

# Brachial Plexus Morphology and Vascular Supply in the Wistar Rat



## Morfologia e Vascularização do Plexo Braquial no Rato Wistar

Maria ANGÉLICA-ALMEIDA<sup>1</sup>, Diogo CASAL<sup>2</sup>, Manuela MAFRA<sup>3</sup>, Luís MASCARENHAS-LEMO<sup>2</sup>, José MARTINS-FERREIRA<sup>3</sup>, Mário FERRAZ-OLIVEIRA<sup>3</sup>, José AMARANTE<sup>4</sup>, João GOYRI-O'NEILL<sup>2,5</sup>  
*Acta Med Port* 2013 May-Jun;26(3):243-250

### ABSTRACT

**Introduction:** The rat is probably the animal species most widely used in experimental studies on nerve repair. The aim of this work was to contribute to a better understanding of the morphology and blood supply of the rat brachial plexus.

**Material and Methods:** Thirty adult rats were studied regarding brachial plexus morphology and blood supply. Intravascular injection and dissection under an operating microscope, as well as light microscopy and scanning electron microscopy techniques were used to define the microanatomy of the rat brachial plexus and its vessels.

**Results:** The rat brachial plexus was slightly different from the human brachial plexus. The arterial and venous supply to the brachial plexus was derived directly or indirectly from neighboring vessels. These vessels formed dense and interconnected plexuses in the epineurium, perineurium, and endoneurium. Several brachial plexus components were accompanied for a relatively long portion of their length by large and constant blood vessels that supplied their epineurial plexus, making it possible to raise these nerves as flaps.

**Discussion:** The blood supply to the rat brachial plexus is not very different from that reported in humans, making the rat a useful animal model for the experimental study of peripheral nerve pathophysiology and treatment.

**Conclusion:** Our results support the homology between the rat and the human brachial plexus in terms of morphology and blood supply. This work suggests that several components of the rat brachial plexus can be used as nerve flaps, including predominantly motor, sensory or mixed nerve fibers. This information may facilitate new experimental procedures in this animal model.

**Keywords:** Animals; Blood Vessels; Brachial Plexus/anatomy and histology; Brachial Plexus/blood supply; Brachial Plexus/surgery; Rats, Wistar.

### RESUMO

**Introdução:** O rato é provavelmente a espécie animal mais utilizada em estudos experimentais de reparação nervosa. Com este trabalho pretendeu-se aprofundar o conhecimento da morfologia e da vascularização do plexo braquial do rato.

**Material e Métodos:** Trinta ratos adultos foram estudados relativamente à morfologia e vascularização do plexo braquial. As técnicas usadas foram a injeção intravascular e dissecação sob microscópio operatório, bem como técnicas de microscopia óptica e microscopia electrónica de varrimento.

**Resultados:** Morfologicamente, o plexo braquial do rato é um pouco diferente do plexo braquial humano. O suprimento arterial e venoso do plexo braquial do rato deriva direta ou indiretamente dos vasos vizinhos. Estes vasos formam plexos vasculares densos e interconectados no epinervo, perinervo e endonervo. Vários componentes do plexo braquial do rato são acompanhados durante um trajeto relativamente longo por vasos sanguíneos relativamente calibrosos e constantes que fornecem o seu plexo epineural, tornando o seu levantamento como retalhos nervosos possível.

**Discussão:** A vascularização do plexo braquial do rato não é muito diferente da reportada na espécie humana, tornando o rato um modelo animal útil para o estudo experimental da fisiopatologia e tratamento da patologia do nervo periférico.

**Conclusão:** Os nossos resultados apoiam a homologia entre o rato e o Homem em termos de morfologia e vascularização do plexo braquial. Este trabalho sugere que vários componentes do plexo braquial do rato podem ser utilizados como retalhos nervosos, incluindo fibras predominantemente motoras, sensitivas ou fibras mistas.

**Palavras-chave:** Animais; Plexo Braquial/anatomia e histologia; Plexo Braquial/cirurgia; Ratos Wistar; Vasos Sanguíneos.

### INTRODUCTION

Peripheral nerve injury affects mainly young people, causing significant social and economic burden.<sup>1-6</sup> Its outcomes can be devastating, particularly if hand function is compromised. The hand is an indispensable tool for work, and participates in most everyday activities, contributing immensely to the wellbeing of the individual.<sup>1-3</sup> Furthermore, the upper limb is essential for interaction with the environment, allowing the study of objects by touch, as well as expression through gestures, painting or music.<sup>1-3</sup>

Although in recent decades there have been remarkable advances in the surgical techniques used in the repair of peripheral nerve lesions, full recovery of function still remains unattainable in many patients.<sup>1-3</sup>

The rat (*Rattus norvegicus*) is probably the animal species most widely used in experimental studies of nerve repair.<sup>7,8</sup> In this species, the rat sciatic nerve has been the focus of new experimental procedures for the repair of the peripheral nervous system.<sup>9</sup> Recent studies suggest that

1. Plastic and Reconstructive Surgery Department and Burn Unit. São José Hospital. Lisbon. Portugal.

2. Anatomy Department. Medical Sciences Faculty, New University of Lisbon. Lisbon. Portugal.

3. Pathology Department. São José Hospital. Lisbon. Portugal.

4. Plastic and Reconstructive Surgery Discipline. Faculdade de Medicina da Universidade do Porto. Porto. Portugal.

5. Centro de Física e Investigação Tecnológica. Departamento de Física. Faculdade de Ciências e Tecnologia. Universidade Nova de Lisboa. Lisboa. Portugal.

