

**PW 33:
Animal models (Part 1)**

PW33-413	<p><u>ELECTROMYOGRAPHIC EVALUATION OF MUSCLE ACTIVITY IN AN ANIMAL MODEL OF DELTA-SARCOGLYCAN DEFICIENCY.</u> GIROUX-METGÈS MA¹, HOGREL JY¹, FISZMAN MY¹, FROMES Y¹ (1) Institut de Myologie, Paris, FRANCE.</p>
<p>To contact the author:: ma.metges@institut-myologie.org.</p>	<p>CHF147 hamster strain is an animal model of delta-sarcoglycan deficiency. Skeletal muscular dystrophy features observed in this model are very similar to those described in humans with more severe disease in proximal than distal muscles. Muscle activation in this limb girdle muscular dystrophy has not yet been studied. Our goal was to develop a standardized protocol for electromyography (EMG) study during either voluntary or electrically elicited contractions.</p> <p>Prior to surgery hamsters were trained to run at different speeds on a motor-driven treadmill. <i>Tibialis anterior</i> (TA) and <i>vastus lateralis</i> (VL) were instrumented in CHF147 and control animals during global anesthesia. In each muscle, electrodes were made up of two teflon insulated multistranded stainless wires (diameter = 110 μm). Recording surfaces were made by removing 1 mm of insulation and the distance between bared regions let to 2-2.5 mm centre-to-centre. The first recordings started at day 6 after surgery. Two kinds of measurements were performed. First, global EMG activity of TA and VL for each animal was recorded during 10-seconds runs at increasing treadmill velocities (from 15 to 35 cm.s⁻¹). Secondly, electrical stimulation on anesthetized animals was performed in order to analyze muscle response during recruitment and fatigue.</p> <p>The methodology used in the present work generally yields EMG signals of good quality either during voluntary or during electrically elicited contractions. Intramuscular wires do not influence leg movements in hamsters. Various results in both contraction modes can be depicted. For example, during repeated stimulation at 20 Hz, compound muscle action potential amplitude decreased while its duration increased associated to spectral compression. The rate of change of such parameters can be used to assess muscle fatigability.</p> <p>This standardized procedure associated to reliable electrophysiological parameters may be useful for the follow-up of animals during the natural progression of the disease or during therapeutic trials.</p>

PW33-414	<p><u>THE ACETYLCHOLINESTERASE DEFICIENCY SEEN AT THE NEUROMUSCULAR JUNCTION IN THE MOUSE MODEL OF SCHWARTZ-JAMPEL SYNDROME IS PROBABLY NECESSARY BUT IS NOT SUFFICIENT TO INDUCE NEUROMYOTONIA</u></p> <p>STUM M¹, BANGRATZ M¹, GIRARD E², BERNARD V³, DAVOINE CS¹, TABTI N¹, WILLER JC⁴, ECHANIZ-LAGUNA A⁵, RENÉ F⁵, MARCEL C⁵, FONTAINE B¹, MOLGO J², KREJCI E³, NICOLE S¹</p> <p>(1) Inserm, U546, Paris, FRANCE. (2) CNRS, UPR9040, Gif sur Yvette, FRANCE. (3) Inserm, U686, Paris, FRANCE. (4) Assistance Publique-Hopitaux de Paris, Paris, FRANCE. (5) Inserm, U692, Strasbourg, FRANCE.</p>
To contact the author:: nicole@chups.jussieu.fr.	<p>Schwartz-Jampel syndrome (SJS) is a recessive disorder characterized by neuromyotonia with complex repetitive discharges at electromyography (EMG). SJS results from hypomorphic mutations of perlecan, a ubiquitous proteoglycan secreted within basement membranes (BM). The muscle hyperactivity in SJS is proposed to result from synaptic acetylcholinesterase (AChE) deficiency as perlecan is crucial for the anchorage of AChE at the neuromuscular junction (NMJ). However, EMG patterns typical of AChE deficiency at the NMJ is not observed in patients with SJS. To determine whether the AChE hypothesis is correct, we studied the mutants of our SJS mouse model (see the abstract of Bangratz et al. for details on the model).</p> <p>Partial AChE deficiency at the NMJ was seen in all striated muscles tested. Lack of pretzel-like organization of postsynaptic acetylcholine receptors, poor branching of nerve terminals, and partially denervated NMJs were also observed. These alterations probably resulted from the major loss of synaptic perlecan as no disorganization of the extrasynaptic muscle BM nor muscle degeneration were seen, that suggested a role of perlecan in the maturation and/or maintenance of the NMJ. However, the AChE deficiency seen at the NMJ was primary to the synaptic deficiency of perlecan and was not due to denervation events as checked by transcriptional and co-staining analyses. EMG performed on the mutants demonstrated the occurrence of neuromyotonic discharges without decrement during repetitive stimulation in all muscles tested except the diaphragm. Despite the absence of neuromyotonic discharges, measures of contractile force and synaptic transmission parameters on isolated hemidiaphragm preparations showed features of AChE deficiency. These results demonstrate an electrophysiological effect of the partial AChE deficiency seen at the NMJ, and suggest that the AChE deficiency is necessary but is not sufficient to induce the neuromyotonia in SJS.</p>

