



Study of neuroprotective and neuroregenerative effects of squaneloyl adenosine nanoparticles in peripheral nerve injury rat model

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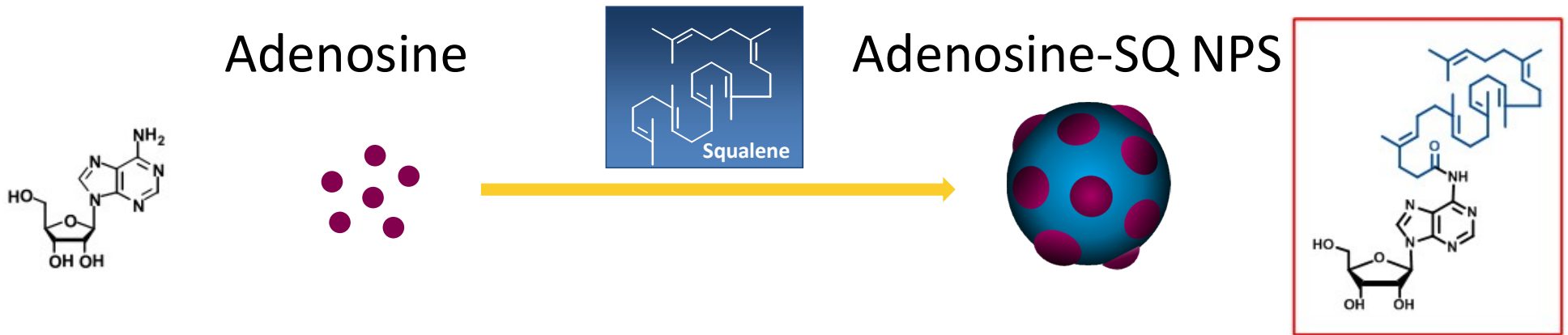
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**Journée Annuelle du Labex NanoSaclay
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Squalenoylation nanotechnology



NPs characterization

(8 mg/ml) :

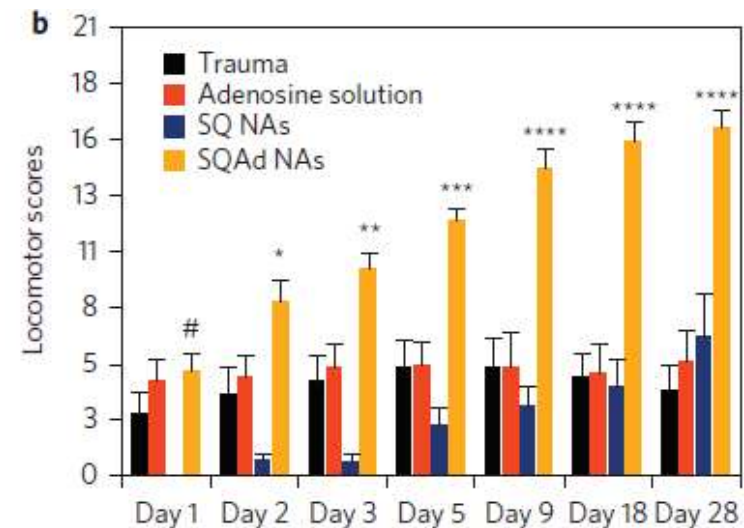
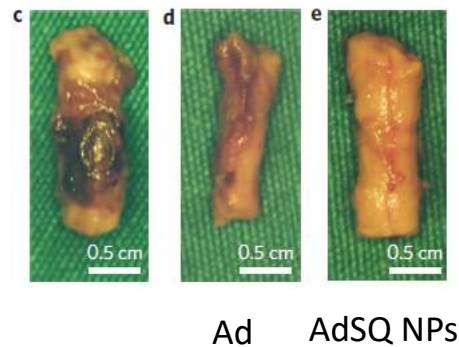
Treatments	Number of preparations	Diameter (nm) (\pm SEM)	PDI	Zeta potentiel (mV)
AdSQ NPs	5	89.7 \pm 6,5	0.05 \pm 0.009	-20.9 \pm 0.8



AdSQ NPs effect on motor recovery in spinal cord trauma :

Gaudin et al. Nature Nanotechnology 2014

- Spinal cord injury on Rats (Sprague Dawley 200 - 250 g)
- AdSQ NPs : 32 mg/kg AdSQ NPs ; 1 injection 5 min post injury
- Behavioral score : Basso, Beattie and Bresnahan grading scale

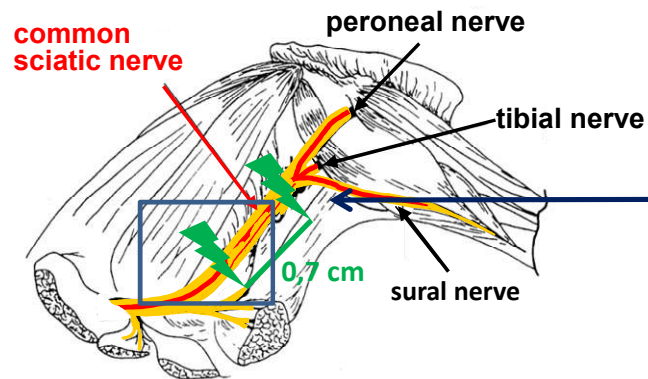


=> Neuroprotective and neuroregenerative effects
on Peripheral nerve injury

In vivo experimental protocol : peripheral nerve injury model

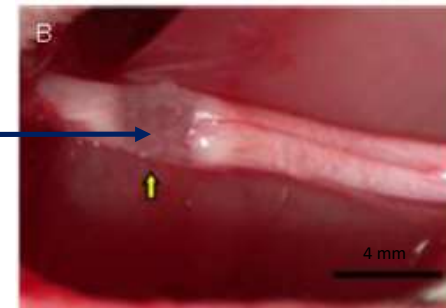
1 Sciatic nerve crush injury (right side)

- Two nerve crush spaced of 0.7 cm apart
- Crush with a round needle holder (5 s)



