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REGULATION AND MODIFICATION OF SYNAPSINS BY LEARNING AND MEMORY FORMATION IN THE MOUSE

Dissertation for obtaining a doctorate degree at the University of Natural Resources and Applied Life Sciences Vienna

Submitted by
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This work is dedicated to my beloved Amma's (Mother's) memory, and is a small way of my appreciation for her endless love, devotion and sacrifices that she dedicated to her children

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I wish to dedicate this thesis to my mother late R. D. Dora for her love and sacrifice. She taught me the good things that really matter in life. The happy memory of my mother still provides a persistent inspiration for my journey in this life.

LIST OF ABBREVIATIONS

2-DE (2-Dimensional Gel Electrophoresis)

CaM Kinase (Ca2+/calmodulin protein kinase)

CE (cognitive enhancement)

DTT (Dithiothreitol)

ELM (Eukaryotic Linear Motif resource)

ESI (Electrospray Ionization)

FA (Formic Acid)

H3PO3 (phosphorous acid)

HCl (hydrogen chloride)

i.p (intraperitoneal)

IMAC (Immobilized Metal Affinity Chromatography).

LTP (long-term potentiation)

MALDI (Matrix-assisted laser desorption/ionization)

MS (mass spectrometry')

MSDB (Mascot Database)

MWM (Morris water maze)

NaCl (sodium chloride)

NCBI (National Center for Biotechnology Information)

PAGE (Polyacrylamide Gel Electrophoresis)

PKA (Protein Kinase A)

PMF (Peptide Mass Fingerprinting)

PTM (Post Translational Modifications)

SDS (Sodium Dodecyl Sulfate)

SGS742 (3-aminopropyl-n-butyl-phosphinic acid)

TFA (Trifluoroacetic acid)

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Preface

The brain is the most sophisticated and complex organ that nature has devised. It controls most of the body activities and is responsible for perception, behavior, cognition memory and consciousness. There are more than 1000 disorders associated with dysfunction of the nervous system such as neurological and psychiatric conditions. Among them certain disease condition have huge social and economic problem. There are an estimated 7.4 million people living with dementia worldwide and at least 3.7 million with Alzheimer's disease (AD). Considering the importance of the nervous system, there are several research in the area of neuroscience is going on around the world to get more insight into brain function and also number of researcher are succeed in discovering novel drug target for the treatment ailments such as dementia, stroke, brain tumors schizophrenia, depression anxiety disorders and addiction.

Large-scale DNA sequencing has transformed biomedical research in a short span of time. With the discovery of most human genes, it is now apparent that a large scale approach to address biological problems is desirable if we are to gain a comprehensive understanding of complex biological processes. The human proteome the collection of all proteins generated by the human genome is estimated to contain 10 million to 20 million proteins about 2-3 orders of magnitude higher than the number of human genes. Proteins direct almost all biological functions, but how any given protein acts is rarely transparent. Proteins do not function independently, but interact in highly complex networks, which in turn influence the intricate network of regulatory mechanisms by which the amount of each protein that is produced is regulated. These regulatory mechanisms, despite many tantalizing clues, remain mysterious and are difficult to model. It is a fundamental problem to determine which proteins interact, and how they fit into a network of interactions and this becomes even more challenging if one wants to do it in an automated way. Many current approaches attempt to integrate together information from a number of sources data from microarrays that give information about which proteins are up and down regulated together, cross-genome analysis about whether the genes for two proteins have evolved together, etc.I n addition to these complexities, to deal with significant presense of protein isoforms due to multiple post-translational modifications (PTMs). Several hundread different types of PTMs exist, such as amino-and carboxy-terminal

cleavage, phosphorylation, glycosylation and myristoylation. It is thus estimated that a protein undergoes between 2 and 20 PTMs in average.

Recent developments have made it possible to use approaches on global scale to decipher the molecular bar code of the brain. One such system-based approach is neuroproteomics*, i.e*., the large-scale profiling and functional annotation of brain proteins. Neuroproteomics is currently on the move from an emerging to a mature technology platform. Considerable development has been achieved in the area of quantitative proteomics. Here in this thesis I used different proteomics techniques including quantitative proteomics, to characterize neuronal proteins synapsin. Synapsin isoforms play an important role in neurotransmission and synaptic plasticity but exact function difficult to pinpoint. First part of thesis deals with structural characterization of individual synapsin isoform in the 2-DE and second part of thesis deals with showing the involvement of synapsin Ia and IIa isoforms but not Ib and IIb in cognitive enhancement of spatial memory. Knowledge of both structural and biological information's could be used to elucidate the role of synapsins isoform during learning and memory formation in the mouse.

Abstract

Synapsins are widely expressed synaptic vesicle phosphoproteins that have been proposed to play an important role in synaptic transmission and synaptic plasticity but structural and biological researches on synapsins are still holding centre stage. Synapsin family isoforms were shows a high level sequence similarity and share many functions although distinct physiological functions of synapsin isoforms have been proposed. Despite extensive research for decades, the exact biological functions of individual synapsin isoforms in neuronal transmission and synaptic plasticity still remain largely unknown. Proteomics has been established as a power tools for understanding a variety of biological problems and especially two-dimensional gel electrophoresis (2-DE) combined with protein identification by mass spectrometry (MS) has been proven to be a powerful, essential and valuable tool for investigation in protein expression and protein modification. Therefore we decided to carryout proteomics technology to identify and characterize synapsins and its post translational modification. We applied functional proteomics technology to gain more insight into how synapsins play a role in cognitive enhancement and spatial memory formation.

Proteins were extracted from hippocampal tissue run on 2-DE and spots were analyzed by MALDI-TOF-TOF, Ion trap and nano-LC-ESI-MS/MS. The IMAC system for phosphopeptide enrichment, Phosphatase treatment to confirm phosphorylation sites, multienzyme digestion as well as de-novo sequencing was employed. Synapsins Ia, IIa and IIb were unambiguously identified and were represented by 15 individual spots on 2-DE. Serine phosphorylation sites were confirmed for Ser-9, Ser-10, Ser 62, Ser-568, T-422 and a novel phosphorylation site was observed at Ser-546 in synapsin IIa. High sequence coverage was generated for synapsins by either MS methods. This additional structural information on these pivotal elements not only

extends protein chemical knowledge but in particular the novel phosphorylation site may have implications for novel function.

Synapsins are essential proteins for synaptic plasticity and there is no information available for their role in cognitive enhancement of spatial memory formation. It was therefore the aim of the study to link individual synapsin proteins and their isoforms to spatial memory formation enhanced by SGS742 in the mouse. Extracted hippocampal proteins from a cognitive study treating OF1 mice with the cognitive enhancer SGS742 and tested in the Morris water maze, were run on two-dimensional gel electrophoresis. Subsequently, protein spots were unambiguously identified by nano-LC-ESI-MS/MS mass spectrometry. Quantification of proteins from four groups (NaCl-treated mice, SGS742-treated mice, SGS742-treated yoked controls and NaCl-treated yoked controls) was carried out according to an in-gel stable isotope labelling method. Synapsin isoforms Ia and IIa were linked to cognitive enhancement of spatial memory by SGS742. Quantitative determination of individual synapsin isoform showed an increase in SGS742-treated mice (mean±SD of ratios between light and heavy stable isotope labelled synapsin protein; SGS742 vs controls: 2.19±0.41 for synapsin Ia, and 1.41 ± 0.81 for synapsin IIa). Synapsins Ib and IIb were not linked to cognitive enhancement. A total of 17 protein spots representing synapsin isoforms were identified and quantified. Using quantification of individual synapsin isoforms showed that these can be clearly assigned to cognitive enhancement by the $GABA_B$ antagonist SGS742. The NaCl –treated controls and the use of yoked controls that were ruling out swimming- and stress-mediated changes of synapsins, unequivocally allow proposing a role for synapsins Ia and IIa in the mechanism of cognitive enhancement of spatial memory formation.

Abstract (German)

Synapsine sind abundante Phosphoproteine der synaptischen Vesikel. Sie sind für ihre wichtige Rolle im Rahmen der synaptischen Weiterleitung und der synaptischen Plastizität bekannt. Isoformen der Synapsin Familie weisen große Deckungsgleichheiten bezüglich ihrer Sequenzen als auch ihrer Funktion auf. Sie unterscheiden sich lediglich aufgrund ihrer physiologischen Funktionen voneinander. Trotz jahrzehntelanger intensiver Forschung konnte die exkakte biologische Funktion der unterschiedlichen Isoformen bis jetzt immer noch nicht vollständig geklärt werden. Die Proteomik ist dabei ein wichtiges Hilfsmittel, um zahlreiche Fragestellungen besser beantworten zu können. Besonders die zweidimensionale Gelelektrophorese (2-DE) gemeinsam mit der Massenspektrometrie (MS), ein leistungsstarkes Gerät um Proteinexpressionen und Proteinmodulierungen zu untersuchen, ist dabei essentiell. Synapsine und ihre posttranslationale Modifikation können mit diesen Methoden identifiziert und charakterisiert werden. Darüberhinaus kann ein besserer Einblick in die Rolle der Synapsine bei der kognitiven Funktion und der Entwicklung eines räumlichen Gedächtnisses zu bekommen werden.

Die Proteine wurden aus dem hippokampalen Gewebe gewonnen und über eine 2-DE laufen gelassen. Anschließend wurden die Spots wurden mittels MALDI-TOF-TOF, Ionenfallen und nano-LC-MS/MS analysiert. Das IMAC-System wurde zur Phosphopeptid-Anreicherung, zur Phosphatase-Aufbreitung, die dazu dient um die Phosphorilierungsstellen nachzuweisen, zur Multienzymverdauung sowie zur de-novo Sequenzierung verwendet. Synapsin Ia, IIa und IIb konnten eindeutig identifiziert und durch 15 einzelne Spots mit der 2-DE dargestellt werden. Serin Phosphorilierungsstellen konnten für Ser-9, Ser-10, Ser-62, Ser-568 und T-422 nachgewiesen werden. Außerdem konnte eine neue Phosphorilierungsstelle bei Ser-546 in Synapsin IIa gezeigt werden.. Die zusätzlich gewonnen Strukturinformation

der Schlüsselelemente, erweitern nicht nur das Wissen über die Proteinchemie, sondern könnten vor allem bisher unbekannte Funktionen aus der neu entdeckten Phosphorilierungsseite offenlegen.

Synapsine sind essentielle Proteine für die synaptische Plastizität und es ist nicht bekannt, welche Rolle sie bei der kognitiven Funktion und der Entwicklung eines räumlichen Gedächtnisses spielen. Aus besagten Gründen war das Ziel der Studie, einzelne Synapsine und ihre Isoformen mit der Entwicklung eines räumlichen Gedächtnisses in Beziehung zu setzen und die Auswirkung von SGS742, einem GABA-B Antagonisten, welcher die Gedächtnisleistung in der Maus verstärken soll, zu untersuchen. Proteine aus dem Hippocampus, die nach einer kognitiven Studie im Morris Wasserlabyrinth von OF1 Mäusen (mit und ohne SGS742 intraperetoneal) entnommen wurden, wurden mit einer 2-DE aufgetrennt. Anschließend wurden die Spots eindeutig mit dem nano-LC-ESI-MS/MS Massenspektrometer identifiziert. Eine Quantifizierung der Proteine wurde bei vier unterschiedlichen Gruppen (NaCl-behandelte Mäuse, SGS742-behandelte Mäuse (Schwimmen im Wasserlabyrinth) und SGS742- und NaCl-behandelte Kontrollen (ausschließlich Schwimmen)) mit einer In-Gel Markierung mit stabilen Isotopen durchgeführt. Die Synapsin Isoformen Ia und IIa konnten mit SGS742 und der Entwicklung eines räumlichen Gedächtnisses in Verbindung gebracht werden. Quantitative Bestimmungen einzelner Synapsin-Isoformen waren in jenen Mäusen erhöht, die mit SGS742 behandelt wurden (Mittelwert ± SD des Verhältnisses zwischen dem niedrig und hoch beladenen Synapsin, das mit stabilen Isotopen markiert wurde; SGS742 vs. Kontrolle: 2.19 ± 0.41 für Synapsin Ia, und 1.41 ± 0.81 für Synapsin IIa). Bei den Synapsinen Ib und IIb konnte in dieser Studie keine Verknüpfung zur Entwicklung eines räumlichen Gedächtnisses hergestellt werden.

Insgesamt konnten 17 Proteinspots, die die Synapsin-Isoformen darstellen, identifiziert und quantifiziert werden. Dadurch konnte eindeutig gezeigt werden, dass diese klar der Entwicklung eines räumlichen Gedächtnisses durch SGS742, zugeordnet werden können. Durch die NaCl-behandelten Kontrollen und durch die Verwendung von Kontrollen die ausschließlich schwimmen mussten, konnte die mögliche schwimm- oder stressmediierte Veränderung ausgeschlossen werden. Den Synapsinen Ia und IIa konnten dadurch eindeutig eine Rolle im Mechanismus der Entwicklung eines räumlichen Gedächtnisses zugesprochen werden.

Keywords: Cognitive enhancer, Phosphorylation, Proteomics, spatial memory, Synapsin

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1 Introduction

1.1 Mass Spectrometric Studies on Mouse Hippocampal Synapsins

Protein isoforms may originate from alternative splicing of mRNA, single-point mutations, and post-translational modifications including proteolytic cleavages. These modifications often introduce a variation in the molecular mass and net charge of the protein. The most efficient technique to separate protein isoforms thus remains 2-Dimensioanl Gel Electrophoresis (2-DE) The identification and localization of the modification can then be obtained using MS/MS analyses of the peptide bearing the modification after proteolysis of the protein. Depending on the nature of the modification to be identified, different strategies to detect the modified peptide can be employed. The choice of this strategy would be facilitated if the molecular mass of the intact protein isoform were known. Indeed, the mass difference between two isoforms or between the isoform and the expected theoretical molecular mass would allow drawing a solid hypothesis on the nature of the protein modification. Here we shown application 2-DE combined with various proteomics techniques to study the molecular heterogenicity of synapsin isoform and their post translational modification.
Synapsins are a family of phosphoproteins specially associated with the cytoplasmic surface of the synaptic vesicle membrane (De Camilli, Cameron et al. 1983; De Camilli, Harris et al. 1983; Huttner, Schiebler et al. 1983). Synapsins are expressed in vertebrates and three genes (synapsin 1, 2 and 3) are observed in mammals (De Camilli, Benfenati et al. 1990; Esser, Wang et al. 1998; Hosaka and Sudhof 1998; Hosaka and Sudhof 1998; Kao, Porton et al. 1998). In vertebrates, synapsins constitute a family of at least five homologous proteins (synapsin Ia, Ib, IIa, IIb, IIIa); collectively referred to as synapsin I and II that are derived by alternative splicing from the primary transcript gene (Sudhof 1989; Sudhof, Baumert et al. 1989; Sudhof, Czernik et al. 1989; Sudhof 1990). All the synapsin isoforms exhibit a high degree of sequence homology in their amino terminal regions (the short A- and B-domains and long C-domain) and divergent carboxy-terminals that are present in different combinations in each of the synapsins (De Camilli, Cameron (De Camilli, Cameron et al. 1983) et al. 1983; De Camilli). The small carboxy-terminal specific to b-type are present in synapsin isoforms (domain F) (Hilfiker, Pieribone et al. 1999). Synapsins are representing one of the abundant families of synaptic proteins, comprising approximately one percent of the total proteins in the brain (Ueda and Greengard

1977; Goelz, Nestler et al. 1981; Browning, Huang et al. 1987). The functional behavior of the synapsins is affected by their phosphorylation states, which are regulated by a variety of physiological and pharmacological messengers that efficiency modifies synaptic function (Nestler and Greengard 1983). Synapsins are major substrates for multiple protein kinases, including cAMP-dependent protein kinase (PKA), Ca2+/calmodulin protein kinases (CaM Kinase) I, II and IV, and mitogen-associated protein kinase/Erk 1/2 (MAPK/Erk). The presence of phosphorylation sites varies from one synapsin isoform to another (Greengard, Valtorta et al. 1993; Jovanovic, Czernik et al. 2000; Sakurada, Kato et al. 2002). All the synapsins are sharing consensus sequences for cAMP-dependent protein kinase and CaM Kinase I, which phosphorylate a serine residue in the amino terminal region (site 1). Synapsin I is an excellent substrate for CaM Kinase II, which phosphorylates two serine residues in the carboxy terminal (site 2 and site 3) (De Camilli, Benfenati et al. 1990). The biological properties of synapsins have been shown by their ability to interact with the synaptic vesicle and the cytoskeleton, actin (Schiebler, Jahn et al. 1986; Greengard, Browning et al. 1987; Benfenati, Valtorta et al. 1992; Fesce, Benfenati et al. 1992; Valtorta, Greengard et al. 1992). Several reports indicated that synapsins are required to sustain neurotransmitter release during high levels of neuronal activity (Li, Rosahl et al. 1995; Pieribone, Shupliakov et al. 1995; Rosahl, Spillane et al. 1995; Takei, Harada et al. 1995; Hilfiker, Schweizer et al. 1998; Chi, Greengard et al. 2001; Humeau, Doussau et al. 2001; Gitler, Xu et al. 2004). This indicates that synapsins are participating in neurotransmitter release by regulating a reserve pool of vesicles. Moreover, synapsins are also involved in regulation of kinetics of release processes (Hilfiker, Schweizer et al. 1998). Synapsin II is involved in synapses formation during nerve cell growth (Han, Nichols et al. 1991; Ferreira, Kosik et al. 1994; Ferreira, Han et al. 1995). Phosphosphorylation of Synapsin II regulates binding of both, synaptic vesicle and actin filaments (Greengard, Valtorta et al. 1993),

In knock out mice analysis it was shown that synapsins are involved in maintaining stable vesicles and required for normal short term plasticity, suggesting that they constitute important regulatory molecules (Rosahl, Spillane et al. 1995) (Rosahl, Geppert et al. 1993) (Ryan, Li et al. 1996). Recently, contradictory to the above finding, it was shown that synapsins are not required for normal vesicle exocytosis and recycling (Ryan, Li et al. 1996). Synapsins regulate synaptic plasticity by a

Ca2+/dependent pathway in the calyx of Held synapse(Sun, Bronk et al. 2006). Recent knock out mice analysis showed that domain E of synapsin accelerates the exo and endocytotic cycle of synaptic vesicles in cerebellar Purkinje cells (Li, Hornshaw et al. 2004).

Synapsin isoforms exhibit significant change of heterogenicity in their protein expressional state. Zhang and co workers observed the presence of different synapsin splice variants in 2-D gels, resolved by different pI and molecular weight (Zhang, Czernik et al. 1994). The nature of many post translational modification associated with synapsin function has been reported. There are several phosphorylation sites confirmed in synapsin I by mass spectrometry analysis in mammals (Matsubara, Kusubata et al. 1996; Maucuer, Le Caer et al. 2000; Wells, Vosseller et al. 2002; McLachlin and Chait 2003; DeGiorgis, Jaffe et al. 2005). In addition to that, a number of O-linked N-acetylglucosamine (O-GlcNAc), modification sites in synapsin I were identified (Luthi, Haltiwanger et al. 1991; Wells, Vosseller et al. 2002; Murrey, Gama et al. 2006). Murrey et al. reported that fucosylation modulates synapsin Ia and Ib expression using synaptic vesicle purification, immunoblots and gel electrophoresis combined with mass spectrometry analysis (Murrey, Gama et al. 2006; Zhu, Doyle et al. 2006). Knowledge of PTMs in Synapsin IIa and IIb are very limited, due to the problems associated with protein purification. Zhu et al reported that Synapsin I and Synapsin II are substrates for protein L-isoaspartyl methyltransferase in mouse brain using a proteomic approach (Zhu, Doyle et al. 2006). A recent global phosphoproteomic study on mouse synaptosomal preparations indicated that synapsin II is phosphorylated at T-422 and S-42646, however the subtype of synapsin II was not defined because in their setting the authors did not identify the isoforms but were rather focusing on identification of phosphopeptides exclusively (Munton, Tweedie-Cullen et al. 2007). Herein we decided to carry out studies on phosphorylation of synapsins qualitative in nature, with and without phosphophorylation enrichment of phosphopeptides by IMAC taken from 2-DE spots, combined with mandatory multiple mass spectrometry analysis thus using an appropriate method to determine PTMs on individual synapsin isoforms.

To our knowledge, no reports on heterogenicity of synapsin isoforms in mouse hippocampus are available and the issue of identifying several isoforms on a 2-DE gel is a major challenge. The aim of this study was to investigate synapsin isoforms

with regard to changes of molecular heterogenicity using 2-DE followed by mass spectrometry. Combination of high-resolution 2-DE with two different mass spectrometry principles (MS) is a reliable method to determine synapsin isoform modifications of sequences and post-translational modifications. Characterisation of synapsin isoforms by MALDI-TOF-TOF following multi-enzyme cleavage of synapsins from mouse hippocampus revealed 15 synapsin expression forms and nano-ESI-LC-MS/MS following enrichment of phosphopeptides identified phosphorylation sites that may form the basis and tools required for future studies on synapsins at the protein level.

1.2 Hippocampal synapsin isoforms levels are linked to cognitive enhancement of memory formation in the mouse

Synapsins are among the most abundant proteins of the presynaptic structure and interacting with synaptic vesicles (Fdez and Hilfiker 2006) (Greengard, Benfenati et al. 1994) (Fdez and Hilfiker 2006). Alternative splicing results into four primary transcripts, synapsins Ia, Ib, IIa and IIb (Cheetham, Hilfiker et al. 2001); (Fdez and Hilfiker 2006); (Hilfiker, Benfenati et al. 2005) (Hilfiker, Pieribone et al. 1999); (Jensen, Walaas et al. 2007) (Hilfiker, Schweizer et al. 1998; Kao, Porton et al. 1999); (Walaas, Hilfiker et al. 2000); (Cheetham, Hilfiker et al. 2001; Fdez and Hilfiker 2006) but 15 well-separated spots identified as synapsins were observed and unambiguously identified by a gel-based proteomic mass spectrometry method (John, Chen et al. 2007)). Synapsins are major substrates for multiple protein kinases, including PKA, CaM kinases I, II and IV, and mitogen-associated protein kinase/Erk 1/2 (MAPK/Erk) (Sudhof 1989); (Jovanovic, Benfenati et al. 1996) Synapsin I and Synapsin II are involved in both, synaptogenesis and plasticity of mature synapses (Chin, Li et al. 1995) (Li, Chin et al. 1995) by controlling synaptic vesicle trafficking at pre- and post-docking levels (Bonanomi, 2005 (Bonanomi, Menegon et al. 2005) (Chi, Greengard et al. 2001) (Chi, Greengard et al. 2003) (Hilfiker, Benfenati et al. 2005) (Hilfiker, Schweizer et al. 1998) (Humeau, Doussau et al. 2001) (Menegon, Bonanomi et al. 2006) (Hilfiker, Schweizer et al. 1998) (Chi, Greengard et al. 2001) (Humeau, Doussau et al. 2001) (Chi, Greengard et al. 2003) (Bonanomi, Menegon et al. 2005) (Hilfiker, Benfenati et al. 2005) (Menegon, Bonanomi et al. 2006). Although Spillane and coworkers (Spillane, Rosahl et al. 1995) originally did not find long-term potentiation (LTP) differences between wild type and knockout mice for both,

synapsin I and II, a clear role for synapsins could be established: mice lacking synapsin II and mice defective in both synapsin I and II showed normal paired-pulse facilitation but lower post-tetanic potentiation (Silva, Rosahl et al. 1996) (Silva, Rosahl et al. 1996). Corradi and coworkers recently showed that both SynI-/- and SynII-/- mice exhibit decreased numbers of synaptic vesicles and synaptic depression upon high-frequency stimulation showing that synapsins have a specific and non-redundant function (Corradi, Zanardi et al. 2008).
Sato and coworkers (Sato, Morimoto et al. 2000) showed that immunoreactivity for synapsin I increased during LTP; likewise, Gomez-Pinilla et al, (Gomez-Pinilla, So et al. 2001) revealed that spatial learning in the Morris water maze (MWM) induced synapsin I mRNA and protein. Michels et al, (Michels, Diegelmann et al. 2005) assigned a role for synapsin (Drosophila has a single gene for synapsin) in associative (olfactory) learning in Drosophila. Kushner and coworkers finally detected that hippocampus-dependent learning stimulated the ERK-dependent phosphorylation of synapsin I and deletion of synapsin I blocked enhancements of learning, presynaptic plasticity and LTP (Kushner, Elgersma et al. 2005).

It was therefore the aim of the study to link hippocampal levels of synapsin and its splice variants to cognitive enhancement in OF1 mice, known to respond to CE by SGS742 (Sunyer, Patil et al. 2008). Using unambiguous mass spectrometric identification of synapsin isoforms along with stable isotope quantification of synapsin proteins. Stringent statistical analysis and the use of sodium chloride-treated mice and drug-treated yoked controls were applied to generate robust data.

2 Objective and definition of the research topic

The overall objective of the PhD thesis was to study and characterize the synapsins in both structural and biological way which could be used to elucidate the role of synapsins isoform during learning and memory formation in the mouse. This was accomplished by application various proteomics techniques including functional proteomics approach, cognitive enhancement and mouse behavioural testing. These methods enabled characterization of hippocampal synapsins role in spatial memory formation.

2.1 Objective of the research topic

2.1.1 Structural study;

Address the Molecular heterogenicity of synapsins

Verify the primary sequence information of synapsin isoforms.

Identify the sites of Post Translational Modifications (PTMs) in synapsins and its splice variants.

2.1.2 Biological Study;

To link hippocampal levels of synapsin and its splice variants to cognitive enhancement in OF1 mice, known to respond to cognitive enhancer by SGS74

2.2 Definition of the research topic

The molecular mechanism of the regulation of transmitter release by synapsins is currently unclear and available literatures are contradictory with each other when assigning the exact function. No reports are available to address the molecular heterogenicity of synapsin protein spots in 2-DE gel. Protein chemical characterization of synapsin II isoforms have not been studied as extensively as those of synapsin I. Studies with animals have shown that an intact hippocampus is required for spatial memory formation Therefore, understanding the hippocampal synapsins molecular heterogenicity and their post translational modification is of important in neurobiological study. This study presents the detailed mapping of protein sequence of synapsin isoforms and their post translational modification by tandem mass spectrometry. There is supportive evidence that hippocampal-dependent spatial cognition can be improved by administration of a GABA (B) receptor antagonist i.e. SGS742 prior to behavioural testing. Using the knowledge of primary structural information of synapsins, this study establishes a functional quantitative approach to propose a role of synapsins in spatial memory enhancement in the OF-1 mouse by GABAB antagonist SGS742.

3 Materials and methods

3.1 Animal Experiment

A total of 40 male OF1 mice, 10-12 weeks old, were used for the experiments. Mice were bred and maintained in cages made of Makrolon and filled with autoclaved woodchips in the Core unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna.

Behavioural tests were performed between 8 a.m. and 1 p.m. by the same experimenter. During the experiments four animals per cage were kept. Experiments were done under licence of the federal ministry of education, science and culture, which includes an ethical evaluation of the project (Project: BMBWK-66.009/0036/BrGT-2006). Housing and maintenance of animals were in compliance with European and national regulations.

Animals (10 per group, total n=40) used for gel-based studies were from MWM studies published recently in this journal (Sunyer, Patil et al. 2008). Animals were treated *i.p*. 40 min before daily testing with SGS742 or with saline solution (0.9% NaCl) as described previously (Sunyer, Shim et al. 2008).

Mice were divided into four different groups. Two groups (SGS742-treated group and NaCl-treated group) were trained in the MWM to find the hidden platform. The dose and time of injection was chosen based upon literature (Froestl, Gallagher et al. 2004).

The other two groups, called yoked controls (non-learning group), were placed in the water maze to swim the same amount of time as their trained partners, but without a platform being present to climb onto. Animals were exposed to the same spatial cues, but without an escape platform, mice did not develop an association between the extra-maze cues and the location of the platform (Sunyer, Patil et al. 2008).

Yoked controls were treated with SGS742 or with saline solution (0.9% NaCl).

SGS742 was kindly supplied by Lundbeck Research Denmark (Ottiliavej 9DK-2500 Copenhagen-Valby, Denmark). The drug solution was freshly prepared every day.

The spatial acquisition phase consisted of four training trials per day (inter-trial interval: 10 min) and four training days.

On the first training day, mice were given an acclimatization training session in the water maze as described before by Sunyer et al. (Sunyer, Patil et al. 2007). Mice were released randomly with their heads facing the pool wall from the four compass locations (NE, NW, SW, and SE), and allowed to swim and search for the hidden platform for 120 s. If the mouse did not reach the platform within 120 s, it was guided to the platform. The time to reach the platform was recorded as the training latency for each trial.

On day five, a single probe trial, in which the platform was removed, was performed. Each mouse was released into the pool after removing the platform and was allowed to swim for 60 s. Time spent in the target quadrant and adjacent quadrants was recorded (Kipnis, Cohen et al. 2004) (Sunyer, Patil et al. 2007).

Six hours after MWM experiments, mice were killed by neck dislocation and hippocampi were dissected. Tissue samples were immediately frozen in liquid nitrogen and stored at −80°C until used for analysis.

All efforts were made to minimise animal suffering and the number of animals used.

3.2 Protein studies

3.2.1 Sample preparation

Hippocampal tissue was powderized and resuspended in 1.0 ml of sample buffer consisting of 7 M urea (Merck, Darmstadt, Germany), 2 M thiourea (Sigma, St. Louis, MO, USA), 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate (CHAPS) (Kelloff and Sigman), 65 mM 1,4-dithioerythritol (Merck, Germany), 1 mM ethylenediaminetraacetic acid (EDTA) (Merck), protease inhibitors complete® (Roche Diagnostics, Basel, Switzerland) and 1 mM phenylmethylsulfonyl chloride. The suspension was sonicated for approximately 15 s. After homogenisation samples were left at 21°C for 1 h and centrifuged at 14,000 x g for 1 h. The supernatant was transferred into Ultrafree-4 centrifugal filter units (Millipore, Bedford, MA), for desalting and concentrating proteins (Weitzdorfer, Hoger et al. 2006). Protein content of the supernatant was determined by the Bradford protein assay system (Bradford

1976) . The standard curve was generated using bovine serum albumin and absorbance was measured at 595 nm.

3.2.2 Two-dimensional gel electrophoresis

Protein extracts prepared were subjected to 2-DE as described elsewhere (Weitzdorfer, Hoger et al. 2008) 700µg of protein were applied on immobilized pH 3–10 nonlinear gradient strips at their basic and acidic ends. Focusing was started at 200 V and voltage was gradually increased to 8000 V over 31 h and then kept constant for a further 3 h (approximately 150,000 V h totally). After the first dimension, strips (18 cm) were equilibrated for 15 min in the buffer containing 6 M urea, 20% glycerol, 2% SDS, 2% DTT and then for 15 min in the same buffer containing 2.5% iodo-acetamide instead of DTT. After equilibration, strips were loaded on 9–16% gradient sodium dodecylsulfate polyacrylamide gels for second-dimensional separation. Gels (180 mm × 200 mm ×1.5 mm) were run at 40 mA per gel. Immediately after the second dimension run, gels were fixed for 18 h in 50% methanol, containing 10% acetic acid, the gels were then stained with Colloidal Coomassie Blue (Novex, San Diego, CA) for 12 h on a rocking shaker. Molecular masses were determined by running standard protein markers (Biorad Laboratories, Hercules, CA) covering the range 10–250 kDa. pI values 3–10 were used as given by the supplier of the immobilized pH gradient strips (Amersham Bioscience, Uppsala, Sweden). Excess of dye was washed out from the gels with distilled water and the gels were scanned with ImageScanner (Amersham Bioscience). Electronic images of the gels were recorded using Adobe Photoshop and Microsoft Power Point Software.