PW33-415	<p><u>INVERSE CORRELATION BETWEEN THE LEVEL OF SECRETED PERLECAN AND THE SEVERITY OF THE ASSOCIATED PHENOTYPE IN A MOUSE MODEL OF SCHWARTZ-JAMPEL SYNDROME</u></p> <p>STUM M¹, BANGRATZ M¹, BERNARD V², DAVOINE CS¹, FONTAINE B¹, KREJCI E², NICOLE S¹</p> <p>(1) Inserm, U546, Paris, FRANCE. (2) Inserm, U686, Paris, FRANCE.</p>
To contact the author:: nicole@chups.jussieu.fr.	<p>Schwartz-Jampel syndrome (SJS) is a recessive disorder with neuromyotonia and chondrodysplasia that appear during childhood and slowly progress until adulthood. SJS results from hypomorphic mutations in the perlecan gene, a proteoglycan secreted within basement membranes, and is allelic to DDSH, a recessive chondrodysplasia lethal at birth. A gene dosage effect is currently proposed to account for the difference in severity: the residual level of perlecan secreted in SJS would rescue the lethality due to the complete lack of perlecan in DDSH. To test this hypothesis and understand the pathophysiology of SJS, we developed one mouse model by introducing the p.C1532Y missense mutation into the perlecan gene by homologous recombination. We developed two mouse lines: one (Neo+) still contained the Neo box in addition to p.C1532Y, and the second (Neo-) contained only the p.C1532Y mutation.</p> <p>Homozygous mutant mice from the two lines were viable. A reduced secretion of perlecan was seen in the Neo+ and Neo- mutants, with a lower level in Neo+ compared to Neo- due to splicing events between the perlecan gene and Neo. In the two lines, the adult mutants were smaller than controls and develop a neuromuscular phenotype with delayed opening of the eyelids and flexion of the hind paw when suspended by the tail. Alterations of neuromuscular junctions with lack of pretzel-like shape and acetylcholinesterase deficiency, and histological changes of skeletal muscles similar to those observed in patients with SJS were observed in the two lines, but they were more severe in Neo+. Altogether, our results argue for an inverse correlation between the level of secreted perlecan and the severity of the phenotype, and propose increasing the level of secreted perlecan by acting on the hypomorph effect of the SJS mutations as a rational therapeutic strategy for SJS.</p>