- Adult male Sprague-Dawley rats
 - 220-250 g on injury day

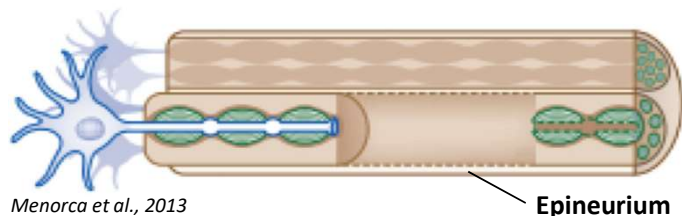
Sciatic nerve crush injury



In rat : *Sung et al., 2012*

proximal

The Seddon and Sunderland classifications of nerve injury : Degree IV



Menorca et al., 2013

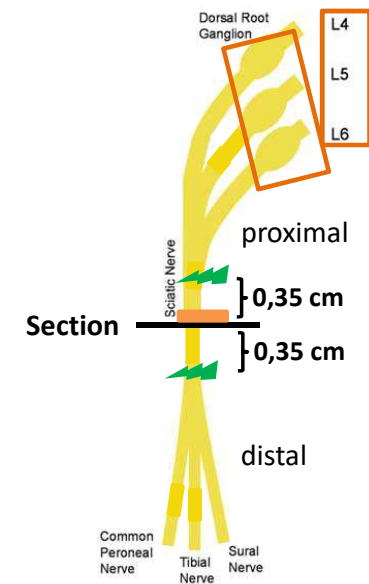
Grade IV
Axonotmesis

- Axon, endoneurium and perineurium damaged with intact epineurium
- Axonal regrowth possible

- **Histological study : nerve regeneration**
- **Behavioral study : motor and sensitive recovery**

HISTOLOGICAL STUDY

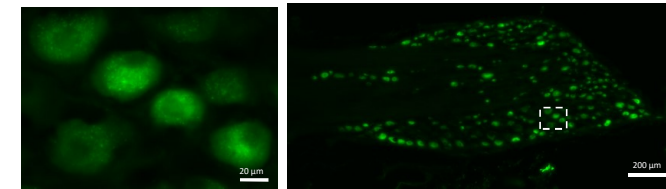
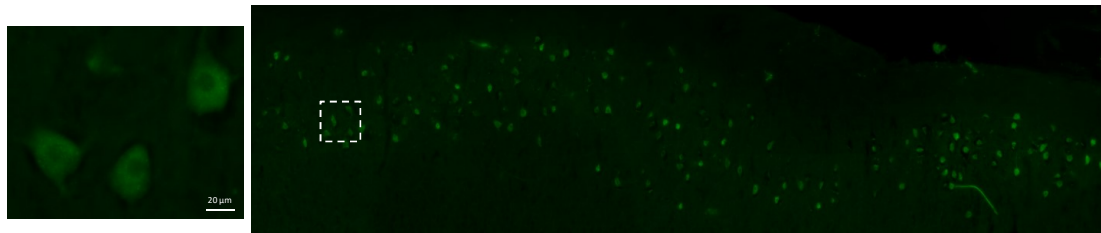
Nerve regeneration



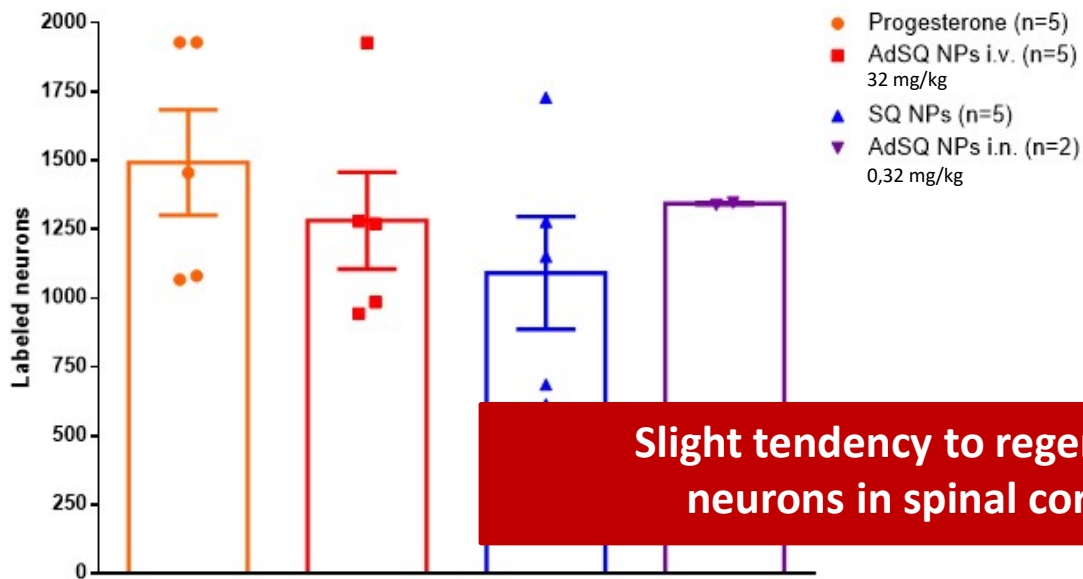
Application of fluorescent retrograde tracer Fluorogold

Effect of ADSQ NPs on regenerated neurons in spinal cord and DRG 20 days after peripheral nerve injury

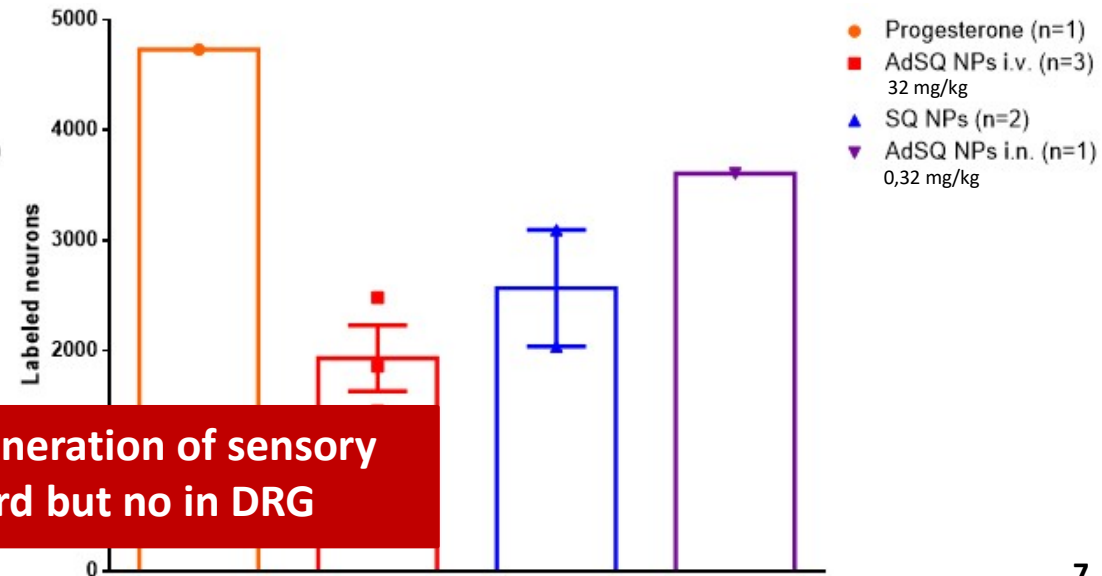
Quantification of neurons labeled by tracer