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models that use the brachial plexus (BP) of the rat may have several advantages over the sciatic nerve, namely a lower incidence of joint contractures and self-mutilation of the operated limb.<sup>9-14</sup> In addition, the model of the BP allows a faster identification of nerve regeneration, since the route to be crossed by growing axons is shorter in the forelimb than in the hindlimb of the rat, due to the greater length of the latter.<sup>9-14</sup> Furthermore, numerous methods have been developed and validated for assessing motor and sensory function of the BP in the rat, giving increasing consistency to the use of experimental models involving the BP of the rat.<sup>6,15-19</sup>

Strangely, however, the literature is very scanty regarding the morphology of the rat BP, and even more so regarding the blood supply to these nerves.<sup>14-17</sup> Hence, the aim of this work was to contribute to a better understanding of the morphology and blood supply of the rat BP.

## MATERIAL AND METHODS

Thirty adult rats weighing between 300 and 350g were subjected to general anesthesia by intraperitoneal injection of a mixture of ketamine and xylazine at doses 90mg / kg and 10mg / kg, respectively.<sup>18</sup> Animals were submitted to a median laparotomy and then euthanized by placing a catheter inside the abdominal aorta and another inside the caudal vena cava, and by exsanguinating animals and replacing the blood with heparinized saline (50 units / ml).

In 25 animals a colloidal suspension of barium sulphate (Micropaque® - Nicholas - Lab) mixed with 10% commercial gelatin (in equal parts), and stained with a red or blue pigment (Pigment Tintolac Super®, Robialac) was injected intravascularly after performing a median laparotomy. The red-colored solution was injected into the abdominal aorta and the blue-stained solution in the caudal vena cava, according to the techniques currently in use at our institution.<sup>19-23</sup>

Rats were dissected with the use of a binocular surgical microscope. The constitution and distribution of the BP and its branches, as well as the origin and termination of the arteries and veins that supplied those nerves was registered. Subsequently, in 20 of the 25 injected rats, nerves were removed and made diaphanous, according to the technique developed by Spalteholz and subsequently modified by Esperança-Pina and Goyri O'Neill.<sup>24-27</sup> These nerves were observed under a binocular loupe, in order to study their vascularization. In addition, in 5 of the intravascularly injected rats, the nerves were fixed in formol and prepared for histological examination, using hematoxylin-eosin and Masson's trichrome stains. Additionally, sections of the nerves were marked with CD31 immunostain, in order to highlight the endothelial lining of vessels.

Finally, in 5 rats a Mercocox® solution was injected through the abdominal aorta, in order to obtain vascular molds that were later observed through scanning electron microscopy, according to the protocols currently used at our institution.<sup>19-22,28</sup>

All the procedures were conducted by researchers certi-

fied in the handling of laboratory animals. The experimental protocol was approved by the Ethical committee at the authors' institution.

## RESULTS

### Brachial Plexus Constituents

The BP in all animals studied was composed of branches originating from the ventral aspect of C4 to C8 and T1. In 57% of cases ( $n = 17$ ) the ventral aspect of T2 established an anastomosis with the ventral aspect of T1, thus contributing to the formation of the BP. This branch from T2, as well as the branch from C4 to the BP, was smaller than the remaining branches that formed the roots of the plexus. The most common disposition of the constituents of the BP and of its terminal and collateral branches is depicted in Fig. 1. A branch from the second and/or the third intercostal nerve to the medial brachial and medial antebrachial cutaneous nerves was found in 87% ( $n = 26$ ) of specimens (Fig. 1C).