PW33-416	<p><u>THE ROLE OF THE DYSTROPHIN-GENE PRODUCT DP71 IN EXCITATORY SYNAPSE ORGANIZATION, GLUTAMATERGIC TRANSMISSION, SYNAPTIC PLASTICITY, AND SELECTIVE BEHAVIORAL FUNCTIONS.</u></p> <p>DAOUD F¹, CANDELARIO-MARTINEZ A³, BILLARD J⁴, AVITAL A⁵, KHELFAOUI M², ROZENVALD Y⁶, GUEGAN M⁷, MORNET D⁹, NUDEL U⁶, CHELLY J¹, MARTINEZ-ROJAS D³, LAROCHE S⁷, YAFFE D⁶, VAILLEND C⁸</p> <p>(1) Institut Cochin, Université Paris-Descartes, CNRS, UMR 8104, PARIS, FRANCE. (2) 2 INSERM U 567, PARIS, FRANCE. (3) CINVESTAV, Physiology, Biophysics and Neurosciences, MEXICO City, MEXICO. (4) Université Paris-Descartes, Faculté de médecine René Descartes, Neurobiologie de la Croissance et de la Sénescence,, PARIS, FRANCE. (5) Weizmann Institute of Science, Neurobiology, Max Stern Yezreel Valley College, Behavioral Sciences,, REHOVOT, ISRAEL. (6) Weizmann Institute of Science, Molecular Cell Biology, REHOVOT, ISRAEL. (7) CNRS, Neurobiologie de l'Apprentissage, de la Mémoire et de la Communication, UMR 8620,, ORSAY, FRANCE. (8) Univ Paris-Sud, ORSAY, FRANCE. (9) 10 INSERM, Equipe ESPRI 25 Muscle et Pathologies, Université de Montpellier I, UFR de Médecine, EA 4202, MONTPELLIER, FRANCE.</p>
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Duchenne muscular dystrophy (DMD) is caused by deficient expression of the cytoskeletal protein, dystrophin. One third of DMD patients also have mental retardation (MR), likely due to mutations preventing expression of dystrophin and other brain products of the DMD gene translated from distinct internal promoters. Loss of Dp71, the major DMD-gene product in brain, is thought to contribute to the severity of MR; however, the specific function of Dp71 is poorly understood. Here we used complementary approaches to explore the role of Dp71 in neuronal function and identify putative mechanisms by which Dp71 loss may impair neuronal and cognitive functions. We used neuronal cell cultures and fractionation studies to assess the subcellular localization of Dp71 and found expression at both pre- and postsynaptic compartments of excitatory synapses. We found that Dp71-associated protein complexes interact with specialized modular scaffolds that cluster glutamate receptors and organize signaling in postsynaptic densities. We further showed that Dp71-null mice display abnormal synapse organization and maturation in vitro, abnormally enhanced glutamatergic transmission and altered synaptic plasticity in CA1 hippocampal area. Analysis of the behavioral phenotype of Dp71-null mice revealed mild behavioral disturbances characterized by reduced exploratory behavior and delayed spatial learning. These findings suggest that Dp71 acts as a key regulator of glutamatergic synapse organization and function. This link between genetic loss of Dp71 and altered glutamatergic synapse function provides a mechanism which may underlie the increased severity of MR when inactivation of Dp71 is associated with that of other brain products of the DMD gene.

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INCLUSION BODY MYOSITIS IN A RHESUS MONKEY.

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Nonhuman primates are the ideal animal models for preclinical transplantation studies. In addition, in the case of myoblast transplantation, the behavior of these cells is similar to humans, in contrast to other animal models. Unfortunately, there are not colonies of myopathic nonhuman primates available for cell transplantation experiments. Here, we report the case of a rhesus (*Macaca mulata*) monkey with a myopathy exhibiting a histological pattern similar to the human inclusion body myositis. The monkey was detected among a group of male rhesus received for studies in our research center. The size and weight of the monkey were less than a half the values of other rhesus of the same age and sex. Since the general state of the animal deteriorated rapidly, the veterinary decided to perform analyses before to proceed to euthanasia. In the biochemical analyses, the values of creatine kinase and LDH were abnormally high. Clinically, we observed generalized severe muscle atrophies and musculotendinous contractures. We performed biopsies in the biceps brachii, quadriceps femoris and gastrocnemius. The biopsies showed several pathological modifications. The most prominent was the presence of several myofibers with rimmed vacuoles and/or eosinophilic hyaline inclusions, together with lymphocyte accumulations around myofibers, necrotic and regenerating myofibers, and thickening of the endomysium. Rimmed vacuoles contained lamellar structures in semithin sections. This veterinary case report point-out the importance to be vigilant in detecting symptoms of neuromuscular diseases in the colonies of nonhuman primates, trying to find candidates to use in neuromuscular research.