Spinal cord - sensory neurons



DRG - motor neurons



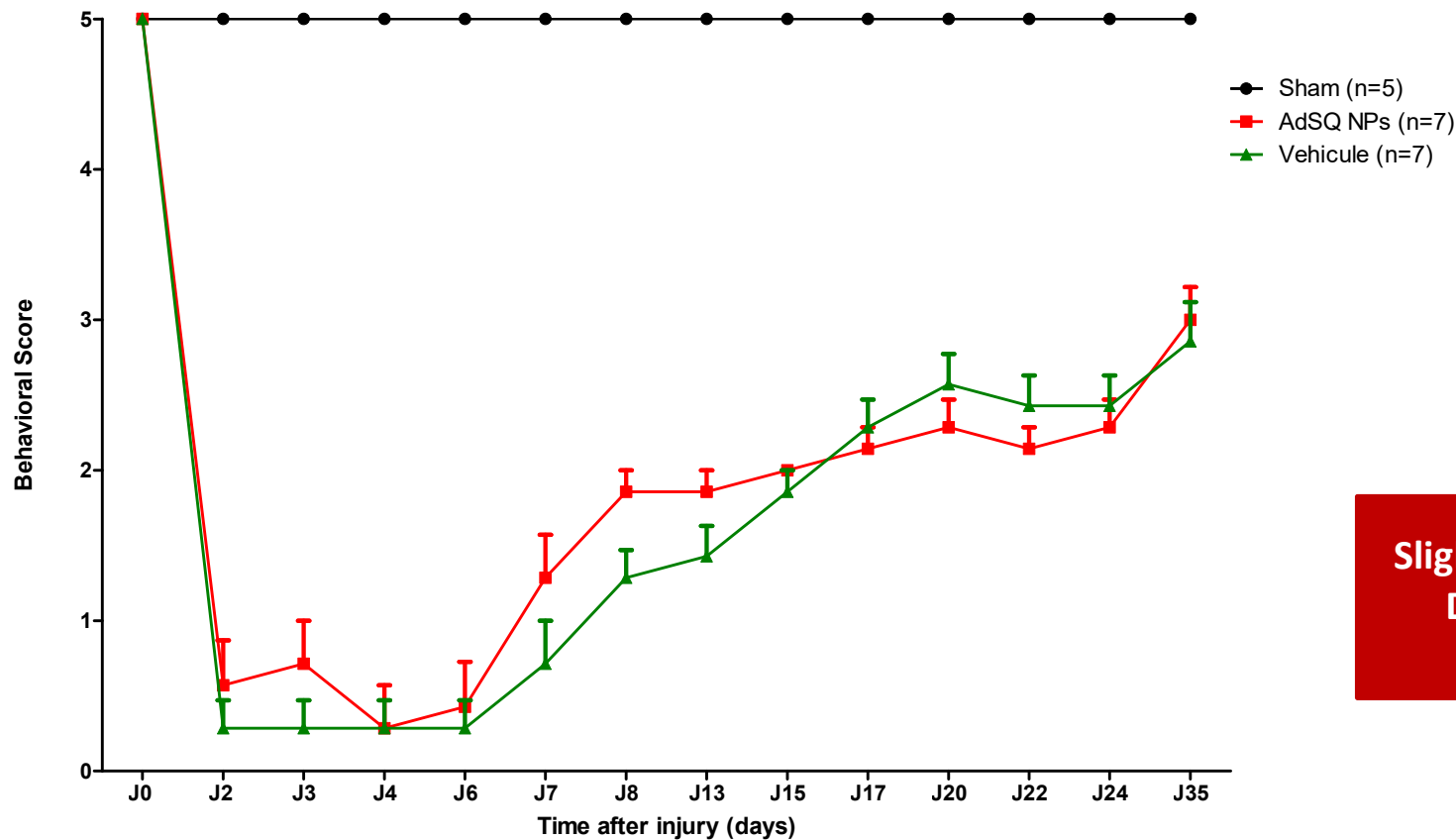
Slight tendency to regeneration of sensory neurons in spinal cord but no in DRG

BEHAVIORAL STUDY

Motor recovery and sensitivity

Motor test : Behavioral score

Effect of AdSQ NPs (32 mg/kg, acute injection) with behavioral score until **35 days** after sciatic nerve injury

