VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 84, No. 1, pp. 61–70
DOI: 10.5603/fm.100615

ISSN 0015–5659
eISSN 1644–3284
journals.viamedica.pl

Intrahepatic distribution of nerves in *Suncus murinus* by whole-mount immunohistological observation

Ke Ren[1]*, Xiangquan Chen[1]*, Ting Yang[2], Rujia Li[2], Shuang-Qin Yi[2]

[1]*Faculty of Physical Education, Qu Jing Normal University, Yun Nan, China*
[2]*Department of Frontier Health Sciences, Graduate School of Human Health Sciences, Tokyo Metropolitan University, Tokyo, Japan*
**Ren K and Chen X contributed equally to this paper.*

[Received: 8 May 2024; Accepted: 25 June 2024; Early publication date: 27 June 2024]

Background: *In recent years, elucidating the actual state of the liver nervous system has attracted attention, owing to clinical needs, such as liver transplantation. Conventional methods for studying the intrahepatic nerve distribution mostly use liver tissue sections, specific markers for immunohistological studies, or anterograde/retrograde tracing in animals. However, knowledge of the three-dimensional structure of intrahepatic innervation is vague or speculative.*

Materials and methods: *In this study,* Suncus murinus *(n = 10) were perfused and fixed, the livers were excised, and the liver parenchyma was carefully removed, leaving only the intrahepatic vasculature. Specimens were prepared to study the three-dimensional structure of* Suncus murinus *intrahepatic and hilar innervation by whole-mount immunohistochemical staining using a neurofilament protein antibody.*

Results: *After the nerves running along the intrahepatic arterial system entered the liver parenchyma from the hepatic hilum, they maintained a relatively rich distribution along the interlobular arteries until the distal end. The innervation of the portal system began to decrease after entering the liver parenchyma and decreased significantly after reaching the deep parts. By the time it reached the end of the interlobular vein, there was very little left. The number of nerves running along the intrahepatic bile duct system was significantly reduced after entering the porta hepatis, and innervation was difficult to observe after completely entering the liver parenchyma.*

Conclusions: *Whole-mount immunohistochemical analyses with an anti-NFP antibody showed that intrahepatic innervation mainly accompanied the hepatic interlobular arteries and extended to their terminal ends. Neuronal regulation is very important in the functional regulation of intrahepatic nutritional vessels. However, there were very few NFP-immunoreactive nerves accompanying the intrahepatic bile duct system, possibly suggesting that the functional regulation of the intrahepatic biliary system mainly relies on hormones and neuropeptides.*
(Folia Morphol 2025; 84, 1: 61–70)

Keywords: intrahepatic innervation, interlobular artery, interlobular vein, interlobular bile duct, porta hepatis, *Suncus murinus*

Address for correspondence: Shuang-Qin Yi, MD, PhD, Professor, Department of Frontier Health Sciences, Graduate School of Human Health Sciences, Tokyo Metropolitan University, 7-2-10, Higashiogu, Arakawa-ku, 116-8551 Tokyo, Japan; e-mail: yittmniu@tmu.ac.jp, tel. +0081-3-3819-1211

INTRODUCTION

The hepatic nerves were historically considered of relatively little importance: "a transplanted liver does well despite its absolute denervation". Animal experiments have since shown that, although the liver function is affected to a certain extent after liver transplantation, severed liver nerves can regenerate and be almost completely repaired [7, 25]. In recent years, elucidating the actual state of the liver nervous system has once again attracted attention due to clinical needs, such as liver transplantation.

A series of studies showed that the hepatic nervous system mainly includes innervation of the extrahepatic biliary tract [3], efferent and sensory hepatic nervous systems [4, 14], and hepatic nervous system development [8]. However, immunohistological studies have typically used conventional methods for studying intrahepatic nerve distribution, mostly involving liver tissue sections and aminergic, cholinergic, nitrergic, and peptidergic nerve-specific markers, such as neuropeptide Y, substance P, vasoactive intestinal peptide, calcitonin gene-related peptide, glucagon-like peptide, somatostatin, neurotensin, galanin, nitric oxide, and serotonin [1, 9, 24], identifying efferent or afferent nerves or sympathetic and parasympathetic nerves [4, 16, 18]. There are also anterograde tracing methods using injections of True Blue or orange fluorescent carbocyanine dye DiI or retrograde tracing studies using injections of horseradish peroxidase to identify vagal innervation of the rat liver [5, 13]. Other studies have been conducted on selective hepatic denervation. Because it is impossible to selectively block the vagus or sympathetic innervation of the liver, it is difficult to produce convincing results [5]. As such, the understanding of the three-dimensional structure of intrahepatic nerve distribution remains vague or speculative.