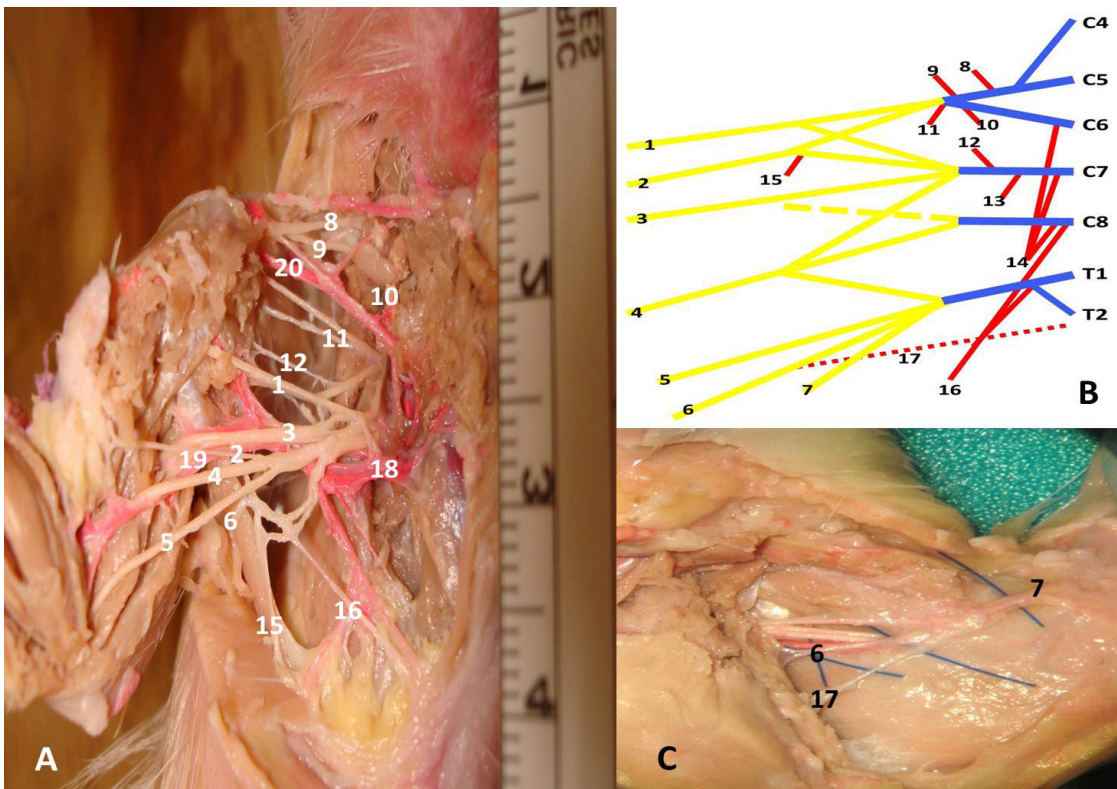
The BP roots emerged between the anterior and middle scalene muscles, forming a flattened BP below the clavicle (Figs 1 and 2). Although there was a clear homology with the elements of the BP in the rat and in man, the origin of the different terminal and collateral branches were found to be different in these two species.<sup>23</sup> (Fig. 1) For example, there were no clear medial, posterior and lateral cords in the rat brachial plexus. In addition, the median nerve, for example, almost always originated from three different roots, representing by far the thickest terminal branch of the brachial plexus of the rat (Fig. 1).

### Macrovascular Blood Supply

The arterial supply to the BP plexus was seen to derive directly or indirectly from the vertebral, axillary, brachial, and median arteries, as well as from arteries arising directly from the aortic arch, and from the acromial and cervical arterial trunks. These last two arteries were analogous to the human thyrocervical trunk. All those arteries gave off several variable branches to the neighbor nerves, forming a conspicuous epineural arterial plexus around roots, trunks, and terminal and collateral branches of the BP (Fig. 2). The arterial branches to each nerve were very variable both in number and caliber, even between both sides of each animal.

The venous drainage followed a similar path to the homonymous arterial structures, draining ultimately in the median, brachial, axillary and cephalic veins (Fig. 3). The veins draining the nerves were more numerous than arteries, but of smaller caliber, making their identification difficult at the epineural level, even at a magnification of 40X.

Nerves not escorted by prominent vessels derived vascular branches (arteries and veins) from small vessels supplying nearby muscles. Sometimes this occurred even where large nutrient vessels existed nearby, with tiny vessels connecting the longitudinal epineural vascular system with the blood supply of nearby muscles. Frequently, blood vessels were seen coming out of the surface muscles accompanying the nerve branches to those muscles. (Fig. 3)



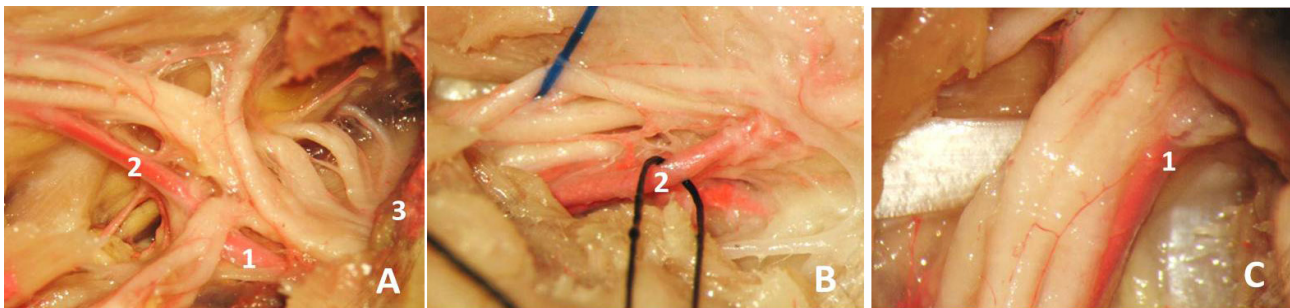
**Figure 1** – Common disposition of the constituents of the brachial plexus (BP) in the Wistar rat.

