.H.S - left front stride; H.B. - hind base; F.B. – front base; R.O.L. - right front and hind limb overlap; L.O.L. - left front and hind limb overlap). Groups of wild type (WT) and hemizygous (HET) transgenic mice were tested. Values statistically different from the wild type values for the same parameter are designated (\*\* $P < 0.01$ , ANOVA test).

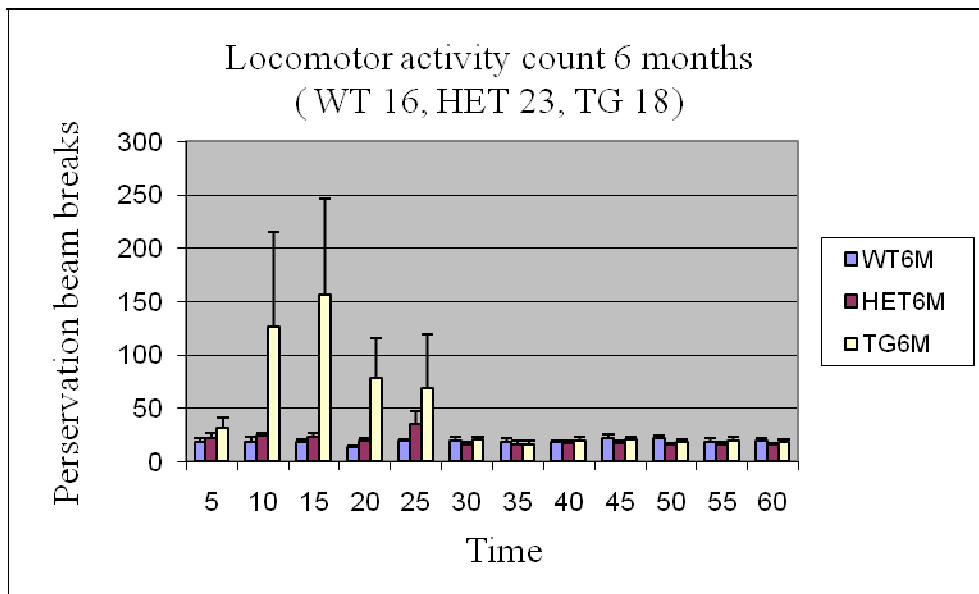
### **3.4 Locomotor activity count test**

The locomotor activity of male mice was assessed during 1 h in 5 min intervals using an automatic movement detection system described in the Material and Methods chapter. All tests were carried out between 10 am and 2 pm.

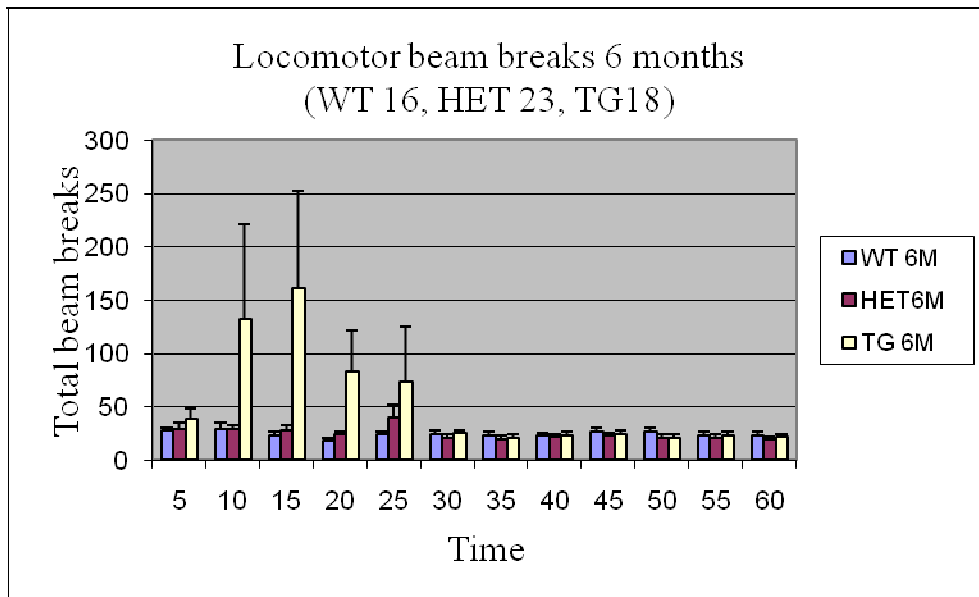
Three groups of animals (wild type, homozygous and hemizygous gamma-synuclein transgenic animals) were tested at 6 and 9 month of age and two groups (wild type and hemizygous gamma-synuclein transgenic animals) – at 12 and 18 month of age. Data were collected for three parameters measured in this test (preservation beam breaks, alternation beam breaks, and total beam breaks) and results are presented in Figures 13a-l. statistical analysis using ANOVA revealed no significant differences between the wild-type and transgenic mouse groups for any parameter and age group.



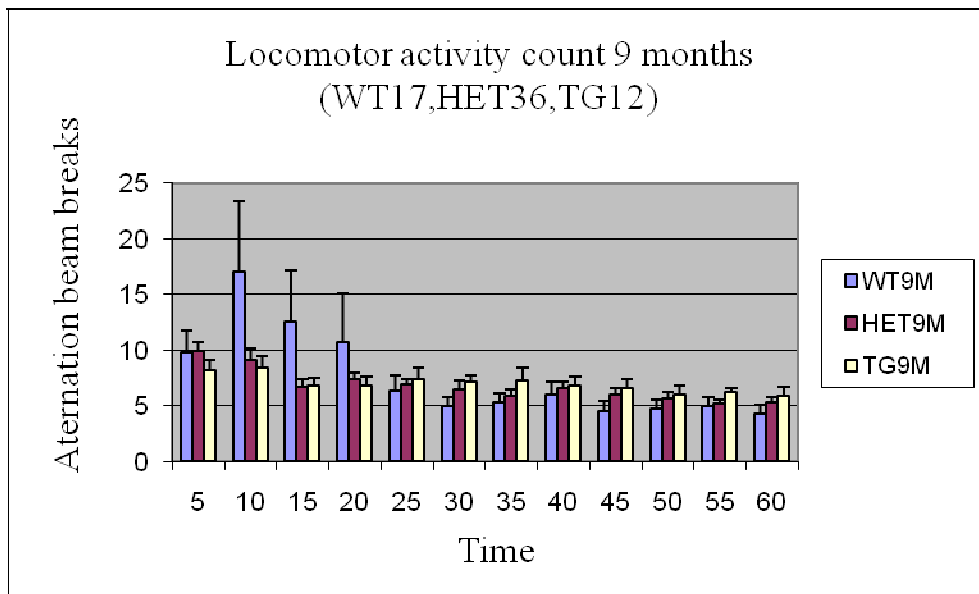
**Figure 13a.** The bar chart shows the number of alternation beam breaks (mean±SEM) during 1 h session. Groups of 6-month old wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice were tested.



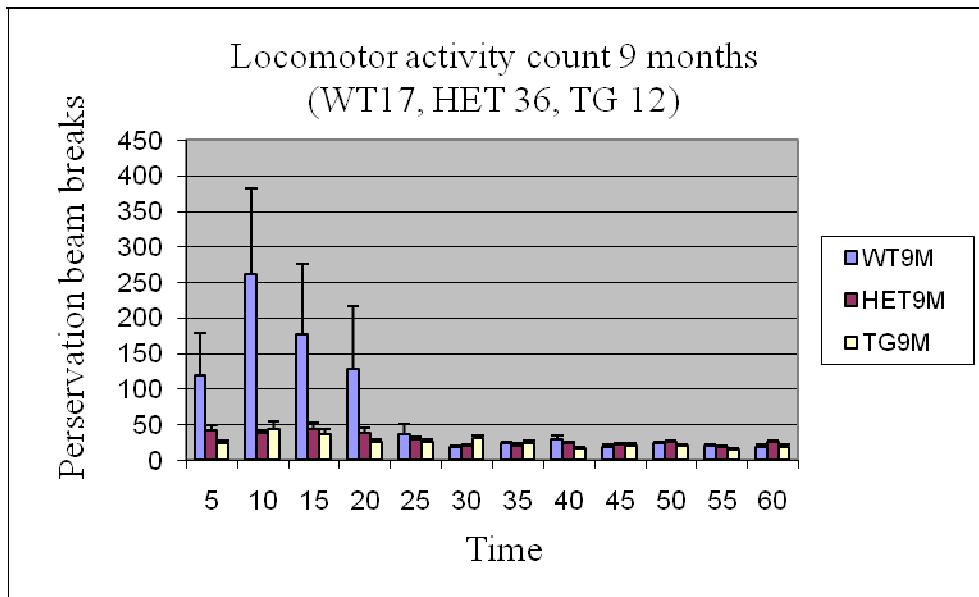
**Figure 13b.** The bar chart shows the number of persistence beam breaks (means±SEM) during 1 h session. Groups of 6-month old wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice were tested.



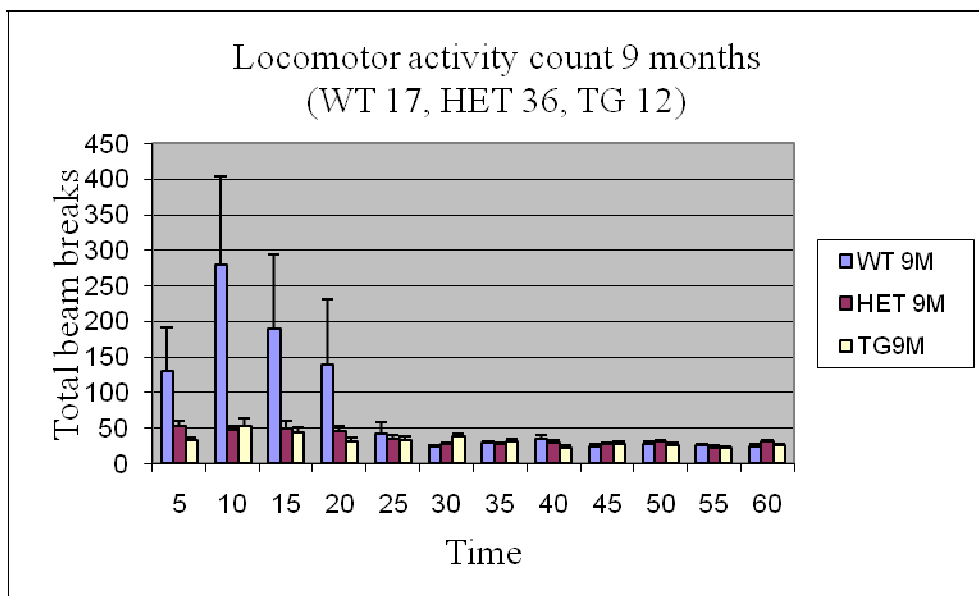
**Figure 13c.** The bar chart shows the total number of beam breaks (means±SEM) during 1 h session. Groups of 6-month old wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice were tested.