Researchers have recently discovered that chronic hepatitis/liver fibrosis/liver cirrhosis [15], biliary atresia (BA) [30], and bisphenol A administration [28] are accompanied by distribution and morphological changes in intrahepatic nerves. Nerve fibres were also shown to be significantly reduced with the progression of fibrosis [15, 30]. Nerve fibres that are distributed to the hepatic parenchyma are derived from a corresponding nervous plexus, and their intrahepatic distribution differs according to the species [23]. The abundance of gap junctions between hepatocytes is assumed to compensate for the lack of direct innervation in rats [21]. It has been demonstrated that nerve fibres are restricted within Glisson's sheath in rats, whereas they extend into the parenchymal region in humans and guinea pigs [12]. Notably, there are distinct species differences in the innervation of the mammalian liver [14].

The house musk shrew, *Suncus murinus*, is one of the most common insectivores. In our previous studies, *Suncus murinus* has been employed for clinico-anatomical and morphological studies of the innervations of the pancreas [19, 36], major duodenal papilla [32], pylorus [34], gallbladder [33], and extrahepatic biliary tract [20, 35] via comparative studies on humans. *Suncus murinus*, as an experimental animal, has been shown to exhibit general morphological characteristics, especially in the visceral system, more similar to those of humans than other currently used laboratory animals, such as mice, rats, and rabbits [17, 29]. These findings suggest that this animal is also more valuable than general laboratory animals in terms of studying the intrahepatic nerve distribution.

In the present study, after experimental *Suncus murinus* specimens were perfused and fixed, white latex was injected into the liver blood vessels, and the liver parenchyma was carefully removed, leaving only the intrahepatic vasculature. The specimen was then prepared, and the three-dimensional structure of the intrahepatic and hilar innervation was investigated by whole-mount immunohistochemical staining using a neurofilament protein antibody. We analysed the actual state of liver innervation, including connections between the extrahepatic and intrahepatic nervous systems.

MATERIALS AND METHODS

Animals

Experiments were performed using adult male *Suncus murinus* (n = 10, weighing 85–100 g, 4–6 months old) from an outbred KAT strain established from a wild population in Kathmandu, Nepal [17], obtained from a closed breeding colony and bred and maintained in our laboratory (Functional Morphology Laboratory, Department of Frontier Health Sciences, Tokyo Metropolitan University, Tokyo, Japan).

Adult *Suncus murinus* were kept individually after weaning (28 days after birth) in plastic cages equipped with a wooden nest box containing paper strips, and kept in a conventionally conditioned animal room: 23 to 27°C, no humidity control, and 12-h light/dark cycle. Commercial trout pellets and water were provided *ad libitum*.