3.2.3 Protein identification by MALDI-TOF/TOF

Extracted peptides were directly applied onto a target (AnchorChipTM, Bruker Daltonics, Bremen, Germany) that was loaded with α-cyano-4-hydroxy-cinnamic acid

(Bruker Daltonics) matrix thinlayer (saturated solution in 100% acetone with 0.1% TFA). The mass spectrometer used in this work was an UltraflexTM TOF/TOF (Bruker Daltonics) operated in positive-ion reflector mode for peptide mass analysis, and the “LIFT” mode was used for tandem mass spectrometry sequencing of peptides using the FlexControlTM 2.4 software (Bruker Daltonics). An accelerating voltage of 25 kV was used for PMF. Calibration of the instrument was performed externally with [M+H]+- ions of angiotensin I, angiotensin II, substance P, bombesin, and adrenocorticotropic hormones (clip 1–17 and clip 18–39). Each spectrum was produced by accumulating data from 200 consecutive laser shots for PMF. Those samples which were analyzed by PMF from MALDI-TOF were additionally analyzed using LIFT-TOF-TOF MS/MS from the same target using LID mode50. In the LID-MS/MS mode using a long-lifetime N2 laser, all ions were accelerated to 8 kV under conditions promoting metastable fragmentation in the TOF1 stage. After selection of jointly migrating parent and fragment ions in a timed ion gate, ions were lifted by 19 kV to high potential energy in the LIFT cell. After further acceleration of the fragment ions in the second ion source, their masses could be simultaneously analyzed in the reflector with high sensitivity. Mass spectra were analyzed using the Flex Analysis 2.4 software. PMF and MS/MS datasets were interpreted with MASCOT (Matrix Science Ltd, London, UK) software searched against MSDB 20051115 database (ftp://ftp.ncbi.nih.gov/repository/MSDB/) via BioTools 2.2® software (Bruker Daltonics). A mass tolerance of 25 ppm and MS/MS tolerance of 0.5 Da and one missing cleavage site were allowed. The probability score calculated by the software was used as criterion for correct identification. Oxidation of methionine, deamidation of Asn and Gln residues were set as variable modifications, carbamidomethylation of cysteine residues as fixed modification. Mascot results were confirmed manually. Unmatched MS/MS spectra were further analysed by de novo sequencing analysis.

MS/MS spectra were sequenced de novo using BioTool 2.2 software with RapiDeNovo® extension (Bruker Daltonics50) and the top high scoring candidate sequences for MS/MS spectra were then submitted to MS BLAST sequence similarity search (http://dove.embl-heidelberg.de/Blast2/msblast.html), which was based on the most likely de novo sequences. Candidate sequences were edited according to MS BLAST conventions and submitted to MS BLAST searches were performed against nr database at http://genetics.bwh.harvard.edu/msblast/ under the following settings: Scoring table, 99; Filter, none; Expect, 1000. Statistical significance of hits was evaluated according to MS BLAST scoring scheme(Shevchenko, Sunyaev et al. 2001).

3.2.4 Protein Identification by LTQ-FT.

The peptides were separated on an UltiMate 3000 dual nano-LC system (Dionex).
After being desalted for 18 min on a precolumn (PepMAP C18, 0.3 × 5 mm, Dionex) (0.1% TFA, 20 µL/min), the peptides wereeluted with a gradient from 0% to 50% B in 90 min and a flowrate of 300 nL/min onto an analytical column (PepMAP *C18,75 × mµ 150 mm,* Dionex) (solvent A, 5% ACN, 0.1% formicacid; solvent B, 80% ACN, 0.08% formic acid). The nano-HPLC system was coupled online to a hybrid linear ion trap/FT-ICR mass spectrometer (LTQ-FT, Thermo Electron, Bremen, Germany)
with a 7 T superconducting magnet via a nano electrospray ionization source (Proxeon Biosystems, Odense, Denmark).The two detectors of the hybrid instrument wereoperated in parallel mode, during one full scan in the FT-ICRcell (resolution 100000,m/z 300-1800). The five most abundantions were subjected to MS/MS and neutral loss triggered MS3analysis in the linear ion trap. Fragmented precursors were

excluded from further fragmentation for 60 s (with 5 ppm massaccuracy), and singly charged peptides were generally excludedfrom MS/MS analysis. Data analysis was performed using theMASCOT (version 2.1.0) software, searching against the NCBI nucleic acid database.

3.2.5 In-Gel Stable-Isotope Labeling and Proteolytic Digestions

Synthesis of N-acetoxy- (1H3) succinimide and N-acetoxy (2H3) succinimide for stable isotope labeling

N-hydroxysuccinimide (Sigma-Aldrich), acetic anhydride (>99% purity, Sigma-Aldrich), and D6-acetic anhydride (>99% purity, Sigma-Aldrich) were used as purchased. N-acetoxy- (1H3) succinimide (light) and N-acetoxy (2H3) succinimide (heavy) were synthetized as described previously (Asara, Zhang et al. 2006). Briefly, 4 g of N-hydroxysuccinimide were mixed with either 19.8 mL of acetic anhydride or 18.6 mL of D6-acetic anhydride. Mixtures were stirred and allowed to react for overnight at 21°C. White crystal products were washed with hexane and dried in vacuum.

Synapsin spots from 2-DE gels were excised using a scalpel to ensure that identical gel volumes were used for both light and heavy isotope labeling reactions. Gel pieces were washed with 50% acetonitrile and dried in a SpeedVac concentrator. Cysteine residues of proteins were reduced with 10 µL of 10 mM DTT at 56 °C for 30 min and alkylated with 10 µL of 55 mM iodoacetamide for 45 min at 21°C. Gel pieces were then washed two times with 150 µL of 50% acetonitrile and dried in a SpeedVac. Approximately 6 mg of light N-acetoxy- (1H3) succinimide were added to dried gel pieces of NaCl treated control sample or NaCl treated yoked control and 6 mg of heavy N-acetoxy (2H3) succinimide were added to dried gel pieces of SGS742-treated samples or SGS742- treated yoked controls. 50 µL of 50 mM HEPES (pH 8.3) were added to each gel piece, vortexed for 1 min to dissolve and subsequently spun

down in a microcentrifuge. Reactions were allowed to incubate for 3 h at 21°C. The gel pieces were washed with 170 µL of 100 mM ammonium bicarbonate followed by 20 µL of 50% hydroxylamine solution for 20 min and incubated at 21°C. Light and heavy labeled gel slices were then washed three times with 150 µL of 100 mM ammonium bicarbonate and two times with 50 µL of ACN prior to drying in a SpeedVac. 12 microliters of 40 ng/µL modified trypsin (sequencing grade; Roche Diagnostic, Basel, Switzerland) was added to the combined gel pieces and placed on ice for 15 min to swell the gel pieces with enzyme solution. Chymotrypsin digestions were performed by addition of 25 mM ammonium bicarbonate containing 25 ng/µL chymotrypsin (sequencing grade; Roche Diagnostic, Basel, Switzerland) and incubated for 2.5 h at 30°C. An additional 45 µL of 50 mM ammonium bicarbonate (pH 8.4) was added and incubated at 37 °C overnight. Peptides were extracted with 35 µL of 20 mM ammonium bicarbonate at 37°C for 15 min, followed by 70 µL of 2% FA and 40% ACN at 37 °C for 15 min, and dried in a SpeedVac to a final volume of 10 µL.

3.2.6 Sample preparation for subtilisin digestion

Subtilisin (Proteinase from Bacillus subtilis var. biotecus A; Fluka) was dissolved at a concentration of 10 µg/µL in 1mM HCl and diluted with 6 M urea and 1 M Tris pH 8.5 at a final concentration of 100 ng/µL. Gel pieces were covered with this concentration and rehydrated for ten minutes at 4º C. Supernatant was removed and replaced by 50 mM ammonium bicarbonate. Digestion was performed for 1 h at 37º C on a shaker. Reaction was stopped by addition of 10% FA to a final concentration of ~1%. After 10 minutes of sonication the supernatant was taken off and peptides were further extracted by adding approximately 20 µL of 5% FA followed by 10 min sonication (procedure was repeated once).

3.2.7 Synapsin identification and quantification by nano-LC-ESI-MS/MS

For synapsin identification and quantification a 2 µL aliquot of tryptic and chymotryptic digested peptides were separated by Ultimate 3000 nano-LC system (Dionex, Amsterdam, Netherlands) and analyzed –both, data-dependent and data-independent , using a QSTAR XL (Applied Biosystems, Foster City, CA) equipped with a nano electrospray ionization source(Chen, Kang et al. 2006). For nano-LC-ESI-MS/MS, the digest was loaded onto PepMap 100 C18 precolumn (300 µm i.d., 5mm long cartridge, from Dionex, Amsterdam, Netherlands) from 0 min to 30 min and then separated by PepMap 100 C18 analytic column (75µ m i.d. 150mm long cartridge, from Dionex, Amsterdam, Netherlands) using a linear gradient of 4% B (Solvent A: 0.1% FA; Solvent B: 80% ACN/0.08% FA) to 60% from 0 min to 30 min, 90% B constant from 30 min 35%, and 4% B from 35 min to 60 min using the Ultimate micropump at a flow rate of 300 nL/min. As peptides eluted from LC, they were electrosprayed into QSTAR XL. Each cycle consisted of one full scan mass spectrum (m/z 350–1600) followed by MS/MS spectra on the three most abundant peptide ions in the full MS scan. The derived mass spectrometry datasets were converted to MASCOT generic format flat files by macot.dll 1.6b21 (Matrix Science, Boston, MA) script supplied with AnalystQS® 1.1 software (Applied Biosystems) and searched against in house licensed MSDB 20051115 and UniProtKB databases with the following differential modifications for lysine residues: unmodified, modified with light reagent (+42.01 on K), and modified with heavy reagent (+45.01 on K) to be sure that all peptides selected for MS/MS analysis could be identified. Abundance ratios of labeled peptides were determined manually using matched peptide information, including m/z value, charge state and retention time from the data

dependent identification run to data independent run. Relative quantification of proteins was obtained by averaging the intensity ratios of multiple derived unique peptides.

For protein identification Oxidation of methionine and deamidation of Gln and Asn were set as variable modifications, carbamidomethylation of cysteine residues as fixed modification.

3.2.8 IMAC Enrichment Using Pierce Phosphopeptide Isolation Kit

Gallium-chelated columns were used according to the manufacture's recommendation with the following modification. Extracted peptides were evaporated by SpeedVac and suspended in 20 µL of 5% acetic acid as binding buffer, the tryptic peptides were transferred to Pierce columns, left for 30 min at room temperature and centrifuged for 1 min at 3000 ×g. The Gallium chelated disc was rinsed once with 50 µL 0.1% (v/v) acetic acid, twice with 50 µL of 100 mM NaCl in 1% acetic acid, twice with 30% (v/v) ACN in 1% acetic acid, and once with water. Phosphopeptides were then eluted by three additions of 20 µL of 100 mM ammonium bicarbonate. Eluates were pooled and evaporated by SpeedVac, and phosphopeptides were suspended in 20 µL of 2% acetonitrile containing 0.1% TFA and the volume was reduced to 5 µL before mass spectrometric analysis.

3.2.9 Phosphatase Treatment

Tryptic digested synapsin IIa samples were subjected to phosphatase treatment. Peptides sample were incubated in a solution of 0.5 µl of Calf intestine alkaline phosphatase (New England Biolabs, Ipswich, MA) in the presence of 100 mM ammonium bicarbonate for 1 h at 37°C. Samples were subsequently analysed by MALDI mass spectrometry.

3.3 Bioinformatics analysis

Protein sequences derived from NCBI protein database were used for protein sequence analysis. ClustalW (http://www.ebi.ac.uk/clustalw/) was used for multiple sequence alignment searches. ELM (http://elm.eu.org) was used for functional domain identification.

3.4 Statistical analysis

Statistical analysis to show group difference between treated and yoked controls were done by using unpaired Student's t-test (Sunyer, 2007 #2; Sunyer, 2007 #6735).

4 Results

4.1 Analysis of synapsin splicing isoform

Alternative splicing of synapsin genes encodes five known synapsins isoforms: Ia, Ib, IIa, IIb and IIIa. The differences in the amino acid sequence among these five splicing isoforms are shown in fig 1. By protein profiling of mouse hippocampal tissue, 15 individual protein spots identified as synapsin I and synapsin II with different molecular weight and pI were observed (fig 2). To determine the sequence of synapsin isoforms and post-translational modifications, a mass spectrometry approach was carried out. Protein spots were excised and digested individually with trypsin, Lys-C and Asp-N and analyzed with MALDI-TOF-TOF, nano-ESI-LC/MS/MS and LTQ-FT.

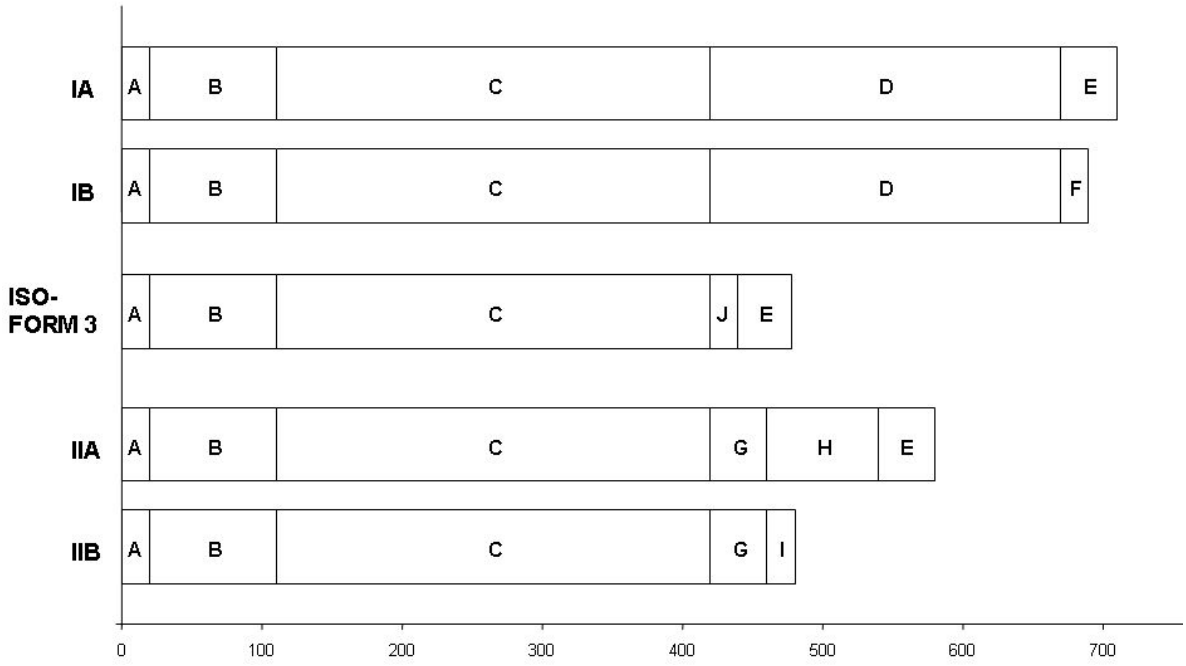


Figure 1: The individual and shared domains of the five isoforms are composed of domains A-C. In the COOH terminal variable region, the proline- and glycine-rich D domain is shared by synapsins Ia and Ib, the proline-rich G domain is shared by synapsins IIa and II b, and the COOH-terminal domain E is present in synapsin Ia and synapsin IIa and Isoform III.

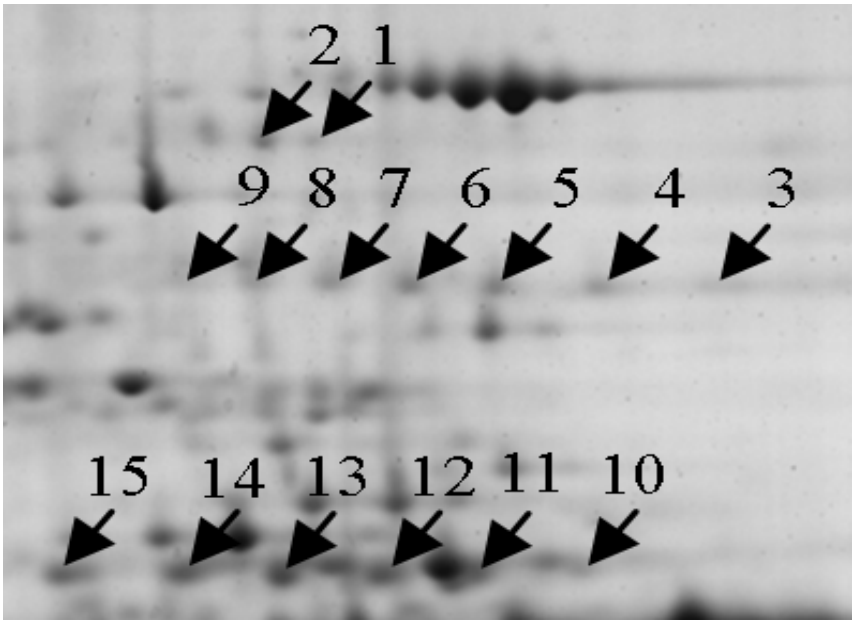


Figure 2: Proteins from mouse hippocampus were subjected to isoelectrofocusing on a 18 cm immobilized pH 3–10 gradient strip and then separated by SDS-PAGE. A 2D gel and partial image of a Coomassie Blue-stained gel image are shown. Synapsin protein spots (marked with arrows) are identified and characterized by mass spectrometry.

4.1.1 Structure of the Synapsins.

The mass spectrometric data of each splicing isoform were compared to identify each synapsin isoforms. Synapsin Ia was identified in spot 1 and 2 (fig 2, table 1). The PMF map indicated that the tryptic peptide SQSLTNAFNLPEPAPPRPSLSQDEVK (amino acid residues 664– 689), which covered the splicing site in synapsin Ia, has an [M+H]+ at *m*/*z* 2822.38 and was identified as its deamidated form in the spot 2 (fig 2, table 2). Nano-ESI-LC/MS/MS analysis of Lys-C digested peptides indicated that peptide $[M+2H]^{2+}$ at *m*/*z* 952.36 SFASLFSD (amino acid residue 699 – 776), which covered the splicing site in synapsin Ia was identified in spot 1 (table 2). MALDI-TOF-TOF analysis of the tryptic peptide SQSLTNAFSFSESSFFR (amino acid residues 544 – 560) which covered the splicing site of synapsin IIa has an [M+H] + at m/z

1941.490 was identified in spot 3-spot 9 (fig 2, fig 7 [upper panel], table 2). Moreover, nano-ESI-LC-MS/MS analysis of tryptic peptides indicated that the C-terminal of the synapsin IIa isoform was identified in all the synapsin IIa protein spots. Nano-ESI-LC-MS/MS and LTQ-FT analysis of Asp-N digested peptides indicated that the peptide [M+2H]2+ at *m/z* 772.87; DCNGIAVGPKQVQAS (amino acid number 465 – 479), covering the splicing site of synapsin IIb, was identified in spots 10-15 with a varying degree of deamidated forms in the individual protein spots (fig 2, table 2).

Data obtained from mass spectrometry indicated that an average of 69% sequence coverage was obtained in both synapsin Ia protein spots (spot 1 and 2), In the case of synapsin IIa (spots 3-9) and synapsin IIb (spot10-15) an average of 78% and 77% was identified with both, the MS and MS/MS approach. In particular, 94% sequence coverage was achieved in spot 12 alone, among this 88% of sequence determined by MS/MS analysis (table 1).

Primary structure of synapsin IIa contains long stretches of 13 serine residues (486-499); neither multiproteolytic nor mass spectrometry approaches could cover this region. To address this ambiguity spot 4 and spot 5 were excised and digested with non-specific enzyme subtilisin and analyzed with nano-ESI-LC/MS/MS analysis and subsequent search analysis with MASCOT identified the peptide at [M+2H] 2+ *m*/*z* 505.82 RLPSGPSLPS (amino acid number 477 – 486). Moreover two peptide sequences corresponding to the sequential removal next two serine residues were identified (477-RLPSGPSLPSS-487, 477-RLPSGPSLPSSS-488) in their [M+2H]2+ state (Supplementary table 1).

4.1.2 Characterization of synapsin isoforms migration differences in 2-D gel

In the 2-DE map of synapsins expression forms (fig 2) many spots had a measurably different pI, but the same molecular weight (Mr); for example, spots 1-2, 3-9 and spots 10-15. Combination of different mass spectrometry and multiproteolytic digest approaches demonstrated that a series of deamidations of glutamine and asparagine residues were identified in the individual protein spots. Due to deamidation, “trains” of spots are proposed to appear in the 2-DE gel (Anderson and Anderson 1991; Sarioglu, Lottspeich et al. 2000). Individual spots digested with different enzymes and analyzed by MALDI-TOF-TOF, nano-ESI-LC/MS/MS and LTQ-FT analyses showed that number of deamidated sites in peptides representing individual protein spots varying from one to another and in some spots the same number of deamidated sites were observed, probably because of relatively low sequence coverage of some individual protein spots (sup table 2, 3 and 4). MS/MS analysis of the individual spots confirmed the exact deamidation site, for example, the MS/MS spectrum at *m*/*z* 3498.716 corresponding to the deamidated glutamine peptide sequence RPPPAQAPAP**Q**PAPQPAPTPSVGSSFFSSLSQAVK (residues 58 -92) (“bold” indicates amino acids that are modified with deamidation), indicated that mass difference between b10 (*m*/*z* 983.335) and b11 (*m*/*z* 1112.166) exactly corresponds to the deamidated glutamine mass (129.04) at amino acid number 69 (fig 3). But not in all the protein spots, a deamidated protein site was observed.

Table 1 - Characterization of Synapsin Isoforms

Spot No.	Protein Name	Exp.pI; M_r	Theor pI; M_r	Average normalized spot volume (Means ± s.d.)	Sequence Coverage		PTMs
					Total	MS/MS	
1	Synapsin Ia	7.86;82,140	9.81; 74097.31	0.256±0.042	62%	39%	Pyro Glu-Q404; Ox-W126(S)
2	Synapsin Ia	7.86; 82,140	9.81; 74097.31	0.233±0.019	44%	13%	P-S9[a], P-S62[a]; Pyro Glu-Q404
3	Synapsin IIa	9; 72,100	8.59; 63372.55	0.171±0.048	61%	38%	Ac-M1[a], Ac-M2[a]
4	Synapsin IIa	8.72; 72,100	8.59; 63372.56	0.216±0.083	70%	45%	Ac-M1[a], Ac-M2[a];P-S10[a]; P-S546[†]; Me-E148
5	Synapsin IIa	8.16; 72,100	8.59; 63372.57	0.306±0.094	71%	44%	Ac-M1, Ac-M2[a]; P-S10[a]; P-S546[†], P-T422, P-S426; Me-E249 Pyro glu-Q94; Ox-W127, Ox-W336
6	Synapsin IIa	7.93; 72,100	8.59; 63372.58	0.283±0.084	75%	45%	Ac-M1, Ac-M2[a]; P-S10[a], P-S546[†]; Pyro glu-Q94; Me-E249
7	Synapsin IIa	7.66; 72,100	8.59; 63372.59	0.238±0.056	78%	41%	Ac-M1[a], Ac-M2[a]; Ox-W127(S); P-S10[a], P-S546[†]
8	Synapsin IIa	7.3; 72,100	8.59; 63372.60	0.233±0.089	66%	43%	P-S546[†]
9	Synapsin IIa	7.1; 72,100	8.59; 63372.61	0.182±0.081	65%	49%	P-S546[†]
10	Synapsin IIb	8.53; 52,720	7.61; 52451.93	0.136±0.029	79%	42%	Ac-M1[a], Ac-M2[a]; Me-E148, Me-E402
11	Synapsin IIb	8.16; 52,720	7.61; 52451.94	0.281±0.078	85%	70%	Ac-M1, Ac-M2[a]; P-S10[a]; Ox-W127(D), Ox-W236(D)
12	Synapsin IIb	7.86; 52,720	7.61; 52451.95	0.345±0.050	94%	88%	Ac-M1, Ac-M2; P-S10[a];P-Ser426, Me-E148, Me-E122; Pyro Glu-Q94; Ox-W127(D&S)
13	Synapsin IIb	7,4; 52,720	7.61; 52451.96	0.541±0.086	81%	51%	Ac-M1[a], Ac-M2[a]; P-S10[a]; P-Ser426; Me-E151; Pyro Glu-Q94; OxW127(D)
14	Synapsin IIb	6.99; 52,720	7.61; 52451.97	0.511±0.131	79%	22%	Ac-M1[a], Ac-M2[a]; P-S10[a]; P-Ser426, T-422; Pyro Glu-Q207
15	Synapsin IIb	6,69; 52,720	7.61; 52451.98	0.389±0.144	44%	7%	nil

[a]indicates modification only identified with PMF data:

Ac-denotes N-acetylation :

P-denotes Phosphorylation :

Me-denotes Methylation:

Pyro glu- denotes modification of N-terminal glutamate.

Ox- denotes oxidation of Tryptophan (D- doubly oxidized; S- singly oxidized):

†) Indicates novel Phosphorylation site Ser-546 identified in all the synapsin IIa protein spots (spot 4-9) except protein spot 3.

Table 2 - Identification of Synapsin isoforms

Synapsin isoform	Peptides that covers the splicing site	Mr(expt)	Mr(calc)	Mr(expt)	Delta	Charge state	Spot no.	Enzyme[1]	MS[2]
Synapsin Ia	[579]SFASLFSD[586]	437.25	872.48	872.39	0.09	$[M+2H]^{2+}$	1	L	E
Synapsin Ia	[664]SQSLTNAFNLPEPAPPRPSLSQDEVK[689, a] Deamidation (NQ)	2823.39	2822.38	2822.41	-0.03	$[M+H]^{+}$	2	T	M
Synapsin IIa	[449]TPPQRPPPQGGPGQPQGMQPPGK[47] Deamidation (NQ); Oxidation (M	2351.16	2350.16	2350.19	-0.03	$[M+H]^{+}$	3-9	T	M
Synapsin IIa	[477]RLPSGPSLPS [486]	505.82	1009.63	1009.56	0.07	$[M+2H]^{2+}$	4, 5	S	E
Synapsin IIa	[477]RLPSGPSLPSS [487]	549.34	1096.66	1096.59	0.08	$[M+2H]^{2+}$	5	S	E
Synapsin IIa	[477]RLPSGPSLPSSS[488]	592.86	1183.70	1183.62	0.08	$[M+2H]^{2+}$	5	S	E
Synapsin IIa	[544]SQSLTNAFSFSESSFFR[560]	1941.90	1940.89	1940.89	-0.00	$[M+H]^{+}$	3-9	T	M
Synapsin IIa	[561]SSANEDEAKAETIR[574]	507.59	1519.76	1519.71	0.05	$[M+2H]^{2}$	3-9	T	E
Synapsin IIa	[565]DEAKAETIRSLRKSFASLFSD[585]	2371.21	2370.21	2370.22	-0.01	$[M+H]^{+}$	5	A	M
Synapsin IIa	[571] ETIRSLRKSFASLFS[585, a]	1742.01	1741.00	1740.95	0.05	$[M+H]^{+}$	5	A	E
Synapsin IIb	[445]DSSKTPPQRPPPQGC[b]LQYIL[464]	1141.85	2281.69	2281.15	0.54	$[M+2H]^{2+}$	10; 11; 13	A	E
Synapsin IIb	[449] TPPQRPPPQGC[b]LQYILDC[b]NGIAVGPK [474] Deamidation (NQ)[a]	2877.48	2876.47	2876.43	0.04	$[M+H]^{+}$	10-15	T	M
Synapsin IIb	[449] TPPQRPPP**Q**GC[b]LQYILDC[b]**N**GIAVGPK [474] 2 Deamidation (NQ)	2878.33	2877.32	2877.41	-0.10	$[M+H]^{+}$	10; 11	T	M
Synapsin IIb	[449]TPPQRPPPQGC[b]LQYILDC[b]NGIAVGPK[474] 4 Deamidation (NQ)[a]	2880.46	2879.46	2879.38	0.07	$[M+H]^{+}$	9	T	M
Synapsin IIb	[465] DC[b]NGIAVGPKQVQAS [479]	773.17	1544.32	1542.75	1.58	$[M+2H]^{2+}$	11	A	E
Synapsin IIb	[465] DC[b]NGIAVGPKQVQAS [479] Deamidation	772.87	1543.73	1543.73	0	$[M+2H]^{2+}$	10	A	LF
Synapsin IIb	[467] **N**GIAVGPK[474] Deamidation (NQ)	756.40	756.40	756.42	-0.02	$[M+H]^{+}$	13	U	E
Synapsin IIb	[467] **N**GIAVGPKQVQAS[479] Deamidation (NQ)	635.34	1268.68	1268.67	0	$[M+2H]^{2+}$	10; 12	A	E; LF
Synapsin IIb	[467]**N**GIAVGPK**Q**VQAS[479] 2 Deamidation (NQ)	635.84	1269.66	1269.66	0	$[M+2H]^{2+}$	12	A	LF

Letters marked in bold indicates the exact site of amino acid modified with deamidation determined by MS/MS analysis.

a) Sequence obtained by PMFdata:

b) Peptides modified with carbamidomethyl:

[1]Used Enzyme T, trypsin; L, Lys-C; A, Asp-N; U, unspecific cleavage peptide:

[2]Used Mass spectrometry M, MALDI-TOF-TOF; E, nano-LC-ESI-MS/MS; LF, LTQ-FT:

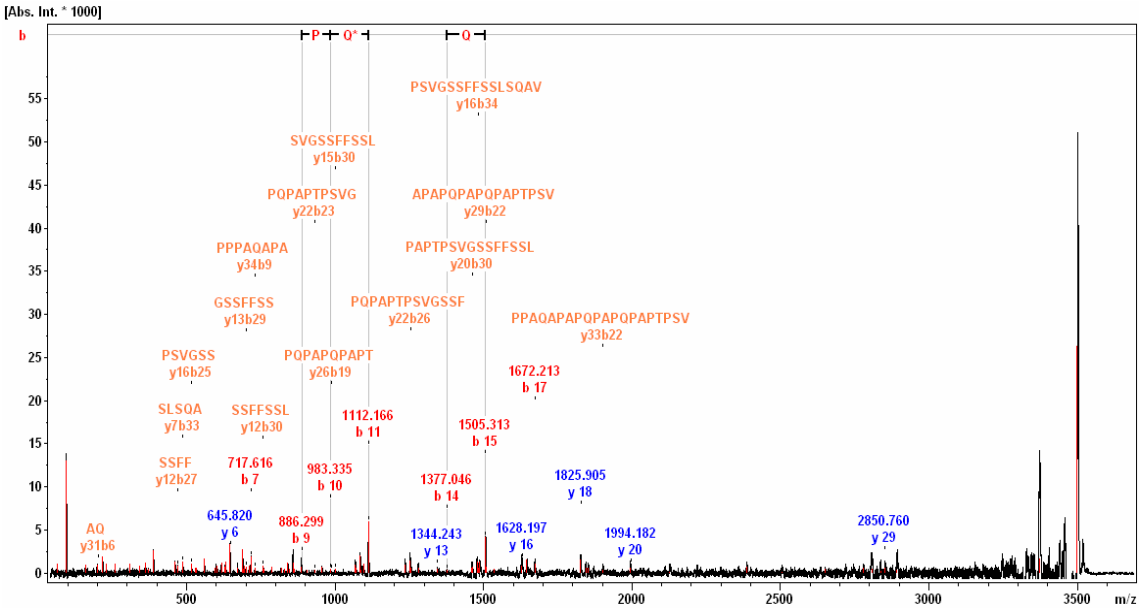


Figure 3: MS/MS spectrum of m/z 3498.716 assignment to sequence 59 - 93: RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAVK, modified by deamidation of a glutamine residue. The *asterisk* indicates that the mass of the b_{10} and b_{11} increased to 129, which corresponds to deamidation of glutamine at amino acid number 69.

4.1.3 N-acetylation at Met-1 or Met-2

By analyzing tryptic and Asp-N digested peptides of synapsin IIa and synapsin IIb (spot 3-15) by MALDI-TOF-TOF and nano-ESI-LC-MS/MS analysis, peptides representing N-terminal residues of these protein primary sequences were determined. MALDI-TOF analysis of protein spots digested with Asp-N shows that mixtures of peptides representing NH4-terminal acetylated methionine (Ac-Met-1) and peptides corresponding to the second methionine (Ac-Met-2) acetylated after cleavage of the initiator methionine were present (fig 4). The corresponding MS spectrum clearly showed that both N-terminally acetylated peptides (Ac-M1MNFLRRRLS, Ac-M2NFLRRRLS) with mass increment of 42 Da exist (fig 4).