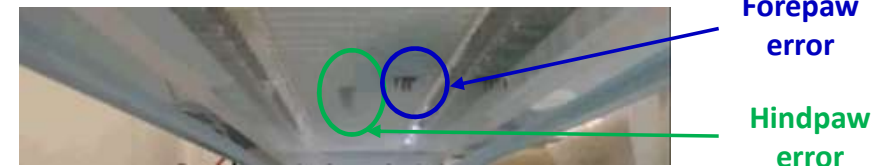
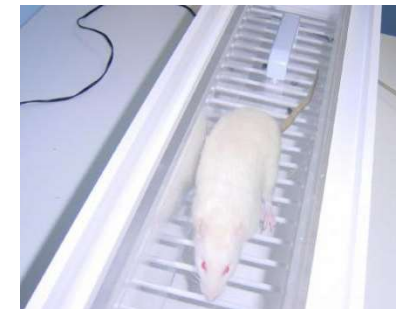
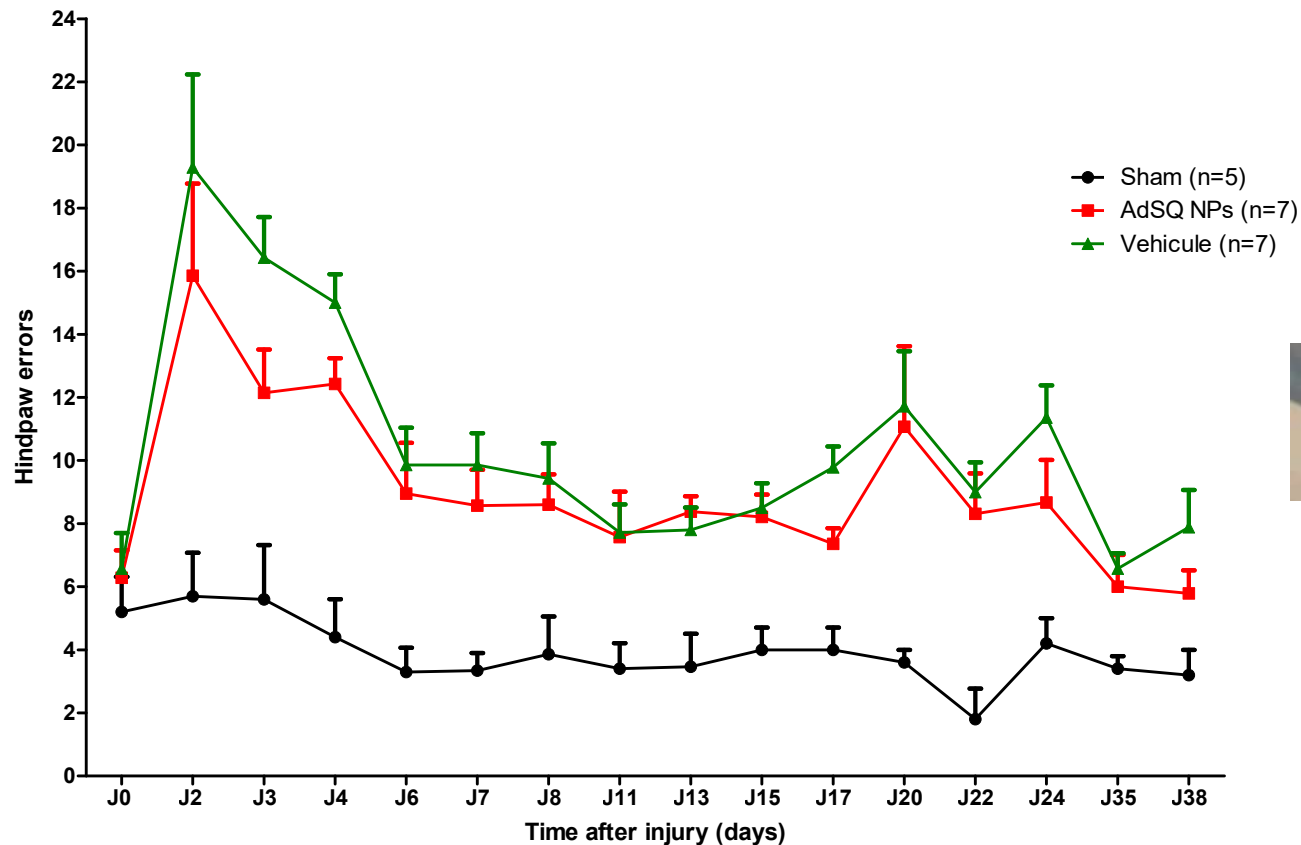


Mobility
Paw position
Hindpaw lift

Slight effect of ADSQ NPs from
D2 to D15 on locomotor
coordination

Motor test : Locotronic

Effect of AdSQ NPs (32 mg/kg, acute injection) on hindpaw errors until **38 days** after sciatic nerve injury

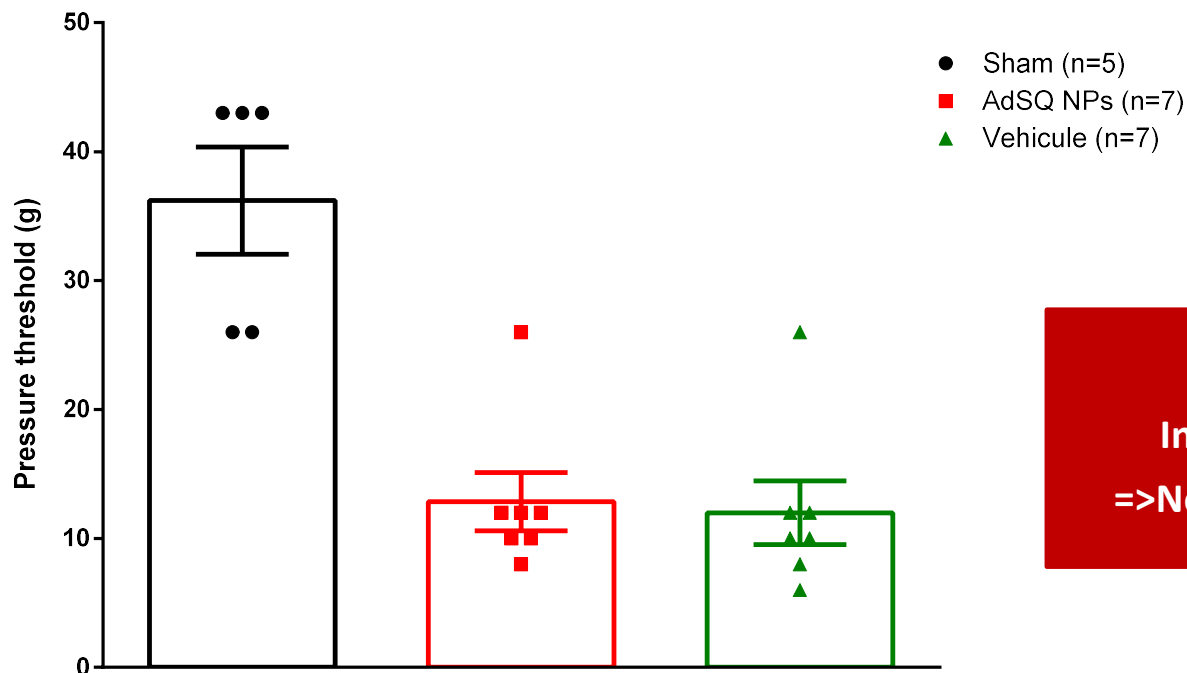


**D2 to D6 after nerve injury :
Slight effect of ADSQ NPs on
locomotor coordination**

Effect of AdSQ NPs - mechanical sensitivity

Von Frey Filament test - mechanical allodynia

Effect of AdSQ NPs (32 mg/kg, acute injection) on hindpaw withdrawal
D 27 after sciatic nerve injury