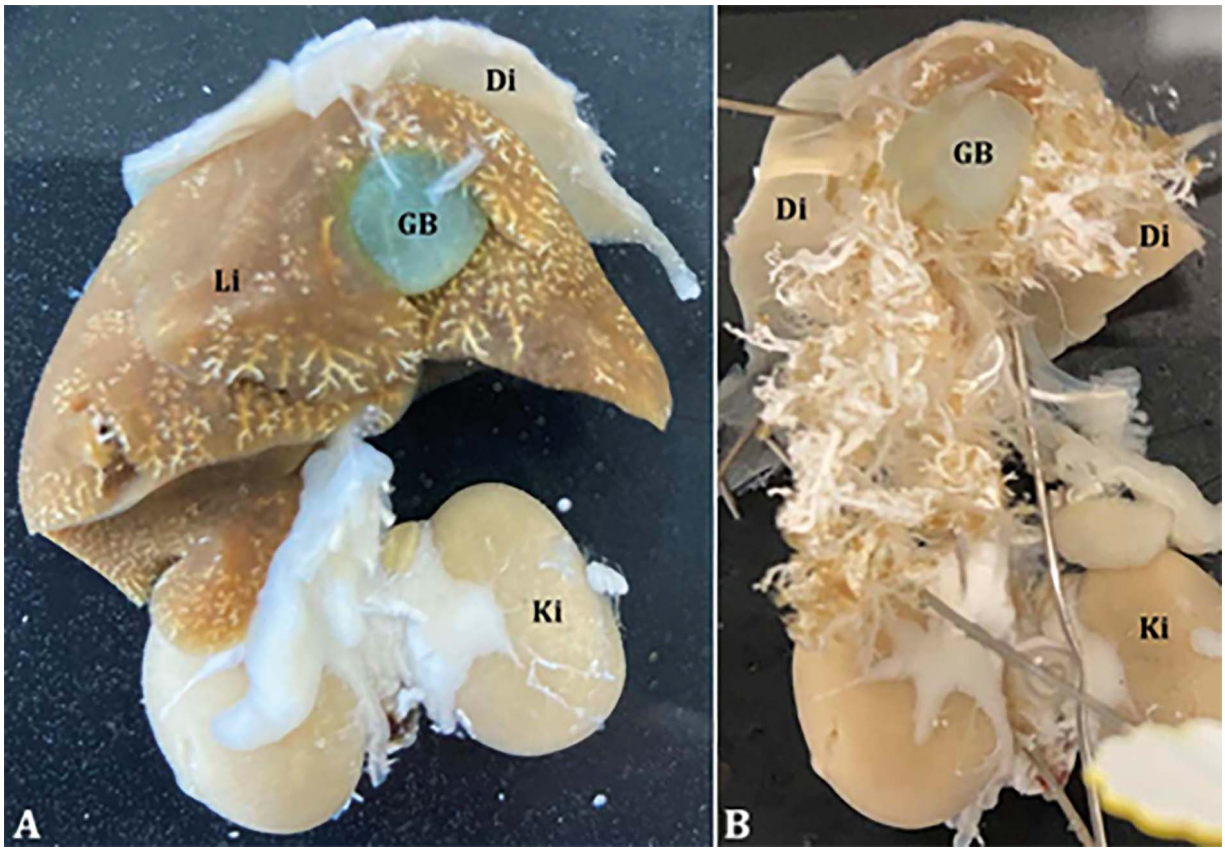

Figure 1. Specimen with blood vessels labelled with white latex, before dissection (**A**) and after dissection (**B**) (liver tissue other than vascular vessels was carefully removed). Arrows, intrahepatic blood vessels injected with white latex. Di — diaphragm; GB — gallbladder; Ki — kidney; Li — liver.

All experimental procedures were approved by the Tokyo Metropolitan University Institutional Animal Care and Use Committee (no. A3-23, A4-20, A5-15, A6-15). Animals were housed and handled in accordance with the Guide for the Care and Use of Laboratory Animals and the Guide for the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

Tissue preparation

Suncus murinus were first euthanised under deep anaesthesia with isoflurane inhalation, and a median abdominal incision was immediately made to open the abdominal cavity. A catheter was inserted retrogradely from the common iliac artery into the abdominal aorta while the inferior vena cava was incised at the same level for bleeding. Perfusion was performed with 4% paraformaldehyde (PFA) buffered with 0.01 M sodium phosphate (pH 7.4). After continuing the pressurised perfusion of white neoprene latex to label the blood vessels until the liver surface turned off-white, the entire abdominal cavity was immersed and fixed overnight with the same fixative (4% PFA) at 4°C.

Subsequently, the upper abdominal organs, including the liver, gallbladder, gastroduodenum, and pancreas, were removed as a whole, together with the abdominal aorta and inferior vena cava, as well as the celiac and superior mesenteric arteries. The collected specimens were further organised, and the gastroduodenum and pancreas were removed (Fig. 1A). The liver was carefully dissected using small pointed forceps. This procedure requires careful and gentle removal of all hepatocellular tissue from the hepatic lobules while avoiding damage to the intrahepatic ductal structures, resulting in complete preservation and exposure of the intrahepatic arterial, portal, biliary, and hepatic venous systems (Fig. 1B). After the dissection was complete, the specimen was used for whole-mount immunostaining, as described below.