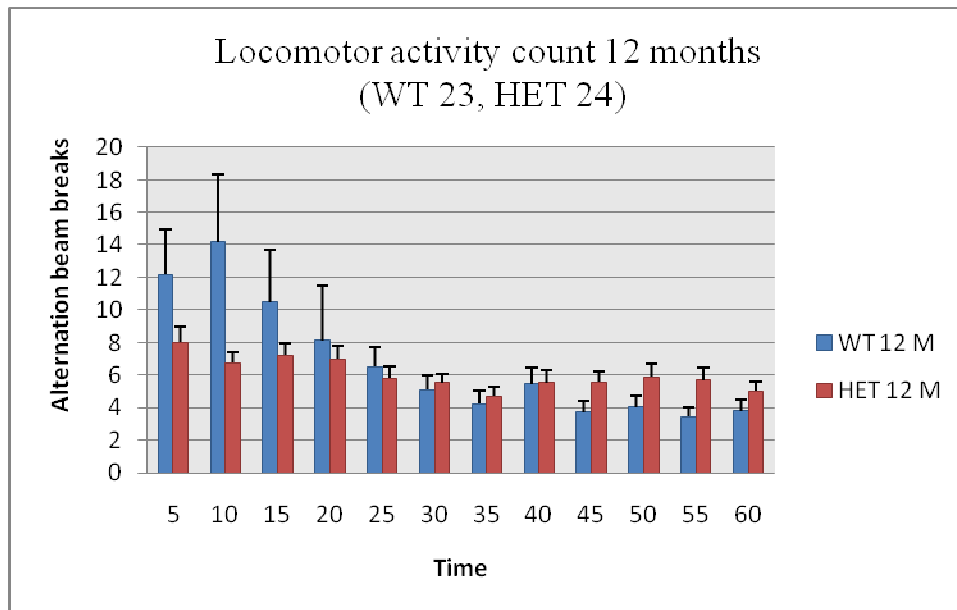
**Figure 13d.** The bar chart shows the number of alternation beam breaks (means±SEM) during 1 h session. Groups of 9-month old wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice were tested.



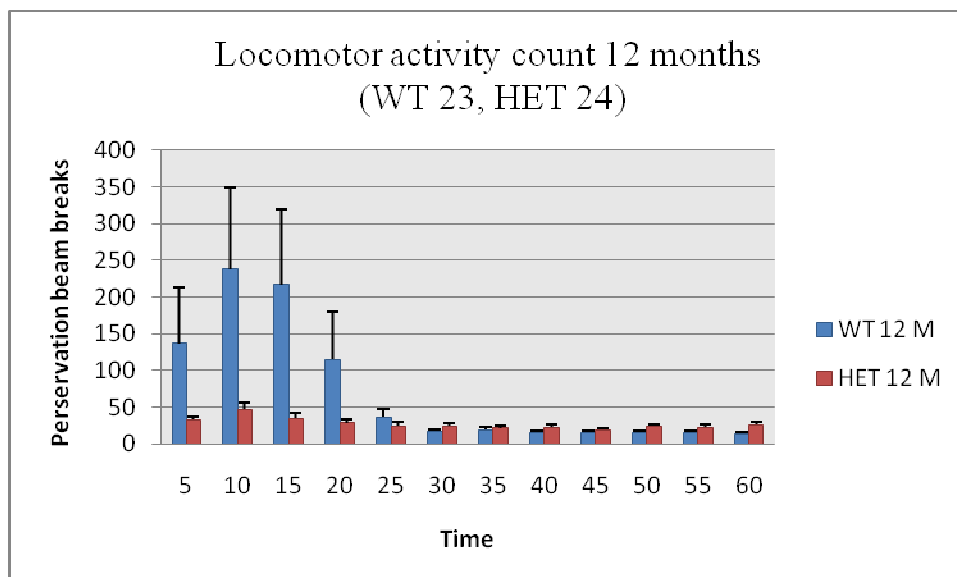
**Figure 13e.** The bar chart shows the number of perservation beam breaks (means±SEM) during 1 h session. Groups of 9-month old wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice were tested.



**Figure 13f.** The bar chart shows the total number of perservation beam breaks (means±SEM) during 1 h session. Groups of 9-month old wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice were tested.

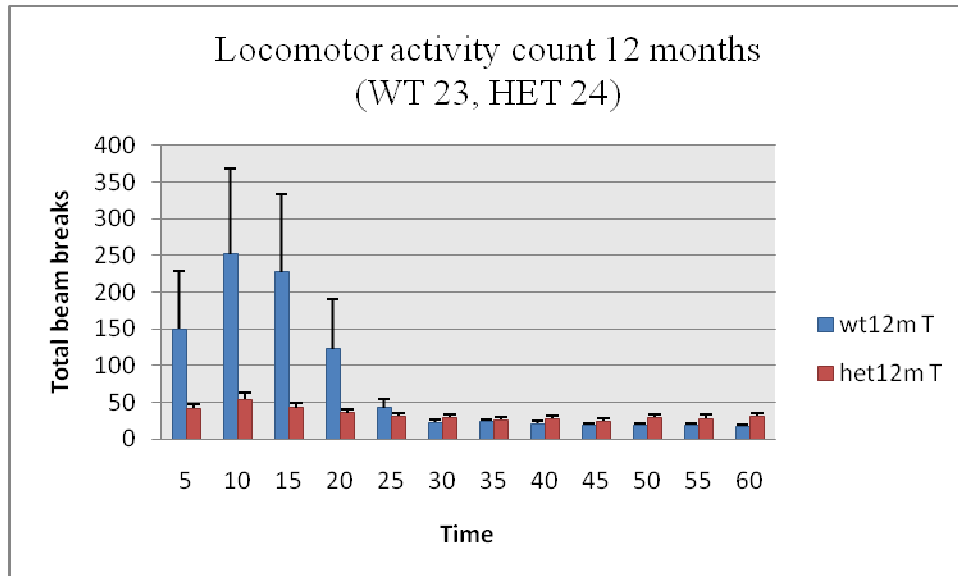


**Figure 13g.** The bar chart shows the number of alternation beam breaks (means±SEM) during 1 h session. Groups of 12-month old wild type (WT) and hemizygous (HET) transgenic mice were tested.

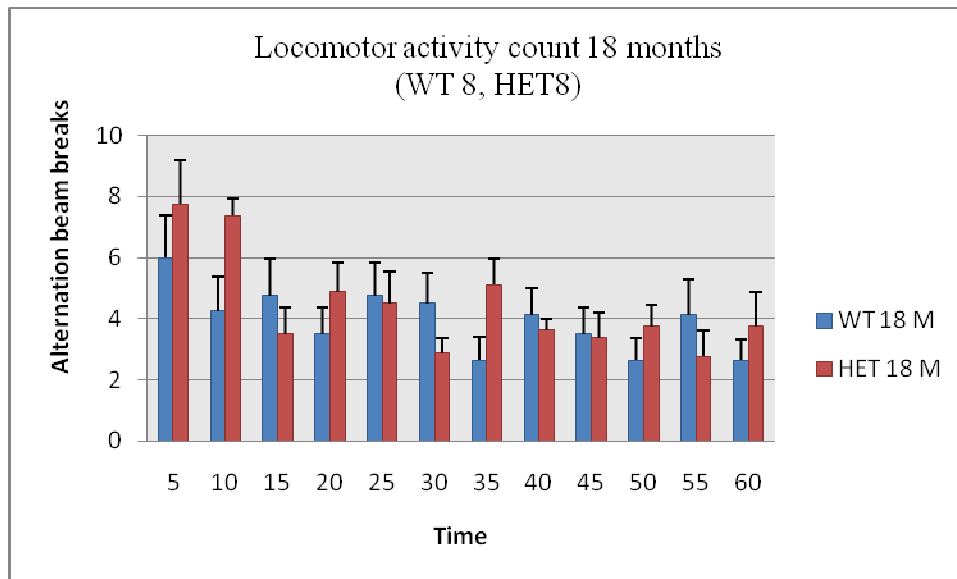


**Figure 13h.** The bar chart shows the number of perservation beam breaks (means±SEM) during 1 h session. Groups of 12-month old wild type (WT) and hemizygous (HET) transgenic mice were tested.

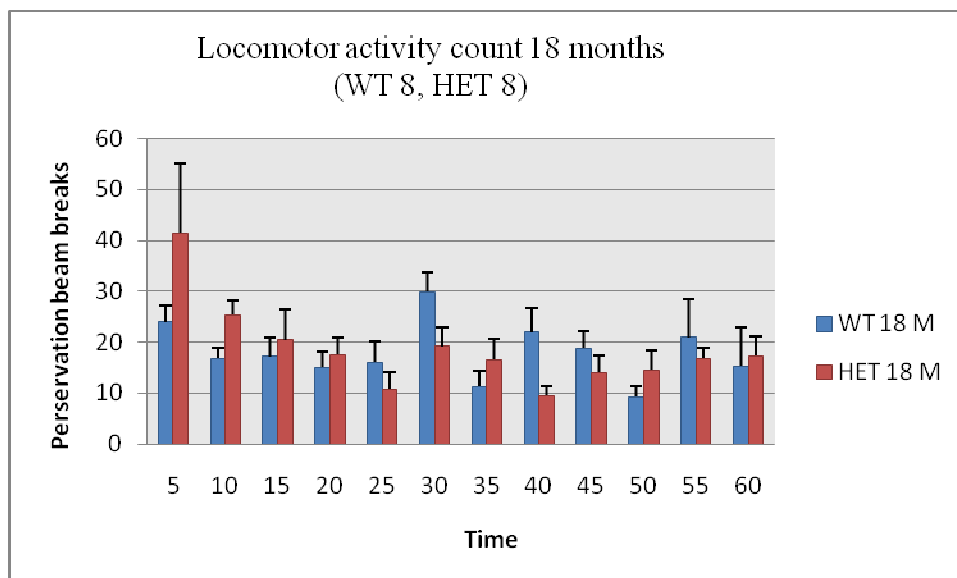




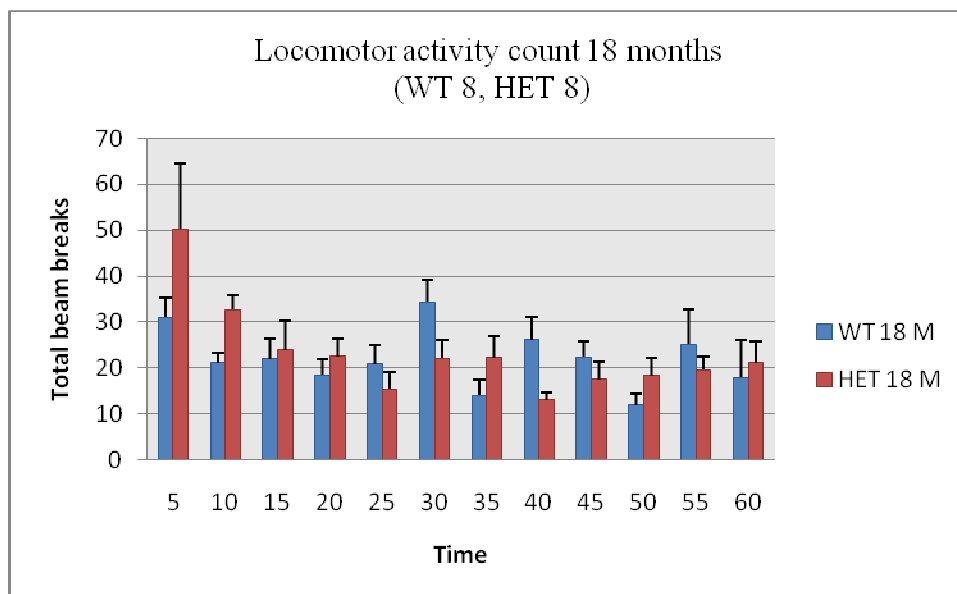
**Figure 13i.** The bar chart shows the total number of beam breaks (means±SEM) during 1 h session. Groups of 12-month old wild type (WT) and hemizygous (HET) transgenic mice were tested.