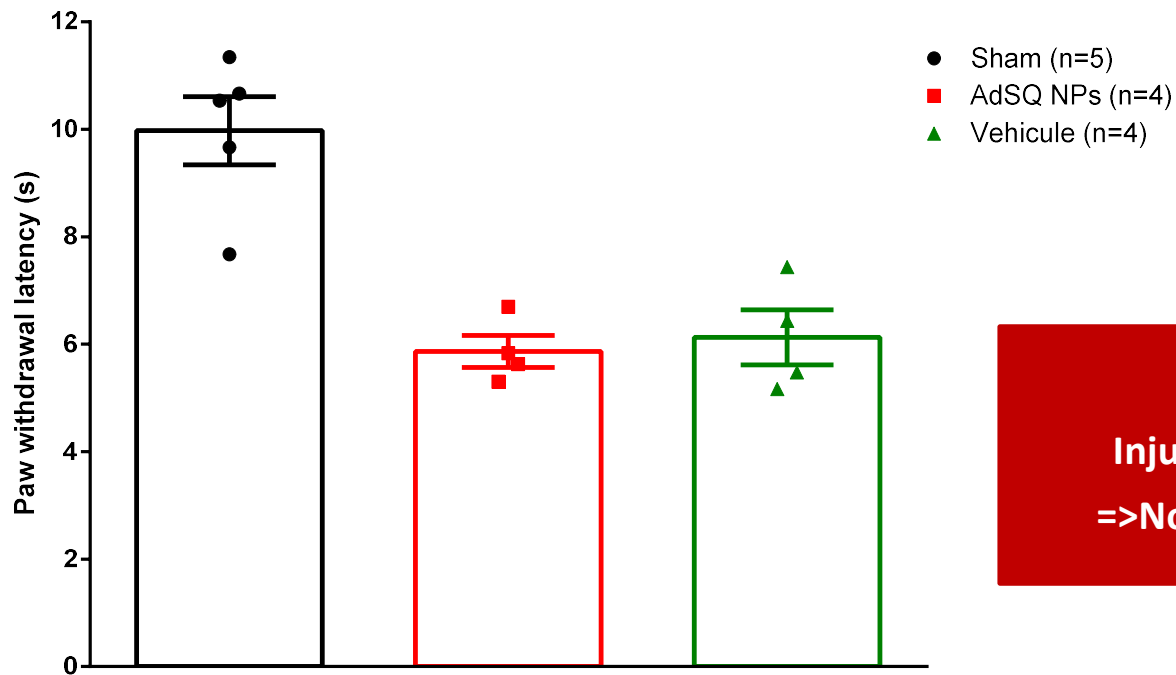


At D27 after nerve injury :
Injured rats still present mechanical allodynia
=>No effect of ADSQ NPs on mechanical sensitivity

Effect of AdSQ NPs - thermal sensitivity

Hargreaves test - thermal hyperalgesia

Effect of AdSQ NPs (32 mg/kg, acute injection) on hindpaw withdrawal latency
D 29 after sciatic nerve injury



At D29 after nerve injury :
Injured rats still present thermal hyperalgesia
=>No effect of ADSQ NPs on thermal sensitivity

CHRONIC TREATMENTS

➤ Behavioral study : **Motor recovery**

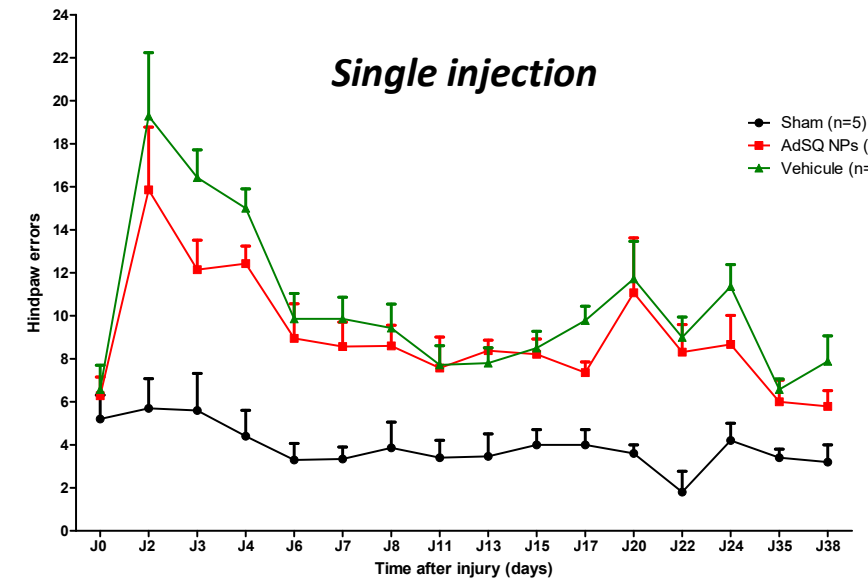
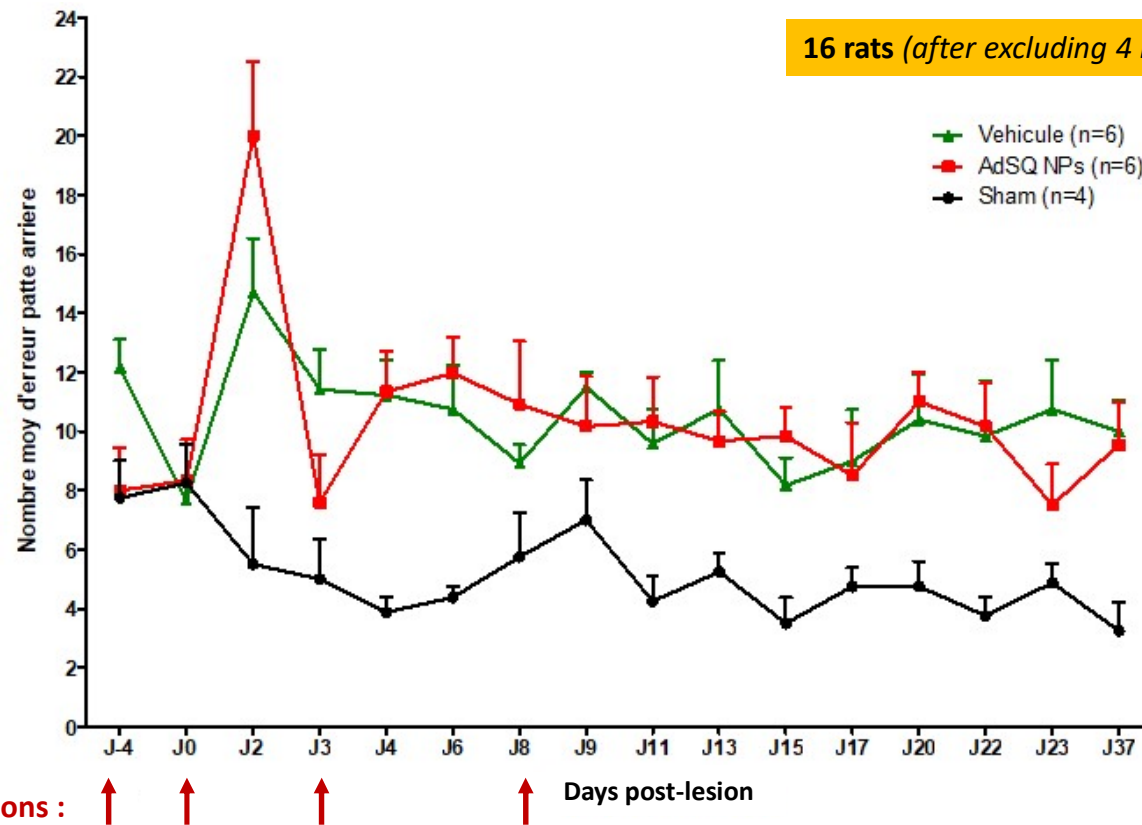
Locotronic test

