ISSN 1220-8841 (Print)
ISSN 2344-4959 (Online)

ROMANIAN
NEUROSURGERY

Vol. XXXIV | No. 4 December 2020

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Andrei Marin,
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DOI: 10.33962/roneuro-2020-081

Romanian Neurosurgery (2020) XXXIV (4): pp. 482-487
DOI: 10.33962/roneuro-2020-081
www.journals.lapub.co.uk/index.php/roneurosurgery

Complications after sciatic nerve defect repair in rats

Andrei Marin[1], Georgiana Gabriela Marin[2], Carmen Giuglea[1,3]

[1] Plastic Surgery Department, "St. John" Emergency Hospital, Bucharest, Romania
[2] Cardiology Department, "C.C. Iliescu" Hospital, Bucharest, Romania
[3] Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Abstract

Experimental microsurgery is a provocative field with great rewards. Nerve microsurgery is particularly challenging because the results of the operation can only be tardily observed. During this time frame, multiple complications can appear to the laboratory rats, which can influence the final results.

For this reason, when experimenting with Wistar rats, one must be familiarized with the possible complications in order to know the suitable solutions for all the issues. Lack of information and henceforth lack of action might result in compromising the final data.

Keywords
sciatic nerve,
rats,
microsurgery

Corresponding author:
Andrei Marin

Plastic Surgery Department, "St. John" Emergency Hospital, Bucharest, Romania

marin_dpt@yahoo.com

Introduction

On a simple internet search with the phrase "sciatic rat nerve", there are a total of over 3,2 million results. [1] This goes to prove that there is a great interest in experimental surgery on the sciatic nerve conducted on laboratory rats.

The rat, particularly the Wistar rat, represents the most suitable candidate for experimental nerve surgery, as it is affordable, easy to manipulate and has a suitable anatomy for different types of experiments. Furthermore, the faster nerve regeneration period (compared to that of humans) represents a major advantage in working with these laboratory animals.

The anatomy of the sciatic nerve in rats is similar to that in humans: the main nerve divides into 3 branches - common peroneal nerve, tibial nerve and sural nerve. [2] The anesthesia is another positive aspect for choosing Wistar rats – it can be performed by the operator by direct intraperitoneal injection. There are more solutions which can be used, depending on the type and length of the operation. [3]

In all experimental surgery performed on animals, a good collaboration with a veterinary is crucial, as he/she provides important expertise regarding anesthesia, post-operative evolution, solutions to problems; it is also stated in the law that in all animal projects, the

ISSN online 2344-4959
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First published
December 2020 by
London Academic Publishing
www.lapub.co.uk

presence of a veterinary in the team is compulsory. Furthermore, all types of animal experimentation require approvals from the Ethics Committee and the Sanitary Veterinary Department. [4]

Material and method.

The complications presented in the article were mostly observed and handled during an experimental project for a PhD thesis, where 4 methods of defect nerve reconstruction were compared:

- nerve graft,
- nerve conduit made from a rat aorta (simple aortic conduit),
- platelet-rich plasma (PRP) inside an aortic conduit,
- stem cells inside the aortic conduit.

Each batch consisted of 10 lab rats and 2 extra lab rats were sacrificed to obtain PRP and aortic conduits.

The anesthesia used was a mixture of xylazine 10mg/kg and ketamine 75mg/kg. After injecting the anesthesia and preparing the operation site (shaving the dorsal gluteal-thigh region), the Wistar rat was placed in prone position and the incision was performed along the femur, 0,5cm inferior to it. A breach was created through the biceps femuri muscle and the sciatic nerve was exposed for a length of 4 cm to be able to observe the emerging branches. 2 separate incisions at a distance of 0,5cm were performed on the sciatic nerve, proximal to the emergence of its branches and the resulting defect was repaired using one of the above-mentioned methods. After nerve reconstruction, the muscle was close using absorbable suture and the skin was close using non-absorbable sutures.

Postoperatory, the rats were placed 2 in a cage, between the 2 existing a separating transparent plastic support. This was to prevent cannibalism between the rats.

The post-operative treatment consisted of enroxil 0,003mg/kg and meloxicam 1mg/kg both injected subcutaneously for 3 days. The wounds were treated locally with betadine solution alternating with baneocin powder.

The rats were monitored for 3 months and afterwards they were euthanized using an overdose of the anesthetic mixture and KCl injected intracardiac.[5] During this period, some rats experienced several complications which were treated accordingly.