**Figure 13j.** The bar chart shows the number of alternation beam breaks (means±SEM) during 1 h session. Groups of 18-month old wild type (WT) and hemizygous (HET) transgenic mice were tested.



**Figure 13k.** The bar chart shows the number of preservation beam breaks (means±SEM) during 1 h session. Groups of 18-month old wild type (WT) and hemizygous (HET) transgenic mice were tested.



**Figure 13l.** The bar chart shows the total number of beam breaks (means±SEM) during 1 h session. Groups of 18-month old wild type (WT) and hemizygous (HET) transgenic mice were tested.

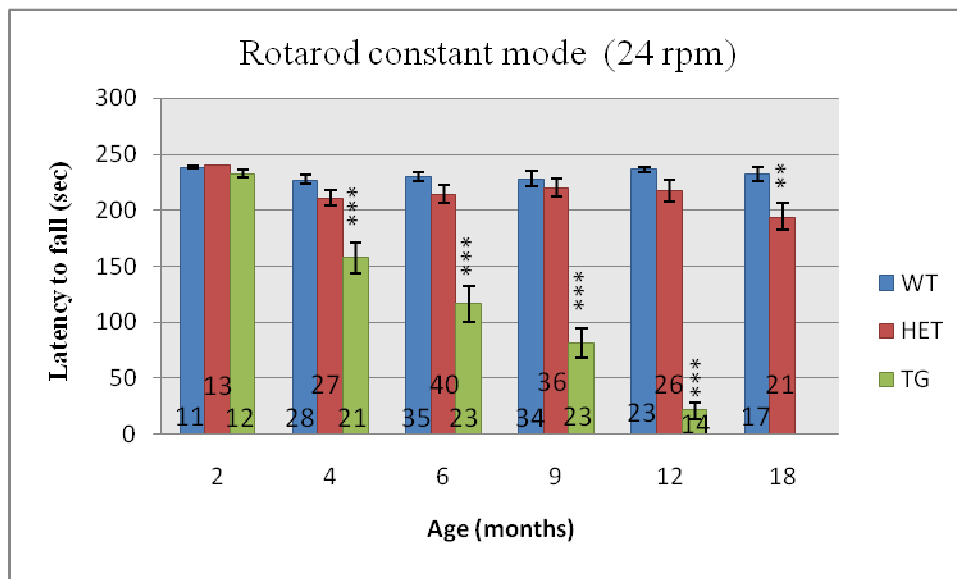
### 3.5 Rota-rod test

The balance and coordination of male mice was tested using both constant speed and accelerating rotarod as described in the Material and Methods chapter. All tests were carried out between 10 am and 2 pm.

Three groups of animals (wild type, homozygous and hemizygous gamma-synuclein transgenic animals) were tested at 2, 4, 6, 9 and 12 month of age and two groups (wild type and hemizygous gamma-synuclein transgenic animals) – at 18 month of age.

Results of the constant speed rotarod analysis are shown in Figure 14a. One way ANOVA analysis revealed that in the performance of homozygous gamma-synuclein transgenic mice is not different from the performance of wild-type mice at the age of

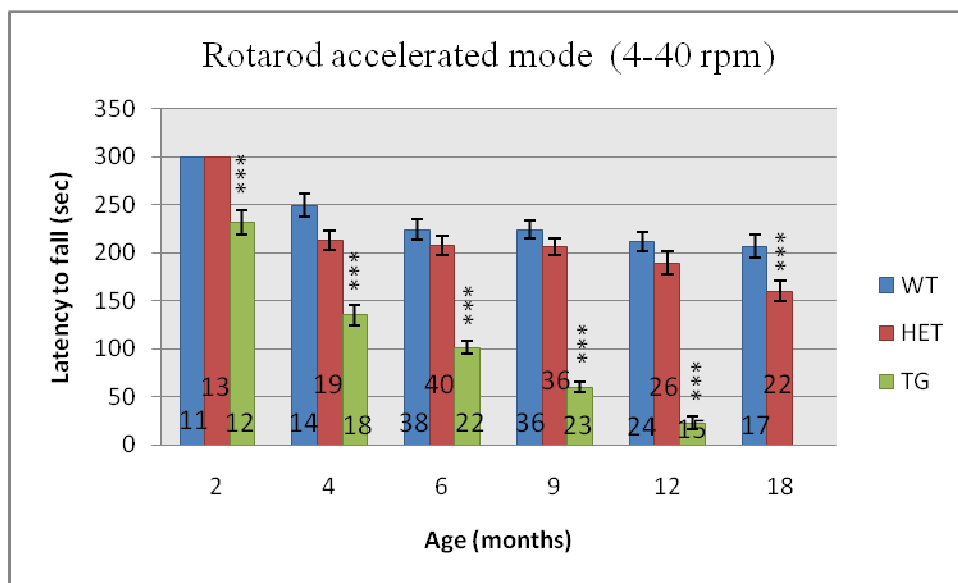
2 months ( $F_{1,22}=1.648$ ,  $P=0.2$ ) but is significantly impeded at the age of 4 months ( $F_{1,47}=29.945$ ,  $P<0.0001$ ), 6 months ( $F_{1,56}=65.742$ ,  $P<0.0001$ ), 9 months ( $F_{1,55}=114.631$ ,  $P<0.0001$ ) and 12 months ( $F_{1,35}=1045.464$ ,  $P<0.0001$ ). In contrast, hemizygous animals exhibited motor dysfunction only at the age of 18 months ( $F_{1,36}=7.211$ ,  $P=0.011$ ).



**Figure 14a.** The bar chart shows means±SEM of the latency to fall of wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice in the constant speed (24 rpm) rotarod test. The number of tested animals in each group is shown. Values statistically different from the wild-type value for the same age group are designated (\*\* $P<0.01$ ; \*\*\* $P<0.001$ ).

Figure 14b shows the results of accelerating rotarod analysis. Similar to the results of the constant speed test, a statistically significant difference with wild-type mice was detected for homozygous gamma-synuclein transgenic mice at the age of 4 months ( $F_{1,30}=49.6$ ,  $P<0.0001$ ), 6 months ( $F_{1,54}=69.494$ ,  $P<0.0001$ ), 9 months ( $F_{1,53}$

= 173.962,  $P < 0.0001$ ) and 12 months ( $F_{1,37} = 177.01$ ,  $P < 0.0001$ ). Surprisingly, this test revealed motor impairments of homozygous mice already at the age of 2 months ( $F_{1,22} = 25.22$ ,  $P < 0.0001$ ). For hemizygous mice the results of the accelerating rotarod test were similar to results of the constant speed test – a difference with wild type mice becomes significant only when animals reach the age of 18 months ( $F_{1,37} = 11.176$ ,  $P = 0.001$ ).



**Figure 14b.** The bar chart shows means±SEM of the latency to fall of wild type (WT), hemizygous (HET) and homozygous (TG) transgenic mice in the accelerating speed rotarod test. The number of tested animals in each group is shown. Values statistically different from the wild-type value for the same age group are designated (\*\*\*) $P < 0.001$ ).

### **3.6 Beam walking test**

The beam walking test was used in this study to test animal balance and coordination. Table 1 shows the number and percent of animals able to turn on a wooden rod and then cross the distance to the escape box (for details see Materials and Methods) as well as average time required for animals from each experimental group to complete these two tasks.

We found that the most useful parameter for analysis of gamma-synuclein transgenic mice performance in this test is percent of animals able to turn on the beam. Only a quarter of homozygous mice was able to perform this task at the age of 4 months, and a further reduction was obvious in groups of 6-month and 9-month old mice. 12-month old homozygous mice were completely unable to turn on the beam. However, at the age of 4 months all animals that successfully turned on the beam were able to cross it. Results for older homozygous animals were unreliable because only a small number of them completed the first part of the test and therefore was not involved in the second, crossing task.

Times required for completing each task significantly varied between individual mice within each genotype group because some animals “freeze” in the middle of the task for significant time periods. Statistically, homozygous animals revealed significant differences at the age of 4 months comparing to their wild-type litter mates for both tasks (ability to turn and ability to cross) ( $P < 0.0384$ ,  $P < 0.0269$ ).

Hemizygous mice displayed a notable decline in performance only at the age of 12 months - only about half of the animals were able to complete both turn and cross tasks at this age. While, statistical analysis of performance of heterozygous animals comparing to wild-type revealed significant differences at the age of 4, 6, 9, and 12

months of age for the ability to turn task (  $P= 0.001$  at 4 months,  $P= 0.0486$  at 6 months,  $P= 0.0353$  at 9 months,  $P= 0.0015$  at 12 months). Due to the large weight of older animals, testing of 18-month and 24-month old heterozygous and wild type mice required a stronger and thicker (17 mm) beam. Statistically, significant differences were observed in the performance of heterozygous mice comparing to their wild type littermate at both ages (18, 24 ) and for both tasks of the test (  $P= 0.004$  at 18 month ,  $P= 0.002$  at 24 month for the ability to turn task) (  $P= 0.0193$  at 18 month,  $P= 0.0005$  for the ability to cross task) (Table 2).

**Table 1. Performance of wild-type (WT), hemizygous (HET) and homozygous (TG) gamma-synuclein transgenic mice in the beam walking test (11 mm beam)**

(\* P< 0.05 , \*\* P< 0.01 , Mann-Whitney test).

4 months	Able to turn	Time required to turn (sec)	Able to cross	Time required to cross (sec)	% able to turn	% able to cross after turn
WT	13 of 14	6.5±2.76	12 of 13	15.3±2.56	92.9	92.3
HET	** 21 of 22	16.8±3.96	* 17 of 21	27.7±3.81	95.5	81
TG	* 5 of 20	12.0±4.44	* 5 of 5	26.0±3.19	25	100

6 months	Able to turn	Time required to turn	Able to cross	Time required to cross	% able to turn	% able to cross after turn
WT	32 of 33	4.5±0.70	25 of 32	26.2±5.57	97	78.1
HET	* 35 of 41	15.3±4.10	25 of 35	32.2±5.18	85.4	71.4
TG	2 of 13	6.0±2.00	0 of 2	N/A	15.4	0

9 months	Able to turn	Time required to turn	Able to cross	Time required to cross	% able to turn	% able to cross after turn
WT	31 of 33	9.8±3.00	28 of 31	33.6±4.95	93.9	90.3
HET	* 27 of 33	11.7±5.20	18 of 27	27.3±4.07	81.8	66.7
TG	1 of 21	26.0±0.00	0 of 1	N/A	4.7	0

12 months	Able to turn	Time required to turn	Able to cross	Time required to cross	% able to turn	% able to cross after turn
WT	19 of 20	3.3±0.76	14 of 19	26.6±4.28	95	73.7
HET	** 13 of 24	10.9±1.81	7 of 13	25.7±4.37	54.2	53.8
TG	0 of 12	N/A	N/A	N/A	0	N/A



**Table 2. Performance of wild-type (WT) and hemizygous (HET) gamma-synuclein transgenic mice in the beam walking test (17 mm beam)**

(\*P< 0.05, \*\*P<0.01, \*\*\*P<0.001, Mann-Whitney test).