**A.** Ventral aspect of a right forepaw dissection showing several of the terminal and collateral branches of the BP, and their intimate association with several major arterial trunks (4X magnification).

**B.** Schematic drawing of the most common disposition of the constituents of the BP according to our study.

**C.** Ventral aspect of a left forepaw dissection showing the usual anastomosis between the second intercostal nerve and the medial brachial and antebrachial cutaneous nerves (10X magnification).

1- Axillary nerve; 2- Musculocutaneous nerve; 3- Radial nerve; 4- Median nerve; 5- Ulnar nerve; 6- Medial brachial cutaneous nerve; 7- Medial antebrachial cutaneous nerve; 8- Dorsal scapular nerve; 9- Suprascapular nerve; 10- Nerve to subclavius muscle; 11- Upper subscapular nerve; 12- Lower subscapular nerve; 13- Thoracodorsal nerve; 14- Long thoracic nerve; 15- Lateral pectoral nerve; 16- Medial pectoral nerve; 17- Intercostobrachial nerve; 18- Axillary artery; 19- Brachial artery; 20- Acromial arterial trunk.

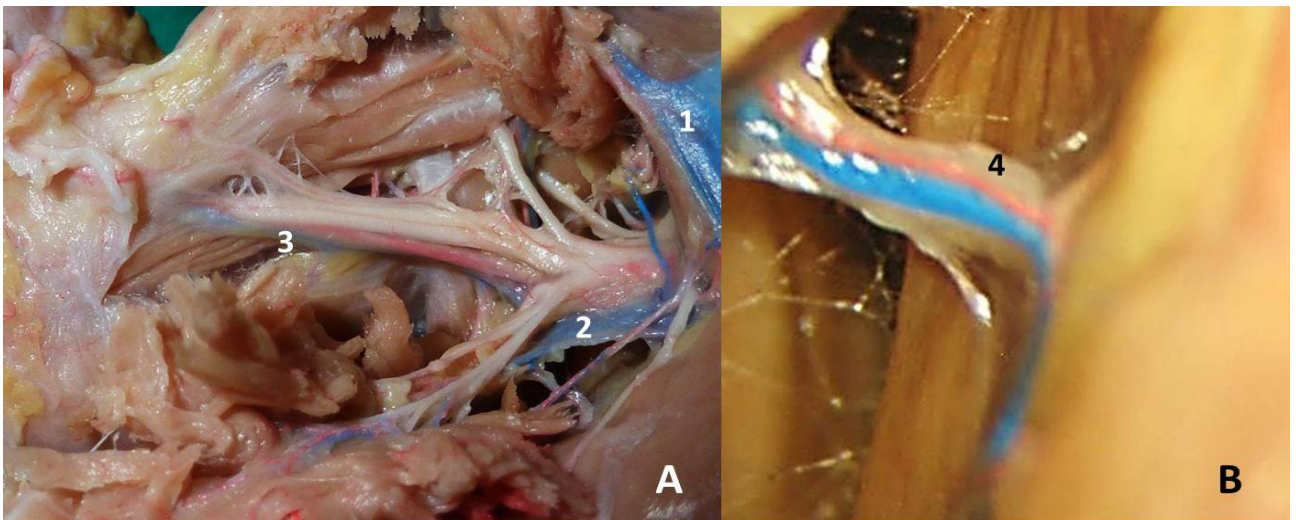


**Figure 2** – Photographs of dissections of the right brachial plexus of the rat showing the arterial blood supply of the plexus from nearby arteries at the level of the roots (A), trunks (B) and terminal branches (C). These small arteries form a network over the surface of nerves – epineural arterial plexus (4X magnification).