Experimental protocols: whole-mount immunohistochemistry

Whole-mount immunostaining for *Suncus murinus* was performed as previously described [20, 32–36]. Briefly, after the fixed specimen was washed

with phosphate-buffered saline (PBS) and rocked gently on the nutator for 4 h, it was treated with 1% (w/v) orthoperiodic acid for 20 min at room temperature (RT) to prevent any intrinsic peroxidase reaction. The specimen was then incubated in freshly prepared 0.004% (w/v) papain in 0.025 mol/L Tris-HCl buffer (pH7.6) at 37°C for 2 h in a constant-temperature bath with gentle rocking. The specimens were washed with PBS for 50–60 min at RT and then stored in 4% PFA at 4°C overnight. The next day, the stored specimens were washed with PBS 4 times for 1 h each at RT, as described above. The specimen was then immersed in 2.5% (w/v), 5% (w/v), and 10% (w/v) sucrose for 30 min each, followed by freezing at –20°C for 30–60 min and thawing at RT, until completely thawed; this cycle was repeated 3 times. The specimens were then stored in 2% Triton X-100 at 4°C overnight. The next day, the specimen was incubated with the primary antibody in PBS containing 0.2% bovine serum albumin, 0.3% Triton X-100, and 0.1% sodium azide for 3 days at 4°C and continuously rocked gently on the nutator. After thorough washing in PBS, the specimens were incubated with the secondary antibody in a dilution buffer containing 0.2% BSA and 0.3% Triton X-100 for 3 days at 4°C. After more thorough washing in PBS, colouration was performed in 0.05 mol/L Tris-HCl buffer containing 0.002% 3,3′-diaminobenzidine (DAB) and 0.1 mL/L H_2O_2 overnight at 4°C. The stained preparations were stored in glycerine to ensure transparency.

The control experiments consisted of the following: (1) omission of the primary antiserum and (2) substitution of the primary antibody with PBS containing 0.2% bovine serum albumin, 0.3% Triton X-100, and 0.1% sodium azide. These controls were performed on the sections at the same time as the primary antibody treatment. The control data are not shown.

After the above staining was completed, the specimens were floated in PBS, and a stereomicroscope (objective lens can magnify 0.8 to 8 times) (Nikon, Tokyo, Japan) was used to observe and analyse the intrahepatic and extrahepatic nerve distribution, with photographs taken (Fig. 2).

Reagents for staining

1. 1% (w/v) orthoperiodic acid: 100 mL distilled water (DW) dissolving 1 g of orthoperiodic acid (Wako, 162-00732, Osaka, Japan).
2. Prepare 0.004% (w/v) papain: 100 mL 0.025 mol/L Tris-HCl buffer (pH 7.6) dissolved in 0.004 g papain (Wako, 164-00172, Osaka, Japan).
3. The primary antibodies used were an anti-neurofilament protein (NFP) antibody (diluted 1:600), a monoclonal mouse anti-all neurofilament consisting of 3 subunit proteins: NF-H (200 kDa), NF-M (160 kDa), and NF-L (70 kDa) (M0762, lot 089, clone: 2F11, Dako). The dilution solution of the primary antibody was 0.2% (w/v) bovine serum albumin (BSA) and 0.3% (v/v) Triton X-100 with 0.01 M PBS (pH 7.4).
4. The secondary antibody labelled with peroxidase-conjugated affinity-purified sheep anti-mouse IgG (HRP, MBL code 330) was diluted to 1:600 with 0.2% (w/v) bovine serum albumin (BSA), 0.3% (v/v) Triton X-100, and 0.1% (w/v) sodium azide with 0.01 M PBS (pH 7.4).

RESULTS

Whole-mount immunohistological staining showed that NFP-immunoreactive positive nerve fibres were completely detected in all parts of the specimen (Fig. 2).

Abundant nerve distribution was observed in the common bile duct, hepatic artery, and portal vein in the extrahepatic area of the hepatic hilum. In the intrahepatic area of the hepatic hilum, the innervation density of each vessel was clearly decreased; in particular, the innervation of the intrahepatic bile duct system was drastically reduced (Fig. 3).

Furthermore, in the liver parenchyma from the hepatic portal, there were abundant nerves along the proximal interlobular artery and vein, with no obvious difference in the distribution density between them. In contrast, nerves along the proximal interlobular bile duct system were not observed (Fig. 4).

Deeper into the liver parenchyma, the nerves along the middle interlobular artery were still abundant, whereas those along the middle interlobular vein were clearly reduced, with the former nerve fibres thicker than the latter. At this level, no nerves were observed in the interlobular bile duct system (Fig. 5).

Nerves corresponding to the thickness of the artery along the peripheral interlobular artery were observed in the peripheral area of the liver parenchyma. Nerves along the peripheral interlobular vein were also observed; however, their density decreased further than that above the deep area, and they were thinner than the nerves of the interlobular artery.