Figures 5a and 5b show the MALDI-TOF/TOF and *de novo* sequencing analysis of singly charged ions at *m*/*z* 1364.71 and *m*/*z* 1233.70 matching the sequence Ac-

M1MNFLRRRLS and Ac-M2NFLRRRLS, respectively. The precursor ions $[M+H]^+$ at *m*/*z* 1364.71 and *m*/*z* 1233.70 were selected for MALDI-TOF-TOF analysis and subsequent de novo sequencing analysis with RapiDeNovo^{TM} software was carried out. De novo sequencing was performed based upon the presence of complementary b and y-ion information from the MS/MS spectrum and de novo sequencing software confirmed the presence of N-terminal acetylation at amino acid Met-1 and Met-2 by generating the peptide sequence Ac-M1MNFLRRRLS, and Ac-M2NFLRRRLS, respectively, with high score (de novo scores, 600 and 268). The generated sequence was subsequently submitted to MS-BLAST search against NCBI nucleic acid database and the sequence perfectly matched to the synapsin IIa (AAH66004) and synapsin IIb (AAC72966) sequence. The MS/MS spectrum [M+H]+ at *m*/*z* 1364.71 for the corresponding peptide sequence Ac-M1MNFLRRRLS indicated the presence of nearly complete b-ions (b1-b9) series and y-ions (y3-y6) information. In particular, observation of a mass of b1 ion at m/z 174 Da, which corresponds to the mass of acetylated methionine (Met-1) and the presence of a1 ion at *m*/*z* 146, was supporting the notion that Met-1 was exclusively acetylated (fig 5a). The MS/MS spectrum $[M+H]^+$ at *m*/*z* 1234.71 for the corresponding peptide sequence Ac-M2NFLRRRLS indicated the presence of b-ions (b1-b8) series and y-ions (y4-y7) information. Moreover, the presence b1 ion at 173.94 Da corresponds to the mass of acetylated Met-2 and the presence of an a1 ion at m/z 145.96, in turn indicating that Met-2 is acetylated (fig 5b). The neutral loss of characteristic acetyl group (-42 Da) from respective acetylated precursor ions clearly indicated the acetylation of these peptide (fig 6a and 6b). This modification was also confirmed in protein spots digested with trypsin analyzed by nano-ESI-LC-MS/MS. The MS/MS spectrum of the precursor ion $[M+2H]^{2+}$ at *m*/*z* 1443.22 and the corresponding peptide sequence Ac-

MMNFLR was identified in synapsin IIa (spot 3) and synapsin IIb (spot 9) in its methionine oxidized state.

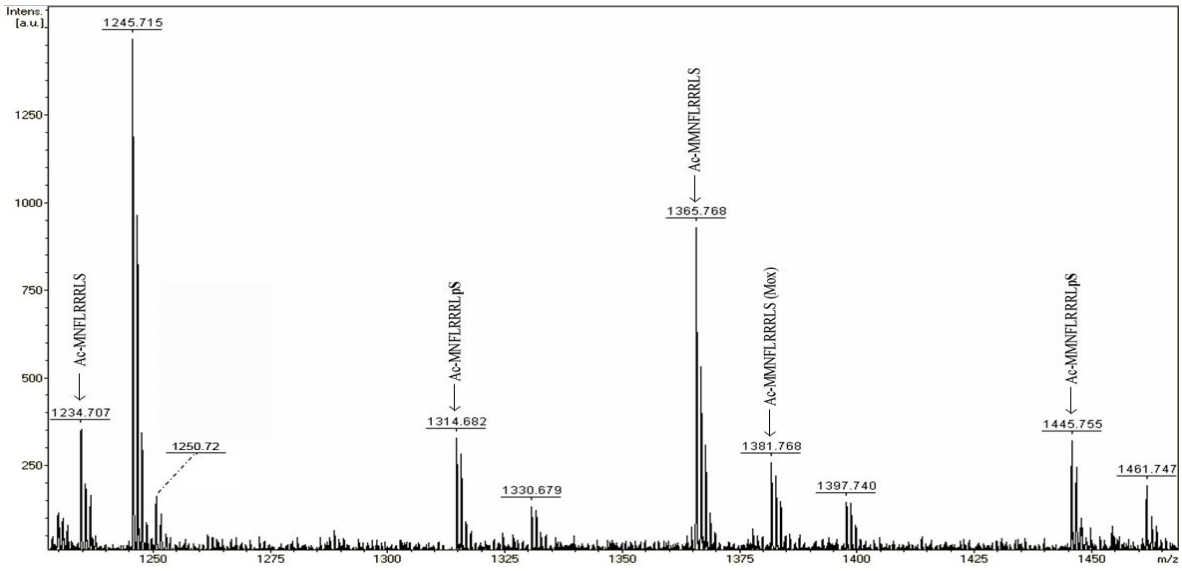


Figure 4: The MS spectrum of synapsin IIb: The mass ions of the acetylated fragments and of its phosphorylated peptides are indicated

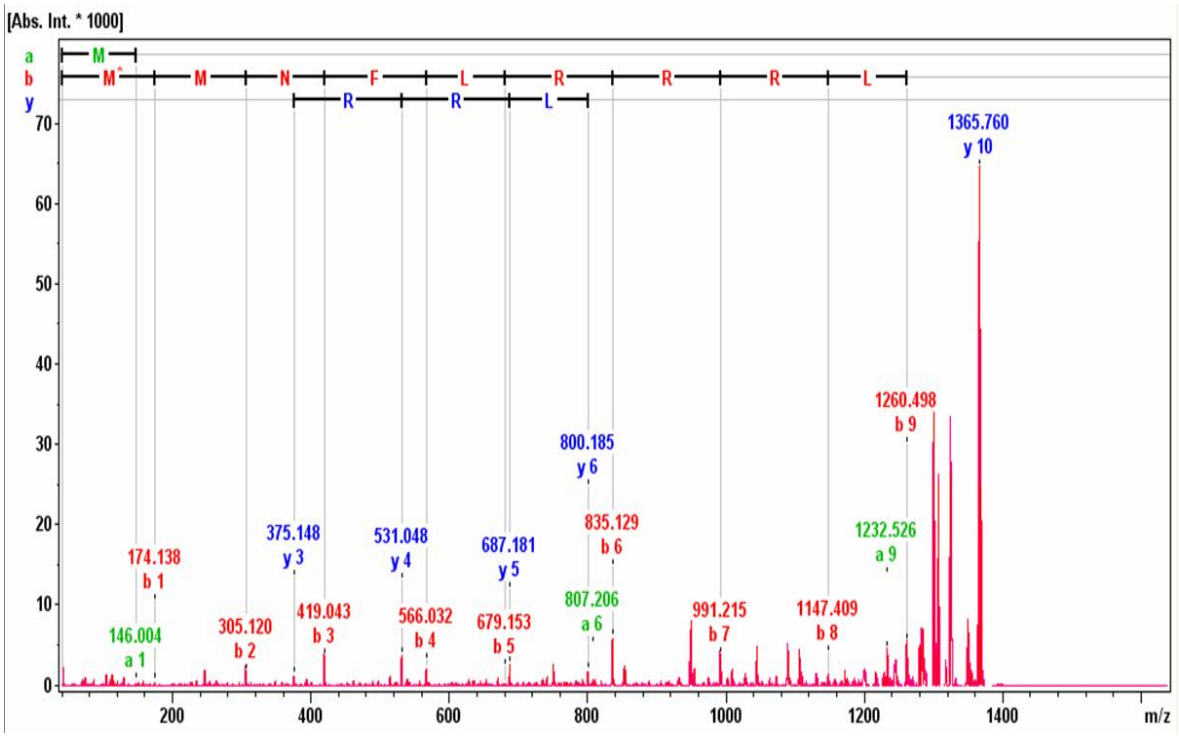
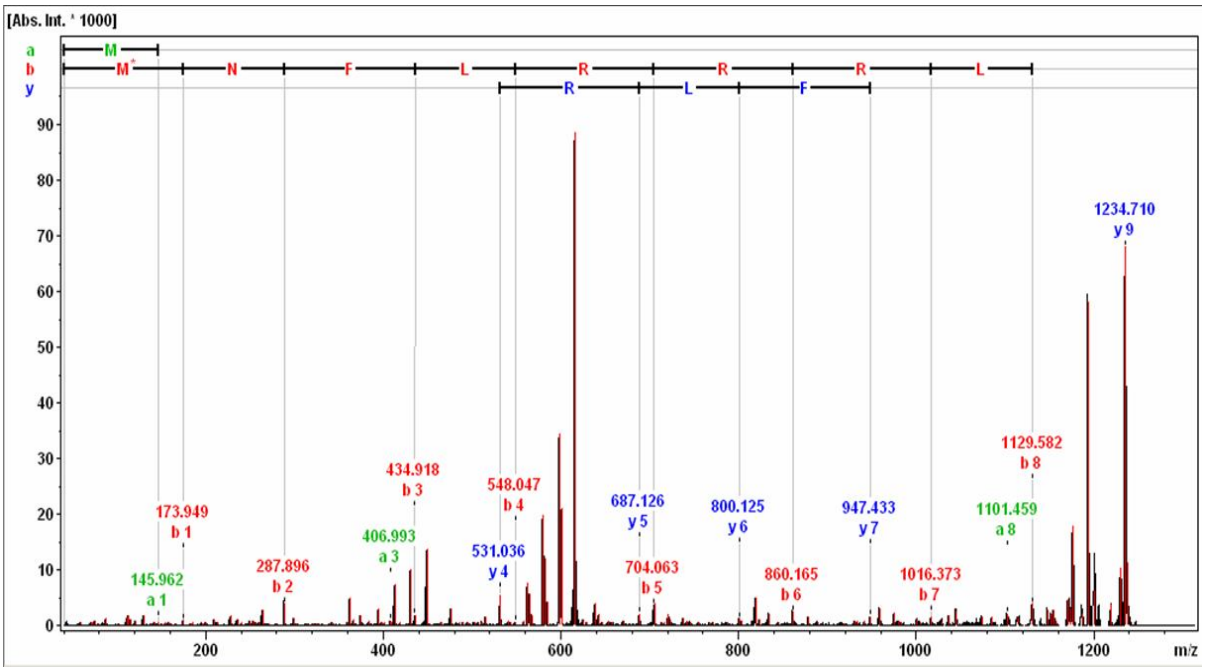


Figure 5a: MS/MS spectrum of m/z 1365.760 (spot 12) assignment of the identified sequence 1–10: MMNFLRRRLS, which contains N-terminally modified methionine. The *asterisk* indicates that the mass of the N-terminal methionine residue was increased to m/z 174.13, which corresponds to Met-1 acetylation.



5b: MS/MS spectrum of m/z 1234,710 (spot 12) assignment of the identified sequence 2–10: MNFLRRRLS, which contains N-terminally modified methionine. The spectrum clearly indicates that Met-1 was removed during posttranslational processing and subsequent addition of an acetyl group. The *asterisk* indicates that the mass of the N-terminal methionine residue was increased to m/z 173.949, which corresponds to Met-2 acetylation

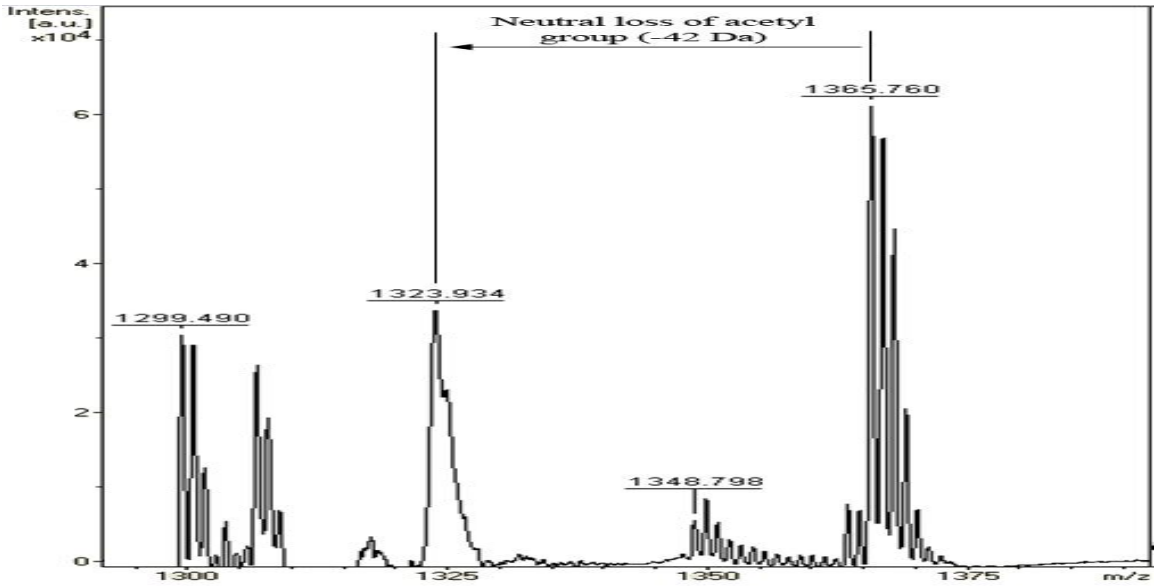


Figure 6a: Identification N-terminal acetylation sites by MALDI-TOF-TOF.
Documentation of the signal at m/z 1323.934 which is corresponding to characteristic neutral loss of acetyl group (-42 Da) from the precursor ion, clearly indicating the Met-1 acetylation**.**

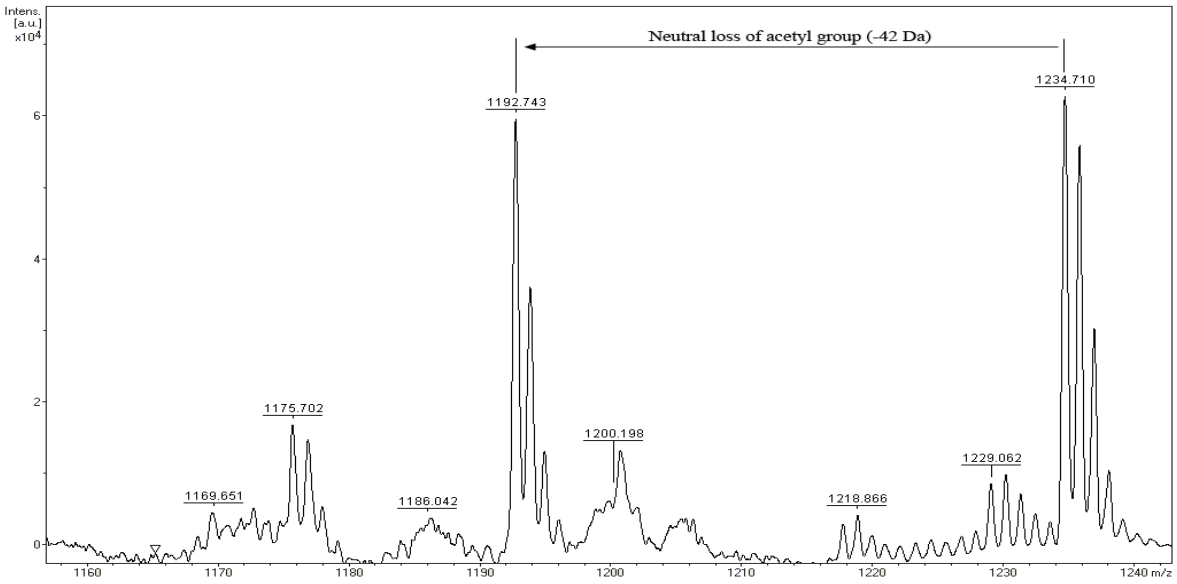


Figure 6b: **Identification N-terminal acetylation sites by MALDI-TOF-TOF.**
The signal at m/z 1192,743 which is corresponding to characteristic neutral loss of an acetyl group (-42 Da) from the precursor ion, clearly indicating Met-2 acetylation.

4.1.4 Identification known and novel phosphorylation sites

PMF analysis of tryptic peptides obtained from synapsin IIa (spot 3-9) indicated an observed ion at *m/z* 2021.86 not detected in spot 3 but present in spots 4-9; this finding shows that spot 3 is the non phosphorylated form of the protein spot and spots 4-9 were phosphorylated forms of synapsin IIa. PMF analysis of Asp- N digested peptides analyzed by MALDI-TOF showed the presence of an ion at m/z ions at 1314.68 and 1445.755 that were not present in spot 3 and 10 but again, present in protein spots 4, 5, 6, 7, 9 ,11,12, 13, 14 and 15, this shows that spot 3 and spot 10 are spots representing unphosphorylated forms of synapsin IIa and synapsin IIb protein spots in the 2-DE gel respectively (fig 2, table 1). Normally, more acidic phosphorylated protein species would be predominantly expected in the left-hand part of the 2-DE-gel (with lower isoelectric point) and the phosphorylated species

would be more prevalent in the right-hand region of the 2-DE-gel (higher isolectric point) (Matsubara, Kusubata et al. 1996).

Our experimental data revealed for the first time a novel phosphorylation site in synapsin IIa in the domain E region, close to the C-terminal of the protein sequence. Figures 6 and 7 show MALDI-TOF-TOF and *de novo* sequencing analysis of singly charged ions at m/z 2021.86 matching the sequence 544-SQp**S**LTNAFSFSESSFFR-560 ("bold" indicates amino acids that are modified with phosphorylation) in synapsin IIa. Because phosphorylation of peptide increases the mass of an ion by 80 Da, PMF data effectively denoted the presence of a phosphopeptide compared to the calculated masses of proteolytic peptides. Protonated molecular ions of tryptic peptide SQSLTNAFSFSESSFFR (residues 544 - 560) were detected in its monophopshosphorylated form ($[M+H]^+$ at m/z 2021.86). The observed mass shift of 80 Da corresponds to the addition of a single phosphate group to the corresponding non-phosphorylated peptide ($[M+H]^+$ at m/z 1941.90 Alkaline phosphatase treatment and MALDI-MS analysis resulted in the disappearance of the monophosphorylated peptide with [M+H]+ at m/z 2021.852 due to dephosphorylation (Figure 7).

The mass of 2021.86 was present in all the synapsin IIa protein spots (spots 4-9) except in non-phosphorylated protein spot 3 (table 1). Observation of the oxidized form of metastable fragment ion at m/z 1936.420 in the MS spectrum strongly suggested that peptide SQSLTNAFSFSESSFFR was modified with phosphorylation (Figure 8). However, this peptide contained 7 potential phosphorylated sites (Ser-544, Ser-546, Thr-548, Ser-552, Ser-554, Ser-556 and Ser-557), so site specific localisation of phosphorylation site is of importance. A precursor ion $[M+H]^+$ at

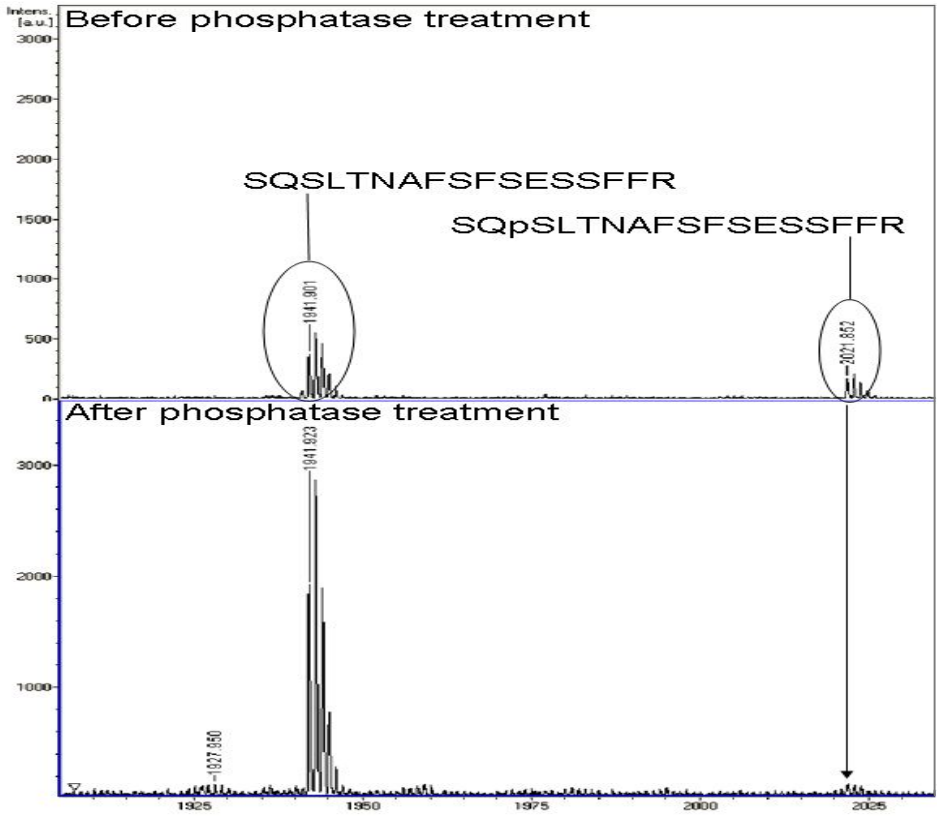


Figure 7; MALDI-MS spectra displaying molecular ions, $[M+H]^+$ of PMF obtained after tryptic digestion of synapsin IIa. A, peptide 544SQpSLTNAFSFSESSFFR560 present in its non- phosphorylated form, [M+H] + at m/z 1941.901 and its mono-phosphorylated form, [M+H] + at m/z 2021.852. B, the same sample after incubation with alkaline phosphatase.

2021.84 was selected for MS/MS analysis and subsequent de novo sequencing analysis with RapiDeNovo^{TM} software was carried out. De novo sequencing was performed based upon the presence of complementary b and y-ion information from the MS/MS spectrum and de novo sequencing software confirmed the presence of phosphorylation at amino acid Ser-456 by generating the peptide sequence 544-SQpSLTNAFSFSESSFFR-560 with high score (de novo score, 8204) (fig 9). The generated sequence was subsequently submitted to MS-BLAST search against NCBI nucleic acid database and as a result, the sequence perfectly matched

to the synapsin IIa sequence. This modification was further verified by the observation of neutral loss of 80 Da (-H3PO3) at m/z 1942.30 as evaluated by MALDI-TOF-TOF analysis (fig 10). A nearly complete series of y-ions (y1-y15) and few b- ions fragment were observed in the MS/MS spectrum, including several ions corresponding to neutral loss of the phosphate group in the b-ion series (m/z 285.00, 498.82, 683.78, and 831.925) (fig 9). Close observation of MS/MS spectrum revealed that the mass difference between y14 and y15 increased to 164, indicating that Ser-546 was specifically phosphorylated as these fragment ion shifted by 80 Da compared with the corresponding fragments generated from the non-phosphorylated peptide (fig 9). In addition, as compared to non-phosphorylated peptide fragments, y1-y13 fragmented masses and b2 ion mass at *m/z* 216.02 were unchanged proposing the unique site phosphorylation site at Ser-546 but not in Ser-544. These results together with presence of [b-Pi3] ion at m/z 285.00: i.e., b3-98 demonstrated that Ser-546 is the only residue phosphorylated in this peptide (fig 9).

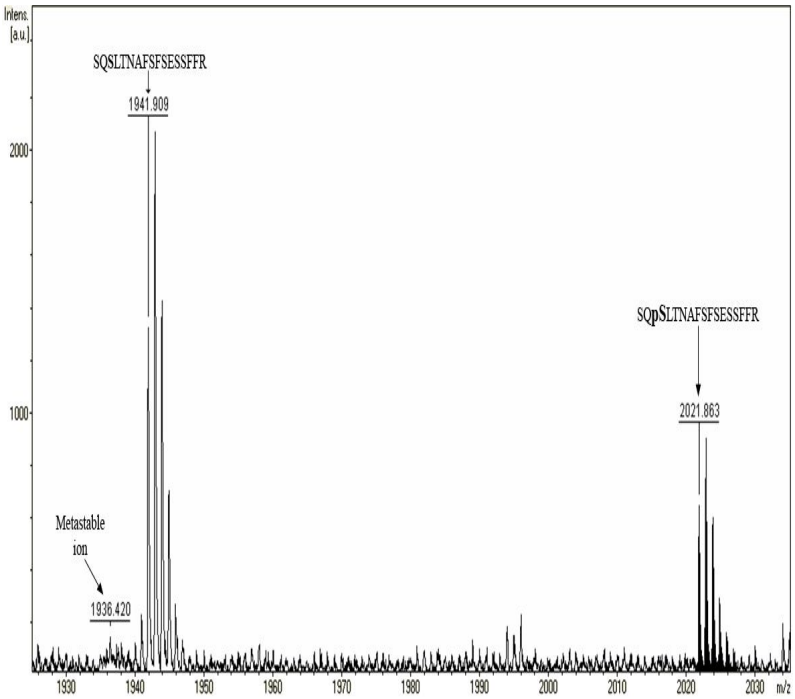


Figure 8: MS spectrum of the phosphorylated peptide of synapsin IIb. The mass ion of the unmodified tryptic fragment and of its phosphorylated peptide is indicated. The signal at m/z 1936.420 may correspond to the oxidized form of the metastable fragment ion.

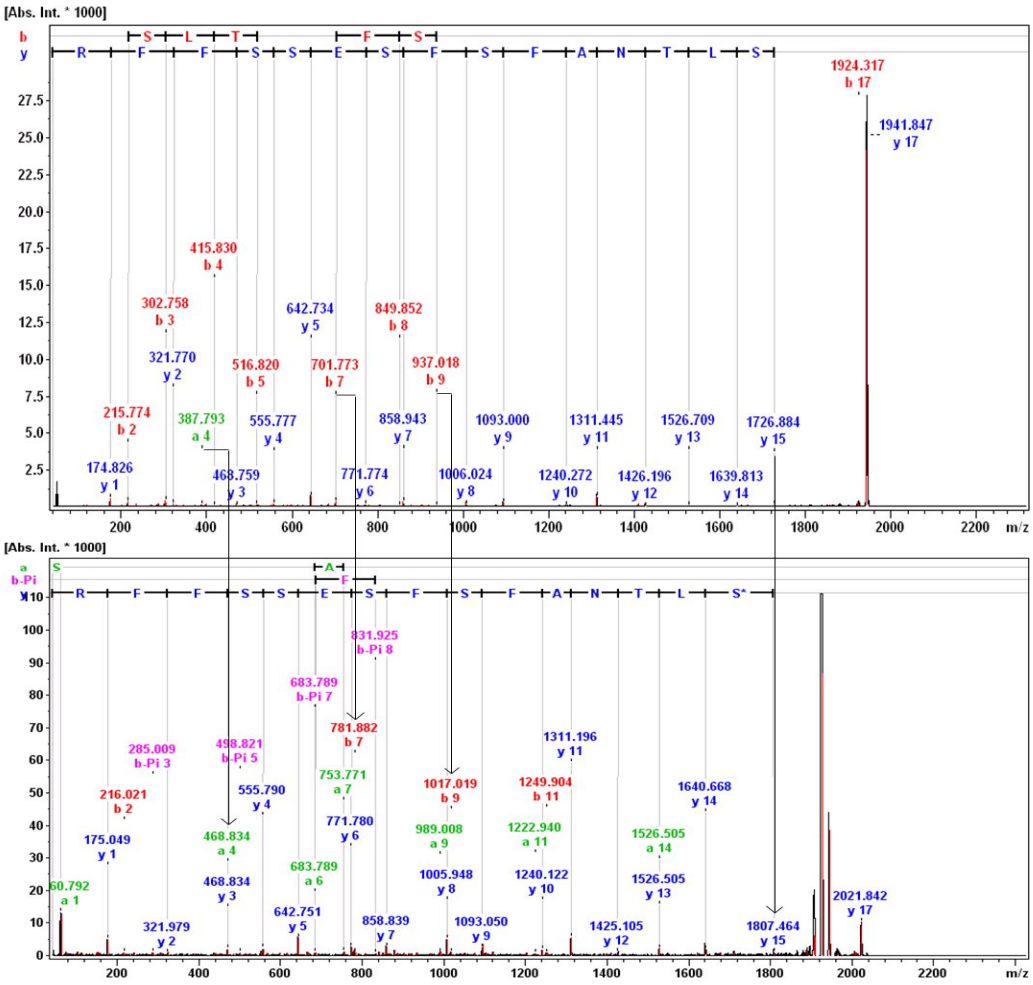


Figure 9: **Identification of a novel phosphorylation site by MALDI-MS/MS** and de novo sequencing analysis. MALDI-TOF-TOF spectra of unphosphorylated peptide SQSLTNAFSFSESSFFR (upper panel) and phosphorylated peptide SQpSLTNAFSFSESSFFR (lower panel). The arrows indicate the 80 Da shifts for the individual fragment ions containing the phosphate moiety. The *asterisk* indicates that the mass of y_{14} and y_{15} increased to 80 Da representing Ser-546 phosphorylation.

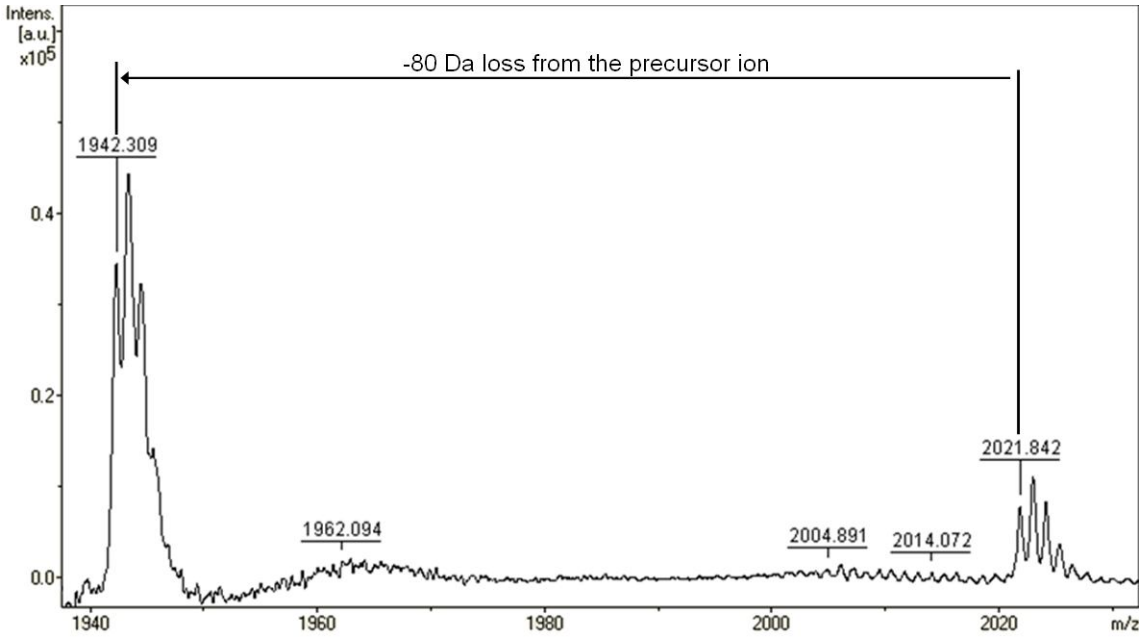


Figure 10: **Identification of the new phosphorylation site by MALDI-TOF/TOF analysis:** Demonstration of neutral loss of 80 Da ($-H_3PO_3$) from the precursor ion.

All four synapsins are known as excellent substrates for cAMP- dependent protein kinase A and CaM kinase I as these contain consensus phosphorylation sites in the domain A region. In the synapsin I isoform, it was experimentally proven that Ser-9 (site 1) was phosphorylated by PKA and CaM kinase I, but there are no mass spectrometrical confirmations available for synapsin IIa and IIb in the mouse organism. Asp-N digested peptides of both, synapsin IIa and synapsin IIb protein spots (spots 4-9; spots11-15) and analysis with MALDI-TOF/TOF confirmed that Ser-9 or Ser-10 were phosphorylated by PKA and CaM kinase I. Protonated molecular ions of Asp-N digested peptides Ac-MMNFLRRRLS (residues 1- 10) and Ac- MNFLRRRLS (residues 2-10) were detected in its monophosphorylated forms $[M+H]^+$ at m/z 1445.75 and m/z 1314.68 respectively (fig 4). The MALDI-MS spectrum obtained from Asp-N digestion is shown in figure 4, the observed mass shift of 80 Da corresponds to the addition of a single phosphate group to the

corresponding non-phosphorylated peptide masses at m/z 1365.76 and m/z 1234.70. This mass increment is in agreement with its observed and theoretical mass of the modified peptides. These mass increments are also observed in oxidized form of these peptides. Precursor ions of phosphorylated peptides ions $[M+H]^+$ at m/z 1445.75 and m/z 1314.68 were subjected to MS/MS analysis. Poor fragmentation patterns in MS/MS spectra were obtained due to the fact, that the acetylated N-terminal and Phosphorylated C-terminal cannot be protonated any more. However, ions corresponding to the loss of H3PO3 (-80 Da) were observed at m/z 1365.95 and m/z 1234.75 from respective phosphorylated peptide ions at m/z 1445.75 and 1314.68 (Data not shown), strongly indicated that Ser-9 or Ser-10 were phosphorylated by PKA and CaM kinase I in the mouse hippocampus.