18 months	Able to turn	Time required to turn	Able to cross	Time required to cross	% able to turn	% able to cross after turn
WT	16 of 16	4.7±1.12	16 of 16	13.9±1.88	100	100
HET	** 20 of 21	12.0±2.05	* 20 of 20	24.0±2.67	95.2	100

24 months	Able to turn	Time required to turn	Able to cross	Time required to cross	% able to turn	% able to cross after turn
WT	8 of 8	8.8±1.60	8 of 8	16.0±1.73	100	100
HET	** 11 of 13	18.3±2.06	*** 11 of 11	40.1±5.34	84.6	100

## Chapter 4- Discussion

The synuclein family consists of three members, alpha-synuclein and beta-synuclein, which are expressed in various regions of the brain, and gamma-synuclein, which is more abundant in the spinal cord and the peripheral nervous system (Akopian et al., 1995, Buchman et al., 1998, Ninkina et al., 2003).

Among these three proteins alpha-synuclein has been studied much more than other two members of the family. To study the role of this protein in pathogenesis of neurodegenerative diseases, knockout mice and different types of transgenic mice over expressing wild type or mutated alpha-synuclein were produced.

The very first alpha-synuclein knock out mouse line was produced by Abeliovich et al. (2000), and the study conducted on these mice revealed no motor dysfunctions. Later several more mouse lines with inactivation of alpha-synuclein gene were produced (Cabin et al., 2002; Schluter et al., 2003; Drolet et al., 2004) and characterisation of their phenotypes confirmed that a constitutive absence of alpha-synuclein has minimal effect on the animal motor function.

In contrast, various transgenic mice expressing wild type or mutated forms of human alpha-synuclein displayed obvious progressive motor dysfunction. Mice expressing wild type human alpha-synuclein under the control of the PDGF-B promoter (Masliah 2000) showed compromised performance on the rotarod at 12 months of age (Hashimoto et al., 2003). Mice with Thy1 promoter-driven expression of A53T mutated human alpha-synuclein displayed early and progressive decline of motor function (Van der Putten et al., 2000). In another study Fleming and his colleagues using a battery of sensorimotor tests, including beam test, pole test, inverted grid, spontaneous activity and gait analysis, also demonstrated early and progressive motor

impairments that could be detected as early as at 2 months in a different mouse line overexpressing human wild type alpha-synuclein under the control of Thy1 promoter (Fleming et al., 2004). However, another set of transgenic mouse lines expressing wild type or A30P alpha-synuclein under the control of Thy1 promoter showed no motor dysfunction (Kahle et al., 2000), probably due to significantly lower levels of transgene expression.

In 2002 two groups independently reported that mice expressing wild type or A53T alpha-synuclein under the control of mouse prion protein gene promoter developed a marked motor deficit at the age of 8 months (Giasson et al., 2002; Lee et al., 2002) that led to premature animal death. Similarly, by using hamster prion protein gene promoter to express wild type, A30P, or A53T alpha-synuclein Gomez-Isla et al. (2003) produced transgenic mice with severe motor dysfunctions.

After many promoters have been used to express human alpha-synuclein in many transgenic mouse lines, it became clear that those lines expressing highest levels show more remarkable motor dysfunctions, which support the idea that expression levels of alpha-synuclein can play an important role in pathogenesis of Parkinson disease and other synucleinopathies (Singleton et al., 2003). However, in all transgenic lines with alpha-synuclein expression driven by general (PDGF-B) or pan-neuronal (Thy1 or prion protein gene) promoters, motor dysfunction was caused predominantly by pathological changes in the spinal cord (Buchman and Ninkina, 2008), which is not typical for alpha-synuclein pathology in human neurodegenerative diseases. In contrast to alpha-synuclein, where expression in neurons of the spinal cord is low, gamma-synuclein expression in spinal motor neurons is high. Therefore, overexpression of gamma-synuclein in these neurons might be more relevant for modelling human motor neuron diseases than overexpression of alpha-synuclein,

although there is no direct evidence of gamma-synuclein involvement in these diseases.

To reveal if pan-neuronal overexpression of gamma-synuclein would cause pathological changes similar to the changes previously observed in mice overexpressing alpha-synuclein, a line of transgenic mice expressing high levels of mouse gamma-synuclein under the control of Thy1 promoter was produced in Vladimir Buchman laboratory in collaboration with Herman van der Putten laboratory (Novartis Pharma AG). In homozygous mice of this line the level of transgene expression was approximately 7 times higher than in neural tissues expressing endogenous gamma-synuclein in wild type mice (Ninkina et al., 2009). It was important to study whether these gamma-synuclein transgenic mice develop motor dysfunction and how the observed behavioural deficiencies progress in ageing animals.

Therefore, in this study five different types of behavioural tests were conducted on three mouse genotype groups (homozygous and hemizygous gamma-synuclein transgenic, and control wild type mice) to assess their balance and coordination as well as general locomotor activity and startle motor response.

The results of gait test, beam test, and rota rod test revealed that there are significant differences between gamma-synuclein transgenic mice (both hemizygous and homozygous) compared to their wild type littermate mice. They also showed that motor dysfunction of homozygous and heterozygous animals is age dependent and correlated directly with the level of gene expression. It was not possible to test homozygous animals after the age of 12 months because even if they survived beyond this age, the mice develop severe motor impairments that prevented reliable testing. For example, pareses and paralyses of limbs led to smears instead of clear footprints

in the footprint test and completely prevented hanging on the inverted grid or staying on the beam/rod.

Using constant mode rotarod test we found no differences between wild type and either hemizygous or homozygous mice at the age of 2 months. But from the age of 4 months statistically significant differences between homozygous and wild type animals have been revealed. In contrast, hemizygous mice exhibited motor dysfunction detectable by this test only at the age of 18 months.

Surprisingly, homozygous animals on accelerating mode of rotarod test revealed motor dysfunction already at the age of 2 months. This suggests that for detection of motor dysfunction caused by overexpression of gamma-synuclein accelerating mode rotarod test is more sensitive than the constant mode. Nevertheless, accelerating mode rotarod test still detected motor impairments in hemizygous mice only at the age of 18 months.

Several parameters were evaluated in the gait test but differences between genotype groups were detected only for the stride length. The width of hind and front paws base as well as the overlaps were the same for genotype groups at each of tested age. Hemizygous gamma-synuclein transgenic mice revealed no gait impairments at 6, 9 and 12 months of age but at 18 months these animals showed significant decrease of the stride length. In contrast, homozygous gamma-synuclein transgenic mice displayed significantly decreased stride length already at 6 and 9 months of age.

Compromised performance in the beam walking test was revealed for 12-month old hemizygous gamma-synuclein transgenic mice, which makes this test more sensitive than rotarod and gait tests for detecting motor deficit in this animals. Homozygous animals display compromised ability to turn on horizontal beam at 4 months of age and to cross the beam - at 6 months of age. Therefore, the ability to turn is a more

sensitive indicator of motor dysfunction in gamma-synuclein transgenic mice than the ability to cross the beam.

In summary, overexpression of gamma-synuclein in neurons of transgenic mice leads to prominent and progressive motor dysfunction that could be detected by using rotarod, gait and beam walking tests.

Although the above tests clearly revealed motor deficits at different age points, locomotor activity count was found unreliable for assessing motor dysfunction in gamma-synuclein transgenic mice. Experimental data obtained in this test was very variable between individual animals even within the same genotype group making results statistically insignificant. One explanation for this is the design of test cages that had only two detector beams. Increased number of beams might produce better and more reliable results.

Another test that produced unreliable results for homozygous gamma-synuclein transgenic mice is prepulse inhibition. The acoustic startle test measures the response of animals to acoustic stimuli characterized by contractions of the major muscles of the body that leads to extension of the forepaws and hind paws followed by muscle flexion into a hunched position. In wild type mice presenting low intensity acoustic stimuli prior to the acoustic startle stimulus attenuates the response to the latter. From experimental data shown in Fig. 11a, b and their statistical analysis it could be concluded that in contrast to wild type mice at all studied ages, 6-month old homozygous gamma-synuclein transgenic mice do not display any prepulse inhibition and at the age of 9 months they show very limited inhibition and only to the highest amplitude prepulse. However, analysis of the initial response to the acoustic startle

stimulus across studied genotype and age groups revealed that it was very low for homozygous gamma-synuclein transgenic mice at all ages (~10-15% of the values for wild type mice, see Table 3). Such low background startle values make the assessment of their inhibition very difficult and obtained results might be misleading.

**Table 3. Response of 6-, 9- and 12-month old wild type (WT), hemizygous (HET) and homozygous (TG) mice to 120Db or 105Db startle stimuli**

6 MONTHS	WT – No stim	HET - No stim	TG - No stim	WT- Startle 105 dB	HET- Startle 105dB	TG- Startle 105dB	WT- Startle 120dB	HET- Startle 120dB	TG- Startle 120dB
MEAN	13.28	14.55	13.23	220.5	57.627	33.07	332.1955	55.009	38.556
STDEV	7.724	7.575	5.722	96.8	34.125	8.486	200.467	25.017	8.824
SQRT	4.69	5.47	4	4.69	5.477	4	4.69	5.477	4
SEM	1.646	1.383	1.43	20.64	6.23	2.12	42.739	4.567	2.206

9 MONTHS	WT – No stim	HET - No stim	TG - No stim	WT- Startle 105 dB	HET- Startle 105dB	TG- Startle 105dB	WT- Startle 120dB	HET- Startle 120dB	TG- Startle 120dB
MEAN	14.4	14.8	10.88	212.6	63.21	33.8	274.06	99.9	42.972
STDEV	7.834	8.621	5.276	97.58	43.6	13.736	134.332	95.22	16.623
SQRT	5	5.09	5	5	5.09	5	5	5.09	5
SEM	1.567	1.69	1.055	19.516	8.55	2.747	26.866	18.674	3.324

12 MONTHS	WT – No stim	HET - No stim	TG – No stim	WT- Startle 105 dB	HET- Startle 105dB	TG- Startle 105dB	WT- Startle 120dB	HET- Startle 120dB	TG- Startle 120dB
MEAN	16.16	16.55	N/A	212.443	58.922	N/A	255.89	81.41	N/A
STDEV	6.618	6.7	N/A	103.7	49.26	N/A	111.353	59.73	N/A
SQRT	4.795	5.196	N/A	4.795	5.196	N/A	4.795	5.196	N/A
SEM	1.379	1.29	N/A	21.631	9.48	N/A	23.218	11.495	N/A

The startle reflex is also substantially decreased in heterozygous gamma-synuclein transgenic mice at all studied ages although to the lesser extent than in homozygous mice (Table 3). Therefore, the decrease of amplitude of the response to the acoustic startle stimulus in gamma-synuclein transgenic mice correlates with the level on transgene expression. The reason of such striking effect seen even in young transgenic mice is not clear. Visual observation of animals during startle test suggested that they react to the stimuli, suggesting that hearing is not affected. However, this reaction was very weak and different from the described above normal reaction of wild type mice. It is possible that although various behavioural tests used in this study revealed very

limited signs of motor dysfunction in 6 month old homozygous and even older heterozygous gamma-synuclein transgenic mice, some of their motor neurons and/or their axons and synapses might be already substantially affected by transgene expression. These pathological changes can be sufficient for preventing normal pattern of muscle contraction in response to the acoustic startle stimulus. Further detailed studies are required to establish exact mechanism of the observed phenomenon.