4 Repeated injections of SQAd NPs (32 mg/kg each; equiv. adenosine 11.5 mg/kg)

D-4 ; D0 ; D3 ; D8

Motor test : Locotronic => locomotor coordination

Effect of **AdSQ NPs (32 mg/kg, repeated injections)** on right hindpaw errors until **38 days** after sciatic nerve injury



**D0 to D37 after nerve injury :
=>No effect of repeated
injections of ADSQ NPs on
locomotor coordination**

CONCLUSION

ACUTE TREATMENT

➤ **Histological** study

- Slight tendency to regeneration of sensitive neurons in spinal cord but no in DRG
- Need to add more groups : Ad, Vehicule, Sham

➤ **Behavioral** study

- Locomotion :
 - Locotronic D2 to D6
 - Behavioral score D2 to D15 } slight effect of AdSQ NPs on locomotor coordination
- Sensitivity to mechanical or thermal stimulation :
 - Injured rats present mechanical allodynia and thermal hyperalgesia

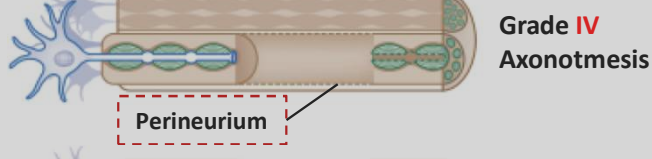
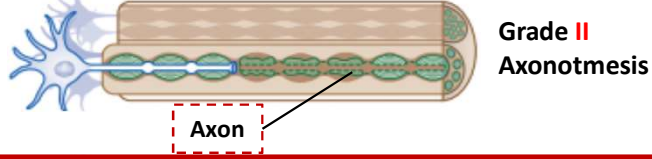
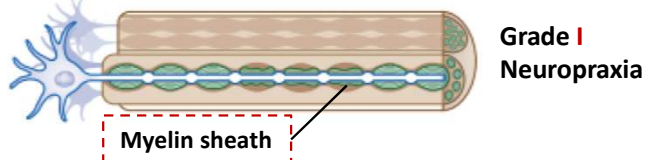
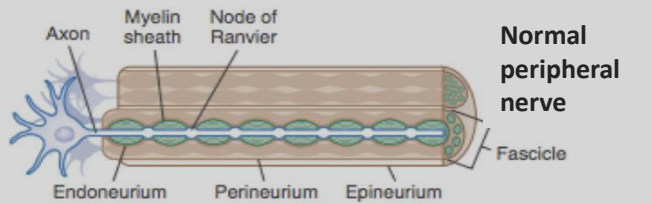
=> No effect of AdSQ NPs at 27 and 29 days after nerve injury

CHRONIC TREATMENT

➤ **Behavioral** study

- Locomotion :
 - No effect of ADSQ NPs on locomotor coordination

Peripheral nerve injury : the Seddon and Sunderland classifications of nerve injury



Seddon	Sunderland	Tissues injured	Description of Injury	Recovery Period
Neurapraxia	Grade I	Myelin Sheath	<ul style="list-style-type: none"> Axonal conduction block Focal segmental demyelination <u>No</u> Wallerian degeneration 	Spontaneous recovery ≤ 3 months
Axonotmesis	Grade II	Myelin Sheath <u>Axon</u>	<ul style="list-style-type: none"> Wallerian degeneration Axonal sprouting 	Spontaneous regenerating 3 cm per month
Axonotmesis	Grade III	Myelin Sheath Axon <u>Endoneurium</u>	<ul style="list-style-type: none"> Intrafascicular fibrosis 	< 2cm per month Determined by degree of fibrosis
Axonotmesis	Grade IV	Myelin Sheath Axon Endoneurium <u>Perineurium</u>	<ul style="list-style-type: none"> Scarring blocks regeneration Neuroma formation 	Surgical intervention usually required
Neurotmesis	Grade V	Myelin Sheath Axon Endoneurium Perineurium <u>Epineurium</u>	<ul style="list-style-type: none"> Nerve discontinuity Nerve gap replaced by scar tissue 	Surgical intervention required

Adapted from Menorca et al., 2013

ACKNOWLEDGMENTS

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Plateau d'Imagerie
Cellulaire et Tissulaire
Olivier Trassard