Results

Out of the 40 rats included initially in the study, 2 rats were excluded due to major complications which could influence the final results – one due to postoperative death, the other one due to sciatic nerve rupture.

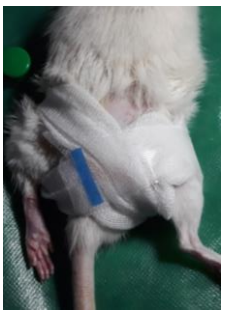
The one death recorded was due to an anesthetic overdose – because the rat would not sleep under anesthesia, multiple doses were administered. This resulted in a profound anesthesia once the drugs took effect, and a prolonged anesthesia time (even after the surgery was over). The rat died the day after the operation.

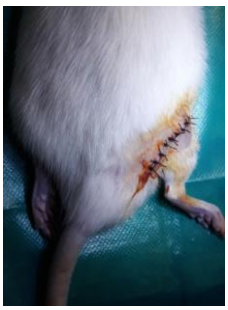
The first postoperative complication observed was wound dehiscence. This occurred in all batches 1-5 days after surgery due to chewing on the surgical knots. Some dehiscence were partial, others were total. 2 different measures were taken, unfortunately without the desired effect.

The first solution was to redo the suture under local anesthesia with the help of an assistant who would firmly restrain the rat not to bite during the procedure. This proved ineffective and dangerous as the rat would bite through the second knots as well.

The second solution was to try to perform a dressing so that the operated rat could no longer reach the wound. This also proved ineffective, as the rat could easily slip out of the dressing and attempt to nibble on the knots.

Special designed plastic cages with a plastic support to create 2 separate compartments





Sutured wound with different types of dressings

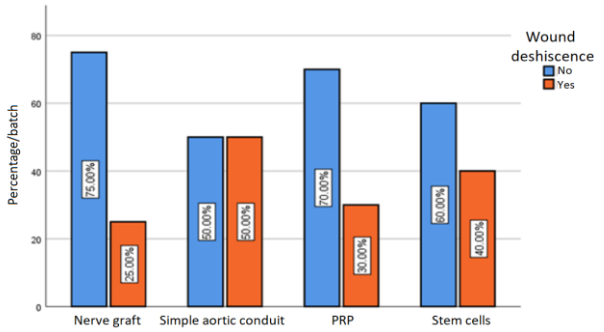
The wounds were treated with different antiseptic solutions and they closed per secundam in maximum one week.

A statistical analysis performed to see which batch presented more wound dehiscence revealed that there was a higher prevalence in the second batch (simple aortic conduit), followed by the stem cells batch. However, this analysis didn't have statistical power (X2 =1.479 (3); p=0.687).

Presence/absence of dehiscence wound in the 4 batches

Batch		Dehiscence		Total rats / batch
		No	Yes	
Nerve graft	No.	6	2	8
	Pct.	75%	25%	100%
Simple aortic conduit	No.	5	5	10
	Pct	50%	50%	100%
PRP	No.	7	3	10
	Pct	70%	30%	100%
Stem cells	No.	6	4	10
	Pct	60%	40%	100%
Total per wound	No.	24	14	38
	Pct	63.2%	36.8%	100%

Graphic of the wound dehiscence distribution in the 4 batches



Another complication encountered was that of self-mutilation of the affected limb. Luckily, the mutilation was limited at the fingernails and like the wound dehiscence it was self-limiting.

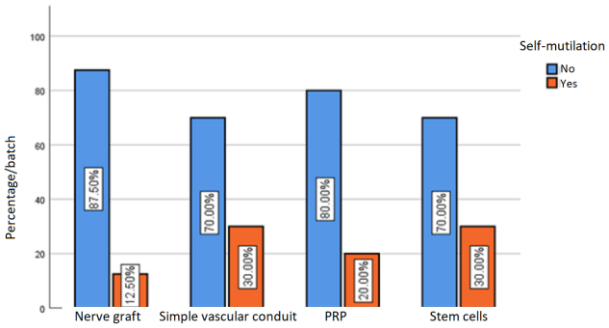
Limb autophagy after sciatic nerve transection – erythema and swelling of the foot and mutilated fingernails (footprint covered with ink due to footprint test).