In conclusion, assessment of animal behaviour in several test clearly demonstrated that overexpression of gamma-synuclein in neurons of transgenic mice leads to the development of significant motor deficit. Observed behavioural changes are progressive and age-dependent. The onset and the degree of motor dysfunction correlate with the dose of transgene. Thus, increased production of gamma-synuclein causes pathological changes in the nervous system that closely resemble changes previously seen in animals overexpressing alpha-synuclein. These results suggest that in addition to alpha-synucleinopathies, certain animal and human neurodegenerative diseases, particularly some forms of motor neuron diseases, might represent gamma-synucleinopathies. Therefore, it is feasible to intensify the search for gamma-synuclein pathology in diseases, which so far was very limited.



## References

- Abeliovich, A., Y. Schmitz, I. Farinas, D. Choi-Lundberg, W. H. Ho, P.E. Castillo, N. Shinsky, J.M. Verdugo, M. Armanini, A. Ryan, M. Hynes, H. Phillips, D. Sulzer, A. Rosenthal. (2000). "Mice lacking alpha-Synuclein Display Functional Deficits in the Nigrostriatal Dopamine System." *Neuron*. 25: 239-252.
- Adamczyk, A.S.J., J.B. Strosznajder. (2005). "Expression of alpha-synuclein in different brain parts of adult and aged rats." *J Physiol Pharmacol*. 56: 29-37.
- Akopian, A.N., J. Wood. (1995). "Peripheral nervous system-specific genes identified by subtractive cDNA cloning." *J Biol Chem*. 270: 21264-21270.
- Al-Wandi, A., N. Ninkina, S. Millership, S.J.M. Williamson, P.A. Jones, V.L. Buchman. (2010). "Absence of alpha-synuclein affects dopamine metabolism and synaptic markers in the striatum of aging mice." *Neurobiol Aging*. 31: 796-804.
- André, C., T.T. Truong, J.F. Robert, Y.C. Guillaume. (2005). "Effect of metals on herbicides- $\alpha$ -synuclein association: A possible factor in neurodegenerative disease studied by capillary electrophoresis." *Electrophoresis*. 26: 3256-3264.
- Arawaka, S., Y. Saito, S. Murayama, H. Mori. (1998). "Lewy body in neurodegeneration with brain iron accumulation type 1 is immunoreactive for alpha-synuclein." *Neurology*. 51: 887-889.
- Arima, K., K. Ueda, N. Sunohara, S. Hirai, Y. Izumiyama, H. Tonozuka-Uehara, M. Kawai. (1998). "Immunoelectron-microscopic demonstration of NACP/alpha-synuclein-epitopes on the filamentous component of Lewy bodies in Parkinson's disease and in dementia with Lewy bodies." *Brain Res*. 808: 93-100.
- Baba, M., S. Nakajo, P.H. Tu, T. Tomita, K. Nakaya, V.M. Lee, J.Q. Trojanowski, T. Iwatsubo. (1998). "Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies." *Am J Pathol*. 152: 879-884.
- Baptista, M.J., C. O'Farrell, S. Daya, R. Ahmad, W.D. Miller, J. Hardy, J.M. Farrer, R.M. Cookson. (2003). "Co-ordinate transcriptional regulation of dopamine synthesis genes by  $\alpha$ -synuclein in human neuroblastoma cell lines." *J Neurochem*. 85: 957-968.
- Baron, M.S., J.L. Vitek, R.A. Bakay, J. Green, W.M. McDonald, S.A. Cole, M.R. DeLong. (2000). "Treatment of advanced Parkinson's disease by unilateral posterior GPi pallidotomy: 4-year results of a pilot study." *Mov Disord*. 15: 230-237.
- Benabid, A.L., S. Chabardes, J. Mitrofanis, P. Pollak. (2009). "Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease." *Lancet Neurol*. 8: 67-81.
- Biere, A.L., S.J. Wood, J. Wypych, S. Steavenson, Y. Jiang, D. Anafi, F.W. Jacobsen, M.A. Jarosinski, G.M. Wu, J.C. Louis, F. Martin, L.O. Narhi, M. Citron. (2000). Parkinson's disease-associated alpha-synuclein is more fibrillogenic than beta- and

gamma-synuclein and cannot cross-seed its homologs. *J. Biol. Chem.* 275:34574–34579.

Bruening, W., B.I.Giasson, A.J.P. Klein-Szanto, V.M.Lee, Q.J.Trojanowski, A.K.Godwin. (2000). "Synucleins are expressed in the majority of breast and ovarian carcinomas and in preneoplastic lesions of the ovary." *Cancer.* 88: 2154-2163.

Buchman, V.L., H.J.Hunter, L.G.Pinon, J.Thompson, E.M.Privalova, N.N.Ninkina, A.M.Davies. 1998. Persin, a member of the synuclein family, has a distinct pattern of expression in the developing nervous system. *J.Neurosci.* 18:9335-9341.

Buchman, V.L., J.Adu, L.G.Pinon, N.N.Ninkina, A.M.Davies. 1998. Persyn, a member of the synuclein family, influences neurofilament network integrity. *Nat. Neurosci.* 1:101–103.

Buchman, V.L., N.N.Ninkina (2008). "Modulation of alpha-synuclein expression in transgenic animals for modelling synucleinopathies--is the juice worth the squeeze." *Neurotox Res.* 14: 329-341.

Burre, J., M.Sharma, T.Tsetsenis, V.L.Buchman, M.R.Etherton, T.C.Sudhof. (2010). "α-Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro." *Science.* 329: 1663-1667.

Cabin, D.E., K.Shimazu, D.Murphy, N.B.Cole, W.Gottschalk, K.L.McIlwain, B.Orrison, A.Chen, C.E.Ellis, R.Paylor, B.Lu, R.L.Nussbaum. (2002). "Synaptic Vesicle Depletion Correlates with Attenuated Synaptic Responses to Prolonged Repetitive Stimulation in Mice Lacking alpha-Synuclein." *J Neurosci.* 22: 8797-8807.

Campion, D., C.Martin, R.Heilig, F.Charbonnier, V.Moreau, M.J.Flaman, L.J.Petit, D.Hannequin, A.Brice, T.Frebourg. (1995). "The NACP/synuclein gene: chromosomal assignment and screening for alterations in Alzheimer disease." *Genomics.* 26: 254-257.

Carter, R.J., L.A.Lione, T.Humby, L.Mangiarini, A.Mahal, G.P.Bates, S.B.Dunnett, A.J.Morton. (1999). "Characterization of Progressive Motor Deficits in Mice Transgenic for the Human Huntington's Disease Mutation." *J Neurosci.* 19: 3248-3257.

Caslake, R., A.Macleod, N.Ives, R.Stowe, C.Counsell. (2009). "Monoamine oxidase B inhibitors versus other dopaminergic agents in early Parkinson's disease." *Cochrane Database Syst Rev.* 4: CD006661.

Chandra, S., F.Fornai, H.B.Kwon, U.Yazdani, D.Atasoy, X.Liu, R.E.Hammer, G.Battaglia, D.C.German, P.E.Castillo, T.C.Sudhof. (2004). "Double-knockout mice for alpha- and beta-synucleins: Effect on synaptic functions." *Proc Natl Acad Sci U S A.* 101: 14966-14971.

Chandra, S., G.Gallardo, R.Fernández-Chacón, O.M.Schlüter, T.C.Sudhof. (2005). "Alpha-Synuclein Cooperates with CSP alpha in Preventing Neurodegeneration." *Cell.* 123: 383-396.

- Clayton,D.F., J.M.George (1999). "Synucleins in synaptic plasticity and neurodegenerative disorders." *J Neurosci Res.* 58: 120-129.
- Conway,K.A., J.D.Harper, P.T.Lansbury.(1998). "Accelerated in vitro fibril formation by a mutant [alpha]-synuclein linked to early-onset Parkinson disease." *Nat Med.* 4: 1318-1320.
- Conway,K.A., J.D.Harper, P.T.Lansbury. (2000). "Fibrils Formed in Vitro from alpha-Synuclein and Two Mutant Forms Linked to Parkinson's Disease are Typical Amyloid." *Biochemistry.* 39: 2552-2563.
- Crawley,J.N., R.Paylor (1997). "A Proposed Test Battery and Constellations of Specific Behavioral Paradigms to Investigate the Behavioral Phenotypes of Transgenic and Knockout Mice." *Horm Behav.* 31: 197-211.
- Da Costa,C.A., E.Maslah, F.Checler. (2003). "Beta-Synuclein Displays an Antiapoptotic p53-dependent Phenotype and Protects Neurons from 6-Hydroxydopamine-induced Caspase 3 Activation." *J Biol Chem.* 278: 37330-37335.
- Danzer,K.M., C.Schnack, A.Sutcliffe, B.Hengerer, F.Gillardon. (2007). "Functional protein kinase arrays reveal inhibition of p-21-activated kinase 4 by  $\alpha$ -synuclein oligomers." *Journal of Neurochemistry.* 103: 2401-2407.
- Dauer,W., N.Kholodilov, M.Vila, A.C.Trillat, R.Goodchild, K.E.Larsen, R.Staal, K.Tieu, Y.Schmitz, C.A.Yuan, M.Rocha, V.Jackson-Lewis, S.Hersch, D.Sulzer, S.Przedborski, R.Burke, R.Hen. (2002). "Resistance of alpha -synuclein null mice to the parkinsonian neurotoxin MPTP." *Proc Natl Acad Sci U S A.* 99: 14524-14529.
- Davidson,W.S., A.Jonas, D.F.Clayton, J.M.George. (1998). "Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes." *J Biol Chem.* 273: 9443-9449.
- Drolet,R.E., B.Behrouz, K.J.Lookingland, J.L.Goudreau, . (2004). "Mice Lacking [alpha]-Synuclein have an Attenuated Loss of Striatal Dopamine Following Prolonged Chronic MPTP Administration." *NeuroToxicology.* 25: 761-769.
- Du,H.-N., L.Tang, X.Y.Luo, H.T.Li, J.Hu, J.W.Zhou, H.Y.Hu. (2003). "A Peptide Motif Consisting of Glycine, Alanine, and Valine Is Required for the Fibrillization and Cytotoxicity of Human alpha-Synuclein " *Biochemistry.* 42: 8870-8878.
- El-Agnaf,O.M.A., J.Ross, D.C.Martin, M.Derek, I.Raffaele, B.Elisabetta, P.Antonello, N.David, W.Andrew. (1998). "Aggregates from mutant and wild-type alpha-synuclein proteins and NAC peptide induce apoptotic cell death in human neuroblastoma cells by formation of beta-sheet and amyloid-like filaments." *FEBS Lett.* 440: 71-75.
- Fan,Y., P.Limprasert, I.V.J.Murray, A.C.Smith, V.M.Y.Lee, J.Q.Trojanowski, B.L.Sopher, A.R.La Spada.(2006). "Beta-synuclein modulates alpha-synuclein

neurotoxicity by reducing alpha-synuclein protein expression." *Hum Mol Genet.* 15: 3002-3011.