The synapsin Ia protein spot (spot 2) digested with trypsin analyzed with MALDI-TOF, confirmed the PKA and CaM kinase I phosphorylation site at Ser-9. The tryptic peptide LSDSNFMANLPNGYMTDLQR (residues 8 – 27) would have an $[M+H]^+$ ion at m/z 2286.04. If Ser-9 were phosphorylated, then it would be at m/z 2366.01. A close examination of all of the PMF data determined an ion at m/z 2366.21 (observed; m/z 2367.21) Therefore, spot 2 probably contains phosphorylation at Ser-9 (table1).

Trypsin digestion of the synapsin Ia protein spot (spot 2) and analyzed by MALDI-TOF, confirmed the MAPK kinase phosphorylation site at Ser-62. The tryptic peptide ASTAAPVASPAAPSPGSSGGGGFFSSLSNAVK (residues 54-85) would have an $[M+H]^+$ ion at m/z 2833.39. If Ser-62 were phosphorylated, then it appeared at *m/z* 2913.35. A close examination of PMF data revealed an ion at *m/z* 2913.48 (observed; *m/z* 2914.48).Therefore, MAPK kinase phosphorylation possibly occur at Ser-62 (table 1).

ESI-LC-MS/MS tandem mass spectra's of phosphorylated peptides obtained after IMAC phosphopeptide enrichment and subsequent nano-ESI-LC-MS/MS anaylsis

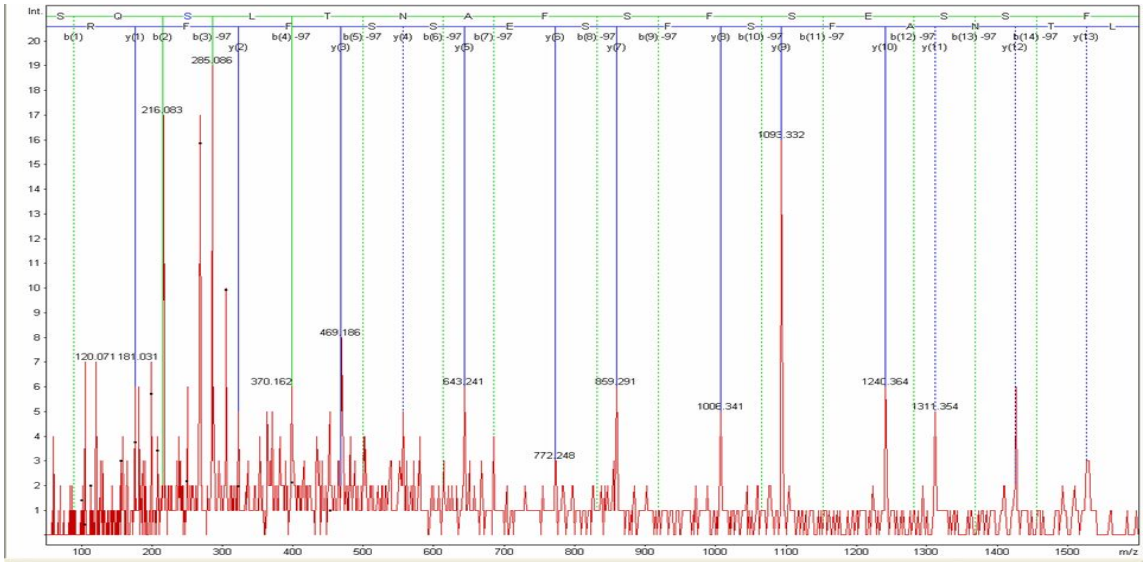


Figure 11a; Tandem mass spectrum of an [M+2H] $^{2+}$ ion at *m/z* 1011.40 SQpSLTNAFSFSESSFFR from tryptic digest of synapsin IIa identified as phosphorylated at S-546.

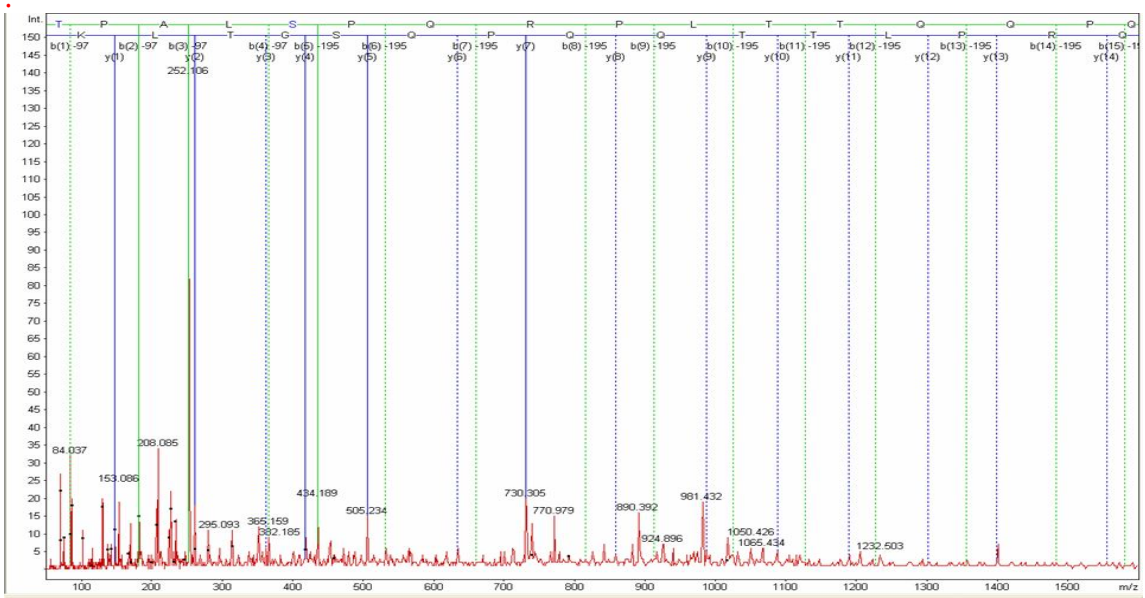


Figure 11b; Tandem mass spectrum of an [M+3H] $^{3+}$ ion at *m/z* 724.96 ^{422}pTPALpSPQRPLTTQQPQSGTLK^{442} from tryptic digest of synapsin IIa identified as di-phosphorylated at T-422, S 426

4.1.5 Identification phosphorylation sites using IMAC and nano-LC-ESI-MS/MS.

To detect minor sites of Synapsin IIa and IIb phosphorylation, peptides generated in the proteolytic digest of trypsin and Asp-N were enriched for phosphopeptides using IMAC (Ga^{3+}) - based preferential enrichment of phosphopeptides and analysed with nano-LC-ESI-MS/MS. Phosphorylated peptides and sites of phosphorylation from the trypsin and Asp-N digest from synapsin IIa and II b are listed in Table 3. The use of IMAC resulted in the confirmation of novel phosphorylatiom at Ser-546 on synapsin IIa (figure 11a). Mass spectra of these peptides clearly showed the doubly and triply charged ions of the phosphorylation forms. Tryptic and Asp-N digested peptides of both, synapsin IIa and synapsin IIb protein spots (spots 5, 12, 13 and 14) were identified as phosphorylated at T-422, S-426. MS/MS spectrum of the triply charged ion at m/z 803.70, 422pTPALSpPQRPLTTQQPQSGTLK442 from the tryptic digests and unspecific cleavage peptide from Asp-N digest $[M+3H]^{3+}$ at *m/z* 724.96 426pSPQRPLTTQQPQSGTLKEP442 were identified as phosphorylated at T-422 and S-426 (figure 11b). However, T-422, S-426 were only identified after IMAC enrichment of phosphopeptides, suggesting that these sites were minor phosphorylation sites. This result supports the recently published phosphorylation sites (T-422 and S-426) on synapsin II isoform in mouse organism (Munton, Tweedie-Cullen et al. 2007).

Table 3 List of phosphorylation peptides and sites of phosphorylation identified after IMAC enrichment

Spot No	Protein Name	Phosphopeptide	Site of Phosphorylation	Observed	Mr(expt)	Mr(calc)	Delta	Expect	Charge	Missed Cleveage	Enzyme[1]	MS[2]
5	Synapsin IIa	^{544}SQ**p**SLTNAFSFSESSFFR^{560}	S-546	1011.40	2020.79	2020.86	-0.07	1.7e-005	2	0	T	E
5	Synapsin IIa	^{544}SQ**p**SLTNAFSFSESSFFR^{560}	S-546	674.52	2020.55	2020.86	-0.31	0.0022	3	0	T	E
5	Synapsin IIa	^{422}TPAL**p**SPQRPLTTQQPQSGTLK^{442}	S-426	776.95	2327.82	2328.18	-0.37	0.39	3	0	T	E
5	Synapsin IIa	^{422}**p**TPAL**p**SPQRPLTTQQPQSGTLK^{442}	T-422, S-426	803.70	2408.08	2408.15	-0.07	0.47	3	0	T	E
14	Synapsin IIb	^{426}**p**SPQRPLTTQQPQSGTLK^{442}	S-426	649.60	1945.78	1945.96	-0.18	0.43	3	0	U	E
14	Synapsin IIb	^{422}**p**TPALSPQRPLTTQQPQSGTLK^{442}	T-422	776.98	2327.93	2328.18	-0.25	2.5	3	0	T	E
13	Synapsin IIb	^{426}**p**SPQRPLTTQQPQSGTLK^{442}	S-426	649.60	1945.79	1945.96	-0.17	1.2	3	0	U	E
12	Synapsin IIb	^{426}**p**SPQRPLTTQQPQSGTLKEP^{442}	S-426	724.96	2171.86	2172.06	-0.20	0.02	3	0	U	E
12	Synapsin IIb	^{426}**p**SPQRPLTTQQPQSGTLK^{442}	S-426	649.61	1945.80	1945.96	-0.16	0.8	3	0	U	E

pS or pT- denotes Phosphorylated Serine or Threonine

[1]Used Enzyme T, trypsin: U, unspecific cleavage peptide from Asp-N digests

[2]Used Mass spectrometry E, nano-LC-ESI-MS/MS.

4.1.6 Confirmation of Pro-44 in synapsin Ia

Based on DNA cloning and sequence analysis of 5' terminal synapsin I gene (exon 1), Chin et al.(Chin, Li et al. 1994) published a partial sequence (1-25) containing leucine instead of proline at position 44 in the mouse organism (GenBank ID; AAA79963) (fig 12). Asp-N digested peptides from spot 1 and spot 2 were analyzed by MALDI-TOF-TOF and confirmed the presence of proline at amino acid 44. MS/MS analysis of the Asp-N digested peptide of spot 1 at *m*/*z* 2623.30 and subsequent MASCOT search perfectly matched to synapsin I, 24 – 51 (DLQRPQPPPPPPSAASPGATPGSATASA) with significant score (ion score 30) and confirmed the presence of proline (fig 13). From spot 2 this peptide was identified in its deamidated form (*m*/*z* 2624.300). The MS/MS spectrum indicated that the presence of ions was corresponding to the several proline containing peptides. The mass difference between y7 and y8 ions corresponds to the mass of proline in the MS/MS spectrum, suggesting the presence of proline at amino acid position 44 in the synapsin Ia primary structure in hippocampal tissue of mouse.

```
sp|O88935|SYN1_MOUSE_Synapsin-      MNYLRRRLSDSNFMANLPNGYMTDLQRPQPPPPPPSAASPGATPGSATAS
gi|1041085|gb|AAA79963.1|_syna      MNYLRRRLSDSNFMANLPNGYMTDLQRPQPPPPPPSAASPGATLGSATAS
                                    ****************************************** ******

sp|O88935|SYN1_MOUSE_Synapsin-      AERASTAAPVASPAAPSPGSSGGGGFFSSLSNAVKQTTAAAAATFSEQVG
gi|1041085|gb|AAA79963.1|_syna      AERASTAAPVASPAAPSPGSSGGGGFFSSLSNAVKQTTAAAAATFSEQVG
                                    **************************************************

sp|O88935|SYN1_MOUSE_Synapsin-      GGSGGAGRGGAAARVLLVIDEPHTD
gi|1041085|gb|AAA79963.1|_syna      GGSGGAGRGGAAARVLLVIDEPHTD
                                    *************************
```

Figure 12; Comparison of partial protein sequence of mouse synapsin Ia (NCBI Accession no: AAH22954), and deduced amino acid amino acid sequences of 5' terminal synapsin I gene (exon 1) (GenBank ID; AAA79963). Sequences were obtained from NCBI protein database and GenBank. The alignment was performed

using the Clustal W program. MSMS data were providing evidence for presence of Proline rather than Lysine in the sequence thus generating a sequence conflict.

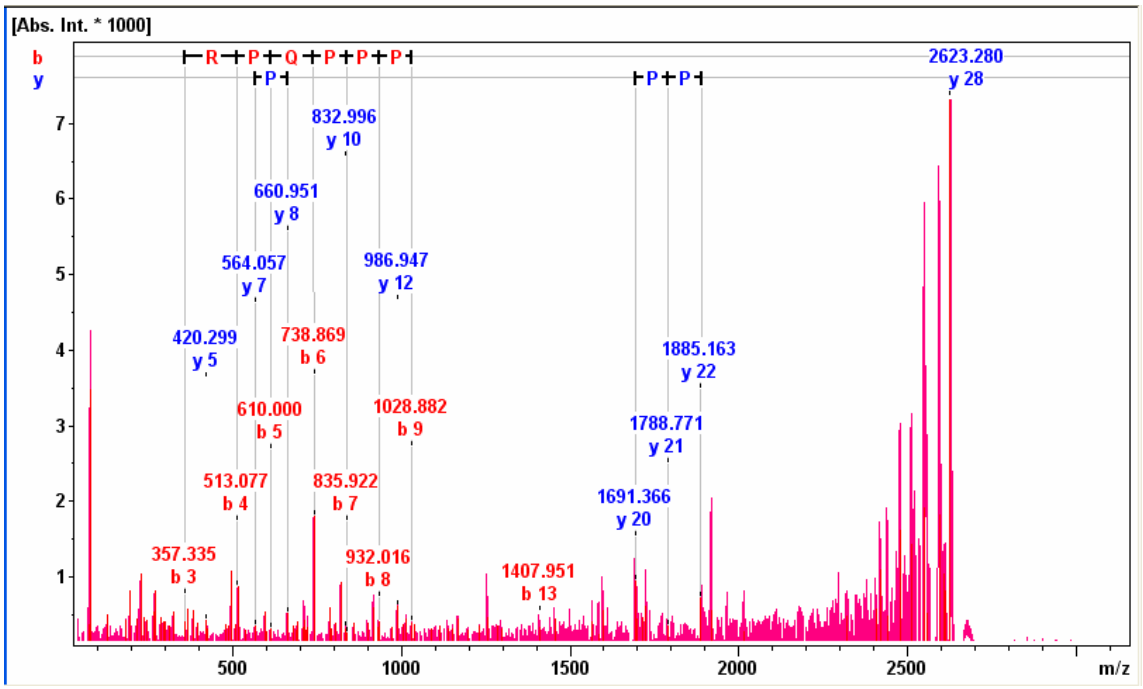


Figure 13; MALDI-TOF/TOF spectrum of *m/z* 2622.30 obtained from peptides resulting from Asp-N digestion of synapsin Ia (spot 1) and subsequent MASCOT analysis confirmed the presence of Proline at position 44.

4.1.7 Identification of Other Modifications in Synapsins Protein Spots

Methylation of the acidic residue glutamic acid (E) and Aspargine (N) were identified in synapsin IIa and synapsin IIb protein spots. Both, tryptic and Asp-N- digested peptides analyzed with MALDI-TOF-TOF and nano-ESI-LC-MS/MS analysis identified methylation of glutamic acid at 148 (Glu-148) in synapsin IIa (spot 4) and synapsin IIb (spot 10 and 12), Glu-249(spot 5 and 6) in synapsin IIa, Glu-151, Glu-122 (spot 13) and Glu-402 (spot 10) in synapsin IIb (table 1)**.** Other modifications include pyro glu at position 404 in synapsin Ia (spot 1 and 2), pyro glu-94 in both synapsin IIa and synapsin IIb (spot 5, 6, 12 and 13) and pyro glu-207 in synapsin IIb (spot 14) and were identified by both mass spectrometry approaches. Tryptophan

oxidation was identified in singly and doubly oxidized states in synapsin I a, synapsin IIa and synapsin IIb protein spots.Wox-126 (spot 1 and 2), Wox-127 (spot 5, 6, 11, 12 and 13) and Wox-336 (spot 5) were observed (table 1).

4.2 Quantitative proteomics analysis of synapsins that were linked to cognitive enhancement and spatial memory formation

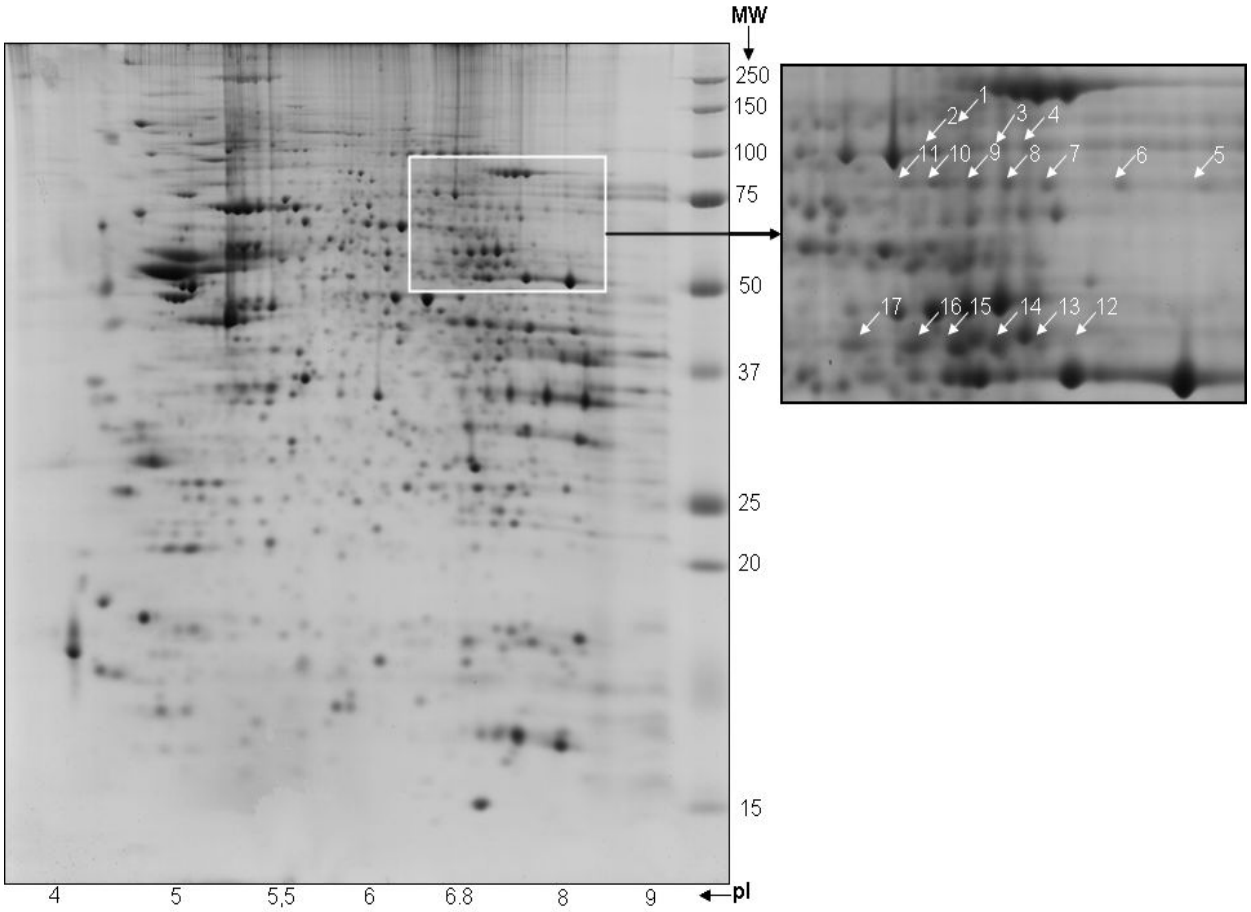


Figure 14; Proteins from mouse hippocampus were subjected to isoelectric focusing on a 18 cm immobilized pH 3-10 gradient strip and then separated by SDS-PAGE. A 2-DE gel and partial image of a Coomassie Blue-stained gel image are shown. Synapsin protein spots (labeled with arrows) are identified and characterized by qQ-TOF mass spectrometry. By protein profiling of mouse hippocampal tissue, 17 individual protein spots identified as synapsin Ia, synapsin Ib, synapsin IIa, and synapsin IIb with different molecular weights and pI values were observed in all the 2-DE gels in the same pattern. Co eluted protein spot used as internal control is represented in spot 10.

The gel- based mass spectrometric approach has proven to be an effective technique to study molecular heterogeneity of synapsin isoforms which revealed that 15 individual synapsin spots were identified and characterized (John, Chen et al. 2007). Herein, protein identification revealed the presence 17 individual protein spots of synapsin isoforms in 2-DE gels of both, SGS742- and NaCl-treated control groups (Figure 14; Supplementary tables 5-7). The relative quantification of synapsin isoform levels was carried out using in-gel stable isotopic labeling (Asara, Zhang et al. 2006) and nano-LC-ESI-MS/MS mass spectrometry. To compare hippocampal synapsin isoform protein levels, 2-DE gel spots containing the synapsin isoforms were excised and differentially labeled. Gel pieces from SGS742-treated group were heavy labeled and from the NaCl-treated group were light labeled. Likewise, SGS742-treated yoked control gel pieces were heavy labeled and NaCl-treated yoked control gel pieces were light labeled. Both heavy and light labeled gel pieces were combined prior to digestion with trypsin or chymotrypsin. For quantitative determination by the in-gel stable isotopic analysis abundance ratios of light and heavy labeled peptides were manually computed by measuring high intensity peptide ions of both, light and heavy labeled peptides. As an example of data generation and handling, figure 15 shows the spectrum of a doubly charged tryptic peptide ion pair from synapsin Ia as well as the corresponding MS/MS spectrum for the sequence SLKPDFVLR with light- and heavy-labeled peptide ion at *m/z* 615.445 and *m/z* 616.955 from NaCl-treated controls and the SGS742- treated gel piece (Figure16a and 16b). Peptide ratios, means ± SD and the resulting statistical data are shown in tables 4, 5.

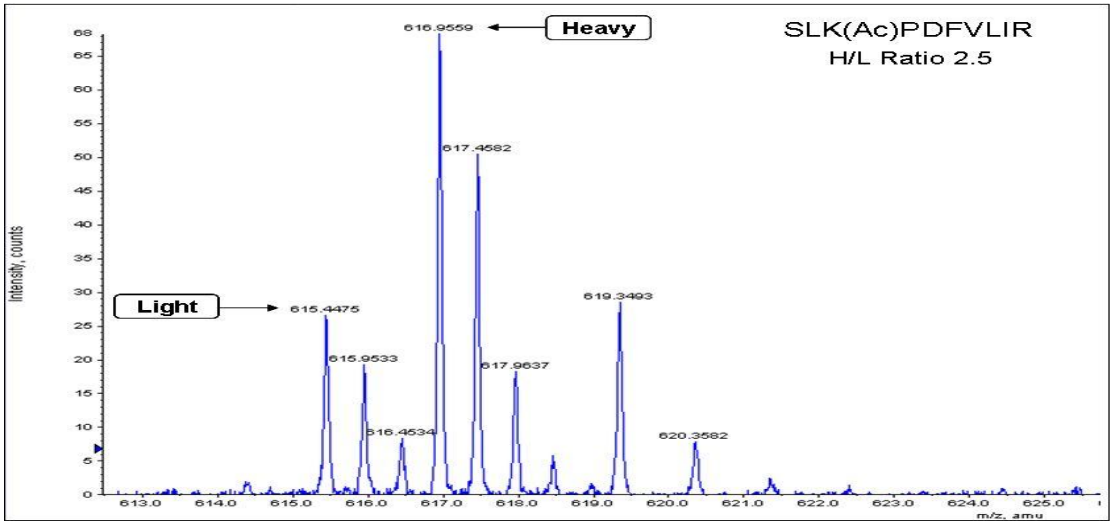


Figure 15; Quantitative analysis of synapsin Ia protein expression in the SGS742- treated and NaCl- treated control sample using the in-gel stable isotopic labeling strategy. Synapsin protein spots from 2-DE gels of hippocampi treated with NaCl and SGS742 were labeled with “light” (L) and “heavy” (H), respectively. An example for mass spectra of light and heavy peptide pairs SLK(Ac)PDFVLIR (m/z 615.44, m/z 616.95) that are assigned to synapsin Ia is shown, revealing a twofold increase in the SGS742-treated sample as compared to the NaCl-treated group.

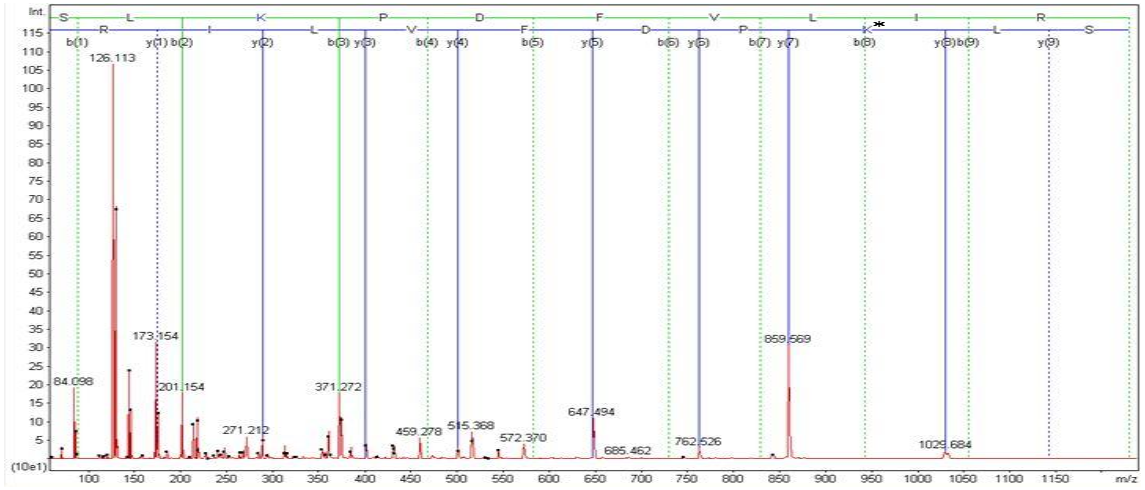


Figure 16a; MS/MS analysis of m/z 615.44 ion and database search revealed a light-labeled tryptic peptide corresponding to the amino acid sequence of SLK (Ac) PDFVLIR. The asterisk indicates that the mass of y7 and y8 increased to 42 Da, representing light- labeled lysine.

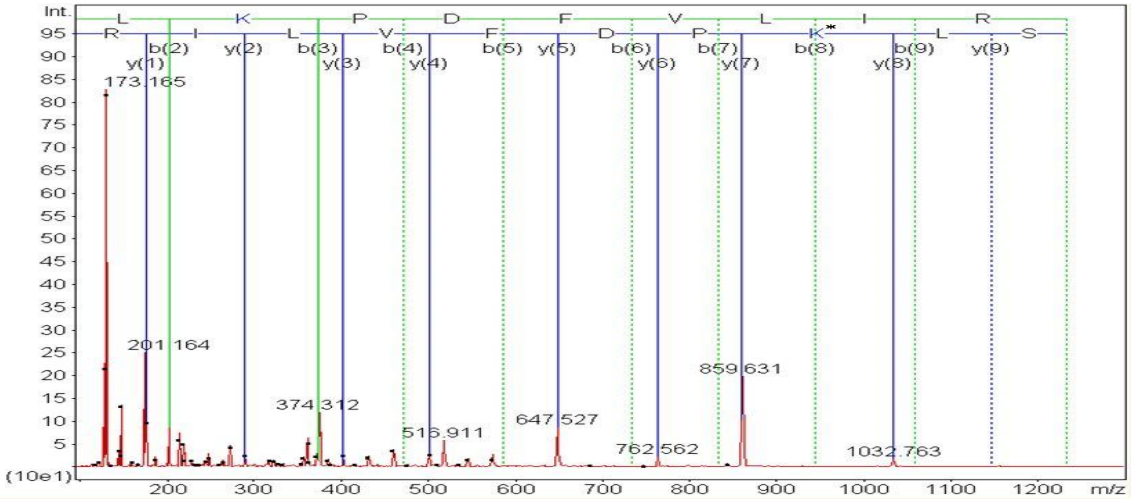


Figure 16b; MS/MS analysis of m/z 616.95 ion with subsequent database search revealed a heavy -labeled tryptic peptide corresponding to the amino acid sequence of SLK (Ac) PDFVLIR. The asterisk indicates that the mass of y7 and y8 increased to 45 Da, representing heavy-labeled lysine.

As an internal standard co-migration of succinyl-CoA: 3-ketoacid-coenzyme A transferase 1 (Q9D0K2) in 2-DE gels was used (Asara, Zhang et al. 2006). In the current study, this enzyme co migrated in 2-DE showing an H/L ratio of 1.01 (Figure 17) and a total mean ratio of 1.13 was obtained in all our experiments. These results demonstrate efficiency of the labeling method and accuracy of mass spectrometry based quantification.

Tables 4 and 5 summarize the quantification of SGS742-treated vs NaCl-treated controls and SGS742-treated yoked controls vs NaCl-treated yoked controls. The demonstration of identification and quantification of peptides is provided in the supplementary table 8.

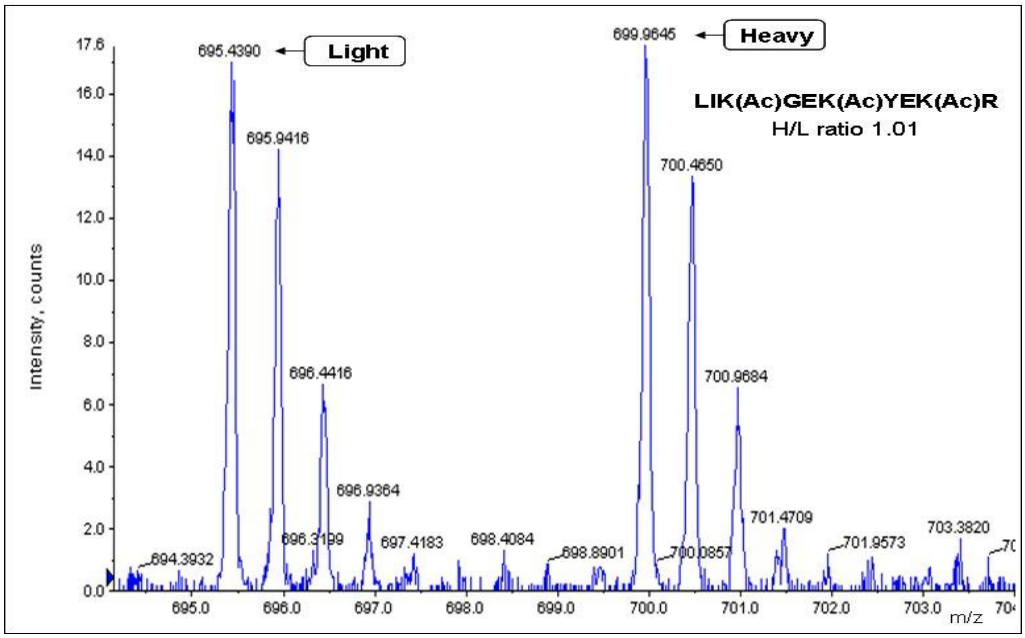


Figure 17. Mass spectrum of co migrating protein succinyl-CoA:3-ketoacid-coenzyme A transferase 1 showing light and heavy peptide pairs LI**K**(Ac)GE**K**(Ac)YE**K**(Ac)R (m/z 695.43, m/z 699.96) and exhibited H/L ratio of 1.01.