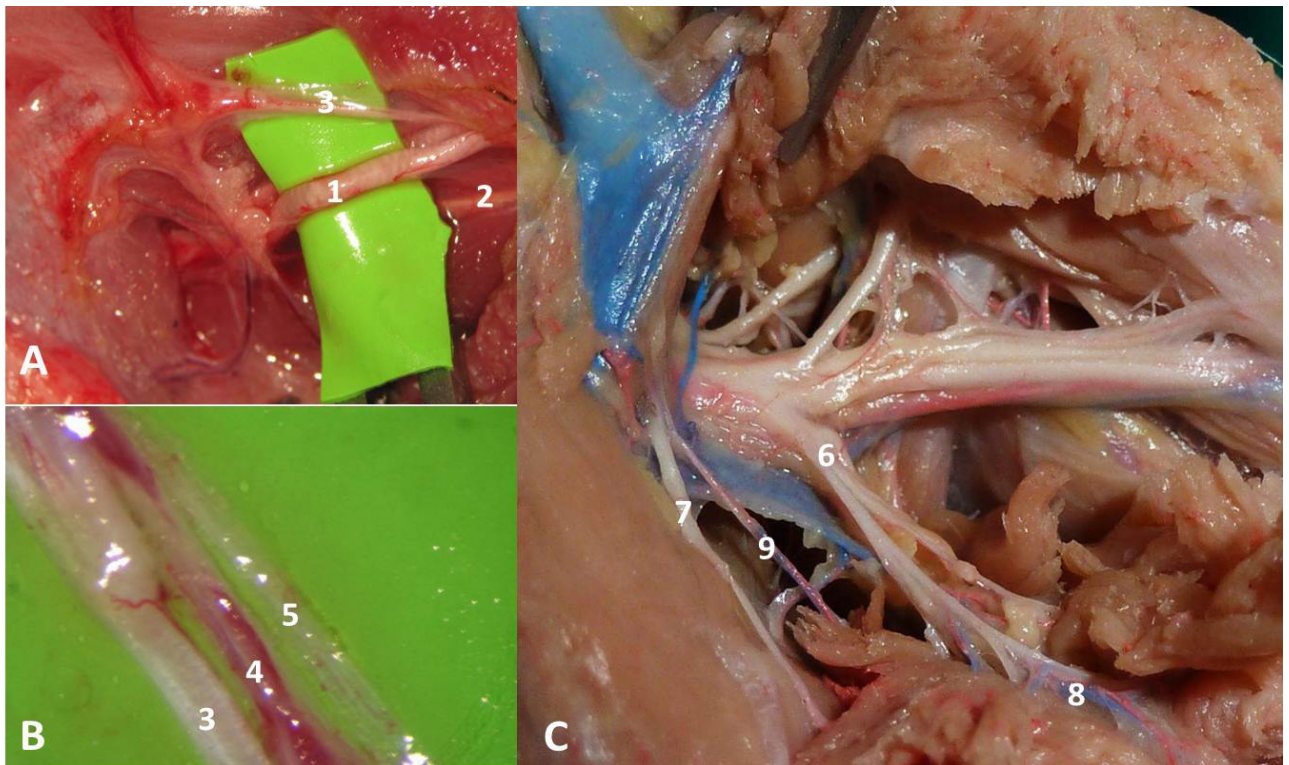
1- Axillary artery; 2- Brachial artery; 3- Vertebral artery.

Some nerves were accompanied by relatively large and constant blood vessels that supplied their epineural plexus, making it possible to raise these nerves as vascular nerve flaps (Fig. 4). Examples of these associations were: the medial brachial cutaneous nerve and branches from the axillary vessels (Fig.s 4A and 4B); the lateral and medial pectoral nerves and their homonymous vessels, which derived from the axillary vessels (Fig. 4C); the thoracodorsal nerve and the thoracodorsal vessels originating from the

subscapular vessels; the radial nerve and the deep brachial vessels given off by the brachial artery in the dorsal aspect of the arm; the musculocutaneous nerve and a descending branch of the ventral circumflex humeral vessels, between the two heads of the biceps brachii; the median nerve and the median vessels at the level of the forearm; and the ulnar nerve and branches of the brachial vessels (equivalent to the human inferior ulnar collateral vessels) at the arm level.



**Figure 3 – A.** Photograph of a dissection of the right brachial plexus showing the arterial (red) and venous (blue) supply to the nerve elements. The vein drainage follows a parallel course to the arterial supply (4X magnification).  
**B.** A closer look at the dorsal scapular nerve illustrating the fact that frequently nerves entering muscles receive arterial and venous branches from the vessels that supply nearby muscles (25X magnification).  
 1-External jugular vein; 2-Axillary vein; 3- Brachial vein; 4- Dorsal scapular nerve.



**Figure 4 –** The parallel path of vessels and elements of the brachial plexus allows the mobilization of several nerves as flaps.  
**A.** Photograph of a dissection of the right forepaw showing the dissection of the medial brachial cutaneous nerve (4X magnification).  
**B.** Photograph of a higher magnification showing the parallel path of the medial brachial cutaneous nerve and an artery and a vein originating and terminating, respectively in the axillary vessels (40X magnification).  
**C.** Dissection of the left BP showing the arterial (red) and venous (blue) supply to the nerve elements. The picture shows the lateral pectoral and the medial pectoral nerve receiving homonymous vessels that originate and terminate in the axillary vessels.  
 1- Median nerve; 2- Ulnar nerve; 3- Medial brachial cutaneous nerve; 4- Artery accompanying the medial brachial cutaneous nerve; 5- Vein accompanying the medial brachial cutaneous nerve; 6- Lateral pectoral nerve; 7- Medial pectoral nerve; 8- Vessels accompanying the lateral pectoral nerve; 9- Vessels accompanying the medial pectoral nerve.