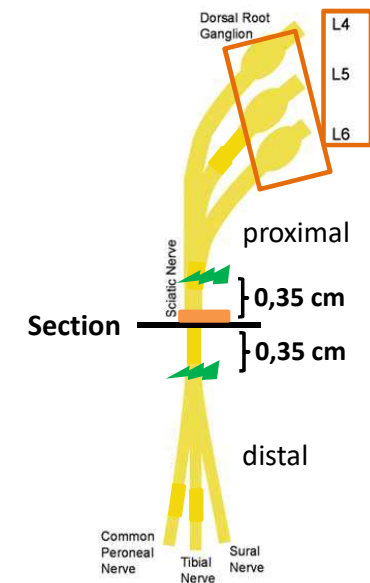
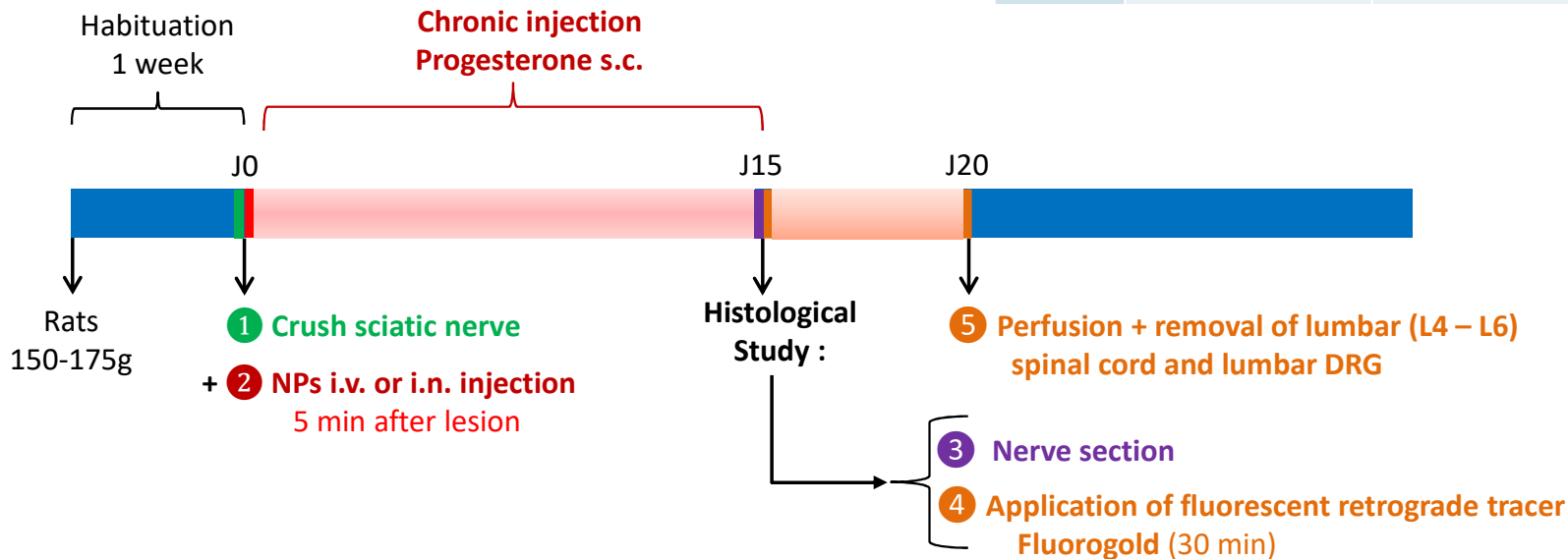
In vivo experimental protocol : general

➤ 20 Male Sprague-Dawley rats

2 Injections treatments

- Acute (AdSQ NPs) : One injection of NPs 5 min after injury
- Chronic (Prog) : One injection of PROG daily during 15 days

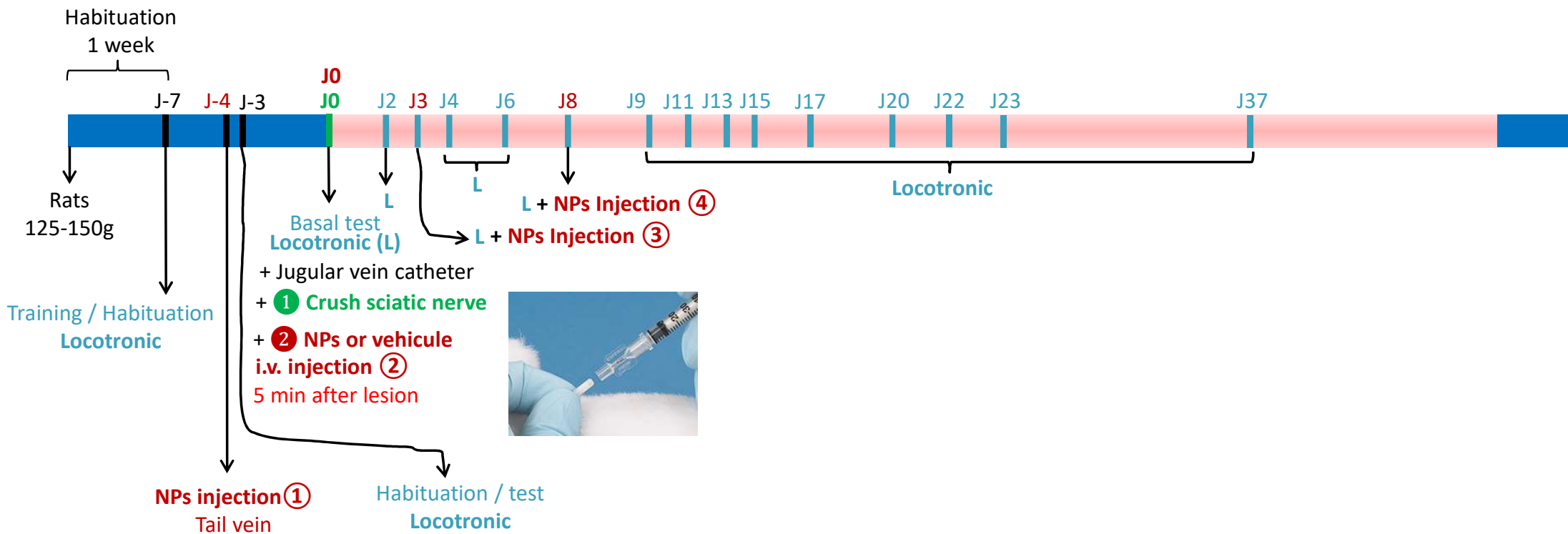
	Treatments	Injection/ volume	Administration	n rats
Groups	AdSQ NPs	i.v. / 1 mL	32 mg/kg	5
	AdSQ NPs	i.n. / 10 µL	0,32mg/kg	5
	SQ NPs	i.v. / 1 mL	20.2 mg/kg	5
	Progesterone (PROG)	s.c.	Chronic 15 d	5



In vivo experimental protocol : chronic treatment

- 16 male Sprague-Dawley rats
- Sham n = 4
 - AdSQ NPs n = 6
 - Vehicule Dextrose 5% n = 6

3 Motor test : Locotronic



In vivo experimental protocol : nanoparticles

2 Injections treatments

- i.v. : tail vein

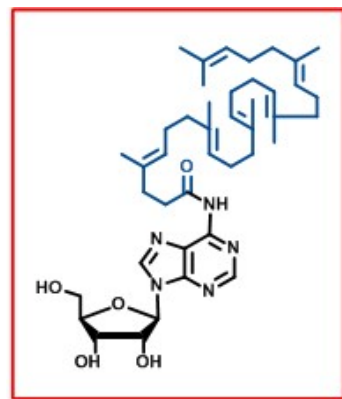
Treatments	Dose	Final concentration	Injection volume
AdSQ NPs	32 mg/kg	8 mg/mL	1 mL i.v.