PW33-418	<p>AN EXPERIMENTAL MODEL OF FIBROSIS IN TIBIALIS ANTERIOR MUSCLE OF MDX MOUSE DESGUERRE I¹, ARNOLD L¹, GHERARDI K¹, FERRY A², CHAZAUD B¹ (1) INSERM U841, Creteil, FRANCE. (2) INSERM U787, Paris, FRANCE.</p>
To contact the author:: benedicte.chazaud@ins erm.fr.	<p>Duchenne muscular dystrophy (DMD) is a progressive disease, with patients gradually losing most of their skeletal muscle strength with increasing fibrosis and fatty tissue infiltration. Endomysial fibrosis is believed to be in part responsible of muscle weakness, since it is correlated to motor deficit.</p> <p>The mdx mouse also lacks dystrophin. From 3 weeks of age, time of a massive myofibre destruction, mdx skeletal muscle presents the same characteristics that DMD muscle including necrotic and regenerating myofibres, surrounded by inflammatory cells. However, excepted the diaphragm muscle that presents similar histology than DMD muscle, limb muscles do not present hallmark of fibrosis, excepted at very old age (at least 20 months). Moreover, adult mdx mouse does not present sign of muscle weakness (excepted after excentric exercise) while diaphragm presents dysfunction and loss of compliance.</p> <p>Our aim was to establish a fibrotic model of dystrophinopathy in adult - not ederyl-mdx mouse by repeated mechanic microinjuries in hindlimb muscle. One week after the end of microinjuries, histological analysis and strength evaluation were performed. Histological stainings and collagene I immunolabellings showed the presence of endomysial fibrosis. Number of centrally nucleated myofibres was unchanged while the diameter of the fibres was decreased in treated muscle. Maximal force was decreased by more than 30% suggesting the involvement of endomysial fibrosis in muscle weakness. After one month resting, endomysial collagene I area was strongly decreased and force raised near control values, suggesting a fibrolytic process.</p> <p>The experimental model of skeletal muscle endomysial fibrosis we propose is transient, showing sequentially the establishment of fibrosis in the interstitial compartment, as it is observed in DMD, then a disappearance of this endomysial fibrosis. This model will be helpfull to study the cellular and molecular mechanisms involved in both fibrogenesis and fibrolysis processes in dystrophic skeletal muscle.</p>

PW33-419	<p>TRIADIN KNOCK-OUT MOUSE : A MODEL FOR CORE MYOPATHIES ? ODDOUX S¹, FAURÉ J¹, BROCARD J¹, BROCARD J², FOUREST A¹, LUNARDI J¹, MARTY I¹ (1) Grenoble Institut des Neurosciences - Inserm U836 - Equipe Muscle et Pathologies - UJF Site Santé - Bat Edmond J Safra - BP 170 - 38042, Grenoble, FRANCE. (2) Grenoble Institut des Neurosciences - Inserm U836 - Equipe Physiopathologie du Cytosquelette - UJF Site Santé - Bat Edmond J Safra - BP 170 - 38042, Grenoble, FRANCE.</p>
To contact the author:: isabelle.marty@ujf-grenoble.fr.	<p>In skeletal muscle, release of calcium leading to contraction (a mechanism also called "excitation contraction coupling") is performed by a macromolecular complex, composed of two calcium channels (ryanodine receptor, RyR, and dihydropyridine receptor, DHPR). A number of associated proteins (triadin, calsequestrin, ...) are also part of this complex, they could regulate the calcium channels. Mutations of RYR1 and DHPR resulting in an alteration of the calcium homeostasis have been associated with various muscle diseases including malignant hyperthermia and core associated congenital myopathies such as Central Core Disease (CCD) or Multi-mini Cores Disease (MmD). However in a number of patients, no mutation has been identified in either of the calcium channels, pointing to potential involvement of other proteins in the physiopathology of these diseases. Our research project aims at understanding the function of triadin in muscle and in the calcium release complex, and its possible involvement in these myopathies. We have previously shown that overexpression of one triadin isoform, Trisk 95, abolishes excitation-contraction coupling, and therefore that the precise stoichiometry of triadin compared to RyR seems to be important for a correct function of the calcium release complex. In order to go deeper in the identification of the function of triadin, we have developed a triadin knock-out mouse, in collaboration with ICS-Strasbourg. The first characterization (Western blot analysis, immunolabelling on muscle section) of this mouse will be presented, and the possible correlation with a human pathology will be discussed.</p>