Presence/absence of self-mutilation in the 4 batches

Batch		Self-mutilation		Total rats/batch
		No	Yes	
Nerve graft	No.	7	1	8
	Pct.	87.5%	12.5%	100%
Simple aortic conduit	No.	7	3	10
	Pct.	70%	30%	100%
PRP	No.	8	2	10
	Pct.	80%	20%	100%
Stem cells	No.	7	3	10
	Pct.	70%	30%	100%
Total self-mutilations	No.	29	9	38
	Pct.	76.3%	23.7%	100%

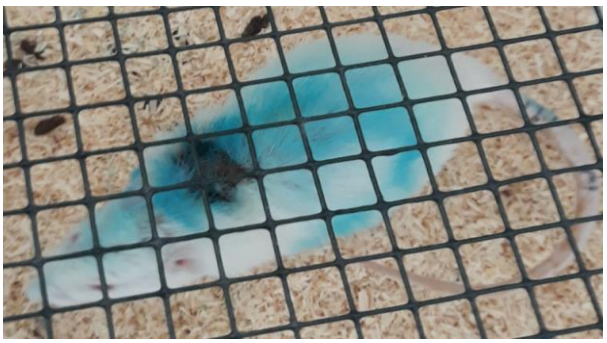
Similar to the wound dehiscence percentage, the self-mutilation of the denervated limb was predominent in the prevalence in the second batch (simple aortic conduit) and the stem cells batch, without statistical difference.

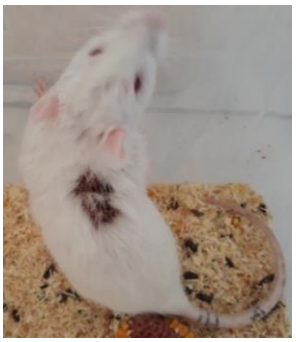
Graphic of self-mutilation distribution in the 4 batches



One month after the beginning of the project, all rats presented severe pruritus. As differential diagnoses, alergic dermititis and parasitologic contamination were taken into consideration. Due to scratching, some rats presented crusts and erosions, especially around the neck and back.

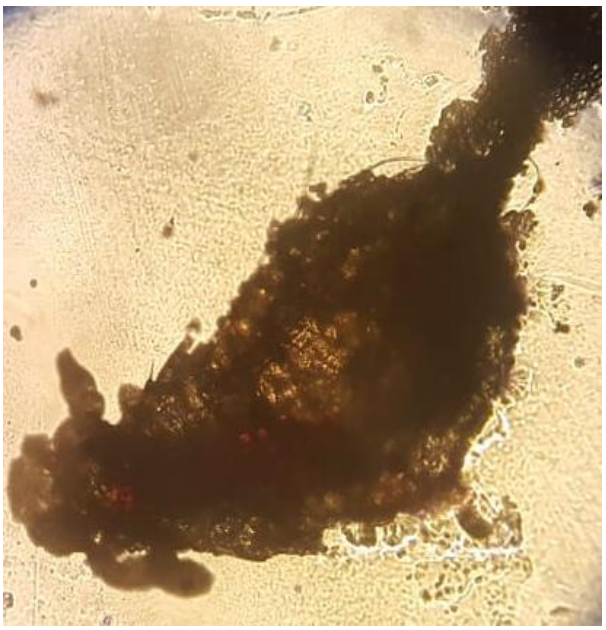
Weight loss occured to all rats experiencing these symptoms. Due to severe scratching, there was an important hair loss in the area with the pruritus. Until the diagnosis was established, the wounds were treated locally with methylene blue, to prevent infection.





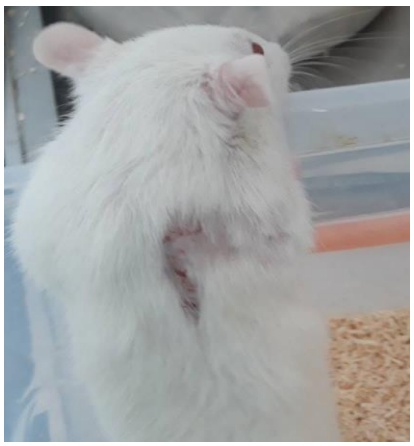
Scratching lesions with hair loss and methylene blue application.

After a dermatologic consult, tape was applied on the skin and the sample was then examined under the microscope. The diagnosis of pediculosis with Polyplax spp. was put and all rats underwent treatment with Stronghold 15mg (Selamectin). This treatment was repeated after 2 weeks. Thorough mechanic sanitation of all cages was performed, as well as of the rooms where the cages laid. Furthermore, the rooms were sterilized for 24 hours using a UV lamp.



Microscopic aspect of the parasite Polyplax spp.