Synapsin Ia was observed as a single spot in all the 2-DE gels analyzed and results show that ratios of SGS742-treated sample vs NaCl-treated samples were increased about two fold (H/L ratio 2.35±0:21). Synapsin IIa was represented by seven individual spots and synapsin IIa total levels were increased by approximately 50% as compared to NaCl-treated controls. The unpaired Student's t-test of the synapsin Ia ratios between SGS742-treated vs NaCl-treated controls and SGS742-treated yoked vs NaCl-treated yoked controls showed that groups were statistically significant ($p < 0.006$). Only spot 7 of synapsin IIa and total synapsin IIa protein were statistically different ($p=0.02$; $p=0.017$). Quantitative proteomics data revealed that synapsin Ib and IIb isoform levels were not altered between SGS742-treated and NaCl-treated group and between both yoked control groups (table 5 and figure 18).

Table 4; Quantification of the relative abundance levels of individual synapsin protein expression forms SGS742-treated samples vs NaCl-treated samples and SGS742-treated yoked controls vs NaCl-treated yoked

Spot No	UniProtKB accession no.	Protein Name	Ratio± SD (SGS742-treated/ NaCl-treated control)	Ratio± SD (SGS742-treated yoked control/ NaCl-treated yoked control)	p-Value[a]
Spot 1	O88935	Synapsin Ia	2.35±0.21	0.66±0.12	0.006
Spot 2	O88935-1	Synapsin Ib	0.90±0.43	0.66±0.13	ns
Spot 3	O88935-1	Synapsin Ib	0.41±0.20	1.01±0.05	x
Spot 4	O88935-1	Synapsin Ib	1.10±0.07	0.92±0.09	x
Spot 5	Q64332	Synapsin IIa	2.20±0.30	0.84±0.16	x
Spot 6	Q64332	Synapsin IIa	1.79±0.54	0.67±0.36	x
Spot 7	Q64332	Synapsin IIa	1.30±0.30	0.84±0.05	0.02
Spot 8	Q64332	Synapsin IIa	1.43±0.73	0.85±0.23	x
Spot 9	Q64332	Synapsin IIa	1.43±0.56	0.82±0.04	x
Spot 10	Q64332	Synapsin IIa	1.26±0.32	0.91±0.08	x
Spot 11	Q64332-2	Synapsin IIa	1.00±0.05	1.07±0.01	x
Spot 12	Q64332-2	Synapsin IIb	1.02±0.42	0.96±0.17	x
Spot 13	Q64332-2	Synapsin IIb	0.87±0.98	0.93±0.01	ns
Spot 14	Q64332-2	Synapsin IIb	0.90±0.27	0.83±0.26	ns
Spot 15	Q64332-2	Synapsin IIb	0.66±0.42	0.83±0.23	ns
Spot 16	Q64332-2	Synapsin IIb	0.76±0.28	0.73±0.02	ns
Spot 17	Q64332-2	Synapsin IIb	0.73±0.08	0.85±0.19	ns

[a]p-values were obtained between mean ratios of SGS742-treated vs NaCl-treated controls and SGS742-treated yoked vs NaCl-treated yoked controls using unpaired Student's t-test.

x - not determined due to low sample amount

ns - not significant

Note that total synapsins Ia and IIa were statistically significantly increased in the SGS742-treated group.

Table 5: Results of synapsin quantification of total synapsin isoforms

Synapsin Isoforms	Number of Spots	Total(SGS742-treated/ NaCl-treated control Ratio± SD	Total (SGS742-treated yoked control/ NaCl-treated yoked control) Ratio± SD	Total p-Value
Synapsin Ia	1	2.35±0.21	0.66±0.12	0.006
Synapsin Ib	3	0.80±0.35	0.86±0.17	ns
Synapsin IIa	7	1.48±0.39	0.87±0.12	0.017
Synapsin IIb	6	0.82±0.13	0.86±0.08	ns

ns – not significant

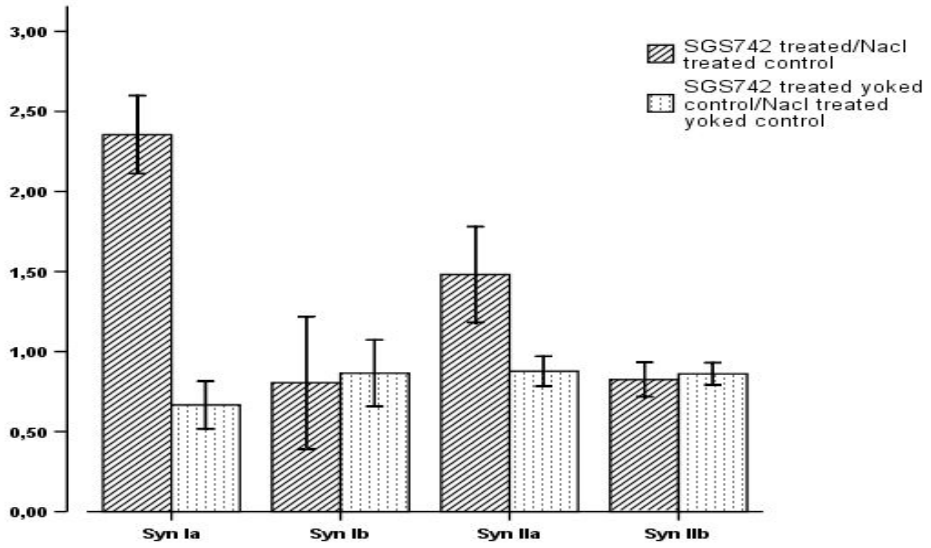


Figure 18; Experimental ratios (means±SD) of SGS742-treated/NaCl-treated control and SGS742-treated - yoked control/NaCl-treated yoked control obtained by Nano-LC-ESI-MS/MS mass spectrometry.

Relative total synapsin isoform expression from OF1 mice with SGS742-treated/NaCl -treated controls and SGS742 -treated yoked controls/NaCl-treated-yoked controls, sacrificed 6 h after the probe trial in the MWM. Each bar represents mean ± SD. Asterisks indicate significance of difference of synapsin Ia and synapsin IIa protein expression in SGS742 -treated mice as compared to yoked controls (paired–Student's t-test; ***P<0.006, *P<0.017).

5 Discussion

5.1 Characterization synapsins and its splice varients using mass spectrometry

Synapsin isoforms and their post-translational modifications play an important role in the regulation of neurotransmission but their precise function has been difficult to pinpoint. Detection of synapsin isoforms as protein spots in 2-DE gels from mouse hippocampus made us investigate the primary structure of synapsin isoforms and their post-translational modifications. Moreover, protein chemical characterization of synapsin II isoforms have not been studied as extensively as those of synapsin I. Combining mass spectrometry (MALDI-TOF-TOF, nano-ESI-LC-MS/MS and LTQ-FT), and enzymatic digestion (trypsin, Asp-N, Lys-C and non-specific enzyme subtilisin) was used and analysis shows the presence of two synapsin Ia protein spots, seven synapsin IIa and six synapsin IIb protein spots in the 2-D gels from mouse hippocampus. Combination of multi-enzyme digestion and mass spectrometry allowed for high sequence coverage of individual synapsin isoforms (table 1), particularly 94 percent of sequence coverage was achieved in the case of synapsin (Siow, Chilcote et al. 1992) IIb protein spots (spot 12).

The isolation of individual isoforms of individual synapsins is technically difficult using conventional purification techniques, because of the extent homology between primary sequences. Moreover, synapsin II is more hydrophobic than synapsin I and it is therefore difficult to purify synapsin II isoforms from brain tissue. These problems have been overcome by the expression of recombinant synapsin IIa in the eukaryotic expression system (Gianazza 1995). Our experimental approach showed that 2-DE based mass spectrometry analysis, may be a complementary approach for characterization of primary structure of synapsin II isoforms *ex-vivo* by obtaining high sequence coverage and post-translational modifications. Deamidation of an asparagine (N9) and glutamine (Q) residues to aspartate (D) and glutamate (E) residues; respectively, is an effect of protein aging, and is often observed in 2-D gels (Tsai, Bruner et al. 1993; Karty, Ireland et al. 2002). Deamidation leads to a series of spots with the same Mr, but measurably different pI (Johnson, Shirokawa et al. 1989). The presence of spot trains in the 2-DE gel can be explained by the existence of a heterogeneous mixture of deamidated protein species. Potential sites of deamidation were identified in "thermally stressed" samples of recombinant human growth hormone (Paranandi, Guzzetta et al. 1994) and recombinant tissue

plasminogen activator (Zhan, Giorgianni et al. 2005). Deamidation pattern (ladder of protein spots) of growth hormone (GH) isoforms in the 2-DE gel were identified and characterized by mass spectrometry (Hilfiker, Benfenati et al. 2005). Both MS and MS/MS analysis of individual protein spots clearly showed that the number of deamidated peptides differs from one spot to the other (sup table 2, 3 and 4). Herein, results clearly show deamidation susceptibility of synapsin isoforms. Deamidation of glutamine to glutamate occurred predominantly in the synapsin IIa and synapsin IIb protein spots (table 2, 3 and 4).

This study presents the detailed mapping of protein sequence of synapsin isoforms by tandem mass spectrometry and PMF. The major focus of this work was to apply mass spectrometry technique to reveal protein characterization and identification of specific synapsin isoforms, as they were not fully shown at the protein level. This was accomplished by analyzing multiple protease digest of synapsin isoforms in the 2-DE followed by MALDI-TOF-TOF, nano-ESI-LC-MS/MS or LTQ-FT. All four synapsins are substrates for protein kinases: cAMP-dependent protein kinase and CaM kinase, which phosphorylate serine residues in the NH2 terminal region. Synapsin I, but not synapsin II, is an excellent substrate for CaM kinase II, which phosphorylates two serine residues in the COOH-terminal region of synapsin I (sites 1 and 2) (De Camilli, Benfenati et al. 1990). Synapsin phosphorylations have been well-studied and were found to regulate function of synapsins (Valtorta, Greengard et al. 1992). Detailed analysis of *ex-vivo* phosphorylation described herein, revealed a new phosphorylation site at Ser-546 in synapsin IIa. This new phosphorylated site is located in the C-terminal domain E of synapsin IIa. All synapsin isoforms are sharing the C-terminal domains of the A-type that plays an important role in both, pre-docking and the post-docking steps of synaptic vesicle exocytosis (Pieribone, Shupliakov et al. 1995; Gitler, Xu et al. 2004) and seem to be required for correct targeting of the synapsins to synaptic vesicles (Fassio, Merlo et al. 2006). How ever, it was stated in a recent report that domain E increases synaptic efficiency by accelerating both the kinetics of exocytosis and the rate of synaptic vesicle cycling (Li, Hornshaw et al. 2004). Interestingly, the equivalent site in bovine synapsin I, Ser-666 was identified as phosphorylated *in-vitro* by CaM kinase II (DeGiorgis, Jaffe et al. 2005) (figure 19). The phosphorylation site Ser-666 was part of the consensus sequence known to be phosphorylated by CaM kinase- II (Hyd- X- R/K- NB-X- S/T-Hyd, where Hyd= hydrophobic, NB= nonbasic, and X may be any residue)(Merrall, Plevin et al. 1993;

Stokoe, Caudwell et al. 1993; Songyang, Lu et al. 1996; White, Kwon et al. 1998). These proposed conserved sequences of CaM Kinase II are extremely conserved throughout all synapsins A-type sequences including synapsin IIa (figure 19). Identification of a new phosphorylated site at Ser-546 in synapsin IIa in the consensus sequence of CaM kinase II, made us conclude that both, synapsin I and synapsin IIa are most likely substrates for CaM kinase II. The exact function of Ser-546 phosphorylation at the E-domain, however, remains to be evaluated in further functional studies. Table 6 indicates identified phosphorylation and predicted consensus sequences for kinases responsible for phosphorylation on Synapsin isoform using web based ELM (EMBL) program.

```
gi|1351166|sp|P17599|SYN1_BOVI      PSQDVPPPATAAAGGPPHPQLNKSQSLTNAFNLPEPAPPRPSLSQDEVKA
gi|6686305|sp|P09951|SYN1_RAT_      PSQDVPPPIIAAAGGPPHPQLNKSQSLTNAFNLPEPAPPRPSLSQDEVKA
gi|73920800|sp|P17600|SYN1_HUM      PSQDVPPPATAAAGGPPHPQLNKSQSLTNAFNLPEPAPPRPSLSQDEVKA
gi|73920802|sp|O88935|SYN1_MOU      PSQDVPPPITAAAGGPPHPQLNKSQSLTNAFNLPEPAPPRPSLSQDEVKA
gi|73920803|sp|Q64332|SYN2_MOU      ----------------HPQLNKSQSLTNAFSFSESSFFRSSANEDEAKA
gi|6685997|sp|Q63537|SYN2_RAT_      ----------------HPQLNKSQSLTNAFSFSESSFFRSSANEDEAKA
gi|6686022|sp|Q92777|SYN2_HUMA      ----------------HPQLNKSQSLTNAFSFSESSFFRSSANEDEAKA
                                    ***************.::*.:  *.* .:**.**
```

Figure 19; Alignment of amino acid sequences of mouse, rat, bovine, and human synapsin I and II isoforms. The consensus sequence predicted to be phosphorylated by CaM kinase-2 (Hyd- X- R/K- NB-X- S/T-Hyd, where Hyd= hydrophobic, NB= nonbasic, and X may be any residue) is marked in the box.

In addition to a new phosphorylation site we confirmed two previously characterized PKA and CaM kinase I phosphorylation sites at Ser-9 (site 1) and CaM kinase II phosphorylation site at Ser-568 in synapsin I through PMF analysis following enzymatic digestion with trypsin. The analysis by MS and MS/MS experiments with Asp-N digest of synapsin IIa and IIb, revealed for the first time unambiguously identified a PKA and CaM kinase I phosphorylation site at Ser-9 or Ser 10 in the mouse organism. A previous study showed that N-terminal phosphorylation of this PKA and CaM kinase I site of all the synapsin, modulates their binding to synaptic vesicle68 (Hosaka, Hammer et al. 1999). Phosphorylation of synapsin Ia at Ser-568 by CaM kinase II abolishes the bundling activity of actin filaments(Polevoda and Sherman 2003).

Table.6 Identified phosphorylation sites and predicted consensus sequence for kinases responsible for phosphorylation on Synapsin isoform

Protein Name	Phosphopeptide	Site of Phosphorylation	Predicted kinases	Consensus pattern
Synapsin Ia	^{8}Lp**S**DSNFMANLPNGYMTDLQR^{27}	Ser-9	CaM kinase I	[RK][RK].[ST]...
Synapsin IIa and Synapsin IIb	Ac-M^{1}MNFLRRRLp**S**^{10}	Ser-10	PKA and CaM kinase I	[RK][RK].[ST]...
Synapsin IIa and Synapsin IIb	Ac-M^{2}NFLRRRLp**S**^{9}	Ser-9	PKA and CaM kinase I	[RK][RK].[ST]...
Synapsin Ia	^{54}ASTAAPVAp**S**PAAPSPGSSGGGGFFSSLSNAVK^{65}	Ser-62	Proline-Directed Kinase (e.g. MAPK)	..([ST])P..
Synapsin IIa	^{544}SQp**S**LTNAFSFSESSFFR^{560}	Ser-546	CaM kinase- II	Hyd- X- R/K- NB-X- S/T-Hyd$^{63-65}$
Synapsin IIa and Synapsin IIb	^{422}p**T**PALp**S**PQRPLTTQQPQSGTLK^{442}	T-422, S-426	Proline-Directed Kinase (e.g. MAPK)	..([ST])P..

Hyd-hyrophobic amino acid
NB-Non-basic aminoacid

N-terminal acetylation, together with N-terminal methionine cleavage, is the most common protein modification in eukaryotic cells. Over 40% of all yeast proteins and almost 90% of mammalian proteins are estimated to be N-terminally acetylated 70 (Driessen, de Jong et al. 1985; Persson, Flinta et al. 1985; Lee, Lin et al. 1989; Polevoda and Sherman 2003). Protein N-terminal methionine cleavage is an essential co-translation process that occurs in the cytoplasm of all organism(Giglione, Boularot et al. 2004). Cleavage of N-terminal methionine is catalyzed by methionine aminopeptidases (Fassio, Merlo et al.) (Kendall, Yamada et al. 1990). There are two types of reported MAPs described in eukaryotes; MAP 1 and MAP 2 (Kendall, Yamada et al. 1990; Li and Chang 1995). However, experimental evidence has been presented that removal of methionine is dependent on whether the penultimate residue has a radius of gyration that is less than 1.29 Å (example bulky, acidic, or hydrophobic amino acids). N-terminal acetylation occurs co-translationally when nascent peptides are between 20 and 50 amino acids long (Driessen, de Jong et al. 1985; Bradshaw, Brickey et al. 1998). The addition of the acetyl group is catalyzed by N-terminal acetyl transferase (NATs) (Polevoda and Sherman 2003). In *Saccharomyces cervisiae* there are known NATs: NatA, NatB, and NatC (Giglione, Boularot et al. 2004). NATs act on substrates with specific but degenerated N-terminal regions, required for the activity of each NAT (Polevoda and Sherman 2000). In the case of NatB an N-terminal sequence of MD-, ME-, MN-, or MM- is required for acetylation (Polevoda, Cardillo et al. 2003). NatB acetylates the first methionine of the nascent chain not subjected to methionine cleavage. The synapsin I isoform contains the N-terminal amino acid sequence MN-. Synapsin IIa and IIb contain MM- in the N-terminal postion. N-terminal acetylation was described in bovine synapsin I by LC-MS analysis (Matsubara, Kusubata et al. 1996) but not in synapsin II isoforms. Our results showed discrepancy in the N-terminal acetylation process by acetylating

first methionine and also acetylating the second methionine after the cleavage of the first methionine in both synapsin II isoforms. On the contrary, our results showed that first methionine could be removed if the penultimate residue has a high radius of gyration value; i.e. Met-2. The loss of methionine could not cause any pI shift and molecular weight change in the 2-DE gels. Finally we conclude that synapsin IIa and IIb proteins, show NH4-terminal acetylated methionine (Ac-Met-1) and a protein corresponding to the second methionine (Ac-Met-2) acetylated after cleavage of the initiator methionine. The biological significance of N-terminal acetylation as well as methionine removal in the amino terminal region close to PKA and CaM kinase I phosphorylation site, certainly deserves further in depth analysis.

In addition to the above mentioned modifications, we detected methylation of certain glutamic acid and aspargine residues. Methylation of acidic moieties can occur for several different reasons. The most common ones are artifacts from electrophoresis, especially in colloidal Coomassie staining of proteins (Haebel, Albrecht et al. 1998), on the other hand, methylation is an important post-translational modifications with biological consequences.

5.2 Hippocampal synapsin isoforms protein levels are linked to cognitive enhancement of memory formation in the mouse

Pharmacological cognitive enhancement is a main topic in neuroscience and SGS742 has been already passing clinical trials (phase II) (Froestl, Gallagher et al. 2004). Although GABAB antagonism is the mechanism proposed and a role for somatostatin was suggested (Pittaluga, Feligioni et al. 2001) downstream cascades and effectors have not been reported to the best of our knowledge and own work could not verify a role for somatostatin in CE (Sunyer, Shim et al. 2008).

Herein, increased hippocampal synapsin Ia and IIa levels are linked to SGS742-mediated spatial memory enhancement in OF1 mice. The gel-based mass spectrometry approach used allowed unambiguous identification of 17 synapsin expression forms (17 well-separated protein spots with individual apparent molecular weights and isoelectric points; supplementary tables 5-7) that represent the four known isoforms Ia, Ib, IIa and IIb along with their modifications. So far existing literature using immunoblotting could not discriminate between these expression forms because of cross-reactivity of the antibodies due to high sequence similarities.

Synapsin Ia and IIa levels were increased following SGS742-induced cognitive enhancement in the MWM in contrast to the Ib and IIb isoforms. Only one isoform for synapsin Ia was observed in OF1-mouse hippocampus and this was linked to CE. Total synapsin IIa isoforms (n=7) were also increased as was an individual expression form for IIa (spot number 7, see table 4). This result may show that individual isoforms may be involved in the mechanism of CE and, moreover, show that the many isoforms may not be redundant.

Assignment to CE is fair because neither hippocampal Ia and IIa isoforms were increased in NaCl-treated mice nor in SGS742-treated yoked mice. SGS742-treated yoked mice were introduced to rule out that increased Ia and IIa synapsin levels were

simply induced by SGS742 treatment. As the MWM did not contain a platform testing yoked controls, memory formation was avoided. In addition, the effect of swim stress on synapsin levels could be ruled out using the yoked control system.

Quantification of a series of unambiguously identified synapsin expression forms (tables 4 and 5) was carried out by a reliable protocol (Asara, Zhang et al. 2006), although not all of the 17 spots could be quantified due to limited amounts of sample available. The method used, in-gel stable isotope labeling for relative quantification using mass spectrometry, however, clearly indicated the ratios between heavy labeled and light labeled synapsin peptides (figure 15). The quantification method revealed an intriguing about two fold increase of synapsin Ia (for synapsin IIa an about 50% increase) six hours following the probe trial in the MWM revealing the rapid and high potential of presynaptic synapsin synthesis at the protein level.

6 Summary and conclusion

In this Ph.D. thesis, proteomics applications were used to study the structural and biological function of synapsins. First part of this PhD study was to mainly focus on to reveal complex molecular heterogeneity of synapsins in the 2-DE gel and identify post transaltional modification of synapsins. Second part this study was deals with the generated structural informations of synapsins were used to link individual synapsin expressional pattern and their involvement in cognitive enhancement and spatial memory formation.

To our knowledge, no reports on heterogenicity of synapsin isoforms in mouse hippocampus are available and the issue of identifying several isoforms on a 2-DE gel is a major challenge. Combination of high-resolution 2-DE with two different mass spectrometry principles (MS) is a reliable method to determine synapsin isoform modifications of sequences and post-translational modifications. Fifteen synapsin isoform protein spots were characterized by mass spectrometry as individual synapsin isoforms, synapsin Ia, synapsin IIa and synapsin IIb. Serine phosphorylation sites were confirmed for Ser-9, Ser-10, Ser-62, S-426, Thr-422 and a novel phosphorylation site was observed at Ser-546 in synapsin IIa. A series of deamidation sites were observed in the individual protein spots. The results may represent a step forward in the protein chemical characterization of these most important neural elements as the molecular basis of synaptic function is of major interest to neuroscience. Knowledge of the individual isoforms and their modifications is a prerequisite for all neurobiological studies at the protein level.

There are several reports were indicated that the role of synapsin in spatial memory formation and knockout mice analysis for synapsins genes showed that impairment in learning and memory formation. But no evidence were available the role of individual splice variants of synapsins in spatial memory enhancement. This was the aim of my

second part of PhD study to link synapsin isoform expression and cognitive enhancement Working on cognitive enhancement (CE) using the cognitive enhancer SGS742 we were therefore interested if cognitive enhancement by this compound would be involving synapsin regulation. SGS742 is a GABAB receptor antagonist (Sunyer, Patil et al. 2008) that has been shown to enhance spatial memory in rodents and even humans (Froestl, Gallagher et al. 2004) (Helm, Haberman et al. 2005) (Mondadori, Jaekel et al. 1993) (Nakagawa and Takashima 1997) Knowledge of primary sequence information that we obtained from first part of my studies were used in this study, combined with quantitative proteomics approach we could able to show dynamics of synapsin expression during cognitive enhancement and spatial memory formation. A role for synapsins Ia and IIa is proposed to play a role in spatial memory enhancement in the OF-1 mouse by GABAB antagonist SGS742. Increased synapsin Ia and IIa hippocampal levels can be assigned to CE and not to drug effects or swim stress as ruled out by the use of two appropriate control systems, NaCl-treatment and drug-yoked controls. It is further suggested to use the shown gel-based proteomic approach for the detection and individual quantification of synapsins that is of utmost importance because individual synapsin expression forms may be representing individual functions.

6.1 Zusammenfassung

In dieser PhD-Arbeit wurde die Proteomiktechnologie verwendet, um die Struktur und Funktion der Synapsine am besten untersuchen zu können. Der erste Teil dieser Arbeit beschäftigt sich hauptsächlich mit der molekularen Heterogenität der Synapsine mit der 2-DE und der posttranslationellen Modifikation. Der zweite Teil der Arbeit bestand darin die daraus gewonnenen Struktur-Informationen mit der kognitiven Funktion und der Entwicklung eines räumlichen Gedächtnisses zu verknüpfen.

Bisher gab es keine Berichte bezüglich der Heterogenität der Synapsin-Isoformen im Hippocampus der Maus und vor allem die Identifizierung der unterschiedlichen Isoformen mittels der 2-DE stellt eine große Schwierigkeit dar.

Die Kombination einer hochauflösenden 2-DE mit zwei unterschiedlichen Massenspektrometriemethoden hat sich als sehr verlässliche Methode herausgestellt um Sequenz- und posttranslationelle Modifikationen der Synapsin-Isoformen zu bestimmen. Serin Phosphorilierungsstellen konnten für Ser-9, Ser-10, Ser-62, Ser-568 und T-422 nachgewiesen werden. Außerdem konnte eine neue Phosphorilierungsstelle bei Ser-546 in Synapsin IIa gezeigt werden. Weiters konnte eine Serie von Deamidierungen in einzelnen Protein-Spots nachgewiesen werden. Diese in meiner Arbeit gewonnenen Resultate könnten ein großer Schritt in Richtung besseren Verständnis und der Proteincharakterisierung eines der wichtigsten neuralen Elemente in der Neurowissenschaft sein. Das Wissen über unterschiedliche Isoformen und deren Modifikationen ist Vorraussetzung für alle neurobiologischen Studien auf dem Proteinlevel.

Unterschiedliche Studien über die Rolle von Synapsin in der Entwicklung des räumlichen Gedächtnisses und mit Synapsin knock-out Mäusen konnten zeigen, dass ein Fehlen von Synapsin große Lernschwierigkeiten zu Folge hat.

Nichtsdestotrotz konnten aber keine direkten Beweise für die Funktion bei der Entwicklung eines räumlichen Gedächtnisses gefunden werden. Das Ziel des zweiten Teils meiner Arbeit war unter anderem auch, den kognitiven Verstärker SGS742 zu untersuchen um zu sehen, welche Rolle dieser bei der Entwicklung eines räumlichen Gedächtnisses spielt. SGS742 ist ein GABA-B Antagonist (Sunyer, Patil et al. 2007), der erwiesenermaßen eine Rolle in der Entwicklung eines räumlichen Gedächtnisses bei Nagetieren und eventuell auch im Menschen spielt (Froestl (Froestl, Gallagher et al. 2004; Helm, Haberman et al. 2005) (Mondadori, Jaekel et al. 1993; Nakagawa and Takashima 1997)In meiner Arbeit konnte gezeigt werden, dass die Synapsine Ia und IIa bei OF-1 Mäusen unter Gabe von SGS742 eine wichtige Rolle spielen. Erhöhte hippokampale Level von Synapsin Ia und IIa können direkt auf die kognitive Funktion zurückgeführt werden. Medikamenteneffekte, sowie Stress durch Schwimmen konnte durch die Verwendung geeigneter Kontrollen ausgeschlossen werden. Weiters sollte darauf hingewiesen werden, dass die verwendete Methode von äußerster Wichtigkeit ist. Einzelne Formen der Synapsinexpression sowie noch unbekannten Funktion könnten daraus abgeleitet werden.

7 Reference

1. Anderson, N. L. and N. G. Anderson (1991). "A two-dimensional gel database of human plasma proteins." Electrophoresis 12(11): 883-906.
2. Asara, J. M., X. Zhang, et al. (2006). "In-Gel Stable-Isotope Labeling (ISIL): a strategy for mass spectrometry-based relative quantification." J Proteome Res 5(1): 155-63.
3. Asara, J. M., X. Zhang, et al. (2006). "In-gel stable isotope labeling for relative quantification using mass spectrometry." Nat Protoc 1(1): 46-51.
4. Benfenati, F., F. Valtorta, et al. (1992). "Interaction of free and synaptic vesicle-bound synapsin I with F-actin." Neuron 8(2): 377-86.
5. Bonanomi, D., A. Menegon, et al. (2005). "Phosphorylation of synapsin I by cAMP-dependent protein kinase controls synaptic vesicle dynamics in developing neurons." J Neurosci 25(32): 7299-308.
6. Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." Anal Biochem 72: 248-54.
7. Bradshaw, R. A., W. W. Brickey, et al. (1998). "N-terminal processing: the methionine aminopeptidase and N alpha-acetyl transferase families." Trends Biochem Sci 23(7): 263-7.
8. Browning, M. D., C. K. Huang, et al. (1987). "Similarities between protein IIIa and protein IIIb, two prominent synaptic vesicle-associated phosphoproteins." J Neurosci 7(3): 847-53.
9. Cheetham, J. J., S. Hilfiker, et al. (2001). "Identification of synapsin I peptides that insert into lipid membranes." Biochem J 354(Pt 1): 57-66.
10. Chen, W. Q., S. U. Kang, et al. (2006). "Protein profiling by the combination of two independent mass spectrometry techniques." Nat Protoc 1(3): 1446-52.
11. Chi, P., P. Greengard, et al. (2001). "Synapsin dispersion and reclustering during synaptic activity." Nat Neurosci 4(12): 1187-93.
12. Chi, P., P. Greengard, et al. (2003). "Synaptic vesicle mobilization is regulated by distinct synapsin I phosphorylation pathways at different frequencies." Neuron 38(1): 69-78.

13. Chin, L. S., L. Li, et al. (1995). "Impairment of axonal development and of synaptogenesis in hippocampal neurons of synapsin I-deficient mice." Proc Natl Acad Sci U S A 92(20): 9230-4.
14. Chin, L. S., L. Li, et al. (1994). "Neuron-specific expression of the synapsin II gene is directed by a specific core promoter and upstream regulatory elements." J Biol Chem 269(28): 18507-13.
15. Corradi, A., A. Zanardi, et al. (2008). "Synapsin-I- and synapsin-II-null mice display an increased age-dependent cognitive impairment." J Cell Sci 121(Pt 18): 3042-51.
16. De Camilli, P., F. Benfenati, et al. (1990). "The synapsins." Annu Rev Cell Biol 6: 433-60.
17. De Camilli, P., R. Cameron, et al. (1983). "Synapsin I (protein I), a nerve terminal-specific phosphoprotein. I. Its general distribution in synapses of the central and peripheral nervous system demonstrated by immunofluorescence in frozen and plastic sections." J Cell Biol 96(5): 1337-54.
18. De Camilli, P., S. M. Harris, Jr., et al. (1983). "Synapsin I (Protein I), a nerve terminal-specific phosphoprotein. II. Its specific association with synaptic vesicles demonstrated by immunocytochemistry in agarose-embedded synaptosomes." J Cell Biol 96(5): 1355-73.
19. DeGiorgis, J. A., H. Jaffe, et al. (2005). "Phosphoproteomic analysis of synaptosomes from human cerebral cortex." J Proteome Res 4(2): 306-15.
20. Driessen, H. P., W. W. de Jong, et al. (1985). "The mechanism of N-terminal acetylation of proteins." CRC Crit Rev Biochem 18(4): 281-325.
21. Esser, L., C. R. Wang, et al. (1998). "Synapsin I is structurally similar to ATP-utilizing enzymes." Embo J 17(4): 977-84.
22. Fassio, A., D. Merlo, et al. (2006). "The synapsin domain E accelerates the exoendocytotic cycle of synaptic vesicles in cerebellar Purkinje cells." J Cell Sci 119(Pt 20): 4257-68.
23. Fdez, E. and S. Hilfiker (2006). "Vesicle pools and synapsins: new insights into old enigmas." Brain Cell Biol 35(2-3): 107-15.
24. Ferreira, A., H. Q. Han, et al. (1995). "Suppression of synapsin II inhibits the formation and maintenance of synapses in hippocampal culture." Proc Natl Acad Sci U S A 92(20): 9225-9.