Farrer, M., J. Kachergus, L. Forno, S. Lincoln, D. S. Wang, M. Hulihan, D. Maraganore, K. Gwinn-Hardy, Z. Wszolek, D. Dickson, J. W. Langston. (2004). "Comparison of kindreds with parkinsonism and  $\alpha$ -synuclein genomic multiplications." *Ann Neurol.* 55: 174-179.

Fine, J., J. Duff, R. Chen, B. Chir, W. Hutchison, A. M. Lozano, A. E. Lang. (2000). "Long-term follow-up of unilateral pallidotomy in advanced Parkinson's disease." *N Engl J Med.* 342: 1708-1714.

Fleming, S. M., J. Salcedo, P. O. Fernagut, E. Rockenstein, E. Masliah, M. S. Levine, M. F. Chesselet. (2004). "Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein." *J Neurosci.* 24: 9434-9440.

Forman, M. S., V. M. Y. Lee, J. Q. Trojanowski. (2005). "Nosology of Parkinson's Disease: Looking for the Way Out of a Quackmire." *Neuron.* 47: 479-482.

Frandsen, P., L. Madsen, C. Bendixen, K. Larsen. (2009). "Porcine  $\gamma$ -synuclein: molecular cloning, expression analysis, chromosomal localization and functional expression." *Mol Biol Rep.* 36: 971-979.

Fujiwara, H., M. Hasegawa, N. Dohmae, A. Kawashima, E. Masliah, M. S. Goldberg, J. Shen, K. Takio, T. Iwatsubo. (2002). "alpha-Synuclein is phosphorylated in synucleinopathy lesions." *Nat Cell Biol.* 4: 160-164.

Gai, W., J. Power, P. Blumbergs, J. Culvenor, P. Jensen. (1999). "Alpha-synuclein immunoprecipitation of glial inclusions from multiple system atrophy brain tissue reveals multiprotein components." *J Neurochem.* 73: 2093-2100.

Galvin, J. E., K. Uryu, V. M. Y. Lee, J. Q. Trojanowski. (1999). "Axon pathology in Parkinson's disease and Lewy body dementia hippocampus contains alpha-, beta-, and gamma-synuclein." *Proc Natl Acad Sci U S A.* 96: 13450-13455.

Gao, N., Y. Li, X. Li, S. Yu, G. L. Fu, B. Chen. (2007). "Effect of alpha-synuclein on the promoter activity of tyrosine hydroxylase gene." *Neurosci Bull.* 23: 53-57.

George, J. M., H. Jin, W. S. Woods, D. F. Clayton. (1995). "Characterization of a novel protein regulated during the critical period for song learning in the zebra finch." *Neuron.* 15: 361-372.

Giasson, B. I., J. E. Duda, I. V. J. Murray, Q. Chen, J. M. Souza, H. I. Hurtig, H. Ischiropoulos, J. Q. Trojanowski, V. M. Lee. (2000). "Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions." *Science.* 290: 985-999.

Giasson, B. I., I. V. J. Murray, J. Q. Trojanowski, V. M. Lee. (2001). "A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly." *J Biol Chem.* 276: 2380-2386.

Giasson, B.I., J.E. Duda, S.M. Quinn, B. Zhang, J.Q. Trojanowski, V.M. Lee. (2002). "Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein." *Neuron*. 34: 521-533.

Giasson, B.I., K. Uryu, J.Q. Trojanowski, V.M. Lee. (1999). "Mutant and wild type human alpha-synucleins assemble into elongated filaments with distinct morphologies in vitro." *J Biol Chem*. 274: 7619-7622.

Goedert, M. (2001). "Alpha-synuclein and neurodegenerative diseases." *Nat Rev Neurosci*. 2: 492-501.

Golts, N., H. Snyder, M. Frasier, C. Theisler, P. Choi, B. Wolozin. (2002). "Magnesium inhibits spontaneous and iron-induced aggregation of alpha-synuclein." *J Biol Chem*. 277: 16116-16123.

Gomez-Isla, T., M.C. Irizarry, A. Mariash, B. Cheung, O. Soto, S. Schrupp, J. Sondel, L. Kotilinek, J. Day, M.A. Schwarzschild, J.H. Cha, K. Newell, D.W. Miller, K. Ueda, A.B. Young, B.T. Hyman, K.H. Ashe. (2003). "Motor dysfunction and gliosis with preserved dopaminergic markers in human alpha-synuclein A30P transgenic mice." *Neurobiol Aging*. 24: 245-258.

Greten-Harrison, B., M. Polydoro, M. Morimoto-Tomita, L. Diao, A.M. Williams, E.H. Nie, S. Makani, N. Tian, P.E. Castillo, V.L. Buchman, S.S. Chandra. (2010). "αβγ-Synuclein triple knockout mice reveal age-dependent neuronal dysfunction." *Proc Natl Acad Sci U S A*. 107: 19573-19578.

Hashimoto, M., L.J. Hsu, A. Sisk, Y. Xia, A. Takeda, M. Sundsmo, E. Masliah. (1998). "Human recombinant NACP/[alpha]-synuclein is aggregated and fibrillated in vitro: Relevance for Lewy body disease." *Brain Res*. 799: 301-306.

Hashimoto, M., E. Rockenstein, M. Mante, M. Mallory, E. Masliah. (2001). "Beta-Synuclein Inhibits alpha-Synuclein Aggregation: A Possible Role as an Anti-Parkinsonian Factor." *Neuron*. 32: 213-223.

Hashimoto, M., R. Edward, E. Masliah. (2003). "Transgenic models of alpha-synuclein pathology: past, present, and future." *Ann N Y Acad Sci*. 991: 171-188.

Hashimoto, M., E. Rockenstein, M. Mante, L. Crews, P. Bar-On, F.H. Gage, R. Marr, E. Masliah. (2004a). "An antiaggregation gene therapy strategy for Lewy body disease utilizing [beta]-synuclein lentivirus in a transgenic model." *Gene Ther*. 11: 1713-1723.

Hashimoto, M., P. Bar-on, G. Ho, T. Takenouchi, E. Rockenstein, L. Crews, E. Masliah. (2004b). "Beta-synuclein regulates Akt activity in neuronal cells. A possible mechanism for neuroprotection in Parkinson's disease." *J Biol Chem*. 279: 23622-23629.

Hassler, R. (1938). "Zur pathologie der paralysis agitans und des postenzephalitischen parkinsonismus." *J Psychol Neurol*. 48: 387-476.

- Irizarry M.C., T.W.Kim, M.McNamara, R.E.Tanzi, D.F.Clyton, J.M.George, B.T.Hyman. (1996). "1996 Characterization of the precursor protein of the non-A beta component of senile plaques (NACP) in the human central nervous system." *J Neuropathol Exp Neurol.* 55: 889–895.
- Ison, J.R., M.K.Taylor, G.P.Bowen, S.B.Schwarzkopf. (1997). "Facilitation and Inhibition of the Acoustic Startle Reflex in the Rat After a Momentary Increase in Background Noise Level." *Behav Neurosci.* 111: 1335-1352.
- Iwai, A., E.Maslah, M.Yoshimoto, N.Ge, L.Flanagan, H.A.Rohan de Silva, A.Kittel, T.Saitoh. (1995). "The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system." *Neuron.* 14: 467-475.
- Iwatsubo, T. (2003). "Aggregation of  $\alpha$ -synuclein in the pathogenesis of Parkinson's disease." *J Neurol.* 250: iii11-iii14.
- Jakes, R., M.G.Spillantini, M.Goedert. (1994). "Identification of two distinct synucleins from human brain." *FEBS Lett.* 345: 27-32.
- Jao, C.C., A.D.Sarkissian., J.Chen, R.Langen, (2004). "Structure of membrane-bound  $\alpha$ -synuclein studied by site-directed spin labeling." *Proc Natl Acad Sci U S A.* 101(22): 8331– 8336.
- Jenco, J.M., A. Rawlingson, B.Daniels, A.J.Morris. (1998). "Regulation of Phospholipase D2: Selective Inhibition of Mammalian Phospholipase D Isoenzymes by  $\alpha$ - and  $\beta$ -Synucleins" *Biochemistry.* 37: 4901-4909.
- Jennifer, A.P. (2007). "Introduction to Parkinson's disease." *Disease-a-month : DM* 53: 195-199.
- Ji, H., Y.E.Liu, T.Jia, M.Wang, J.Liu, G.Xiao, B.K.Joseph, C.Rosen, Y.E.Shi. (1997). "Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing." *Cancer Res.* 57: 759-764.
- Jones, B.J., D.J.Roberts. (1968). "The quantitative measurement of motor incoordination in naive mice using an accelerating rotarod." *J Pharm Pharmacol.* 20: 302–304.
- Kahle, P.J., M.Neumann, L.Ozmen, V.Muller, H.Jacobsen, A.Schindzielorz, M.Okochi, U.Leimer, H.van der Putten, A.Probst, E.Kremmer, H.A.Kretschmar, C.Haass. (2000). "Subcellular localization of wild-type and Parkinson's disease-associated mutant  $\alpha$ -synuclein in human and transgenic mouse brain." *J Neurosci.* 20: 6365-6373.
- Kelly, P.J., J.E.Ahlskog, S.J.Goerss, J.R.Daube, J.R.Duffy, B.A.Kall. (1987). "Computer-assisted stereotactic ventralis lateralis thalamotomy with microelectrode recording control in patients with Parkinson's disease." *Mayo Clin Proc.* 62: 655-664.

- Kholodilov, N.G., T.F.Oo, R.E.Burke. (1999). "Synuclein expression is decreased in rat substantia nigra following induction of apoptosis by intrastriatal 6-hydroxydopamine." *Neurosci Lett.* 275: 105-108.
- Kruger, R., L.Schls, T.Müller, W.Kuhn, D.Woitalla, H.Przuntek, J.T.Epplen, O.Riess. (2001). "Evaluation of the gamma-synuclein gene in German Parkinson's disease patients." *Neuroscience Letters* 310: 191-193.
- Kruger, R., W.Kuhn, T.Muller, D.Woitalla, M.Graeber, S.Kosel, H.Przuntek, J.T.Epplen, L.Schols, O.Riess. (1998). "Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease." *Nat Genet.* 18: 106-108.
- Lavedan, C. (1998). "The synuclein family." *Genome Res.* 8: 871– 880.
- Lee, M., H.Dong-Hoon, H.Barry, J.Peter. (2001). "Effect of the overexpression of wild-type or mutant alpha-synuclein on cell susceptibility to insult." *J Neurochem.* 76: 998-1009.
- Lee, M., W.Stirling, Y.Xu, X.Xu, D.Qui, A.S.Mandir, T.M.Dawson, N.G.Copeland, N.A.Jenkins, D.L.Price. (2002). "Human alpha-synuclein-harboring familial Parkinson's disease-linked Ala-53 --> Thr mutation causes neurodegenerative disease with alpha-synuclein aggregation in transgenic mice." *Proc Natl Acad Sci U S A.* 99: 8968-8973.
- Lee, V.M.Y., J.Q.Trojanowski. (2006). "Mechanisms of Parkinson's Disease Linked to Pathological [alpha]-Synuclein: New Targets for Drug Discovery." *Neuron.* 52: 33-38.
- Lewy, F. (1912). "The first description of the inclusions that define Parkinson's disease at a neuropathological level." *Handbuch der Neurologie.* 3: 920-933.
- Li, J.Y., J.P.Henning, A.Dahlstrom. (2002). "Differential localization of [alpha]-, [beta]- and [gamma]-synucleins in the rat CNS." *Neuroscience.* 113: 463-478.
- Liu, D., L.Jin, H.Wang, H.Zhao, C.Zhao, C.Duan, L.Lu, B.Wu, S.Yu, P.Chan, Y.Li, H.Yang. (2008). "Silencing  $\alpha$ -Synuclein Gene Expression Enhances Tyrosine Hydroxylase Activity in MN9D Cells." *Neurochem Res.* 33: 1401-1409.
- Marie-Laure, M., J.H.Marjorie, M.M.Katherine, S.Tanya, W.R.Mark. (2007). "Acute effects of a lateral postural assist on voluntary step initiation in patients with Parkinson's disease." *Mov Disord.* 22: 20-27.
- Maroteaux, L., R.H.Scheller. (1991). "The rat brain synucleins; family of proteins transiently associated with neuronal membrane." *Mol Brain Res.* 11: 335–343.
- Maroteaux, L., J.T.Campanelli, R.H.Scheller. (1988). "Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal." *J Neurosci.* 8: 2804-2815.