## Microvascular Blood Supply

The hematoxylin-eosin and Masson's trichrome stained sections as well as the CD-31 immunostained sections of the BP showed multiple blood vessels at the epineurium, perineurium and endoneurium, at all levels, from the proximal aspect of the roots to the distal portion of the terminal and collateral branches (Fig. 5). Vessels were scarcer and of larger caliber in the epineurium than in the perineurium, and in turn fewer and larger in the perineurium than in the endoneurium. (Fig. 5)

The scanning microscopy specimens corroborated these findings (Fig. 6). In addition, they showed that the epineurial, perineurial and endoneurial vascular systems were profusely anastomosed throughout the length and width of the elements of the BP.

## DISCUSSION

In humans, the systematic study of the anatomy of the BP dates back to at least to the late nineteenth century, with the work of JF Wash, who characterized the so called 'normal variety' of the BP, based on an extensive dissection study.<sup>29</sup> To these studies were added several others, notably those of Herringham, Kerr, Wilfred Harris, Ruth Miller, Alnot Hueten, Narakas, Bonnel, Adolphi and Ko Hirasawa, and more recently those of Pandey and Shukla.<sup>30-38</sup> These works abundantly documented the high variability of the BP constituents.<sup>14-17</sup>

In contrast, the morphological studies of the rat's BP are much fewer.<sup>14,39</sup> Interestingly, similarly to Green and Chiasson, we found that the BP in this species was formed from anastomoses between the ventral rami of C4 to T2 in the majority of specimens.<sup>14,40</sup> This contrasts with the work of Bertelli that disabowed the contribution of T2 in the genesis of the rat BP, after dissecting 42 specimens.<sup>14</sup> Also in contrast to the latter author, and in accordance with the two former authors, we found the BP of the rat to be significantly different from the human BP in several ways, namely not being easily divisible into lateral, medial and posterior cords, and frequently having a different origin of its terminal and collateral branches (Fig. 1).<sup>14,40</sup> Concerning the differences in the terminal branches of the BP, the median nerve, for example, almost always originated from three different roots derived from C7 to T2, and represented by far the thickest terminal branch of the BP of the rat. In humans, the median nerve is usually formed from the reunion of a lateral and a medial cord.<sup>23</sup> In addition, in the absence of a posterior cord in the rat BP, the axillary and the radial nerves originated from separate dorsal branches from the C4-C6 and from the C7-C8 nerve roots, respectively. In humans the axillary and the radial nerves derive from the posterior cord.<sup>32</sup> The common absence of this cord in the rat also determined that the upper and lower subscapular nerves, as well as the thoracodorsal nerve, which usually are given off by the posterior cord in humans, had a different origin in the rat.<sup>41</sup> Thus, in our series the upper subscapular nerve usually originated from the C5-C6 roots whereas the lower subscapular and the thoracodorsal nerves were derived from the C7 nerve

root. However, taking into consideration that all the mentioned series as well as our own, are relatively small, we believe that further studies are warranted to clarify the anatomy of the rat BP.

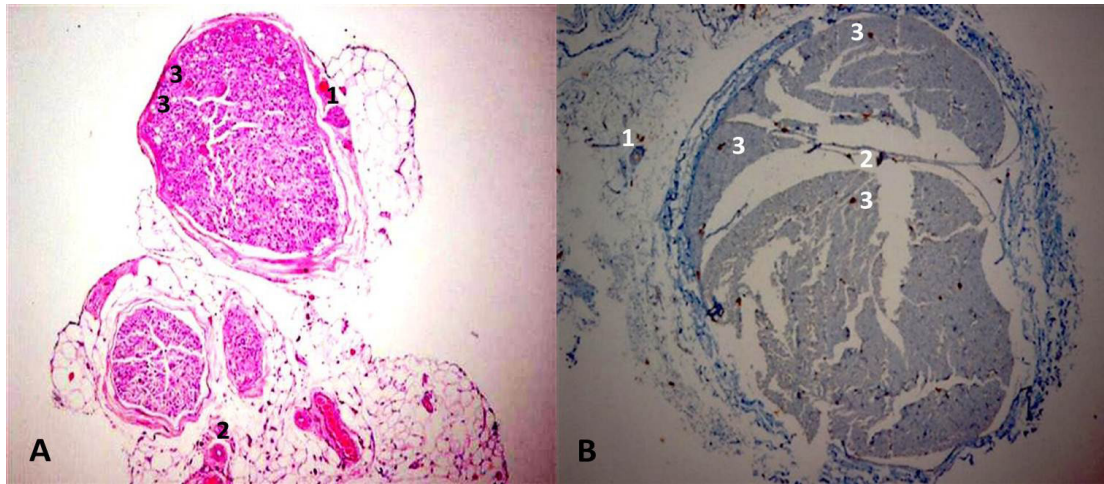
In animals, an in particular in rats, the study of the blood supply of nerves is very limited.<sup>7,14,39,42-44</sup> Notwithstanding, this knowledge may be of great use in defining and implementing surgical techniques involving the BP and its collateral branches and terminals.<sup>7</sup> Bertelli et al. presented the largest study we could find in the literature regarding the blood supply of the rat BP.<sup>14</sup> However, this author's report was based exclusively on the macroscopic blood supply of these nerves after injecting a colored latex solution in the arterial system of 10 rats.<sup>14</sup> Therefore, as far as we could determine, the present work, which involved 30 rats, represents the largest work published regarding the macro and microvascular blood supply to the rat BP and its branches. One of the main limitations of the present work was its mainly qualitative nature. In fact, we did not have the proper resources to perform a detailed evaluation of the caliber of the fine vessels that supply the rat BP constituents. Notwithstanding, this knowledge is not indispensable to the surgical approach to the rat BP. As far as we could determine, this subject has not been addressed by other authors. Thus, this limitation in the knowledge of the blood supply to the rat BP requires further studies.