After the first application of Selamectin, there was a complete relief of the pruritus and a significant improvement of the scratching lesions. All rats recovered both locally (regaining the lost hair) as well as in terms of general state (in the following weeks there was a progressive weight gain).



3rd week after Selamectin treatment

Discussions

Proper anesthesia is the key to a successful surgery with optimal results. While it would help to sedate the animals using inhaling substances such as isoflurane before injecting them, direct intraperitoneal injection with ketamine, xylazine or acepromazine (or a mixture of these solutions) can be performed even without sedation but with proper contention. [6,7,8]

Correct calculation of the proper dose is crucial for the outcome. If a rat doesn't fall asleep in 5 minutes after the normal dose is administrated, this doesn't mean it has drug resistance, but rather that it takes the anesthetic a longer time to come into effect. Therefore, if a rat doesn't respond in the 5-minute time frame, it should be left aside and another rat should be prepared for surgery. Otherwise, administrating a new dose of anesthetic might result in a prolonged anesthesia or even death.

After the rat is under anesthesia, another important aspect is the handling or moving of the rat. Taking into consideration the muscle relation induced by the anesthesia, the rat is susceptible to aspiration if it is grabbed by the tail, with the head down. If this occurs, one should try freeing the airways using a small cannula attached to a vacuum. The typical symptoms a rat experiences in such cases are hiccup-like movement, with desaturation; if no prompt intervention is performed, this will also lead to its death. The best way to prevent this is to manipulate the rat by grabbing it by the back of its neck, heads up. Although Britto et all believe that total fasting or only-solids deprivation does not induce gastric emptying in mice, a preoperative total fasting for 6-8 hours could reduce this risk. [9]

Cannibalism immediately after surgery is another issue to be considered. The rats need to be separated a few days after the operation until the wound is closed. Afterwards, they can be placed together and it would also be recommend not keeping them separated as they have a tendency to self-mutilate by chewing on the operated, senseless leg when left alone.

One difficult complication to treat is self-mutilation – either limb mutilation (in case of peripheral nerve injuries) or wound dehiscence caused by biting the threads which hold the wound tight. Hindlimb autotomy/autophagy represents the mutilation of the anesthetic foot by the animal which doesn't feel the limb and therefore doesn't recognize it as part of its body. Wall et all described degrees of hindlimb autotomy differentiating on the type of nerve injury (cut nerve and encapsulated in polythene tube, sectioning with immediate repair, nerve ligation and nerve crush). [10]

Prevention of self-mutilation until nerve regeneration occurs is therefore a necessity. Plastic head collars could be useful for dogs or other large animals but are not appropriate for the rat, as it can easily get out of this restraint due to its neck anatomy. Repelling solutions (such as quinine) applied on the limb or on the wound could prove effective because of its bitter taste. Al-Adawi et all also proved the efficiency of 6-hydroxydopamine injected in the ascending noradrenergic bundle 1 week prior to transection or N-(2-) Chloroethyl-N-ethyl-2-bromobenzylamine (DSP4) injection 24 h prior to transection. [11] Picric acid is another solution that showed better results in reducing limb autophagy compared to the commercial bite-deterrent chemical (denatonium benzoate). [12]

Another possible complication which fortunately did not occur in this project is infection. Although rodents are quite resistant to infection, it would be recommended that postoperative antibiotic be given and sterile conditions during the operation be used.

Graft versus host disease is another possible complication when using foreign allogen tissue. Although no such reaction was noted during the project, even without the use of immunosuppressant medication, this could become a serious problem when transplanting tissues which determine a strong antigenic reaction.

Even though the project was conducted in a restricted environment in a building located in the veterinary university campus especially dedicated to this experiment, rat contamination occurred. There were 2 possible explanations. One would be the human factor – the people who come in contact with these animals to feed and clean after the rats. The second explanation, although unlikely, could be the 2 windows located at 2,5m altitude in the rooms where the experiments took place. A fast diagnosis and rapid intervention were the key in saving the animals and the project in this case.

Conclusions

Animal experimentation requires not only feeding and cleaning, but also attending to the possible

complications generated by human intervention. When it comes to nerve surgery, the most frequent complications are self-mutilation (either wound dehiscence or autotomy of a limb that in no longer sensitive innervated).

This study shows that the rats that presented wound dehiscence were predominantly in the simple aortic conduct batch; the same batch manifested self-mutilation of the denervated inferior limb.

The laboratory animals present all possible complication of the surgical intervention, as well as health complications specific to the animals. However, some solutions which may apply to human patients may not be a proper solution for animals.

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