- Masliah,E., E.Rockenstein, I.Veinbergs, M.Mallory, M.Hashimoto, A.Takeda, Y.Sagara, A.Sisk, L.Mucke. (2000). "Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders." *Science*. 287: 1265-1269.
- Mezey,E., A.Deheijia, G.Harta, M.I.Papp, M.H.Polymeropoulos, M.J.Brownstein. (1998). "Alpha-synuclein in neurodegenerative disorders: murderer or accomplice?" *Nat Med*. 4: 755–757.
- Miyasaki,J.M. (2006). "New practice parameters in Parkinson's disease." *Nat Clin Pract Neuro*. 2: 638-639.
- Moore,D.J., A.B.West, V.L.Dawson, T.M.Dawson. (2005). "Molecular pathophysiology of Parkinson's disease." *Ann Rev Neurosci*. 28: 57-87.
- Mori,F., K.Tanji, M.Yoshimoto, H.Takahashi, K.Wakabayashi. (2002). "Demonstration of [alpha]-Synuclein Immunoreactivity in Neuronal and Glial Cytoplasm in Normal Human Brain Tissue Using Proteinase K and Formic Acid Pretreatment." *Exp Neurol*. 176: 98-104.
- Mori,F., M.Nishie, M.Yoshimoto, H.Takahashi, K.Wakabayashi. (2003). "Reciprocal accumulation of beta-synuclein in alpha-synuclein lesions in multiple system atrophy." *Neuroreport*. 14: 1783-1786.
- Murphy,D.D., S.M.Rueter, J.Q.Trojanowski, V.M.Y.Lee. (2000). "Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons." *J Neurosci*. 20: 3214-3220.
- Nakajo,S., S.Shioda, Y.Nakai, K.Nakaya. (1994). "Localization of phosphoneuroprotein 14 (PNP 14) and its mRNA expression in rat brain determined by immunocytochemistry and in situ hybridization." *Brain Res. Mol. Brain Res*. 27: 81-86.
- Nakajo,S., K.Tsukada, H.Kameyama, Y.Furuyama, K.Nakaya. (1996). "Distribution of phosphoneuroprotein 14 (PNP 14) in vertebrates: its levels as determined by enzyme immunoassay." *Brain Res*. 741: 180-184.
- Narabayshi,H. (1982). "Generating mechanism and treatment of parkinsonian symptoms." *Rinsho Shinkeigaku*. 22: 1055-1062.
- Narhi,L., S.J.Wood, S.Steavenson, Y.Jiang, G.M.Wu, D.Anafi, S.A.Kaufman, F.Martin, K.Sitney, P.Denis, J.C.Louis, J.Wypych, A.L.Biere, M.Citron. (1999). "Both familial Parkinson's disease mutations accelerate alpha-synuclein aggregation." *J Biol Chem*. 274: 9843-9846.
- Nemani,V.M., W. Lu, V.Berge, K.Nakamura, B.Onoa, M.K.Lee, F.A.Chaudhry, R.A.Nicoll, R.H.Edwards. (2010). "Increased Expression of alpha-Synuclein Reduces Neurotransmitter Release by Inhibiting Synaptic Vesicle Reclustering after Endocytosis." *Neuron*. 65: 66-79.



- Nguyen, J.V., I.Soto, K-Y.Kim, E.A.Bushong, E.Oglesby, F.J.Valiente-Soriano, Z.Yang, C.O.Davis, J.L.Bedont, J.L.Son, J.O.Wei, V.L.Buchman, D.J.Zack, M.Vidal-Sanz, M.H.Ellisman, N.Marsh-Armstrong. (2011). "Myelination transition zone astrocytes are constitutively phagocytic and have synuclein dependent reactivity in glaucoma." *Proc Natl Acad Sci U S A*. 108: 1176-1181.
- Ninkina, N., K.Papachroni, D.C.Robertson, O.Schmidt, L.Delaney, F.O'Neill, F.Court, A.Rosenthal, S.M.Fleetwood-Walker, A.M.Davies, V.L.Buchman. (2003). "Neurons expressing the highest levels of gamma-synuclein are unaffected by targeted inactivation of the gene." *Mol Cell Biol*. 23: 8233-8245.
- Ninkina, N., O.Peters, S.Millership, H.Salem, H.van der Putten, V.L.Buchman. (2009). "Gamma-synucleinopathy: neurodegeneration associated with overexpression of the mouse protein." *Hum Mol Genet*. 18: 1779-1794.
- Ninkina, N., M.V.Alimova-Kost, J.W.Paterson, L.Delaney, B.B.Cohen, S.Imreh, N.V.Gnuchev, A.M.Davies, V.L.Buchman. (1998). "Organization, expression and polymorphism of the human persyn gene." *Hum Mol Genet*. 7: 1417-1424.
- Ninkina, N.N., E.M.Privalova, L.G.P.Pinõn, A.M.Davies, V.L.Buchman. (1999). "Developmentally Regulated Expression of Persyn, a Member of the Synuclein Family, in Skin." *Exp Cell Res*. 246: 308-311.
- Nishioka, K., C.Wider, C.Vilarino-Guell, A.I.Soto-Ortolaza, S.J.Lincoln, J.M.Kachergus, B.Jasinska-Myga, O.A.Ross, A.Rajput, C.A.Robinson, T.J.Ferman, Z.K.Wszolek, D.W.Dickson, M.J.Farrer. (2010). "Association of alpha-, beta-, and gamma-Synuclein with diffuse lewy body disease." *Arch Neurol*. 67: 970-975.
- Norris, E.H., B.I.Giasson, V.M.Y.Lee, P.S.Gerald. (2004). [alpha]-Synuclein: Normal Function and Role in Neurodegenerative Diseases. *Current Topics in Developmental Biology*, Academic Press. Volume 60: 17-54.
- Ohtake, H., P.Limprasert, Y.Fan, O.Onodera, A.Kakita, H.Takahashi, L.T.Bonner, D.W.Tsuang, I.V.Murray, V.M.Lee, J.Q.Trojanowski, A.Ishikawa, J.Idezuka, M.Murata, T.Toda, T.D.Bird, J.B.Leverenz, S.Tsuji, A.R.La Spada. (2004). "Beta-synuclein gene alterations in dementia with Lewy bodies." *Neurology* 63: 805-811.
- Olanow C.W., J.H. Kordower, T.B. Freeman. (1996). "Fetal nigral transplantation as a therapy for Parkinson's disease." *Trends in Neurosciences*. 19:102-109
- Olanow, C.W., M.Stern, K.Sethi. (2009). "The scientific and clinical basis for the treatment of Parkinson disease." *Neurology*. 72: S1-136.
- Oort, P.J., T.A.Knotts, M.Grino, N.Naour, J.P.Bastard, K.Clament, N.Ninkina, V.L.Buchman, P.A.Permana, X.Luo, G.Pan, T.N.Dunn, S.H.Adams. (2008). "Gamma-synuclein is an adipocyte-neuron gene coordinately expressed with leptin and increased in human obesity." *J Nutr*. 138: 841-848.

- Ostrerova-Golts, N., L. Petrucelli, M. Farrer, N. Mehta, P. Choi, J. Hardy, B. Wolozin. (1999). "alpha-Synuclein shares physical and functional homology with 14-3-3 proteins." *J Neurosci.* 19: 5782-5791.
- Ostrerova-Golts, N., L. Petrucelli, J. Hardy, J. M. Lee, M. Farrer, B. Wolozin. (2000). "The A53T alpha-synuclein mutation increases iron-dependent aggregation and toxicity." *J Neurosci.* 20: 6048-6054.
- Pallone, J. A. (2007). "Introduction to Parkinson's Disease." *Disease-a-month* : DM 53: 195-199.
- Park, J.-Y., P. T. Lansbury. (2003). "Beta-Synuclein Inhibits Formation of alpha-Synuclein Protofibrils: a Possible Therapeutic Strategy against Parkinson's Disease " *Biochemistry* 42: 3696-3700.
- Parkinson, J. (1817). "An Essay on the Shaking Palsy " London: Whittingham and Rowland.
- Peng, X. M., R. Tehranian, P. Dietrich, L. Stefanis, R. G. Perez. (2005). "Alpha-synuclein activation of protein phosphatase 2A reduces tyrosine hydroxylase phosphorylation in dopaminergic cells." *J Cell Sci.* 118: 3523-3530.
- Perez, R. G., J. Waymire, E. Lin, J. J. Liu, F. Guo, M. J. Zigmond. (2002). "A role for alpha-synuclein in the regulation of dopamine biosynthesis." *J Neurosci.* 22: 3090-3099.
- Perrin, R. J., W. S. Woods, D. F. Clayton, J. M. George. (2000). "Interaction of human alpha-Synuclein and Parkinson's disease variants with phospholipids. Structural analysis using site-directed mutagenesis." *J Biol Chem.* 275: 34,393–34,398.
- Polymeropoulos, M. H., J. J. Higgins, L. I. Golbe, W. G. Johnson, S. E. Ide, G. Di Iorio, G. Sanges, E. S. Stenroos, L. T. Pho, A. A. Schaffer, A. M. Lazzarini, R. L. Nussbaum, R. C. Duvoisin. (1996). "Mapping of a gene for Parkinson's disease to chromosome 4q21-q23." *Science.* 274: 1197-1199.
- Polymeropoulos, M. H., C. Lavedan, E. Leroy, S. E. Ide, A. Dehejia, A. Dutra, B. Pike, H. Root, J. Rubenstein, R. Boyer, E. S. Stenroos, S. Chandrasekharappa, A. Athanassiadou, T. Papapetropoulos, W. G. Johnson, A. M. Lazzarini, R. C. Duvoisin, G. Di Iorio, L. I. Golbe, R. L. Nussbaum. (1997). "Mutation in the alpha-synuclein gene identified in families with Parkinson's disease." *Science.* 276: 2045-2047.
- Robertson, D. C., O. Schmidt, N. Ninkina, P. A. Jones, J. Sharkey, V. L. Buchman. (2004). "Developmental loss and resistance to MPTP toxicity of dopaminergic neurons in substantia nigra pars compacta of  $\gamma$ -synuclein,  $\alpha$ -synuclein and double  $\alpha/\gamma$ -synuclein null mutant mice." *Journal of Neurochemistry* 89: 1126-1136.
- Rochet, J. C., T. Outeiro, K. A. Conway, T. T. Ding, M. J. Volles, H. A. Lashuel, R. M. Bieganski, S. L. Lindquist, P. T. Lansbury. (2004). "Interactions among alpha-synuclein, dopamine, and biomembranes: some clues for understanding neurodegeneration in Parkinson's disease." *J Mol Neurosci.* 23: 23-34.