In humans, the first major contributor to the knowledge of the blood supply to peripheral nerves was Sir Sydney Sunderland.<sup>45-47</sup> He concluded, after carefully studying the topography and morphology of the *vasa nervorum*, that the location, number and caliber of these vessels were highly variable.<sup>45-47</sup> He summed up his findings by saying that any vessel in the vicinity of a nerve would send or receive branches from that nerve.<sup>45-47</sup>

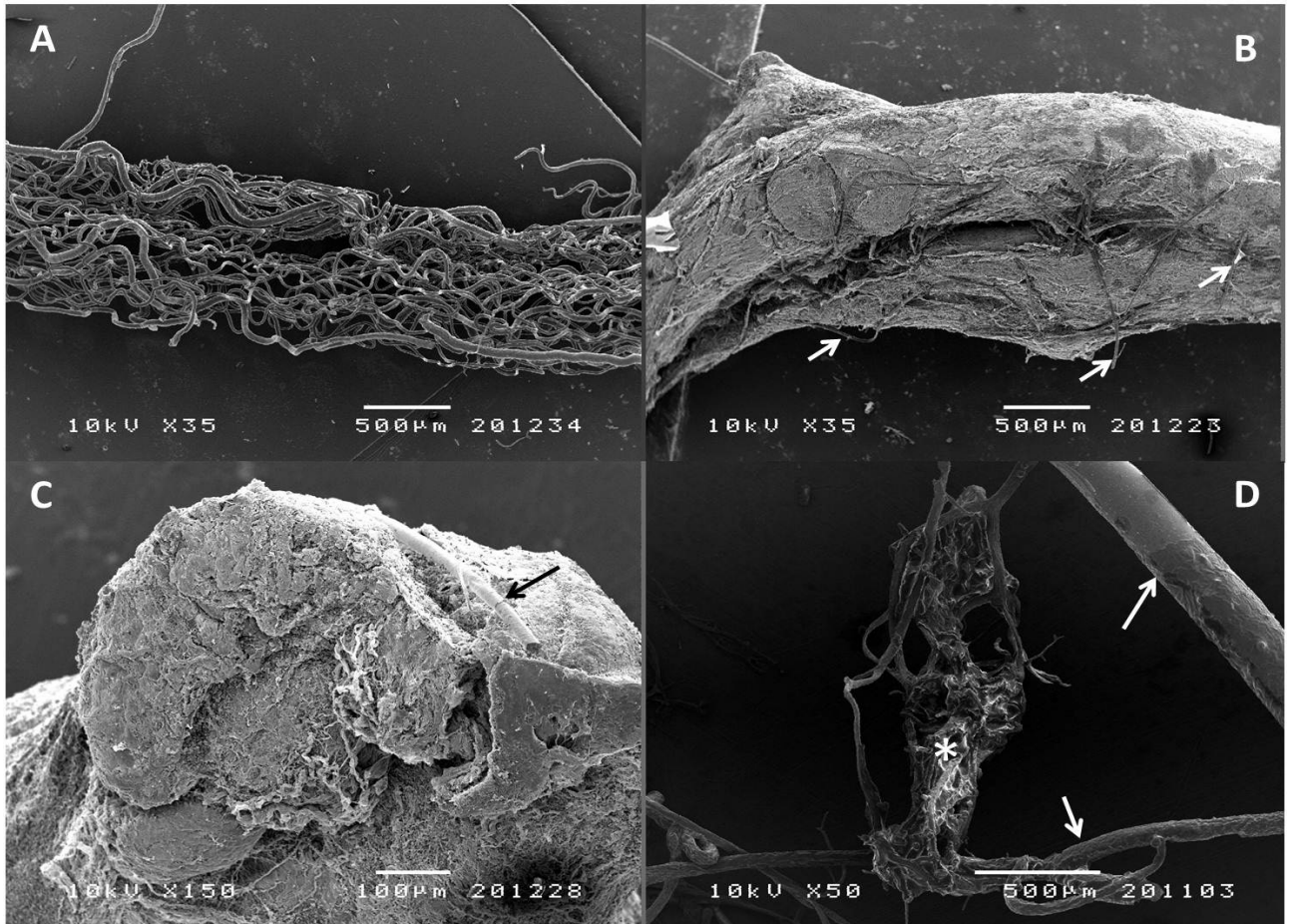
Recently, Taylor's group reported that, in humans, upper and lower limb nerves not escorted by prominent vessels receive an important contribute from vessels supplying nearby muscles.<sup>45,46</sup> According to this author, this would sometimes occur even where large nutrient vessels exist nearby, with tiny vessels connecting the longitudinal anastomotic system of the nerve and the blood supply to the muscle.<sup>45,46</sup> We confirmed Sunderland's and Taylor's findings in this study, which gives strong support for the use of the rat as model for ischemic insults to the peripheral nervous system.