PW33-420	<p>LOSS OF SKELETAL MUSCLE STRENGTH IN JP-45 KNOCK OUT MICE DELBONO O¹, XIA J², TREVES S², WANG ZM¹, JIMENEZ-MORENO R¹, PAINE A¹, MESSI LM¹, NISHI M⁴, BRIGUET A³, SCHAEERER F³, TAKESHIMA H⁴, ZORZATO F² (1) Wake Forest School of Medicine, Winston-Salem, USA. (2) University Basel Hospital, Basel, SWITZERLAND. (3) Santhera Parmaceuticals, Liestal, SWITZERLAND. (4) Kyoto University, Kyoto, JAPAN.</p>
	<p>Skeletal muscles from young adult mice containing homozygous deletion of the gene for the sarcoplasmic reticulum protein JP-45 were studied and compared to age-matched wild-type litter mates. Analysis of spontaneous motor activity with a running wheel revealed that dark phase running distance ran by the JP-45 KO mice was significantly lower compared to wild type. <i>In vitro</i> EDL and soleus muscle mechanical property analysis showed slower twitch and tetanic absolute and specific force in JP-45 KO mice compared to wild-type, while muscle size, wet weight and cross sectional area of muscle fibers were not different. Isolated sarcotubular membranes isolated from 3 month and 6 months old JP-45 KO mouse muscles exhibited reduced [³H]PN200-110 binding indicating down regulation of Ca_v1.1 protein. SR Ca²⁺ release in voltage-clamped muscle fibers of JP-45 KO was significantly reduced compared to wild-type.</p> <p>Our results show that ablation of JP-45 reduces functional Ca_v1.1 protein expression and/or targeting to the t-tubule membrane, impairing the EC coupling signal between Ca_v1.1 and RyR1, thereby reducing skeletal muscle force</p>

PW33-421	<p><u>IMPAIRED MTOR SIGNALING IN MOUSE SKELETAL MUSCLE LEADS TO A SEVERE MYOPATHY</u></p> <p>GANGLOFF YG¹, RISSON V¹, CORNELOUP C¹, MAZELIN L¹, SANCHEZ H², ROCERRI M³, RICHARD H², VIGNAUD A⁴, BAUCHE S⁵, HANTAI D⁵, MUELLER M⁶, KOZMA S⁷, THOMAS G⁷, FERRY A⁴, PENDE M³, BIGARD X², KOULMANN N², SCHAEFFER L¹</p> <p>(1) CNRS-UMR5239-IFR128, Ecole Normale Supérieure, Lyon, FRANCE. (2) CRSSA, Grenoble, FRANCE. (3) INSERM U810, Faculté de médecine Necker, Paris, FRANCE. (4) UMR 787, Université Pierre et Marie Curie-Paris 6, Paris, FRANCE. (5) INSERM U582, Institut de Myologie, Paris, FRANCE. (6) Novartis Pharma AG, Basel, SWITZERLAND. (7) Department of Genome Science, Cincinnati, USA.</p>
To contact the author:: yann-gael.gangloff@ens-lyon.fr.	<p>The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that coordinates cellular responses to nutritional and growth factor conditions by controlling transcription, translation, cell size, cytoskeletal organization and autophagy. mTOR is encoded by an essential gene and its function <i>in vivo</i> has been mainly inferred by employing the mTOR inhibitor, rapamycin. By this approach, mTOR signaling has been shown to be necessary for skeletal muscle growth. However, mTOR is part of two distinct complexes, mTORC1 and mTORC2, which display differential sensitivity to rapamycin. Therefore, we generated and characterized skeletal muscle-specific mTOR knock-out mice to identify the full spectrum of mTOR functions in the post-natal development of skeletal muscle. We show that these mice develop a severe myopathy displaying characteristics of dystrophies and metabolic myopathies as they exhibit muscle fiber regeneration, increased glycogen content, decreased glycolytic capacity as well as altered oxidative metabolism. We find that mTOR-depleted muscles are weaker and that they show slower contractile properties and reduced fatigue resistance. Moreover, our data show significantly altered expression of genes critical for muscle structure and energy metabolism in mutant muscles that could underlie the pathology. These results demonstrate a critical role for mTOR in maintenance of muscle fiber integrity. Our findings suggest that alterations of mTOR-mediated physiological processes could contribute to the pathogenesis of a broad range of myopathies and emphasizes the therapeutic interest in developing activators of this pathway.</p>