- Rockenstein,E., L.A.Hansen, M.Mallory, J.Q.Trojanowski, D.Galasko, E.Maslah. (2001). "Altered expression of the synuclein family mRNA in Lewy body and Alzheimer's disease." *Brain Res.* 914: 48-56.
- Saito,Y., M.Kawai, K.Inoue, R.Sasaki, H.Arai, E.Nanba, S.Kuzuhara, Y.Ihara, I.Kanazawa, S.Murayama. (2000). "Widespread expression of alpha-synuclein and [tau] immunoreactivity in Hallervorden-Spatz syndrome with protracted clinical course." *J Neurol Sci.* 177: 48-59.
- Savitt,J.M., V.L.Dawson, T.M.Dawson. (2006). "Diagnosis and treatment of Parkinson disease: molecules to medicine." *J Clin Invest.* 116: 1744-1754.
- Schluter,O.M., F.Fornai, M.G.Alessandri, S.Takamori, M.Geppert, R.Jahn, T.C.Südhof. (2003). "Role of [alpha]-synuclein in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in mice." *Neuroscience* 118: 985-1002.
- Serpell,L.C., J.Berriman, R.Jakes, M.Goedert, R.A.Crowther. (2000). "Fiber diffraction of synthetic alpha-synuclein filaments shows amyloid-like cross-beta conformation." *Proc Natl Acad Sci U S A.* 97: 4897-4902.
- Sharon,R., M.Goldberg, I.Bar-Josef, R.A. Betensky, J.Shen, D.J.Selkoe. (2001). "alpha-Synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins." *Proc Natl Acad Sci U S A.* 98: 9110-9115.
- Shibasaki,Y., D.Baillie, D.St Clair, A.J.Brookes. (1995). "High-resolution mapping of SNCA encoding alpha-synuclein, the non-A beta component of Alzheimer's disease amyloid precursor, to human chromosome 4q21.3-->q22 by fluorescence in situ hybridization." *Cytogenet Cell Genet.* 71: 54-55.
- Shibayama-Imazu,T., O.Kyoko, H.Yuko, N.Shigeo, S.Seiji, O.Hidehiko, N.Yasumitsu, N.Kazuyasu. (1998). "Distribution of PNP 14 (beta-synuclein) in neuroendocrine tissues: localization in Sertoli cells." *Mol Reprod Dev.* 50: 163-169.
- Singleton,A.B., M.Farrer, J.Johnson, A.Singleton, S.Hague, J.Kachergus, M.Hulihan, T.Peurainna, A.Dutra, R.Nussbaum, S.Lincoln, A.Crawley, M.Hanson, D.Maraganore, C.Adler, M.R.Cookson, M.Muenter, M.Baptista, D.Miller, J.Blancato, J.Hardy, K.Gwinn-Hardy. (2003). "alpha-Synuclein locus triplication causes Parkinson's disease." *Science.* 302: 841.
- Snyder,H., K.Mensah, C.Hsu, M.Hashimoto, I.G.Surgucheva,B.Festoff, A.Surguchov, E.Maslah, A.Matouschek, B.Wolozin. (2005). "beta-Synuclein reduces proteasomal inhibition by alpha-synuclein but not gamma-synuclein." *J Biol Chem.* 280: 7562-7569.
- Sopher,B.L., K.L.Koszdin, M.E.McClain, S.B.Myrick, R.A.Martinez, A.C.Smith, A.R.La Spada. (2001). "Genomic organization, chromosome location, and expression analysis of mouse beta-synuclein, a candidate for involvement in neurodegeneration." *Cytogen Genome Res.* 93: 117-123.

Spillantini, M.G., A. Divane, M. Goedert. (1995). "Assignment of Human alpha-Synuclein (SNCA) and beta-Synuclein (SNCB) Genes to Chromosomes 4q21 and 5q35." *Genomics* 27: 379-381.

Spillantini, M.G., R. Crowther, R. Jakes, M. Hasegawa, M. Goedert. (1998). "alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies." *Proc Natl Acad Sci U S A.* 95: 6469-6473.

Spillantini, M.G., M. Schmidt., V.M. Lee, J.Q. Trojanowski, R. Jakes, M. Goedert. (1997). "Alpha-synuclein in Lewy bodies." *Nature.* 388: 839-840.

Surgucheva, I., B. McMahan, F. Ahmed, S. Tomarev, M.B. Wax, A. Surguchov. (2002). "Synucleins in glaucoma: Implication of  $\gamma$ -synuclein in glaucomatous alterations in the optic nerve." *J Neurosci Res.* 68: 97-106.

Surgucheva, I., A. Surguchov, B. McMahan, E. Masliah. (2001). "Synucleins in ocular tissues." *J Neurosci Res.* 65: 68-77.

Surguchov, A., I. Surgucheva, E. Solessio, W. Baehr. (1999). "Synoretin--A New Protein Belonging to the Synuclein Family." *Mol Cell Neurosci.* 13: 95-103.

Tehrani, R., S.E. Montoya, A.D. Van Laar, T.G. Hastings, R.G. Perez. (2006). "Alpha-synuclein inhibits aromatic amino acid decarboxylase activity in dopaminergic cells." *J Neurochem.* 99: 1188-1196.

Tobe, T., S. Nakajo, A. Tanaka, A. Mitoya, K. Omata, K. Nakaya, M. Tomita, Y. Nakamura. (1992). "Cloning and characterization of the cDNA encoding a novel brain-specific 14-kDa protein." *J Neurochem.* 59: 1624-1629.

Tretiakoff, C. (1919). "Contribution a l etude de l anatomie pathologique du locus niger de soemmering avec quelques deductions relatives a la pathogenie des troubles du tonus musculaire et de la maladie de Parkinson." Thesis, univ. Paris.

Tu, P. H., J. Galvin., M. Baba, B. Giasson, T. Tomita, S. Leight, S. Nakajo, T. Iwatsubo, J.Q. Trojanowski, V.M. Lee. (1998). "Glial cytoplasmic inclusions in white matter oligodendrocytes of multiple system atrophy brains contain insoluble alpha-synuclein." *Ann Neurol.* 44: 415-422.

Ueda, K., H. Fukushima, E. Masliah, Y. Xia, A. Iwai, M. Yoshimoto, D.A. Otero, J. Kondo, Y. Ihara, T. Saitoh. (1993). "Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease." *Proc Natl Acad Sci U S A.* 90: 11282-11286.

Uversky, V.N., J. Li, A.L. Fink. (2001). "Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular NK between Parkinson's disease and heavy metal exposure." *J Biol Chem.* 276: 44284-4496.

Uversky, V.N., J.Li, P.Souillac, I.S.Millett, S.Doniach, R.Jakes, M.Goedert, A.L.Fink. (2002). "Biophysical properties of the synucleins and their propensities to fibrillate: inhibition of alpha-synuclein assembly by beta- and gamma-synucleins." *J Biol Chem.* 277: 11970-11978.

van der Putten, H., K.-H.Wiederhold, A.Probst, S.Barbieri, C.Mistl, S.Danner, S.Kauffmann, K.Hofele, W.P.J.M.Spooren, M.A.Ruegg, S.Lin, P.Caroni, B.Sommer, M.Tolnay, G.Bilbe. (2000). "Neuropathology in mice expressing human alpha-synuclein." *J Neurosci.* 20: 6021-6029.

Wakabayashi, K., S.Hayashi, A.Kakita, M.Yamada, Y.Toyoshima, M.Yoshimoto, H.Takahashi. (1998). "Accumulation of alpha-synuclein/NACP is a cytopathological feature common to Lewy body disease and multiple system atrophy." *Acta Neuropathol.* 96: 445-452.

Wang, Y.-L., A.Takeda, H.Osaka, Y.Hara, A.Furuta, R.Setsuie, Y.-J.Sun, J.Kwon, Y.Sato, M.Sakurai, M.Noda, Y.Yoshikawa, K.Wada. (2004). "Accumulation of [beta]- and [gamma]-synucleins in the ubiquitin carboxyl-terminal hydrolase L1-deficient gad mouse." *Brain Res.* 1019: 1-9.

Wersinger, C., A.Sidhu. (2009). "Partial regulation of serotonin transporter function by gamma-synuclein." *Neurosci Lett.* 453: 157-161.

Windisch, M., B.Hutter-Paier, E.Schreiner, R.Wronski. (2004). "β-synuclein-derived peptides with neuroprotective activity." *J Mol Neurosci.* 24: 155-165.

Woong, C., S.Zibae, R.Jakes, L.C.Serpell, B.Davletov, R.A.Crowther, M.Goedert. (2004). "Mutation E46K increases phospholipid binding and assembly into filaments of human alpha-synuclein." *FEBS Lett.* 576: 363-368.

Yamin, G., L.A.Munishkina, M.A.Karymov, Y.L.Lyubchenko, V.N.Uversky, A.L.Fink. (2005). "Forcing Nonamyloidogenic beta-Synuclein To Fibrillate " *Biochemistry* 44: 9096-9107.

Yu, S., X.Zuo, Y.Li, C.Zhang, M.Zhou, Y.-L.Zhang, K.Uéda, P.Chan. (2004). "Inhibition of tyrosine hydroxylase expression in alpha-synuclein-transfected dopaminergic neuronal cells." *Neurosci Lett.* 367: 34-39.

Zarranz, J.J., J.Alegre, J.C.Gomez-Esteban, E.Lezcano, R.Ros, I.Ampuero, L.Vidal, J.Hoenicka, O.Rodriguez, B.Atares, V.Llorens, E.Gomez Tortosa, T.del Ser, D.G.Munoz, J.G.de Yébenes. (2004). "The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia." *Ann Neurol.* 55: 164-173.

Zhou, W., J.Milder, C.R.Freed. (2008). "Transgenic mice overexpressing tyrosine-to-cysteine mutant human alpha-synuclein: a progressive neurodegenerative model of diffuse Lewy body disease." *J Biol Chem.* 283: 9863-9870.

Neuropsychiatr Dis Treat. 2008 October; 4(5): 835–845. PMID: PMC2626922  
Potential cellular and regenerative approaches for the treatment of Parkinson's disease  
Emma L Lane, Olivia J Handley, Anne E Rosser, and Stephen B Dunnett  
Brain Repair Group, School of Biosciences, Cardiff University, CF10 3US, UK  
Correspondence: Emma Lane, Brain Repair Group, School of Biosciences, Cardiff  
University, Cardiff, CF10 3US, UK, Tel +44 29 2087 4112, Fax +44 29 2087 6749,  
Email laneel@cf.ac.uk  
Author information ▼ Copyright and License information ▼  
Copyright © 2008 Dove Medical Press Limited. All rights reserved