There is some evidence that vascularized nerve grafts (also known as 'nerve flaps') may be superior to the traditional nerve grafts (devoid of a blood supply of their own) in several ways, especially if the local blood supply is poor, as it is the case of fibrosis or previous radiotherapy.<sup>45,46,48</sup> These vascularized nerve grafts may promote invasion of nerve grafts by macrophages which stimulate the removal of myelin fragments and contribute to maintaining the Schwann cells, which in turn could make nerve regeneration faster and more complete.<sup>49</sup>

We observed that several of the BP branches were accompanied by relatively large and constant blood vessels



**Figure 5** – Optical microscopy images of the blood supply to the brachial plexus.  
**A.** Photograph of a hematoxylin-eosin stained section of the C6 ventral rootlets of the rat brachial plexus showing the epineural, perineural and endoneural blood vessels (4X magnification).  
**B.** Photograph of a CD-31 immunostained section of the upper trunk of the rat BP showing the epineural, perineural and endoneural blood vessels (4X magnification).



**Figure 6** – Scanning electron microscopy images of the blood supply to the rat brachial plexus.  
**A.** In a segment of a corroded vascular cast of the upper trunk it is possible to observe a dense intraneural capillary network – the intraneural vascular plexus.  
**B.** An uncorroded segment of the upper trunk of the brachial plexus showing a numerous vessels (arrows) supplying the surface of the nerve trunk and forming an epineural vascular plexus.  
**C.** An uncorroded segment of a transverse section of the C7 root of the BP showing an epineural artery (arrow) coursing obliquely over the nerve and forming part of the epineural vascular plexus.  
**D.** In a transverse section of a corroded vascular cast of the upper trunk it is possible to observe the dense intraneural vascular plexus (\*) connected to neighbor extraneural vessels (arrows).

that supplied their epineural plexus, making it possible to raise these nerves as nerve flaps. Hence, our work suggests that the following components of the rat BP can be mobilized as flaps: the thoracodorsal nerve in the lateral thorax; the medial pectoral nerve, and the lateral pectoral nerve in the ventral aspect of the thorax; the medial brachial cutaneous nerve in the arm; the radial nerve in the dorsal aspect of the arm; the musculocutaneous nerve between the two heads of the biceps brachii; the median nerve at the level of the forearm; and the ulnar nerve at the arm level. The experimental utility of some of these flaps is quite promising. For example, the medial brachial cutaneous nerve could be used as a model for sensory nerve flaps and the medial and lateral pectoral nerves, and the thoracodorsal nerve could provide models of predominantly motor nerve flaps.<sup>17</sup> Most other nerves mentioned above could potentially be used as mixed nerve flaps.

Bertelli et al had already alluded to the anatomical possibility of using the ulnar and the medial brachial and medial antebrachial cutaneous nerves as nerve flaps.<sup>14</sup> However, as far as the authors could determine the concept of using the remaining nerves as flaps is new in the literature. This information could be used for example to compare the efficacy of nerve repair using motor, sensory or mixed nerve flaps in the Wistar rat model.

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## CONCLUSION

Although the BP of the rat is not exactly identical to that of human morphologically, it shares a significant homology. In addition, its blood supply is not significantly different from the pattern reported in the human species, making it a useful model for the experimental study of peripheral nerve pathology and treatment.

This work suggests that several components of the rat BP can be used as nerve flaps, including predominantly motor, sensory or mixed nerve fibers. This information may facilitate new experimental procedures in this animal model.

## ACKNOWLEDGMENTS

The authors are grateful to all Technical Staff members of the Department of Anatomy at their institution, particularly to Mr. Carlos Lopes and to Mr. Marco Costa.

## CONFLICT OF INTERESTS

None stated.

## FUNDING SOURCES

One of the authors (Diogo Casal) received a scholarship from "The Programme for Advanced Medical Education" sponsored by "Fundação Calouste Gulbenkian, Fundação Champalimaud, Ministério da Saúde and Fundação para a Ciência e Tecnologia, Portugal."

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