PW33-422	<p><u>ASSESSMENT OF GAIT QUALITY IN DYSTROPHIN-DEFICIENT DOGS USING ACCELEROMETRY</u> BARTHELEMY I¹, BARREY E³, THIBAUD JL¹, URIARTE A¹, BLOT S¹, HOGREL JY² (1) Laboratoire de Neurobiologie - ENVA, Maisons-Alfort, FRANCE. (2) Institut de Myologie - GH Pitié-Salpêtrière, Paris, FRANCE. (3) Laboratoire d'Etude de la Physiologie de l'Exercice - INSERM 902 - Genopole, Evry, FRANCE.</p>
To contact the author:: ibarthelemy@vet-alfort.fr.	<p>Dystrophin-deficient dogs (GRMD, LRMD) exhibit similar pathophysiological and clinical features as Duchenne patients and represent therefore the best animal model to evaluate a therapeutic benefit. Emerging systemic approaches and the associate necessity to assess their clinical efficiency have led us to develop a quantitative, discriminating and non-invasive method to analyse locomotion. Accelerometry, a gait analysis technique based on 3D recording of accelerations, seems to be easy to perform in dogs and a source of several quantified parameters.</p> <p>In order to test this method, 11 dystrophin-deficient and 5 healthy adult dogs were encouraged to walk or trot along a 20 metres corridor, as spontaneously as possible. Three axial accelerations were recorded close to the center of gravity, the accelerometer being placed under the sternum, using a light elastic belt tightened around the thorax (Equimetrix device ©). Recorded data were then analysed using specific gait analysis software on ten seconds-samples of steady state locomotion.</p> <p>The accelerometer device was well tolerated by the dogs. The trot was spontaneously adopted by healthy, and by less affected dystrophic dogs, whereas gait of severely affected animals was restricted to walk. The mean regularity of dorso-ventral accelerations was found to be significantly higher in healthy than in trotting dystrophic dogs. Total mechanical power of gait (W/kg) was also observed to be significantly decreased in dystrophic dogs. Interestingly, the medio-lateral component of the power was significantly increased in dystrophic dogs, demonstrating a swaying component in the gait of these dogs.</p> <p>These preliminary results show that accelerometry is a simple and non-invasive method of functional evaluation of dog's gait and is efficient to quantitatively discriminate dystrophic from healthy dogs, using specific signal processing methods. Longitudinal studies are ongoing in order to describe gait evolutions of healthy versus dystrophic dogs at various ages, with various phenotypes and after treatment.</p>