25. Ferreira, A., K. S. Kosik, et al. (1994). "Aberrant neurites and synaptic vesicle protein deficiency in synapsin II-depleted neurons." Science 264(5161): 977-9.
26. Fesce, R., F. Benfenati, et al. (1992). "Effects of the neuronal phosphoprotein synapsin I on actin polymerization. II. Analytical interpretation of kinetic curves." J Biol Chem 267(16): 11289-99.
27. Froestl, W., M. Gallagher, et al. (2004). "SGS742: the first GABA(B) receptor antagonist in clinical trials." Biochem Pharmacol 68(8): 1479-87.
28. Gianazza, E. (1995). "Isoelectric focusing as a tool for the investigation of post-translational processing and chemical modifications of proteins." J Chromatogr A 705(1): 67-87.
29. Giglione, C., A. Boularot, et al. (2004). "Protein N-terminal methionine excision." Cell Mol Life Sci 61(12): 1455-74.
30. Gitler, D., Y. Xu, et al. (2004). "Molecular determinants of synapsin targeting to presynaptic terminals." J Neurosci 24(14): 3711-20.
31. Goelz, S. E., E. J. Nestler, et al. (1981). "Distribution of protein I in mammalian brain as determined by a detergent-based radioimmunoassay." Proc Natl Acad Sci U S A 78(4): 2130-4.
32. Gomez-Pinilla, F., V. So, et al. (2001). "Spatial learning induces neurotrophin receptor and synapsin I in the hippocampus." Brain Res 904(1): 13-9.
33. Greengard, P., F. Benfenati, et al. (1994). "Synapsin I, an actin-binding protein regulating synaptic vesicle traffic in the nerve terminal." Adv Second Messenger Phosphoprotein Res 29: 31-45.
34. Greengard, P., M. D. Browning, et al. (1987). "Synapsin I, a phosphoprotein associated with synaptic vesicles: possible role in regulation of neurotransmitter release." Adv Exp Med Biol 221: 135-53.
35. Greengard, P., F. Valtorta, et al. (1993). "Synaptic vesicle phosphoproteins and regulation of synaptic function." Science 259(5096): 780-5.
36. Haebel, S., T. Albrecht, et al. (1998). "Electrophoresis-related protein modification: alkylation of carboxy residues revealed by mass spectrometry." Electrophoresis 19(5): 679-86.
37. Han, H. Q., R. A. Nichols, et al. (1991). "Induction of formation of presynaptic terminals in neuroblastoma cells by synapsin IIb." Nature 349(6311): 697-700.
38. Helm, K. A., R. P. Haberman, et al. (2005). "GABAB receptor antagonist SGS742 improves spatial memory and reduces protein binding to the cAMP

response element (CRE) in the hippocampus." Neuropharmacology 48(7): 956-64.

39. Hilfiker, S., F. Benfenati, et al. (2005). "Structural domains involved in the regulation of transmitter release by synapsins." J Neurosci 25(10): 2658-69.
40. Hilfiker, S., V. A. Pieribone, et al. (1999). "Synapsins as regulators of neurotransmitter release." Philos Trans R Soc Lond B Biol Sci 354(1381): 269-79.
41. Hilfiker, S., F. E. Schweizer, et al. (1998). "Two sites of action for synapsin domain E in regulating neurotransmitter release." Nat Neurosci 1(1): 29-35.
42. Hosaka, M., R. E. Hammer, et al. (1999). "A phospho-switch controls the dynamic association of synapsins with synaptic vesicles." Neuron 24(2): 377-87.
43. Hosaka, M. and T. C. Sudhof (1998). "Synapsin III, a novel synapsin with an unusual regulation by Ca2+." J Biol Chem 273(22): 13371-4.
44. Hosaka, M. and T. C. Sudhof (1998). "Synapsins I and II are ATP-binding proteins with differential Ca2+ regulation." J Biol Chem 273(3): 1425-9.
45. Humeau, Y., F. Doussau, et al. (2001). "Synapsin controls both reserve and releasable synaptic vesicle pools during neuronal activity and short-term plasticity in Aplysia." J Neurosci 21(12): 4195-206.
46. Huttner, W. B., W. Schiebler, et al. (1983). "Synapsin I (protein I), a nerve terminal-specific phosphoprotein. III. Its association with synaptic vesicles studied in a highly purified synaptic vesicle preparation." J Cell Biol 96(5): 1374-88.
47. Jensen, V., S. I. Walaas, et al. (2007). "A delayed response enhancement during hippocampal presynaptic plasticity in mice." J Physiol 583(Pt 1): 129-43.
48. John, J. P., W. Q. Chen, et al. (2007). "Mass spectrometric studies on mouse hippocampal synapsins Ia, IIa, and IIb and identification of a novel phosphorylation site at serine-546." J Proteome Res 6(7): 2695-710.
49. Johnson, B. A., J. M. Shirokawa, et al. (1989). "Formation of isoaspartate at two distinct sites during in vitro aging of human growth hormone." J Biol Chem 264(24): 14262-71.
50. Jovanovic, J. N., F. Benfenati, et al. (1996). "Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions." Proc Natl Acad Sci U S A 93(8): 3679-83.

51. Jovanovic, J. N., A. J. Czernik, et al. (2000). "Synapsins as mediators of BDNF-enhanced neurotransmitter release." Nat Neurosci 3(4): 323-9.
52. Kao, H. T., B. Porton, et al. (1998). "A third member of the synapsin gene family." Proc Natl Acad Sci U S A 95(8): 4667-72.
53. Kao, H. T., B. Porton, et al. (1999). "Molecular evolution of the synapsin gene family." J Exp Zool 285(4): 360-77.
54. Karty, J. A., M. M. Ireland, et al. (2002). "Artifacts and unassigned masses encountered in peptide mass mapping." J Chromatogr B Analyt Technol Biomed Life Sci 782(1-2): 363-83.
55. Kelloff, G. J. and C. C. Sigman (2005). "New science-based endpoints to accelerate oncology drug development." Eur J Cancer 41(4): 491-501.
56. Kendall, R. L., R. Yamada, et al. (1990). "Cotranslational amino-terminal processing." Methods Enzymol 185: 398-407.
57. Kipnis, J., H. Cohen, et al. (2004). "T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions." Proc Natl Acad Sci U S A 101(21): 8180-5.
58. Kushner, S. A., Y. Elgersma, et al. (2005). "Modulation of presynaptic plasticity and learning by the H-ras/extracellular signal-regulated kinase/synapsin I signaling pathway." J Neurosci 25(42): 9721-34.
59. Lee, F. J., L. W. Lin, et al. (1989). "N alpha-acetyltransferase deficiency alters protein synthesis in Saccharomyces cerevisiae." FEBS Lett 256(1-2): 139-42.
60. Li, K. W., M. P. Hornshaw, et al. (2004). "Proteomics analysis of rat brain postsynaptic density. Implications of the diverse protein functional groups for the integration of synaptic physiology." J Biol Chem 279(2): 987-1002.
61. Li, L., L. S. Chin, et al. (1995). "Localization of the synapsin II (SYN2) gene to human chromosome 3 and mouse chromosome 6." Genomics 28(2): 365-6.
62. Li, X. and Y. H. Chang (1995). "Amino-terminal protein processing in Saccharomyces cerevisiae is an essential function that requires two distinct methionine aminopeptidases." Proc Natl Acad Sci U S A 92(26): 12357-61.
63. Li, X., T. W. Rosahl, et al. (1995). "Mapping of synapsin II (SYN2) genes to human chromosome 3p and mouse chromosome 6 band F." Cytogenet Cell Genet 71(3): 301-5.
64. Luthi, T., R. S. Haltiwanger, et al. (1991). "Synapsins contain O-linked N-acetylglucosamine." J Neurochem 56(5): 1493-8.

65. Matsubara, M., M. Kusubata, et al. (1996). "Site-specific phosphorylation of synapsin I by mitogen-activated protein kinase and Cdk5 and its effects on physiological functions." J Biol Chem 271(35): 21108-13.
66. Maucuer, A., J. P. Le Caer, et al. (2000). "Specific Ser-Pro phosphorylation by the RNA-recognition motif containing kinase KIS." Eur J Biochem 267(14): 4456-64.
67. McLachlin, D. T. and B. T. Chait (2003). "Improved beta-elimination-based affinity purification strategy for enrichment of phosphopeptides." Anal Chem 75(24): 6826-36.
68. Menegon, A., D. Bonanomi, et al. (2006). "Protein kinase A-mediated synapsin I phosphorylation is a central modulator of Ca2+-dependent synaptic activity." J Neurosci 26(45): 11670-81.
69. Merrall, N. W., R. J. Plevin, et al. (1993). "Mitogen-activated protein kinase (MAP kinase), MAP kinase kinase and c-Mos stimulate glucose transport in Xenopus oocytes." Biochem J 295 (Pt 2): 351-5.
70. Michels, B., S. Diegelmann, et al. (2005). "A role for Synapsin in associative learning: the Drosophila larva as a study case." Learn Mem 12(3): 224-31.
71. Mondadori, C., J. Jaekel, et al. (1993). "CGP 36742: the first orally active GABAB blocker improves the cognitive performance of mice, rats, and rhesus monkeys." Behav Neural Biol 60(1): 62-8.
72. Munton, R. P., R. Tweedie-Cullen, et al. (2007). "Qualitative and quantitative analyses of protein phosphorylation in naive and stimulated mouse synaptosomal preparations." Mol Cell Proteomics 6(2): 283-93.
73. Murrey, H. E., C. I. Gama, et al. (2006). "Protein fucosylation regulates synapsin Ia/Ib expression and neuronal morphology in primary hippocampal neurons." Proc Natl Acad Sci U S A 103(1): 21-6.
74. Nakagawa, Y. and T. Takashima (1997). "The GABA(B) receptor antagonist CGP36742 attenuates the baclofen- and scopolamine-induced deficit in Morris water maze task in rats." Brain Res 766(1-2): 101-6.
75. Nestler, E. J. and P. Greengard (1983). "Protein phosphorylation in the brain." Nature 305(5935): 583-8.
76. Paranandi, M. V., A. W. Guzzetta, et al. (1994). "Deamidation and isoaspartate formation during in vitro aging of recombinant tissue plasminogen activator." J Biol Chem 269(1): 243-53.

77. Persson, B., C. Flinta, et al. (1985). "Structures of N-terminally acetylated proteins." Eur J Biochem 152(3): 523-7.
78. Pieribone, V. A., O. Shupliakov, et al. (1995). "Distinct pools of synaptic vesicles in neurotransmitter release." Nature 375(6531): 493-7.
79. Pittaluga, A., M. Feligioni, et al. (2001). "Potentiation of NMDA receptor function through somatostatin release: a possible mechanism for the cognition-enhancing activity of GABA(B) receptor antagonists." Neuropharmacology 41(3): 301-10.
80. Polevoda, B., T. S. Cardillo, et al. (2003). "Nat3p and Mdm20p are required for function of yeast NatB Nalpha-terminal acetyltransferase and of actin and tropomyosin." J Biol Chem 278(33): 30686-97.
81. Polevoda, B. and F. Sherman (2000). "Nalpha -terminal acetylation of eukaryotic proteins." J Biol Chem 275(47): 36479-82.
82. Polevoda, B. and F. Sherman (2003). "N-terminal acetyltransferases and sequence requirements for N-terminal acetylation of eukaryotic proteins." J Mol Biol 325(4): 595-622.
83. Rosahl, T. W., M. Geppert, et al. (1993). "Short-term synaptic plasticity is altered in mice lacking synapsin I." Cell 75(4): 661-70.
84. Rosahl, T. W., D. Spillane, et al. (1995). "Essential functions of synapsins I and II in synaptic vesicle regulation." Nature 375(6531): 488-93.
85. Ryan, T. A., L. Li, et al. (1996). "Synaptic vesicle recycling in synapsin I knock-out mice." J Cell Biol 134(5): 1219-27.
86. Sakurada, K., H. Kato, et al. (2002). "Synapsin I is phosphorylated at Ser603 by p21-activated kinases (PAKs) in vitro and in PC12 cells stimulated with bradykinin." J Biol Chem 277(47): 45473-9.
87. Sarioglu, H., F. Lottspeich, et al. (2000). "Deamidation as a widespread phenomenon in two-dimensional polyacrylamide gel electrophoresis of human blood plasma proteins." Electrophoresis 21(11): 2209-18.
88. Sato, K., K. Morimoto, et al. (2000). "Increased synapsin I immunoreactivity during long-term potentiation in rat hippocampus." Brain Res 872(1-2): 219-22.
89. Schiebler, W., R. Jahn, et al. (1986). "Characterization of synapsin I binding to small synaptic vesicles." J Biol Chem 261(18): 8383-90.
90. Shevchenko, A., S. Sunyaev, et al. (2001). "Charting the proteomes of organisms with unsequenced genomes by MALDI-quadrupole time-of-flight

mass spectrometry and BLAST homology searching." Anal Chem 73(9): 1917-26.

91. Silva, A. J., T. W. Rosahl, et al. (1996). "Impaired learning in mice with abnormal short-lived plasticity." Curr Biol 6(11): 1509-18.
92. Siow, Y. L., T. J. Chilcote, et al. (1992). "Synapsin IIa: expression in insect cells, purification, and characterization." Biochemistry 31(17): 4268-75.
93. Songyang, Z., K. P. Lu, et al. (1996). "A structural basis for substrate specificities of protein Ser/Thr kinases: primary sequence preference of casein kinases I and II, NIMA, phosphorylase kinase, calmodulin-dependent kinase II, CDK5, and Erk1." Mol Cell Biol 16(11): 6486-93.
94. Spillane, D. M., T. W. Rosahl, et al. (1995). "Long-term potentiation in mice lacking synapsins." Neuropharmacology 34(11): 1573-9.
95. Stokoe, D., B. Caudwell, et al. (1993). "The substrate specificity and structure of mitogen-activated protein (MAP) kinase-activated protein kinase-2." Biochem J 296 (Pt 3): 843-9.
96. Sudhof, T. C. (1989). "Synaptic vesicles." Curr Opin Cell Biol 1(4): 655-9.
97. Sudhof, T. C. (1990). "The structure of the human synapsin I gene and protein." J Biol Chem 265(14): 7849-52.
98. Sudhof, T. C., M. Baumert, et al. (1989). "A synaptic vesicle membrane protein is conserved from mammals to Drosophila." Neuron 2(5): 1475-81.
99. Sudhof, T. C., A. J. Czernik, et al. (1989). "Synapsins: mosaics of shared and individual domains in a family of synaptic vesicle phosphoproteins." Science 245(4925): 1474-80.
100. Sun, J., P. Bronk, et al. (2006). "Synapsins regulate use-dependent synaptic plasticity in the calyx of Held by a Ca2+/calmodulin-dependent pathway." Proc Natl Acad Sci U S A 103(8): 2880-5.
101. Sunyer, B., S. Patil, et al. (2008). "Strain-dependent effects of cognitive enhancers in the mouse." Amino Acids 34(3): 485-95.
102. Sunyer, B., S. Patil, et al. (2007). "Strain-dependent effects of SGS742 in the mouse." Behav Brain Res 181(1): 64-75.
103. Sunyer, B., K. S. Shim, et al. (2008). "Hippocampal levels of phosphorylated protein kinase a (phosphor-S96) are linked to spatial memory enhancement by SGS742." Hippocampus.

104. Sunyer, B., K. S. Shim, et al. (2008). "The cognitive enhancer SGS742 does not involve major known signaling cascades in OF1 mice." Neurochem Res 33(7): 1384-92.

105. Takei, Y., A. Harada, et al. (1995). "Synapsin I deficiency results in the structural change in the presynaptic terminals in the murine nervous system." J Cell Biol 131(6 Pt 2): 1789-800.

106. Tsai, P. K., M. W. Bruner, et al. (1993). "Origin of the isoelectric heterogeneity of monoclonal immunoglobulin h1B4." Pharm Res 10(11): 1580-6.

107. Ueda, T. and P. Greengard (1977). "Adenosine 3':5'-monophosphate-regulated phosphoprotein system of neuronal membranes. I. Solubilization, purification, and some properties of an endogenous phosphoprotein." J Biol Chem 252(14): 5155-63.

108. Valtorta, F., P. Greengard, et al. (1992). "Effects of the neuronal phosphoprotein synapsin I on actin polymerization. I. Evidence for a phosphorylation-dependent nucleating effect." J Biol Chem 267(16): 11281-8.

109. Walaas, S. I., S. Hilfiker, et al. (2000). "Decrease in phorbol ester-induced potentiation of noradrenaline release in synapsin I-deficient mice." Synapse 36(2): 114-9.

110. Weitzdorfer, R., H. Hoger, et al. (2008). "Differences in hippocampal protein expression at 3 days, 3 weeks, and 3 months following induction of perinatal asphyxia in the rat." J Proteome Res 7(5): 1945-52.

111. Weitzdorfer, R., H. Hoger, et al. (2006). "Changes of hippocampal protein levels during postnatal brain development in the rat." J Proteome Res 5(11): 3205-12.

112. Wells, L., K. Vosseller, et al. (2002). "Mapping sites of O-GlcNAc modification using affinity tags for serine and threonine post-translational modifications." Mol Cell Proteomics 1(10): 791-804.

113. White, R. R., Y. G. Kwon, et al. (1998). "Definition of optimal substrate recognition motifs of Ca2+-calmodulin-dependent protein kinases IV and II reveals shared and distinctive features." J Biol Chem 273(6): 3166-72.

114. Zhan, X., F. Giorgianni, et al. (2005). "Proteomics analysis of growth hormone isoforms in the human pituitary." Proteomics 5(5): 1228-41.

115. Zhang, W., A. J. Czernik, et al. (1994). "Matrix-assisted laser desorption mass spectrometric peptide mapping of proteins separated by two-dimensional gel electrophoresis: determination of phosphorylation in synapsin I." Protein Sci 3(4): 677-86.

116. Zhu, J. X., H. A. Doyle, et al. (2006). "Protein repair in the brain, proteomic analysis of endogenous substrates for protein L-isoaspartyl methyltransferase in mouse brain." J Biol Chem 281(44): 33802-13.

8 Appendix

List of Supplementary tables

Supplementary table 1

Tandem MS of Peptides from the Subtilisin digest of Synapsin IIa (spots no 4 and 5)and the corresponding molecular masses:

Supplementary table 2

Identification of deamidated peptides and their site from synapsin Ia isoforms by a combination of MALDI-TOF-TOF and nano-ESI-LC-MS/MS mass spectrometry analysis:

Supplementary table 3

Identification of deamidated peptides and their sites from synapsin IIa isoforms by a combination of mass spectrometry analysis

Supplementary table 4

Identification of deamidated peptides and their sites from synapsin IIb isoforms by a combination of mass spectrometry analysis

Supplementary table 5

Identification of synapsin Ia and Ib isoforms by mass spectrometry analysis

Supplementary table 6

Identification of synapsin IIa protein by mass spectrometry analysis

Supplementary table 7

Identification of synapsin IIb protein by mass spectrometry analysis.

Supplementary table 8

ISIL-labeled peptides were used for quantification of the synapsin isoforms. Peptides were identified by nano-LC-ESI-MS/MS.

Supplementary table 1-Tandem MS of Peptides from the Subtilisin digest of Synapsin IIa (spots no 4 and 5)and the corresponding molecular masses

Spot No	Start-End	Experimental $[M+H]^+$ ion (m/z)	Calculated $[M+H]^+$ ion (m/z)	peptides
Spot 4	118 - 126	1023.56, 2^+	1023.49	LVVDEPHTD
	180 - 187	1014.63, 2^+	1014.60	RPDFVLIR
	477 - 486	1009.63, 2^+	1009.56	RLPSGPSLPS[a]
Spot 5	59 - 79	2072.25, 3^+	2072.08	RPPPAQAPAPQPAPQPAPTPS
	59 - 81	2228.31, 3^+	2228.17	RPPPAQAPAPQPAPQPAPTPSVG
	59 - 82	2315.36, 3^+	2315.20	RPPPAQAPAPQPAPQPAPTPSVGS
	118 - 126	1023.56, 2^+	1023.49	LVVDEPHTD
	180 - 187	1014.67, 2^+	1014.60	RPDFVLIR
	241 - 249	988.58, 2^+	988.52	GGEKFPLIE
	256 - 270	1782.13, 3^+	1781.99	HREMLTLPTFPVVVK Oxidation (M)
	258 - 270	1488.91, 2^+	1488.83	EMLTLPTFPVVVK Oxidation (M)
	261 - 270	1099.74, 2^+	1099.66	TLPTFPVVVK
	283 - 293	1349.68, 2^+	1349.59	VENHYDFQDIA
	422 - 431	1078.69, 2^+	1078.61	TPALSPQRPL.
	477 - 486	1009.62, 2^+	1009.56	RLPSGPSLPS[a]
	477 - 487	1096.66, 2^+	1096.59	RLPSGPSLPSS[a]
	477 - 488	1183.70, 2^+	1183.62	RLPSGPSLPSSS[a]

a) Peptides that cover the polyserine region

Supplementary table 2 Identification of deamidated peptides and their site from synapsin Ia isoforms by a combination of MALDI-TOF-TOF and nano-ESI-LC-MS/MS mass spectrometry analysis

Spot No	Protein Name	Peptides	Experimental $[M+H]^+$ ion (m/z)	Calculated $[M+H]^+$ ion (m/z)	Number of Deamidation site	Enzyme[b]	Mass Spectrometry[c]
Spot 1	Synapsin Ia	[447]QTSQQPAGPPAQQRPPPQGGPPQPGPGPQR[a 476]	2833.38	2833.39	2	T	M
		[508] LPSPTAAPQQSASQATPVTQGQGR[531, a]	2378.18	2378.18	1	T	M
Spot 2	Synapsin Ia	[24] DL**Q**RPQPPPPPPSAASPGATPGSATASA[51]	2623.30	2623.28	1	A	M
		[447]QTSQQPAGPPAQQRPPPQGGPPQPGPGPQR[a 476]	3039.48	3039.49	2	T	M
		[477]QGPPLQQRPPPQGQQHLSGLGPPAGSPLPQR[a 507]	3222.65	3221.64	2	T	M
		[508]LPSPTAAPQQSASQATPVTQGQGR[a 531]	2378.18	2378.18	1	T	M
		[588]QGPPQKPPGPAGPTR[a 602]	1485.80	1485.76	2	T	M
		[664]SQSLTNAFNLPEPAPPRPSLSQDEVK[a 689]	2822.38	2822.41	1	T	M

Letters marked in bold represent the identification of the exact site of deamidation.

a) Sequence obtained from PMF analysis.

b) Used enzyme T, trypsin; A, Asp-N.

Supplementary table 3 - Identification of deamidated peptides and their sites from synapsin IIa isoforms by a combination of mass spectrometry analysis

Spot No	Protein Name	Peptides	Experimental $[M+H]^+$ ion (m/z)	Calculated $[M+H]^+$ ion (m/z)	Enzyme[b]	Mass Spectrometry[c]	No of deamidation sites
3	synapsin IIa	[58]RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAVK[a 92]	3497.71	3497.79	T	M	1
		[165]DM$_{ox}$QVLRNGTKVVRSFRP[a 181]	2018.99	2019.07	A	M	1
		[414] MNQLLSRTPALSPQRPLTTQQPQSGTLK[a 441]	3109.63	3109.61	L	M	3
		[449] TPPQRPPPQGGPGQPQGMQPPGK[471]	2350.08	2350.15	T	M	1(Q464)
4	synapsin IIa	[58]RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAVK[92]	3497.71	3497.79	T	M	1 (Q69)
		[422] TPALSPQRPLTTQQPQSGTLKEPDSSK[a 448]	2893.50	2893.43	T	M	2
		[449] TPPQRPPPQGGPGQPQGMQPPGK [471]	2351.17	2351,13	T	M	2
5	synapsin IIa	[58] RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAVK[a 92]	3497.81	3497.79	T		1
		[283] VENHYDFQDIASVVALTQTYATAEPFIDAK[a 312]	3356.67	3356,61	T	M	1
		[421] TPALSPQRPLTTQQPQSGTLKEPDSSK[a 447]	2892.46	2892.48	T	M	1 (Q464)
		[449] TPPQRPPPQGGPGQPQGM$_{ox}$QPPGK [471]	2350.16	2350.19	T	M	2
		[449]TPPQRPPPQGGPGQPQGMQPPGK[a 471]	2335.13	2335.14	T	M M	1

6	synapsin IIa	[281]VKVENHYDFQDIASVVALTQTYATAEPFIDAK[a 312]	3583.86	3583.77	T	M	2
		[383] DYIFEVMDCSM$_{ox}$PLIGEHQVEDRQLITDLVISK[a 414]	3793.89	3793.83	t	M	1
		[415] M$_{ox}$NQLLSRTPALSPQRPLTTQQPQSGTLK[a 442]	3108.91	3108.62	L	M	2
		[449] TPPQRPPPQGGPGQPQGM$_{ox}$QPPGKVLPPR[a 476]	2913.50	2914.49	T	M	2
7	synapsin IIa	[59] RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAVK[a 93]	3497.16	3497.79	T	M	1
		[165] DMQVLRNGTKVVRSFRP[a 181]	2003.13	2003.07	A	M	1
		[283] VENHYDFQDIASVVALTQTYATAEPFIDAK[a 312]	3357.65	3357.59	L	M	1
		[338] TNTGSAMLEQIAMSDR[a 353]	1724.79	1724.77	T	M	1
		[415] M$_{ox}$NQLLSRTPALSPQRPLTTQQPQSGTLK[a 442]	3091.71	3091.64	L	M	1
		[422] TPALSPQRPLTTQQPQSGTLK[a 442]	2250.18	2250.19	T	M	2
		[449] TPPQRPPPQGGPGQPQGM$_{ox}$QPPGK[a 471]	2352.13	2352.12	T	M	3

8	synapsin IIa						
		[59] RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAV	3497.85	3497.79	T	M	1
		[422] TPALSPQRPLTTQQPQSGTLKEPDSSK[a 448]	2895.42	2895.43	T	M	4
		[449] TPPQRPPPQGGPGQPQGM$_{ox}$QPPGK[a 471]	2353.12	2353.10	T	M	4
9	synapsin IIa						
		[59] RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAV	3497.83	3497.89	T	M	1
		[239] TLGGEKFPLIEQTYYPNHR[a 257]	2264.07	2264.13	T	M	2
		[415] MNQLLSRTPALSPQRPLTTQQPQSGTLK[a 442]	3092.56	3093.63	T	M	2
		[422] TPALSPQRPLTTQQPQSGTLKEPDSSK[a 448]	2250.11	2250.19	T	M	2
		[449] TPPQRPPPQGGPGQPQGM$_{ox}$QPPGK[a 471]	2336.12	2336.12	T	M	3

Letters marked in bold represent the identification of the exact site of deamidation.

Mox denotes Methionine oxidation.

a) Sequence obtained from PMF analysis.

b) Used enzyme T, trypsin; L, Lys-C; A, Asp-N.

c) Used Mass spectrometry M, MALDI-TOF-TOF; E, nano-ESI-LC-MS/MS.

Supplementary table 4 - Identification of deamidated peptides and their sites from synapsin IIb isoforms by a combination of mass spectrometry analysis

Spot No	Protein Name	Peptides	Experimental $[M+H]^+$ ion (m/z)	Calculated $[M+H]^+$ ion (m/z)	Enzyme[b]	Mass Spectrometry[c]	No of deamidation sites
spot 10	synapsin IIb	^{25}DL**Q**RPEPQ^{32,}	982.47	982.47	U	LF	1 (Q27)
		^{59}RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAVK$^{a\ 93}$	3497.84	3497.79	T	M	1
		^{90}**Q**AVKQTAASAGLV^{102}	1243.68	1243.68	A	LF	1 (Q90)
		^{94}**Q**TAASAGLV^{102}	817.42	817.42	A	LF	1 (Q94)
		^{141}DIKVE**Q**A^{147}	802.41	802.41	A	LF	1 (Q146)
		^{165}DMQVLRNGTKVVRSFRP$^{a\ 181}$	2004.07	2004.06	A	M	2
		^{417}**Q**LLSRTPAL^{425}	998.58	998.58	U	LF	1 (Q417)
		^{422}TPALSPQRPLTTQQPQSGTLK$^{a\ 442}$	2249.18	2249.20	T	M	1
		^{449}TPPQRPPPQGCLQYILDC$^{\#}$NGIAVGPK$^{a\ 474}$	2876.41	2876.43	T	M	1
		^{465}DC$^{\#}$**N**GIAVGPKQVQAS^{479}/ ^{467}**N**GIAVGPKQVQAS^{479}	1543.73/	1543.73/	A/U	LF	1 (467)

spot 11	synaps in IIb	^{25}DL**Q**RPEPQQPPPAPGPGAATA^{45}	2094.63	2095.03	A	E	1 (Q27)
		^{59}RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAVK$^{a\ 93}$	3497.90	3497.79	T	M	1
		^{90}**Q**AVKQTAASAGLV^{102}	1243.95	1243.68	U	E	1 (Q90)
		^{198}DFRHLVIGM**Q**YAGLPSINSL^{217}	2230.43	2231.14	A	E	1 (Q207)
		^{383}DYIFEVMDC$^{\#}$SMPLIGEHQVEDR$^{a\ 404}$	2683.21	2683.16	T	M	1
		^{414}MoxNQLLSR$^{421,\ SD\text{-}35}$	877.48	877.43	T	E	1 (N415)
		^{422}TPALSPQRPLTTQQPQSGTLK$^{a\ 442}$	2250.24	2250.19	T	M	2
		^{426}SPQRPLTTQQPQSGTLK^{442}	1866.58	1866.98	U	E	1(Q435) or (Q434)
		^{449}TPPQRPPPQGC$^{\#}$LQYILDC$^{\#}$NGIAVGPK$^{a\ 474}$	2879.46	2879.38	T	M	4
spot 12	synaps in IIb	^{25}DL**Q**RPEPQ^{32}	982.47	982.47	U	FA	1(Q27)
		^{59}RPPPAQAPAP**Q**PAPQPAPTPSVGSSFFSSLSQAVK^{93}	3497.82	3497.79	T	M	1(Q69)
		^{69}**Q**PAPQPAPTPSVGSSFFSSL^{88}	2002.97	2002.95	U	FA	2 (Q69, Q73)
		^{90}QAVKQTAASAGLV^{102}	1243.68	1243.68	U	FA	1(Q90)
		^{165}DMQVLRNGTKVVRSFRP$^{a\ 181}$	2004.11	2004.06	A	M	2
		^{182}DFVLIR**Q**HAF^{191}	1245.65	1245.65	A	FA	1 (Q188)
		^{206}M$_{ox}$**Q**YAGLPSINSL^{217}	1309.62	1309.62	U	FA	1 (Q207)
		^{291}DIASVVALT**Q**TY^{302}	1280.65	1280.65	U	FA	1 (Q300)
		^{389}TNTGSAMLEQIAMoxSAR^{353}	1756:80	!756:76	T	M	1
		^{416}NQLLSRTPAL^{425}	1112.62	1112.62	U	FA	1 (N416 or Q417)
		^{422}TPALSPQRPLTTQQPQSGTLKEPDSSK$^{a\ 448}$	2249.19	2249.20	T	M	1
		^{443}EPDSSKTPPQRPPPQGC$^{\#}$LQYIL^{464}	2509.24	2509.22	A	M	2

		[449]TPPQRPPP**Q**GC[#]LQYILDC[#]**N**GIAVGPK[474] [467]**N**GIAVGPK**Q**VQAS[479]	2877.32 1269.66	2877.42 1269.66	T U	M FA	2 (Q457, N467) 2 (N467, Q475)
Spot 13	Synaps in IIb	[90]**Q**AVKQTAASAGLV[102] [182] DFVLIRQHAFGM$_{ox}$AE**N**E[197] [188]**Q**HAFGMAENE[197] [198] DFRHLVIGM$_{ox}$QYAGLPSI**N**SL [217] [283]VENHYDFQDIASVVALTQTYATAEPFIDAK[a 312] [291] DIASVVALT**Q**TY[302] [338]TNTGSAM$_{ox}$LEQIAM$_{ox}$SDR[a 353] [417] QLLSRTPALSPQRPLTT [433] [420] SRTPALSPQRPLTTQQP**Q**SGTLK[442]	1243.69 1892.91 1133.50 2247.18 3356.68 1280.69 1756.80 1879.09 2493.38	43.68 1892.87 1133.44 2247. 14 3356.61 1280.65 1756.76 1879.05 2493.32	U U U U T U T U U	E E E E M E M E E	 1(Q-90) 1(Q-196) 1(Q-188) 1(Q- 215) 1 1(Q- 300) 1 1(Q- 417) 2(Q-429) and (Q-437)
Spot 14	Synaps in IIb	[59]RPPPAQAPAPQPAPQPAPTPSVGSSFFSSLSQAVK[a 93] [165]DMQVLRNGTKVVRSFRP[181] [415]MN**Q**LLSR[421] [422]TPALSPQRPLTTQQPQSGTLKEPDSSK[a 448] [426]SPQRPLTT**Q**[434] [445]DSSKTPQRPPPQGCLQYIL[467]	3497.87 2004.11 861.47 2892.47 1027.59 22821.17	3497.79 2004.09 861.44 2892.48 1027.53 2282.14	T A T T U A	M M E M FA M	1 2 1(Q417) 1 1(Q434) 1

Spot 15	Synapsin IIb	^{449}TPPQRPPP**Q**GC$^{\#}$LQYILDC$^{\#}$**N**GIAVGPK^{474}	2877.32	2877.42	T	M	2(Q457, N467)

Letters marked in bold represent the identification of the exact site of deamidation.