➤ NPs preparation (scale-up):

- Nanoprecipitation with 39 mg AdSQ

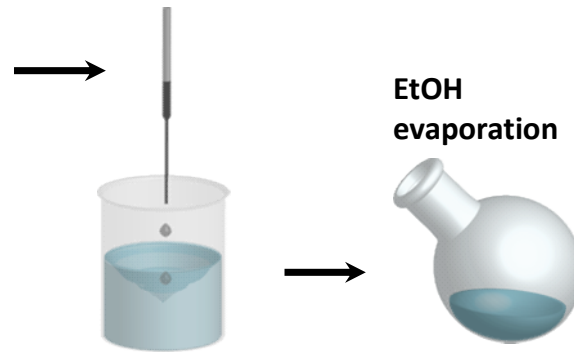
Treatments	Number of preparations	Number of rats	Quantity of AdSQ (mg)	Diameter (nm)	PDI	Zeta potentiel (mV)
AdSQ NPs ①	1	4	39.71	119.433	0.089	-15.1
AdSQ NPs ②	1	4	38.29	143.467	0.064	-18.9

Ad-SQ nanoparticles



Adenosine-Squalene
(solubilized in EtOH)

Nanoprecipitation



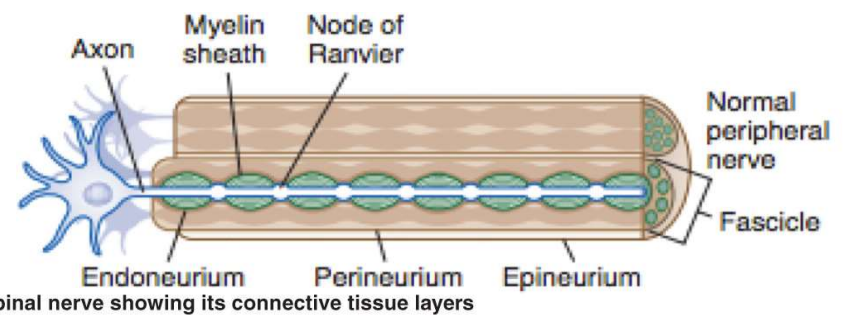
SQd NPs at 8 mg/mL

NPs characterization (8 mg/ml) :

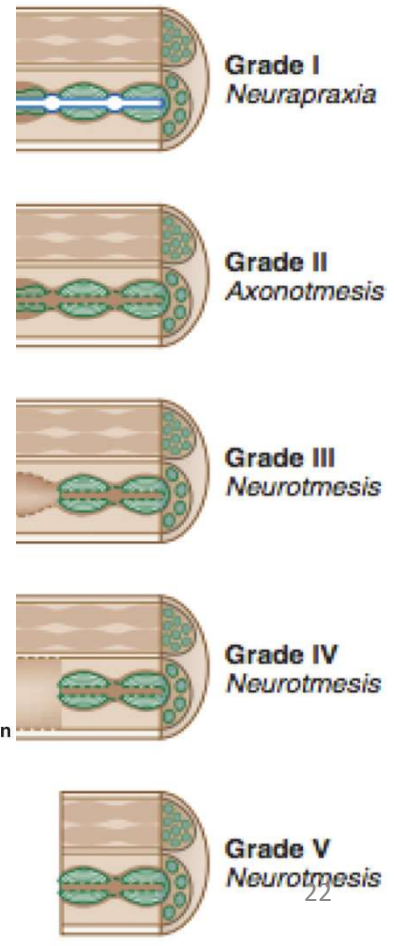
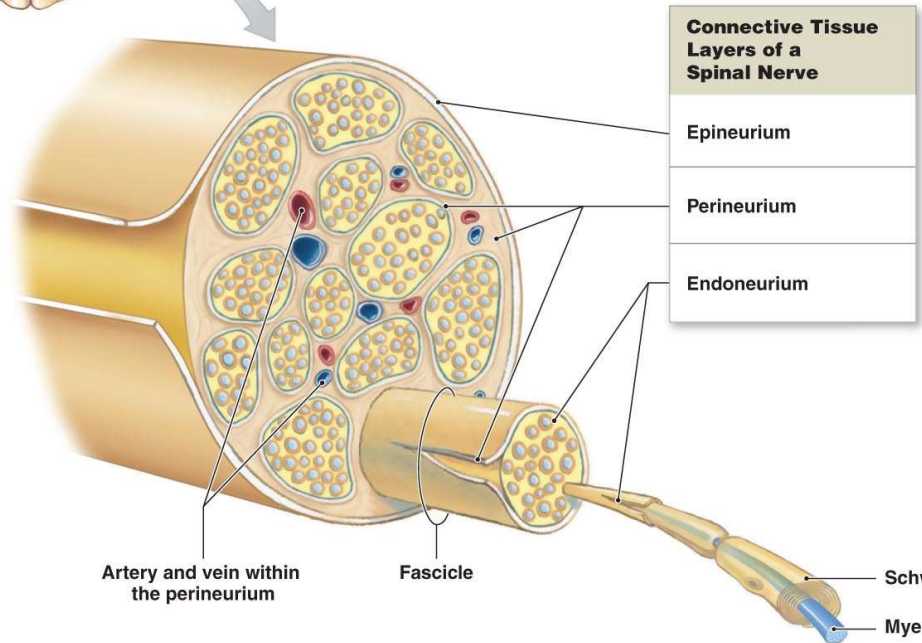
Treatments	Number of preparations	Diameter (nm) (\pm SEM)	PDI	Zeta potentiel (mV)
AdSQ NPs	5	89.7 \pm 6,5	0.05 \pm 0.009	-20.9 \pm 0.8

Seddon's Classification	Sunderland's Classification	Tissues Injured
Neurapraxia	Grade I	Myelin
Axonotmesis	Grade II	Myelin, axon
Neurotmesis	Grade III: Axon continuity is disrupted by loss of endoneurial tubes, but perineurium is protected	Myelin, axon, endoneurium
	Grade IV: Nerve fascicle is damaged, but sheath continuity is maintained	Myelin, axon, endoneurium, perineurium
	Grade V: Substantial perineural hemorrhage and scarring occur	Myelin, axon, endoneurium, perineurium, epineurium

Source: References 3, 5.



in. 2013 Aug; 29(3): 317-330. doi: 10.1016/j.hcl.2013.04.002 - Mozilla Firefox (Navigation privée)
 n.nih.gov/pmc/articles/PMC4408553/table/T2/
 Author manuscript: available in PMC 2015 Apr 24.
 in final edited form as:
 in. 2013 Aug; 29(3): 317-330.
 1016/j.hcl.2013.04.002
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Classification of Nerve Injury

Injury
Focal segmental demyelination
Axon damaged with intact endoneurium
Axon and endoneurium damaged with intact perineurium
Axon, endoneurium, and perineurium damaged with intact epineurium
Complete nerve transection.
MacKinnon & Dellon) Mixed levels of injury along the nerve

Fig. 1.
Classification of nerve trauma