PW33-423	<p>PHYSIOPATHOLOGICAL CONSEQUENCES OF EXCITATION-CONTRACTION UNCOUPLING PIÉTRI-ROUXEL F¹, GENTIL C¹, FERRY A¹, VIGNAUD A¹, GARCIA L¹ (1) UMRS 787 Inserm-UPMC, Institut de Myologie, Paris, FRANCE.</p>
To contact the author:: pietri@chups.jussieu.fr.	<p>Adipose infiltration is a characteristic common to many muscular dystrophies. In certain cases, the infiltration is such that all of the muscle fibers are replaced by adipocytes, preventing or at least limiting, the rational of gene or cell therapies. Our previous studies on denervated muscle have demonstrated an unsuspected plasticity of muscle fibres. Indeed, the muscle syncytium, reserved the capacity to convert into mononucleated cells able to develop into adipocytes. These results enable us to re-examine the physiopathology of Duchenne myopathy and the progressive emergence of adipose metaplasia. The <i>primum movens</i> remains the absence of dystrophin, which is at the origin of the extreme fragility of DMD muscle fibres. This fragility results in spontaneous microlesions. The number and repetition of necrotic events are such that certain microlesions probably escape the repair process thus causing the electric disconnection of distal parts of segmented fibers. These distal segments would then develop like denervated muscle, resulting in a fatty transformation. The rational mentioned above encouraged us to explore the consequences of an interruption of the functional activity of muscle fibers without affecting the nerve-muscle communication. This can be carried out by destroying the skeletal excitation-contraction coupling system. The excitation-contraction coupling is guaranteed at the level of the triads, forming "electric synapses" between membranes of the transverse tubules and the sarcoplasmic reticulum. It has been known that the alpha1s sub-unit of the slow Ca²⁺ channel (DHP-R) ensures the "voltage sensor". To study the morphogenetic consequences related to an inactivation of this <i>sensor</i>, we have developed AAV vectors harboring constructions coding snRNA-U7 directed against exons crucial for the synthesis of the alpha1s sub-unit. Kinetics of muscle atrophy, tissue modifications and expression of molecular markers following this type of paralysis were analyzed.</p>

PW33-424	<p><u>GLIAL CELLS AT NEUROMUSCULAR JUNCTION PARTICIPATE TO THE MOTOR END-PLATE PHENOTYPE.</u> MUSARELLA M¹, BELLOUZE S¹, CAILLOL G¹, AUTILLO-TOUATI A¹ (1) UMR641, INSERM, Faculté de médecine Nord, Université de la Méditerranée, Marseille, FRANCE.</p>
To contact the author:: amapola.autillo-touati@univmed.fr.	<p>Mouse mutants provide good models for the study of degenerative neuromuscular diseases in human. Although recent data indicate a pivotal role for Terminal Schwann Cells (TSC) in maintaining synaptic structure and function, their role in pathologies affecting the neuromuscular junction (NMJ) has been poorly investigated until now. The study we conducted help to re-evaluate the importance of TSC both in the normal junction and in diseased individuals.</p> <p>In <i>med</i> mice (motor endplate disease), neuromuscular transmission is severely impaired and death occurs 20 days after birth. The homozygous <i>med</i> mice are voltage-gated sodium channel Nav1.6 null mutants.</p> <p>As published (Musarella et al., 2006) :</p> <ol style="list-style-type: none"> 1. In wild-type mice peripheral nervous system, this isoform is not only expressed at nodes of Ranvier but also in TSC. 2. The number of TSC per NMJ is dramatically decreased at terminal stage of <i>med</i> pathology (in the wild-type, 86 % of NMJ had associated TSC whereas only 36 % in the mutant). <p>In the present work, we investigate the time course of the loss of TSC, at a pre-clinical stage (P7), at the onset of the disease (P10) and at the terminal stage (P19). By confocal microscopy on sterno-mastoid muscle sections (40µm), we quantified the number of TSC per NMJ identified by S100 immunostaining in vicinity to alpha-bungarotoxin staining and the number of apoptotic nuclei localised by " In Situ Cell Death Détection Kit, Fluorescein (Roche) ".</p> <p>Progressive loss of TSC occurs before the onset of the phenotype and is due to an increase of glial cell apoptosis.</p> <p>These results suggest that defects in Terminal Schwann Cells contribute to the <i>med</i> phenotype.</p>