Mox denotes Methionine oxidation.

a) Sequence obtained from PMF analysis.

b) Used enzyme T, trypsin; L, Lys-C; A, Asp-N.

c) Used Mass spectrometry M, MALDI-TOF-TOF; E, nano-ESI-LC-MS/MS.

Supplementary table 5 - Identification of synapsin Ia and Ib isoforms by mass spectrometry

Protein Name	Spot no.	Start - End	Observed	Mr(expt)	Mr (calc)	Delta	Miss	Sequence
Synapsin Ia	1	86 - 108	684.69	2051.05	2050.97	0.08	0	QTTAAAAATFSEQVGGGSGGAGR
		177 - 186	594.37	1186.72	1186.71	0.01	1	SLKPDFVLIR
		239 - 256	717.06	2148.17	2148.05	0.12	0	LGTEEFPLIDQTFYPNHK
		257 - 269	735.42	1468.82	1468.75	0.07	0	EMLSSTTYPVVVK
		282 - 299	667.37	1999.09	1999.02	0.09	0	VDNQHDFQDIASVVALTK
		300 - 311	663.86	1325.7	1325.65	0.05	0	TYATAEPFIDAK
		319 - 324	361.71	721.4	721.38	0.03	0	IGQNYK
		329 - 336	439.71	877.41	877.43	-0.02	0	TSVSGNWK
		337 - 352	586.28	1755.82	1755.78	0.05	0	TNTGSAMLEQIAMSDR ($2M_{ox}$)
		414 - 420	416.72	831.43	831.43	0	0	MTQALPR (M_{ox})
		431 - 446	514.59	1540.76	1540.76	0	0	GSHSQSSSPGALTLGR
		508 - 531	793.47	2377.4	2377.2	0.2	0	LPSPTAAPQQSASQATPVTQGQGR
		535 - 556	679.07	2034.17	2034.08	0.1	1	PVAGGPGAPPAARPPASPSPQR
		557 - 565	450.22	898.42	898.46	-0.04	0	QAGAPQATR
		566 - 576	528.79	1055.56	1055.56	0	0	QASISGPAPTK
		577 - 587	513.26	1024.5	1024.5	0	0	ASGAPPGGQQR
		588 - 602	495.6	1483.77	1483.79	-0.02	1	QGPPQKPPGPAGPTR
		603 - 612	484.74	967.46	967.48	-0.02	0	QASQAGPGPR
		613 - 622	541.78	1081.54	1081.55	-0.01	0	TGPPTTQQPR
		623 - 631	398.22	794.43	794.42	0.03	0	PSGPGPAGRP
		698 - 706	501.29	1000.56	1000.49	0.07	1	KSFASLFSD
Synapsin Ib	2	282 - 299	667.49	1999.44	1999	0.44	0	VDNQHDFQDIASVVALTK
		431 - 446	514.65	1540.93	1540.76	0.17	0	GSHSQSSSPGALTLGR
		557 - 565	450.28	898.54	898.46	0.08	0	QAGAPQATR

		566 - 576	528.86	1055.72	1055.56	0.15	0	QASISGPAPTK
		603 - 612	484.8	967.58	967.48	0.1	0	QASQAGPGPR
		613 - 622	541.85	1081.69	1081.55	0.14	0	TGPPTTQQPR
Synapsin Ib	3	28 - 53	799.49	2395.45	2395.21	0.23	0	PQPPPPPPSAASPGATPGSATASAER
		86 - 108	684.71	2051.11	2051	0.1	0	QTTAAAAATFSEQVGGGSGGAGR
		187 - 194	482.23	962.45	962.48	-0.03	0	QHAFSMAR
		282 - 299	667.37	1999.09	1999.04	0.05	0	VDNQHDFQDIASVVALTK
		300 - 311	663.86	1325.72	1325.61	0.1	0	TYATAEPFIDAK
		319 - 324	361.71	721.41	721.41	0	0	IGQNYK
		329 - 336	447.21	892.4	892.39	0.01	0	TSVSGNWK
		431 - 446	514.6	1540.78	1540.8	-0.01	0	GSHSQSSSPGALTLGR
		508 - 531	793.49	2377.45	2377.23	0.22	0	LPSPTAAPQQSASQATPVTQGQGR
		535 - 556	679.08	2034.23	2034.11	0.12	1	PVAGGPGAPPAARPPASPSPQR
		557 - 565	450.23	898.45	898.5	-0.05	0	QAGAPQATR
		566 - 576	520.28	1038.55	1038.53	0.01	0	QASISGPAPTK
		566 - 576	528.8	1055.58	1055.6	-0.01	0	RQASISGPAPTK
Synapsin Ib	4	115 - 128	545.9795	1634.9167	1634.8668	-0.0499	0	VLLVIDEPHTDWAK
		177 - 186	594.3823	1186.7501	1186.7074	-0.0427	1	SLKPDFVLIR
		300 - 311	663.8771	1325.7395	1325.6503	-0.0892	0	TYATAEPFIDAK
		557 - 565	450.2403	898.4661	898.4621	-0.004	0	QAGAPQATR
		566 - 576	528.8028	1055.5911	1055.5611	-0.03	0	QASISGPAPTK
		577 - 587	513.2699	1024.5253	1024.505	-0.0203	0	ASGAPPGGQQR
		588 - 602	495.6143	1483.821	1483.7896	-0.0314	1	QGPPQKPPGPAGPTR
		603 - 612	484.7528	967.491	967.4835	-0.0075	0	QASQAGPGPR

Supplementary table 6 - Identification of synapsin IIa by mass spectrometry analysis

Protein name	Spot No	Start - End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence
Synapsin IIa	5	94 - 112	581.01	1740	1739.88	0.12	0	QTAASAGLVDAPAPSAASR
		116 - 129	541.31	1620.92	1620.85	0.07	0	VLLVVDEPHTDWAK
		136 - 143	468.76	935.5	935.5	0	0	ILGDYDIK
		178 - 187	625.39	1248.77	1248.7	0.07	1	SFRPDFVLIR
		239 - 257	755.09	2262.25	2262.14	0.11	1	TLGGEKFPLIEQTYYPNHR
		245 - 257	559.99	1676.96	1676.83	0.12	0	FPLIEQTYYPNHR
		320 - 325	354.71	707.42	707.36	0.06	0	IGNNYK
		330 - 337	446.74	891.46	891.44	0.01	0	TSISGNWK
		405 - 414	565.38	1128.74	1128.68	0.06	0	QLITDLVISK
		422 - 442	750.5	2248.47	2248.22	0.25	1	TPALSPQRPLTTQQPQSGTLK
		561 - 574	507.6	1519.77	1519.71	0.06	1	SSANEDEAKAETIR
		578 - 586	501.27	1000.52	1000.49	0.03	1	KSFASLFSD
Synapsin IIa	6	93 - 111	581	1739.96	1739.88	0.08	0	QTAASAGLVDAPAPSAASR
		135 - 142	468.76	935.5	935.5	0	0	ILGDYDIK
		177 - 186	625.38	1248.74	1248.7	0.04	1	SFRPDFVLIR
		244 - 256	559.98	1676.92	1676.83	0.08	0	FPLIEQTYYPNHR
		244 - 256	559.98	1676.92	1676.83	0.09	0	FPLIEQTYYPNHR
		319 - 324	354.72	707.42	707.36	0.06	0	IGNNYK
		404 - 413	565.36	1128.7	1128.68	0.03	0	QLITDLVISK

		421 - 428	435.25	868.48	868.48	0	0	TPALSPQR
		560 - 573	507.58	1519.72	1519.71	0.01	1	SSANEDEAKAETIR
		577 - 585	501.26	1000.51	1000.49	0.03	1	KSFASLFSD
		93 - 111	581	1739.96	1739.88	0.08	0	QTAASAGLVDAPAPSAASR
		135 - 142	468.76	935.5	935.5	0	0	ILGDYDIK
		177 - 186	625.38	1248.74	1248.7	0.04	1	SFRPDFVLIR
		244 - 256	559.98	1676.92	1676.83	0.08	0	KFPLIEQTYYPNHR
		244 - 256	559.98	1676.92	1676.83	0.09	0	FPLIEQTYYPNHR
		94 - 112	581.06	1740.15	1739.88	0.27	0	QTAASAGLVDAPAPSAASR
		116 - 129	541.36	1621.06	1620.85	0.21	0	VLLVVDEPHTDWAK
		136 - 143	468.79	935.57	935.5	0.08	0	ILGDYDIK
		178 - 187	625.45	1248.88	1248.7	0.18	1	SFRPDFVLIR
		188 - 200	775.95	1549.89	1550.66	-0.77	0	QHAFGMAENEDFR
		245 - 257	560.03	1677.06	1676.83	0.23	0	FPLIEQTYYPNHR
		320 - 325	354.74	707.47	707.36	0.11	0	IGNNYK
		330 - 337	446.76	891.51	891.44	0.07	0	TSISGNWK
		405 - 414	565.41	1128.81	1128.68	0.13	0	QLITDLVISK
		422 - 442	750.52	2248.54	2248.22	0.33	1	TPALSPQRPLTTQQPQSGTLK
		561 - 574	507.63	1519.86	1519.71	0.15	1	SSANEDEAKAETIR
		578 - 586	501.29	1000.57	1000.49	0.08	1	KSFASLFSD
Synapsin IIa	7	94 - 112	581.02	1740.04	1739.88	0.16	0	QTAASAGLVDAPAPSAASR

		116 - 129	541.33	1620.97	1620.85	0.12	0	VLLVVDEPHTDWAK
		136 - 143	468.77	935.53	935.5	0.03	0	ILGDYDIK
		178 - 187	625.42	1248.83	1248.7	0.13	1	SFRPDFVLIR
		245 - 257	560	1676.98	1676.83	0.15	0	FPLIEQTYYPNHR
		320 - 325	354.72	707.43	707.36	0.07	0	IGNNYK
		330 - 337	446.75	891.48	891.44	0.04	0	TSISGNWK
		405 - 414	565.36	1128.71	1128.68	0.04	0	QLITDLVISK
		422 - 442	750.5	2248.48	2248.22	0.27	1	TPALSPQRPLTTQQPQSGTLK
Synapsin IIa	8	94 - 112	581.02	1740.05	1739.88	0.17	0	QTAASAGLVDAPAPSAASR
		116 - 129	541.34	1621	1620.85	0.15	0	VLLVVDEPHTDWAK
		135 - 143	532.85	1063.68	1063.59	0.09	1	KILGDYDIK
		136 - 143	468.78	935.55	935.5	0.05	0	ILGDYDIK
		178 - 187	625.42	1248.83	1248.7	0.14	1	SFRPDFVLIR
		245 - 257	560.01	1676.99	1676.83	0.16	0	FPLIEQTYYPNHR
		320 - 325	354.73	707.44	707.36	0.08	0	IGNNYK
		330 - 337	446.76	891.5	891.44	0.06	0	TSISGNWK
		405 - 414	565.4	1128.78	1128.68	0.11	0	QLITDLVISK

		415 - 421	439.26	876.5	876.45	0.05	0	MNQLLSR
		422 - 442	750.51	2248.52	2248.22	0.3	1	TPALSPQRPLTTQQPQSGTLK
		430 - 442	699.97	1397.93	1397.75	0.17	0	PLTTQQPQSGTLK
		449 - 471	784.17	2349.5	2349.16	0.34	1	TPPQRPPPQGGPGQPQGMQPPGK
		478 - 503	813.51	2437.52	2437.12	0.4	0	LPSGPSLPSSSSSSSSSSSSSSAPQR
		561 - 574	507.61	1519.81	1519.71	0.1	1	SSANEDEAKAETIR
		578 - 586	501.29	1000.56	1000.49	0.07	1	KSFASLFSD
Synapsin IIa	9	94 - 112	581.1	1740.29	1739.88	0.41	0	QTAASAGLVDAPAPSAASR
		116 - 129	541.36	1621.07	1620.85	0.22	0	VLLVVDEPHTDWAK
		136 - 143	468.81	935.6	935.5	0.1	0	ILGDYDIK
		178 - 187	625.43	1248.85	1248.7	0.15	1	SFRPDFVLIR
		188 - 200	775.97	1549.93	1550.66	-0.73	0	QHAFGMAENEDFR
		245 - 257	560.03	1677.08	1676.83	0.25	0	FPLIEQTYYPNHR
		320 - 325	354.78	707.54	707.36	0.18	0	IGNNYK
		330 - 337	446.79	891.57	891.44	0.12	0	TSISGNWK
		405 - 414	565.42	1128.83	1128.68	0.16	0	QLITDLVISK
		449 - 471	784.28	2349.8	2350.15	-0.35	1	TPPQRPPPQGGPGQPQGMQPPGK

		561 - 574	507.65	1519.94	1519.71	0.22	1	SSANEDEAKAETIR
Synapsin IIa	10	94 - 112	581	1739.97	1739.88	0.09	0	QTAASAGLVDAPAPSAASR
		136 - 143	468.75	935.49	935.5	-0.01	0	ILGDYDIK
		178 - 187	625.4	1248.79	1248.7	0.09	1	SFRPDFVLIR
		188 - 200	523.28	1566.81	1566.65	0.16	0	QHAFGMAENEDFR (M_{ox})
		245 - 257	559.96	1676.87	1676.83	0.04	0	FPLIEQTYYPNHR
		258 - 270	745.46	1488.9	1488.83	0.07	0	EMLTLPTFPVVVK
		330 - 337	446.73	891.44	891.44	0	0	TSISGNWK
		338 - 353	586.29	1755.86	1755.78	0.09	0	TNTGSAMLEQIAMSDR (M_{ox})
		405 - 414	565.36	1128.7	1128.68	0.03	0	QLITDLVISK
		561 - 574	507.59	1519.74	1519.71	0.03	1	SSANEDEAKAETIR
Synapsin IIa	11	94 - 112	581	1739.98	1739.88	0.1	0	QTAASAGLVDAPAPSAASR
		94 - 113	623.71	1868.09	1867.98	0.12	1	QTAASAGLVDAPAPSAASRK
		116 - 129	541.32	1620.92	1620.85	0.07	0	VLLVVDEPHTDWAK
		136 - 143	468.76	935.5	935.5	0	0	ILGDYDIK
		178 - 187	417.28	1248.83	1248.7	0.13	1	SFRPDFVLIR

		245 - 257	559.97	1676.88	1676.83	0.05	0	FPLIEQTYYPNHR
		320 - 325	354.71	707.41	707.36	0.05	0	IGNNYK
		330 - 337	446.73	891.45	891.44	0.01	0	TSISGNWK
		405 - 414	565.37	1128.72	1128.68	0.05	0	QLITDLVISK
		561 - 574	507.59	1519.74	1519.71	0.03	1	SSANEDEAKAETIR

M_{ox} - denotes methionine oxidation

Supplementary table 7 -Identification of synapsin IIb protein by mass spectrometry analysis.

Protein Name	Spot no.	Start - End	Observed	Mr(expt)	Mr (calc)	Delta	Miss	Sequence
Synapsin IIb	12	94 - 112	581.03	1740.06	1739.88	0.18	0	QTAASAGLVDAPAPSAASR
		116 - 129	541.34	1621	1620.85	0.14	0	VLLVVDEPHTDWAK
		135 - 143	532.85	1063.68	1063.59	0.09	1	KILGDYDIK
		136 - 143	468.78	935.55	935.5	0.05	0	ILGDYDIK
		178 - 187	625.42	1248.83	1248.7	0.13	1	SFRPDFVLIR
		188 - 200	523.27	1566.79	1566.65	0.14	0	QHAFGMAENEDFR (M_{ox})
		239 - 257	755.18	2262.53	2262.14	0.39	1	TLGGEKFPLIEQTYYPNHR
		245 - 257	560.01	1677	1676.83	0.17	0	FPLIEQTYYPNHR
		320 - 325	354.73	707.44	707.36	0.08	0	KIGNNYK
		330 - 337	446.75	891.49	891.44	0.05	0	TSISGNWK
		338 - 353	586.33	1755.96	1755.78	0.18	0	TNTGSAMLEQIAMSDR ($2M_{ox}$)
		405 - 414	565.4	1128.78	1128.68	0.11	0	QLITDLVISK
		415 - 421	439.26	876.5	876.45	0.05	0	MNQLLSR (M_{ox})
		422 - 429	435.27	868.53	868.48	0.05	0	TPALSPQR
		422 - 442	750.52	2248.53	2248.22	0.31	1	TPALSPQRPLTTQQPQSGTLK
		430 - 442	699.96	1397.91	1397.75	0.16	0	PLTTQQPQSGTLK
Synapsin IIb	13	94 - 112	581.04	1740.11	1739.88	0.23	0	QTAASAGLVDAPAPSAASR
		116 - 129	541.35	1621.03	1620.85	0.18	0	VLLVVDEPHTDWAK
		135 - 143	532.86	1063.7	1063.59	0.11	1	KILGDYDIK

		136 - 143	468.79	935.56	935.5	0.06	0	ILGDYDIK
		178 - 187	625.44	1248.86	1248.7	0.16	1	SFRPDFVLIR
		188 - 200	517.95	1550.82	1550.66	0.16	0	QHAFGMAENEDFR
		239 - 257	755.19	2262.55	2262.14	0.41	1	TLGGEKFPLIEQTYYPNHR
		245 - 257	560.01	1677.02	1676.83	0.19	0	FPLIEQTYYPNHR
		258 - 270	737.52	1473.03	1472.83	0.2	0	EMLTLPTFPVVVK
		271 - 280	497.79	993.56	993.48	0.08	0	IGHAHSGMGK
		283 - 312	1119.71	3356.12	3355.62	0.5	0	VENHYDFQDIASVVALTQTYATAEPFIDAK
		320 - 325	354.74	707.46	707.36	0.1	0	IGNNYK
		330 - 337	446.77	891.52	891.44	0.07	0	TSISGNWK
		338 - 353	863.05	1724.09	1723.79	0.3	0	TNTGSAMLEQIAMSDR
		383 - 404	895.21	2682.62	2682.18	0.44	0	DYIFEVMDCSMPLIGEHQVEDR
		405 - 414	565.41	1128.8	1128.68	0.12	0	QLITDLVISK
		405 - 414	565.41	1128.8	1128.68	0.13	0	QLITDLVISK
		415 - 421	431.27	860.53	860.45	0.07	0	MNQLLSR
		422 - 429	435.28	868.55	868.48	0.07	0	TPALSPQR
		422 - 442	750.53	2248.57	2249.2	-0.64	1	TPALSPQRPLTTQQPQSGTLK
		430 - 442	699.99	1397.97	1397.75	0.22	0	PLTTQQPQSGTLK
		430 - 448	681.46	2041.35	2041.03	0.32	1	PLTTQQPQSGTLKEPDSSK
Synapsin IIb	14	94 - 112	581.04	1740.11	1739.88	0.23	0	QTAASAGLVDAPAPSAASR
		94 - 113	623.77	1868.28	1867.98	0.3	1	QTAASAGLVDAPAPSAASRK

		116 - 129	541.36	1621.06	1620.85	0.21	0	VLLVVDEPHTDWAK
		135 - 143	532.86	1063.71	1063.59	0.12	1	KILGDYDIK
		136 - 143	468.79	935.57	935.5	0.08	0	ILGDYDIK
		178 - 187	625.45	1248.88	1248.7	0.18	1	SFRPDFVLIR
		188 - 200	775.95	1549.88	1550.66	-0.77	0	QHAFGMAENEDFR
		188 - 200	517.95	1550.84	1550.66	0.18	0	QHAFGMAENEDFR
		239 - 257	755.2	2262.57	2262.14	0.43	1	TLGGEKFPLIEQTYYPNHR
		245 - 257	560.03	1677.05	1676.83	0.22	0	FPLIEQTYYPNHR
		258 - 270	737.54	1473.07	1472.83	0.24	0	EMLTLPTFPVVVK
		271 - 280	497.8	993.58	993.48	0.1	0	IGHAHSGMGK
		320 - 325	354.74	707.47	707.36	0.11	0	IGNNYK
		330 - 337	446.77	891.53	891.44	0.09	0	TSISGNWK
		338 - 353	575.68	1724.03	1723.79	0.24	0	TNTGSAMLEQIAMSDR
		338 - 353	863.06	1724.11	1723.79	0.32	0	TNTGSAMLEQIAMSDR
		383 - 404	895.27	2682.78	2682.18	0.6	0	DYIFEVMDCSMPLIGEHQVEDR
		405 - 414	565.41	1128.81	1128.68	0.14	0	QLITDLVISK
		405 - 414	565.42	1128.82	1128.68	0.15	0	QLITDLVISK

		415 - 421	431.28	860.54	860.45	0.09	0	MNQLLSR
		422 - 429	435.29	868.57	868.48	0.09	0	TPALSPQR
		422 - 442	750.55	2248.62	2248.22	0.4	1	TPALSPQRPLTTQQPQSGTLK
		430 - 442	700	1397.98	1397.75	0.22	0	PLTTQQPQSGTLK
		449 - 474	959.98	2876.93	2876.43	0.49	1	TPPQRPPPQGCLQYILDCNGIAVGPK
Synapsin IIb	15	94 - 112	580.99	1739.96	1739.88	0.08	0	QTAASAGLVDAPAPSAASR
		94 - 113	623.69	1868.06	1867.98	0.09	1	QTAASAGLVDAPAPSAASRK
		116 - 129	541.3	1620.88	1620.85	0.03	0	VLLVVDEPHTDWAK
		135 - 143	532.81	1063.6	1063.59	0.01	1	KILGDYDIK
		136 - 143	468.75	935.48	935.5	-0.02	0	ILGDYDIK
		178 - 187	417.26	1248.75	1248.7	0.05	1	SFRPDFVLIR
		178 - 187	625.38	1248.75	1248.7	0.05	1	SFRPDFVLIR
		188 - 200	517.9	1550.67	1550.66	0.01	0	QHAFGMAENEDFR
		245 - 257	559.97	1676.88	1676.83	0.04	0	FPLIEQTYYPNHR
		271 - 280	497.75	993.48	993.48	0	0	IGHAHSGMGK
		320 - 325	354.7	707.39	707.36	0.03	0	IGNNYK

		330 - 337	446.72	891.43	891.44	-0.01	0	TSISGNWK
		338 - 353	575.62	1723.84	1723.79	0.06	0	TNTGSAMLEQIAMSDR
		405 - 414	565.35	1128.69	1128.68	0.02	0	QLITDLVISK
		415 - 421	431.23	860.45	860.45	-0.01	0	MNQLLSR
		422 - 429	435.24	868.46	868.48	-0.01	0	TPALSPQR
		422 - 442	750.46	2248.37	2248.22	0.15	1	TPALSPQRPLTTQQPQSGTLK
		430 - 442	699.93	1397.85	1397.75	0.1	0	PLTTQQPQSGTLK
Synapsin IIb	16	94 - 112	581.02	1740.04	1739.88	0.16	0	QTAASAGLVDAPAPSAASR
		94 - 113	623.74	1868.2	1867.98	0.22	1	QTAASAGLVDAPAPSAASRK
		116 - 129	541.33	1620.97	1620.85	0.12	0	VLLVVDEPHTDWAK
		135 - 143	532.84	1063.67	1063.59	0.08	1	KILGDYDIK
		136 - 143	468.78	935.54	935.5	0.04	0	ILGDYDIK
		178 - 187	625.42	1248.83	1248.7	0.13	1	SFRPDFVLIR
		188 - 200	517.93	1550.77	1550.66	0.11	0	QHAFGMAENEDFR
		239 - 257	755.16	2262.47	2262.14	0.32	1	TLGGEKFPLIEQTYYPNHR
		245 - 257	560.01	1676.99	1676.83	0.16	0	FPLIEQTYYPNHR

Synapsin IIb	17	94 - 112	581.01	1739.99	1739.88	0.11	0	QTAASAGLVDAPAPSAASR
		116 - 129	541.32	1620.93	1620.85	0.08	0	VLLVVDEPHTDWAK
		135 - 143	532.83	1063.64	1063.59	0.04	1	KILGDYDIK
		136 - 143	468.76	935.51	935.5	0.01	0	ILGDYDIK
		178 - 187	625.4	1248.79	1248.7	0.09	1	SFRPDFVLIR
		188 - 200	517.91	1550.72	1550.66	0.06	0	QHAFGMAENEDFR
		258 - 270	737.49	1472.96	1472.83	0.13	0	EMLTLPTFPVVVK
		320 - 325	354.72	707.42	707.36	0.06	0	IGNNYK
		330 - 337	446.74	891.46	891.44	0.02	0	TSISGNWK
		338 - 353	863.01	1724	1723.79	0.21	0	TNTGSAMLEQIAMSDR
		383 - 404	900.51	2698.5	2698.17	0.33	0	DYIFEVMDCSMPLIGEHQVEDR
		405 - 414	565.37	1128.72	1128.68	0.04	0	QLITDLVISK
		415 - 421	431.25	860.48	860.45	0.02	0	MNQLLSR

Supplementary table 9.

ISIL-labeled peptides were used for quantification of the synapsin isoforms. Peptides were identified by nano-LC-ESI-MS/MS. Synapsin isoform peptides from different gels per group are provided.

Spot no.	Protein name	Gel no.	Light labeled peptide	Heavy labeled peptide	Charge state	Enzyme	Identified peptide sequence	Light	Heavy	H/L Ratio	Mean	Norm.	Total protein Mean	Total protein SD
SGS742-treated vs NaCl-treated														
1	Synapsin Ia	1	615.41	616.92	2+	Trypsin	SLK(Ac)PDFVLIR	6	13.9	2.32	2.32	2.19	2.19	0.41
		2	615.41	616.91	2+	Trypsin	SLK(Ac)PDFVLIR	23	57	2.48	2.48	2.28		
		3	615.44	616.95	2+	Trypsin	SLK(Ac)PDFVLIR	19.5	34	1.74	2.94	2.59		
			407.78	409.29	2+	Trypsin	NGVK(Ac)VVR	0.58	2.4	4.14				
		4	615.44	616.95	2+	Trypsin	SLK(Ac)PDFVLIR	16	28	1.75	2.01	1.88		
			407.78	409.29	2+	Trypsin	NGVK(Ac)VVR	1.1	2.5	2.27				
		5	615.47	616.98	2+	Trypsin	SLK(Ac)PDFVLIR	30	49	1.63	1.63	1.58		
		6	408.83	409.32	2+	Trypsin	NGVK(Ac)(Ac)VVR	2.9	7.8	2.69	2.78	2.65		
			615.45	616.96	2+	Trypsin	SLK(Ac)PDFVLIR	28	68	2.43				
			567.04	569.04	3+	Trypsin	VQK(Ac)IGQNYK(Ac)AYMR	5.8	18.7	3.22				
2	Synapsin Ib	1	615.42	616.93	2+	Trypsin	SLK(Ac)PDFVLIR	149	85	0.57	0.63	0.59	0.89	0.44
			509.65	510.66	3+	Trypsin	QGPPQK(Ac)PPGPAGPTR	21	13	0.62				
			702.42	703.43	3+	Trypsin	QASISGPAPTK(Ac)ASGAPPGGQQR	36	25	0.69				
		2	615.41	616.92	2+	Trypsin	SLK(Ac)PDFVLIR	10.5	7.2	0.69	0.60	0.53		
		3	407.33	408.84	2+	Trypsin	NGVK(Ac)VVR	17	16	0.94	0.99	0.91		
			615.45	616.95	2+	Trypsin	SLK(Ac)PDFVLIR	200	203	1.02				
			567.04	569.05	3+	Trypsin	VQK(Ac)IGQNYK(Ac)AYMR	16.8	16.9	1.01				
		4	615.47	616.97	2+	Trypsin	SLK(Ac)PDFVLIR	112	177	1.58	1.58	1.51		
3	Synapsin Ib	1	616.92	569.00	3+	Trypsin	SLK(Ac)PDFVLIR	64	15	0.23	0.28	0.25	0.41	0.20
		2	407.78	409.29	2+	Trypsin	NGVK(Ac)VVR	28	9	0.32	0.36	0.34		
			566.99	569.01	3+	Trypsin	VQK(Ac)IGQNYK(Ac)AYMR	45	18	0.40				
		3	615.41	616.92	2+	Trypsin	SLK(Ac)PDFVLIR	79	44	0.56	0.65	0.63		
			702.42	703.43	3+	Trypsin	QASISGPAPTK(Ac)ASGAPPGGQQR	20.3	15.2	0.75				
4	Synapsin Ib	1	407.79	409.29	2+	Trypsin	NGVK(Ac)VVR	18.1	26	1.44	1.31	1.16	1.10	0.08
			615.41	616.91	2+	Trypsin	SLK(Ac)PDFVLIR	108	140	1.30				
			567.00	569.00	3+	Trypsin	VQK(Ac)IGQNYK(Ac)AYMR	22.5	27	1.20				
		2	615.4070	616.91	2+	Trypsin	SLK(Ac)PDFVLIR	15.4	19	1.23	1.12	1.01		
			407.7860	409.29	2+	Trypsin	NGVK(Ac)VVR	3.8	4.8	1.26				
			509.63	510.64	3+	Trypsin	QGPPQK(Ac)PPGPAGPTR	11	9.5	0.86				
		3	407.7860	409.29	2+	Trypsin	NGVK(Ac)VVR	8.4	10.5	1.25	1.18	1.14		

			615.4070	616.91	2+	Trypsin	SLK(Ac)PDFVLIR	11.3	14.8	1.31					
			509.63	510.64	3+	Trypsin	QGPPQK(Ac)PPGPAGPTR	3.5	3.4	0.97					
5	Synapsin IIa	1	522.24	523.75	2+	Trypsin	K(Ac)SFASLFSD	2	5.1	2.55	2.55	2.41	1.96	0.64	
		4	522.23	523.75	2+	Trypsin	K(Ac)SFASLFSD	2.2	3.4	1.55	1.55	1.50			
6	Synapsin IIa	1	522.22	523.74	2+	Trypsin	K(Ac)SFASLFSD	2.3	5.5	2.39	2.39	2.26	1.79	0.54	
		2	408.76	410.28	2+	Trypsin	NGTK(Ac)VVR	8.9	11.5	1.29	1.29	1.20			
		3	781.85	783.36	2+	Trypsin	SSANEDEAK(Ac)AETIR	5.1	13	2.55	2.30	2.23			
			562.30	564.28	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	3.9	8	2.05					
		4	781.98	783.47	2+	Trypsin	SSANEDEAK(Ac)AETIR	1.8	2.8	1.56	1.56	1.46			
			1007.15	1008.67	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	2.5	3.9	1.56					
7	Synapsin IIa	1	562.26	564.27	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	16.5	21	1.27	1.27	1.20	1.31	0.29	
		2	781.84	783.85	2+	Trypsin	SSANEDEAK(Ac)AETIR	13.5	27.5	2.04	2.04	1.80			
		3	781.89	783.41	2+	Trypsin	SSANEDEAK(Ac)AETIR	11.5	15	1.30	1.30	1.27			
		4	408.75	410.26	2+	Trypsin	NGTK(Ac)VVR	11	12	1.09	1.09	1.04			
		5	677.47	678.47	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR ox	10.3	14	1.36	1.29	1.23			
			522.31	523.82		Trypsin	K(Ac)SFASLFSD	18	25	1.39					
			891.52	892.53	3+	Trypsin	TSISGNWK(Ac)TNTGSAMLEQIAMSDR	31	35	1.13					
8	Synapsin IIa	1	522.24	523.75	2+	Trypsin	K(Ac)SFASLFSD	5.4	4.2	0.78	0.85	0.75	1.43	0.72	
			781.85	783.36	2+	Trypsin	SSANEDEAK(Ac)AETIR	35.1	34.5	0.98					
			562.29	564.30	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	15	12	0.80					
			410.23	410.24	2+	Trypsin	NGTK(Ac)VVR	15	12.5	0.83					
		2	781.85	783.36	2+	Trypsin	SSANEDEAK(Ac)AETIR	13	23	1.77	1.48	1.40			
			671.71	672.73	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	22.5	27	1.20					
		3	408.76	410.25	2+	Trypsin	NGTK(Ac)VVR	6.5	17	2.62	2.62	2.54			
		4	667.39	670.41	2+	Trypsin	GK(Ac)K(Ac)ILGDYDIK(Ac)	10	17.3	1.73	1.73	1.62			
		5	781.88	783.40	2+	Trypsin	SSANEDEAK(Ac)AETIR	32	27	0.84	0.85	0.82			
			408.75	410.26	2+	Trypsin	NGTK(Ac)VVR	13.9	9.9	0.71					
			522.25	523.74	2+	Trypsin	K(Ac)SFASLFSD	2.3	1.63	0.71					
			562.28	564.30	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	12.7	9.5	0.75					
			891.41	892.42	3+	Trypsin	TSISGNWK(Ac)TNTGSAMLEQIAMSDR	9	11	1.22					
9	Synapsin IIa	1	522.24	523.76	2+	Trypsin	K(Ac)SFASLFSD	4	8	2.00	2.25	2.13	1.43	0.56	
			562.30	564.28	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	5	12.5	2.50					
		2	408.73	410.24	2+	Trypsin	NGTK(Ac)VVR	42	45	1.07	1.18	1.15			
			672.06	673.07	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	34	44	1.29					
		3	671.73	672.72	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR (pyro)	21	37	1.76	1.58	1.48			
			408.74	410.26	2+	Trypsin	NGTK(Ac)VVR	20	28	1.40					
		4	408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	12	12.5	1.04	2.31	2.24			
			671.73	672.74	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	3.5	12.5	3.57					
		5	408.75	410.26	2+	Trypsin	NGTK(Ac)VVR	19	24	1.26	1.26	1.20			

		6	553.30	554.80	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	1.95	2.1	1.08	1.11	1.03		
			644.32	645.83	2+	Trypsin	IGNNYK(Ac)AYMR	2.8	3.2	1.14				
			677.40	678.41	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	6.3	7	1.11				
		7	1007.13	1008.16	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	11.7	8.8	0.75	0.90	0.79		
			671.80	672.80	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR (pyro)	8.9	9.4	1.06				
10	Synapsin IIa	1	522.26	523.77	2+	Trypsin	K(Ac)SFASLFSD	3.5	4.2	1.20	1.20	1.14	1.26	0.32
		2	677.47	678.47	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	26	23.4	0.90	0.92	0.86		
			522.31	523.82	2+	Trypsin	K(Ac)SFASLFSD	5	5.1	1.02				
			1007.18	1008.64	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR (pyro)	4	3.7	0.93				
			677.47	678.47	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR ox	27.8	25.6	0.92				
			846.88	849.91	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	13.9	11.9	0.86				
		3	522.29	523.80	2+	Trypsin	K(Ac)SFASLFSD	33	47	1.42	1.54	1.49		
			781.96	783.47	2+	Trypsin	SSANEDEAK(Ac)AETIR	33	45	1.36				
			562.35	564.35	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	29	49	1.69				
			846.87	849.89	2+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	75	125	1.67				
			408.79	410.30	2+	Trypsin	NGTK(Ac)VVR	4	6.2	1.55				
		4	522.31	523.82	2+	Trypsin	K(Ac)SFASLFSD	44	68	1.55	1.66	1.54		
			781.98	783.50	2+	Trypsin	SSANEDEAK(Ac)AETIR	18	32	1.78				
11	Synapsin IIa	1	408.79	410.30	2+	Trypsin	NGTK(Ac)VVR	10.5	12.9	1.23	1.16	1.10	1.01	0.05
			522.30	523.81	2+	Trypsin	K(Ac)SFASLFSD	16.2	17.8	1.10				
			562.35	564.35	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	17	22	1.29				
			846.88	849.90	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	35	36	1.03				
		2	408.81	410.32	2+	Trypsin	NGTK(Ac)VVR	6.8	8	1.18	1.02	0.97		
			522.32	523.83	2+	Trypsin	K(Ac)SFASLFSD	72	55	0.76				
			677.50	678.51	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	52	57	1.10				
			846.92	849.93	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	53	55	1.04				
		3	408.79	410.29	2+	Trypsin	NGTK(Ac)VVR	11.6	10	0.86	1.04	0.98		
			667.46	670.48	2+	Trypsin	GK(Ac)K(Ac)ILGDYDIK(Ac)	16.4	19	1.16				
			562.35	564.37	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	3.8	3.6	0.95				
			1007.17	1008.67	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	8.5	8	0.94				
			682.81	683.81	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	2.5	2.8	1.12				
			891.53	892.54	3+	Trypsin	TSISGNWK(Ac)TNTGSAMLEQIAMSDR	15.3	18.9	1.24				
		4	408.79	410.29	2+	Trypsin	NGTK(Ac)VVR	4.4	4.9	1.11	1.11	0.98		
		5	408.79	410.29	2+	Trypsin	NGTK(Ac)VVR	10.5	11.3	1.08	1.07	1.02		
			522.31	523.82	2+	Trypsin	K(Ac)SFASLFSD	31	34	1.10				
			562.68	564.37	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	21.5	22.5	1.05				
			843.54	846.55	2+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	42.2	44.8	1.06				
12	Synapsin IIb	2	408.76	410.25	2+	Trypsin	NGTK(Ac)VVR	4.5	6	1.33	1.39	1.32	1.02	0.42
			677.41	678.42	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	10.6	15.4	1.45				

		4	408.82	410.32	2+	Trypsin	NGTK(Ac)VVR	33	21	0.64	0.82	0.73		
			1007.22	1008.70	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	24.1	17	0.71				
			677.50	678.50	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	155	175	1.13				
13	Synapsin IIb	2	408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	2.3	3.2	1.39	0.43	0.41	0.87	0.98
		3	408.79	410.30	2+	Trypsin	NGTK(Ac)VVR	36	9	0.25	0.22	0.21		
			846.88	849.89	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	200	39	0.20				
		4	562.34	564.36	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	31	73	2.35	2.27	2.00		
			846.89	849.90	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	110	250	2.27				
			408.80	410.30	2+	Trypsin	NGTK(Ac)VVR	22	48	2.18				
14	Synapsin IIb	1	408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	32	23	0.72	0.72	0.68	0.90	0.27
		2	556.97	558.98	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	3.3	3.3	1.00	1.25	1.19		
			677.41	678.41	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	10	14.9	1.49				
		3	887.10	886.10	3+	Trypsin	TSISGNWK(Ac)TNTGSAMLEQIAMSDR	23	28	1.22	1.22	1.14		
		4	667.42	670.44	3+	Trypsin	GK(Ac)K(Ac)ILGDYDIK(Ac)	7.3	11.9	1.63	1.40	1.24		
			672.43	673.44	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	52	61	1.17				
		5	408.77	410.28	2+	Trypsin	NGTK(Ac)VVR	45.1	22.3	0.49	0.72	0.67		
			677.44	678.44	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	190.1	180.2	0.95				
		6	408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	10.4	8.1	0.78	0.84	0.73		
			553.29	554.80	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	3.8	2.8	0.74				
			644.31	645.80	2+	Trypsin	IGNNYK(Ac)AYMR	2.8	2.8	1.00				
			562.28	564.29	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	30	23.5	0.78				
			677.39	678.40	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	4.2	4.2	1.00				
			846.79	849.80	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	16.9	13	0.77				
		7	408.78	410.29	2+	Trypsin	NGTK(Ac)VVR	6	3.8	0.63	0.75	0.67		
			553.37	554.88	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	3.8	3.08	0.81				
			562.36	564.37	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	70	16	0.23				
			1007.04	1008.66	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	23.9	21.2	0.89				
			677.47	678.47	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	90	105	1.17				
15	Synapsin IIb	1	408.75	410.25	2+	Trypsin	NGTK(Ac)VVR	46	28	0.61	0.61	0.58	0.68	0.45
		2	408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	49	24	0.49	0.52	0.49		
			644.33	645.83	2+	Trypsin	IGNNYK(Ac)AYMR	17.9	9.8	0.55				
		3	408.75	410.26	2+	Trypsin	NGTK(Ac)VVR	37	52	1.41	1.48	1.43		
			644.35	645.84	2+	Trypsin	IGNNYK(Ac)AYMR	4	6.8	1.70				
			564.32	562.30	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	37	49	1.32				
		4	408.75	410.26	2+	Trypsin	NGTK(Ac)VVR	21	4	0.19	0.19	0.18		
		5	408.74	410.26	2+	Trypsin	NGTK(Ac)VVR	20	28	1.40	1.33	1.17		
			553.28	554.79	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	5.2	6.1	1.17				
			562.26	564.28	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	19	27	1.42				
		6	408.76	410.26	2+	Trypsin	NGTK(Ac)VVR	24	10.3	0.43	0.50	0.44		

			562.28	564.30	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	43	18	0.42					
			677.73	678.74	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	6	5	0.83					
			846.78	849.80	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	42	14	0.33					
		7	408.78	410.29	2+	Trypsin	NGTK(Ac)VVR	8.6	4	0.47	0.60	0.50			
			553.37	554.88	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	4.5	2.7	0.60					
			562.35	564.37	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	51	37	0.73					
16	Synapsin IIb	1	408.75	410.26	2+	Trypsin	NGTK(Ac)VVR	38	19.9	0.52	0.62	0.59	0.65	0.09	
			562.32	562.32	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	20.8	15	0.72					
		2	812.46	816.99	2+	Trypsin	DICAVK(Ac)AVHGK(Ac)DGK(Ac)	35	27	0.77	0.77	0.71			
		4	408.78	410.29	2+	Trypsin	NGTK(Ac)VVR	19.9	12.8	0.64	0.59	0.57			
			667.46	670.48	2+	Trypsin	GK(Ac)K(Ac)ILGDYDIK(Ac)	11.9	5.9	0.50					
			677.47	678.47	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	75	64	0.85					
			846.88	849.90	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	65	25	0.38					
		5	408.77	410.29	2+	Trypsin	NGTK(Ac)VVR	10	8.2	0.82	0.76	0.73			
			553.37	554.87	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	5.4	3.15	0.58					
			843.04	846.05	2+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR ox	50	27	0.54					
			851.03	854.05	2+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	25.3	19	0.75					
			1007.00	1008.15	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	40	35	0.88					
			671.80	672.80	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR (pyro)	32	31	0.97					
17	Synapsin IIb	1	408.74	410.26	2+	Trypsin	NGTK(Ac)VVR	14.7	13.5	0.92	0.92	0.81	0.73	0.09	
		2	408.77	410.26	2+	Trypsin	NGTK(Ac)VVR	16.8	15.3	0.91	0.89	0.78			
			667.40	670.42	2+	Trypsin	GK(Ac)K(Ac)ILGDYDIK(Ac)	36.3	17	0.47					
			846.82	849.84	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	66.3	85.3	1.29					
		3	812.49	817.01	2+	Trypsin	DICAVK(Ac)AVHGK(Ac)DGK(Ac)	8.5	7.1	0.84	0.84	0.80			
		4	408.76	410.27	2+	Trypsin	NGTK(Ac)VVR	3	2.7	0.90	0.80	0.76			
			553.31	554.82	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	1.5	1.1	0.73					
			644.33	645.84	2+	Trypsin	IGNNYK(Ac)AYMR	1.52	1.13	0.74					
			562.29	564.30	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	5.5	4.6	0.84					
		5	408.79	410.29	2+	Trypsin	NGTK(Ac)VVR	11	10	0.91	0.88	0.82			
			562.36	564.36	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	15.8	12	0.76					
			1007.17	1008.67	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	10.7	10.4	0.97					
		6	408.78	410.29	2+	Trypsin	NGTK(Ac)VVR	10.8	6.8	0.63	0.74	0.69			
			553.36	554.88	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	2.1	1.8	0.86					
		7	408.79	410.31	2+	Trypsin	NGTK(Ac)VVR	44	38	0.86	0.85	0.75			
			562.34	564.36	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	102	86	0.84					
			1007.00	1008.64	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	45	42	0.93					
			846.88	849.90	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	280	210	0.75					
		8	408.81	410.31	2+	Trypsin	NGTK(Ac)VVR	27	17	0.63	0.71	0.62			
			562.35	564.36	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	76	55	0.72					

			677.48	678.48	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	197	179	0.91					
			846.90	849.92	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	260	150	0.58					
		9	408.81	410.31	2+	Trypsin	NGTK(Ac)VVR	15	10.5	0.70	0.67	0.58			
			562.35	564.36	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	51	37	0.73					
			846.90	849.91	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	124	73	0.59					
SGS742-treated yoked vs NaCl-treated yoked															
1	Synapsin Ia	1	615.42	616.93	2+	Trypsin	SLK(Ac)PDFVLIR	169	149	0.88	0.74	0.75	0.67	0.13	
			407.27	409.27	2+	Trypsin	NGVK(Ac)VVR	64	44	0.69					
			597.87	599.38	2+	Trypsin	AVEALHGK(Ac)DGR	74	49	0.66					
		2	407.27	409.26	2+	Trypsin	NGTK(Ac)VVR	3.5	1.8	0.51	0.51	0.52			
		3	614.77	615.87	2+	Trypsin	SLK(Ac)PDFVLIR	12	9	0.75	0.75	0.73			
2	Synapsin Ib	1	615.39	616.87	2+	Trypsin	SLK(Ac)PDFVLIR	32	19	0.59	0.56	0.57	0.66	0.13	
			566.94	568.97	3+	Trypsin	VQK(Ac)IGQNYK(Ac)AYMR	17	9	0.53					
		4	745.83	748.84	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEF	40	30	0.75	0.75	0.76			
3	Synapsin Ib	1	615.43	616.87	2+	Trypsin	SLK(Ac)PDFVLIR	85	88	1.04	1.04	1.05	0.94	0.13	
		2	566.94	568.97	3+	Trypsin	VQK(Ac)IGQNYK(Ac)AYMR	17.1	13.5	0.79	0.79	0.80			
		4	634.33	635.84	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	12	8.5	0.71	0.96	0.97			
4	Synapsin Ib	1	615.39	616.89	2+	Trypsin	SLK(Ac)PDFVLIR	5	6	1.20	1.06	1.07	1.03	0.05	
			566.98	568.99	3+	Trypsin	VQK(Ac)IGQNYK(Ac)AYMR	3.5	3.2	0.91					
		4	634.39	635.90	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	105	100	0.95	1.00	0.99			
			720.44	721.96	2+	Chymotrypsin	K(Ac)LWVDTCSEIF	41	43	1.05					
			637.80	639.81	3+	Chymotrypsin	RNGVK(Ac)VVRSLK(Ac)PDFVL	46.5	46.5	1.00					
5	Synapsin IIa	1	408.75	410.25	2+	Trypsin	NGTK(Ac)VVR	26	16.2	0.62	0.72	0.73	0.85	0.17	
			562.34	564.35	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	36.75	23	0.63					
			677.44	678.44	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	28.6	26.1	0.91					
		2	408.78	410.29	2+	Trypsin	NGTK(Ac)VVR	3.9	3.8	0.97	0.97	0.96			
6	Synapsin IIa	2	408.26	409.76	2+	Trypsin	NGTK(Ac)VVR	12	5	0.42	0.42	0.41	0.67	0.37	
		4	640.37	641.38	3+	Chymotrypsin	RSSANEDEAK(Ac)AETIRSL	27	25	0.93	0.92	0.93			
			697.38	698.39	3+	Chymotrypsin	K(Ac)TNTGSAMLEQIAMSDRY	11.5	10.5	0.91					
7	Synapsin IIa	1	408.75	410.26	2+	Trypsin	NGTK(Ac)VVR	32	31.5	0.98	0.82	0.83	0.85	0.06	
			522.24	523.75	2+	Trypsin	K(Ac)SFASLFSD	107	82.5	0.77					
			553.29	554.80	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	16.2	10.3	0.64					
			562.28	564.30	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	47	41	0.87					
			677.40	678.40	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	71.5	60.05	0.84					
			846.79	849.81	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	25.05	20.05	0.80					
		2	846.82	849.84	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	43	40	0.93	0.93	0.93			
		3	408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	29	24	0.83	0.89	0.81			
			781.89	783.40	2+	Trypsin	SSANEDEAK(Ac)AETIR	72	68	0.94					
			562.32	564.33	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	36	32	0.89					

		4	855.52	858.53	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEFSEL	39	36	0.92	0.85	0.84		
			748.81	745.79	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEF	47.5	36	0.76				
			634.36	635.87	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	104	91	0.88				
8	Synapsin IIa	2	408.09	410.26	2+	Trypsin	NGTK(Ac)VVR	67	47	0.70	0.70	0.69	0.86	0.23
		4	855.56	858.57	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEFSEL	55	54	0.98	1.01	1.02		
			745.83	748.85	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEF	130	135	1.04				
			640.40	641.41	3+	Chymotrypsin	RSSANEDEAK(Ac)AETIRSL	47	58	1.23				
			904.57	907.59	2+	Chymotrypsin	K(Ac)TLGGEK(Ac)FPLIEQTY	280	220	0.79				
			723.93	722.42	2+	Chymotrypsin	K(Ac)LWVDACSEMF	84	104	1.24				
			681.45	682.96	2+	Chymotrypsin	DIRVQK(Ac)IGNNY	76	66	0.87				
			634.38	635.89	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	150	145	0.97				
			603.41	604.92	2+	Chymotrypsin	RNGTK(Ac)VVRSF	15	14.9	0.99				
			490.77	492.27	2+	Chymotrypsin	K(Ac)TNTGSAML	28	27.5	0.98				
9	Synapsin IIa	1	408.77	410.25	2+	Trypsin	NGTK(Ac)VVR	7.5	5	0.67	0.79	0.80	0.83	0.05
			671.74	672.75	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	15	15	1.00				
			677.41	678.43	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	14	10	0.71				
			846.82	849.83	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	12	9.3	0.78				
		3	408.80	410.31	2+	Trypsin	NGTK(Ac)VVR	72	55	0.76	0.87	0.86		
			677.48	678.49	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	35.33	34.38	0.97				
			781.90	783.41	3+	Trypsin	SSANEDEAK(Ac)AETIR	240	210	0.88				
10	Synapsin IIa	1	522.26	523.77	2+	Trypsin	K(Ac)SFASLFSD	63	46	0.73	0.85	0.86	0.92	0.08
			781.90	783.42	3+	Trypsin	SSANEDEAK(Ac)AETIR	60	49	0.82				
			562.31	564.32	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	63	52	0.83				
			677.42	678.42	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	109	109	1.00				
			846.81	849.83	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	84	67	0.80				
			408.76	410.27	2+	Trypsin	NGTK(Ac)VVR	40	37	0.93				
		2	408.27	410.26	2+	Trypsin	NGTK(Ac)VVR	44	43	0.98	0.98	0.97		
			562.34	564.35	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	115	114	0.99				
11	Synapsin IIa	1	522.26	523.78	2+	Trypsin	K(Ac)SFASLFSD	9.2	10.5	1.14	1.06	1.07	1.07	0.01
			553.32	554.82	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	7.5	7.4	0.99				
			781.90	783.41	2+	Trypsin	SSANEDEAK(Ac)AETIR	23	26	1.13				
			846.81	849.84	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	46	45	0.98				
		4	490.75	492.27	2+	Chymotrypsin	K(Ac)TNTGSAML	11	12.8	1.16	1.09	1.08		
			603.39	604.91	2+	Chymotrypsin	RNGTK(Ac)VVRSF	6.3	7.5	1.19				
			634.37	635.88	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	40	44	1.10				
			745.81	748.83	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEF	53	53	1.00				
			855.52	858.54	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEFSEL	65	65	1.00				
12	Synapsin IIb	1	408.77	410.28	2+	Trypsin	NGTK(Ac)VVR	12	11	0.92	0.84	0.85	0.97	0.17
			562.32	564.33	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	15.4	11.8	0.77				

		2	408.74	410.24	2+	Trypsin	NGTK(Ac)VVR	15	16.5	1.10	1.10	1.09		
13	Synapsin IIb	1	408.77	410.28	2+	Trypsin	NGTK(Ac)VVR	40	39.99	1.00	0.84	0.83	0.94	0.10
			553.32	554.83	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	27	15	0.56				
			644.36	645.86	2+	Trypsin	IGNNYK(Ac)AYMR	19.2	13.7	0.71				
			562.31	564.32	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	60	59.99	1.00				
			846.84	849.85	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	90	85	0.94				
		2	408.73	410.24	2+	Trypsin	NGTK(Ac)VVR	42	42	1.00	0.97	0.89		
			562.26	564.28	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	50	49	0.98				
			846.76	849.77	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	106	100.1	0.94				
		3	408.81	410.32	2+	Trypsin	NGTK(Ac)VVR	63	73	1.16	1.16	1.05		
			843.04	846.07	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	53	51	0.96				
			826.21	827.90	3+	Trypsin	K(Ac)TLGGEK(Ac)FPLIEQTYYPNHR	57	77	1.35				
		4	634.38	635.89	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	35	34	0.97	0.97	0.98		
14	Synapsin IIb	1	846.76	849.77	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	95	105	1.11	1.12	1.13	0.84	0.26
			562.27	564.28	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	40	45.05	1.13				
			553.29	554.80	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	7	7.5	1.07				
			408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	41	45	1.10				
			1006.97	1008.50	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	13	15.5	1.19				
		3	408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	146	131	0.90	0.70	0.70		
			553.83	554.85	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	120	70	0.58				
			843.45	846.46	2+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	100	68	0.68				
			562.32	564.34	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	116.75	88	0.75				
			672.42	673.44	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	29.86	15.5	0.52				
			677.76	678.76	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	104	82.33	0.79				
		4	634.39	635.89	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	180	175	0.97	0.69	0.68		
			681.45	682.96	2+	Chymotrypsin	DIRVQK(Ac)IGNNY	90	59	0.66				
			722.41	723.91	2+	Chymotrypsin	K(Ac)LWVDACSEMF	280	230	0.82				
			904.56	907.58	2+	Chymotrypsin	K(Ac)TLGGEK(Ac)FPLIEQTY	368	200	0.54				
			745.82	748.84	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEF	310	178	0.57				
			855.54	858.57	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEFSEL	420	230	0.55				
15	Synapsin IIb	1	846.76	849.78	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	215	179	0.83	1.01	1.02	0.81	0.18
			562.28	564.29	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	26	27	1.04				
			644.31	645.82	2+	Trypsin	IGNNYK(Ac)AYMR	6.8	7.8	1.15				
		3	408.81	410.32	2+	Trypsin	NGTK(Ac)VVR	90	87	0.97	0.77	0.67		
			843.04	846.06	2+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	200	148	0.74				
			945.61	948.63	3+	Trypsin	EMLTLPTFPVVVK(Ac)IGHAHSGMGK(Ac)VK(Ac)	218	134	0.61				
		4	490.78	492.28	2+	Chymotrypsin	K(Ac)TNTGSAML	78	54	0.69	0.74	0.74		
			603.41	604.92	2+	Chymotrypsin	RNGTK(Ac)VVRSF	68	34	0.50				
			634.39	635.88	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	200	185	0.93				

			904.57	907.58	2+	Chymotrypsin	K(Ac)TLGGEK(Ac)FPLIEQTY	119	95	0.80				
			745.83	748.85	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEF	231	185	0.80				
			855.55	858.57	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEFSEL	350	260	0.74				
16	Synapsin IIb	1	408.77	410.28	2+	Trypsin	NGTK(Ac)VVR	63	48	0.76	0.71	0.72	0.73	0.02
			562.31	564.32	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	118	90	0.76				
			846.83	849.85	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	240	145	0.60				
		3	408.25	409.76	2+	Trypsin	NGTK(Ac)VVR	120	75	0.63	0.77	0.73		
			562.32	564.33	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	99	68	0.69				
			677.42	678.42	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	91	91.33	1.00				
			766.10	767.10	3+	Trypsin	SQAVK(Ac)QTAASAGLVDAPAPSAASR	103.33	78.11	0.76				
		4	603.40	604.92	2+	Chymotrypsin	RNGTK(Ac)VVRSF	45	44.5	0.99	0.75	0.75		
			634.36	635.87	2+	Chymotrypsin	ATAEPFIDAK(Ac)Y	220	160	0.73				
			855.55	858.57	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEFSEL	320	175	0.55				
17	Synapsin IIb	1	408.77	410.28	2+	Trypsin	NGTK(Ac)VVR	36	46	1.28	1.10	1.11	0.85	0.19
			553.33	554.84	2+	Trypsin	VQK(Ac)IGNNYK(Ac)	14	15	1.07				
			636.36	637.87	2+	Trypsin	IGNNYK(Ac)AYMR	5.4	5	0.93				
			556.99	559.00	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	9.9	9.5	0.96				
			846.83	849.85	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	190	240	1.26				
		2	408.77	410.28	2+	Trypsin	NGTK(Ac)VVR	48	48	1.00	0.89	0.89		
			562.31	564.33	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	33	31	0.94				
			1007.09	1008.58	2+	Trypsin	QLITDLVISK(Ac)MNQLLSR	29	24	0.83				
			846.83	849.85	3+	Trypsin	K(Ac)AK(Ac)VLLVVDEPHTDWAK(Ac)CFR	105	85	0.81				
		3	408.74	410.25	2+	Trypsin	NGTK(Ac)VVR	211	153	0.73	0.72	0.69		
			644.35	645.86	2+	Trypsin	IGNNYK(Ac)AYMR	54	40	0.74				
			562.31	564.32	3+	Trypsin	VQK(Ac)IGNNYK(Ac)AYMR	155	103.75	0.67				
			678.08	679.09	3+	Trypsin	QLITDLVISK(Ac)MNQLLSR	176	125	0.71				
			891.41	892.42	3+	Trypsin	TSISGNWK(Ac)TNTGSAMLEQIAMSDR	246	191	0.78				
		4	603.40	604.92	2+	Chymotrypsin	RNGTK(Ac)VVRSF	63	55	0.87	0.79	0.72		
			697.42	698.42	3+	Chymotrypsin	K(Ac)TNTGSAMLEQIAMSDRY	55	63	1.15				
			745.83	748.84	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEF	310	180	0.58				
			855.55	858.58	3+	Chymotrypsin	RGK(Ac)K(Ac)ILGDYDIK(Ac)VEQAEFSEL	155	90	0.58				

Ac-denotes acetylation of lysine light and heavy labelled.

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Presentations and conferences

9TH INTERNATIONAL CONGRESS ON AMINO ACIDS AND PROTEINS, VIENNA AUSTRIA – 8. AUGUST 2005

[poster] "characterization of human stomatin like protein-2 (slp-2) a novel homologue of stomatin, by using mass spectrometry"

5TH Forum of European Neuroscience, vienna austria -8 july 2006

[poster] "DYSREGULATION OF SIGNALING AND CHAPERONE PROTEINS EIGHT HOURS FOLLOWING SPINAL CORD INJURY IN THE RAT"

UNIVERSITÄTSVORLESUNG vienna austria -21 june 2007

[poster] "Nitric Oxide and Oxygen Radical Attack on GDP-Dissociation Inhibitor 2 (GDI-2) in Spinal Cord Injury of the Rat"

Publications

1. JOHN JP, LUBEC G. COMPLETE SEQUENCING AND OXIDATIVE MODIFICATION OF TWO PROTEINS REPRESENTING MN-SOD (SOD2) IN HUMAN MEDULLOBLASTOMA CELLS DAOY (IN PREPARATION)
2. JOHN JP, LUBEC G. HIPPOCAMPAL SYNAPSIN ISOFORMS LEVELS ARE LINKED TO COGNITIVE ENHANCEMENT OF MEMORY FORMATION IN THE MOUSE (ACCEPTED IN HIPPOCAMPUS, 2008)
3. JOHN JP, OH JE, POLLAK A, LUBEC G. IDENTIFICATION AND CHARACTERISATION OF ARSENITE (+3 OXIDATION STATE) METHYLTRANSFERASE (AS3MT) IN MOUSE NEUROBLASTOMA CELL LINE N1E-115.AMINO ACIDS. 2007 DEC 21;
4. JOHN JP, CHEN WQ, POLLAK A, LUBEC G. MASS SPECTROMETRIC STUDIES ON MOUSE HIPPOCAMPAL SYNAPSINS IA, IIA, AND IIB AND IDENTIFICATION OF A NOVEL PHOSPHORYLATION SITE AT SERINE-546.J PROTEOME RES. 2007 JUL;6(7):2695-710. 2007 .
5. JOHN JP, PINTSOV O, PETTER-PUCHNER A, REDL H, POLLAK A, CHEN WQ, LUBEC G. NITRIC OXIDE AND OXYGEN RADICAL ATTACK ON GDP-DISSOCIATION INHIBITOR 2 (GDI-2) IN SPINAL CORD INJURY OF THE RAT. J PROTEOME RES. 2007 APR;6(4):1500-9.
6. BIERCZYNSKA-KRZYSIK A, JOHN JP, SILBERRING J, KOTLINSKA J, DYLAG T, CABATIC M, LUBEC. PROTEOMIC ANALYSIS OF RAT CEREBRAL CORTEX, HIPPOCAMPUS AND STRIATUM AFTER EXPOSURE TO MORPHINE.INT J MOL MED. 2006 OCT;18(4):775-84
7. POLLAK DD, JOHN JP, HOEGER H, LUBEC G. AN INTEGRATED MAP OF THE MURINE HIPPOCAMPAL PROTEOME BASED UPON FIVE MOUSE STRAINS. ELECTROPHORESIS. 2006 JUL; 27(13):2787-98.
8. POLLAK DD, JOHN JP, BUBNA-LITTITZ H, SCHNEIDER A, HOEGER H, LUBEC G. COMPONENTS OF THE PROTEIN QUALITY CONTROL SYSTEM ARE EXPRESSED IN A STRAIN-DEPENDENT MANNER IN THE MOUSE HIPPOCAMPUS. NEUROCHEM INT. 2006 OCT; 49(5):500-7.
9. JOHN JP, ANRATHER D, POLLAK A, LUBEC G MASS SPECTROMETRICAL VERIFICATION OF STOMATIN-LIKE PROTEIN 2 (SLP-2) PRIMARY STRUCTURE. PROTEINS. 2006 AUG;64(2):543-51
10. POLLAK DD, JOHN JP, SCHARL T, LEISCH F, SCHNEIDER A, HOEGER H, LUBEC G. STRAIN-DEPENDENT REGULATION OF NEUROTRANSMISSION AND ACTIN-REMODELLING PROTEINS IN THE MOUSE HIPPOCAMPUS.GENES BRAIN BEHAV. 2006 MAR;5(2):200-4.
11. POLLAK DD, JOHN JP, SCHNEIDER A, HOEGER H, LUBEC G. STRAIN-DEPENDENT EXPRESSION OF SIGNALING PROTEINS IN THE MOUSE HIPPOCAMPUS. NEUROSCIENCE. 2006;138 (1):149-58.
12. LUBEC G, AFJEHI-SADAT L, YANG JW, JOHN JP. SEARCHING FOR HYPOTHETICAL PROTEINS: THEORY AND PRACTICE BASED UPON ORIGINAL DATA AND LITERATURE. PROG NEUROBIOL. 2005 SEP-OCT;77 (1-2):90-127. REVIEW.