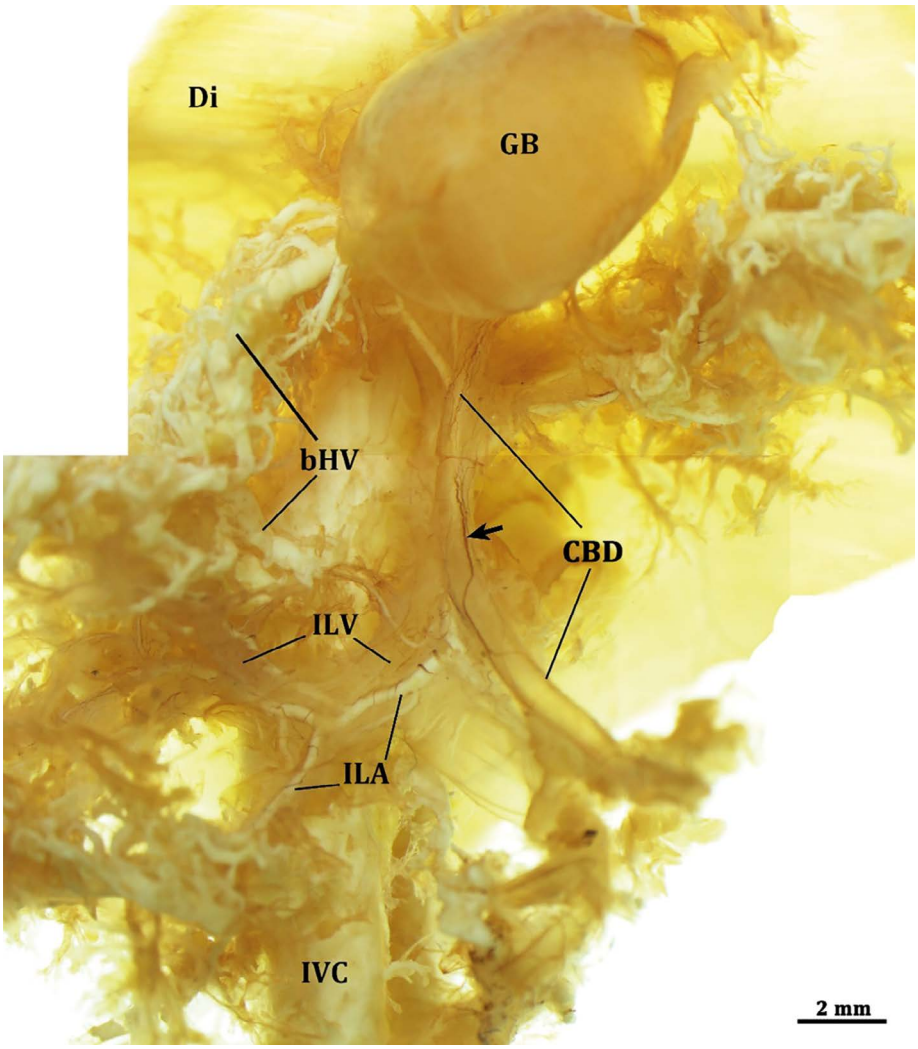

Figure 2. Overall view after whole-mount immunohistological staining with NFP antibody. Arrow, showing the NFP-positive nerve fibre. CBD — common bile duct; Di — diaphragm; GB — gallbladder; ILA — interlobular artery; ILV — interlobular vein; IVC — inferior vena cava, bHV — branches of hepatic vein.

Interlobular bile ducts were not observed near the terminal area (Fig. 6).

DISCUSSION

In this study, the intrahepatic and hilar nerve distribution in *Suncus murinus* was investigated using whole-mount immunohistochemistry. The hepatic artery, portal vein, and bile duct system were accompanied by abundant innervation at the hepatic hilum. After the nerves running along the intrahepatic arterial system entered the liver parenchyma, they maintained a relatively rich nerve distribution along the interlobular arteries until their end. The innervation of the portal venous system began to decrease after entering the liver parenchyma. It decreased significantly after reaching the deep part and remained very little when it reached the end of the interlobular vein. The nerves running along the intrahepatic bile duct system were significantly reduced near the porta hepatis, and the innervation was difficult to observe after completely entering the liver parenchyma.

The liver receives both sympathetic and parasympathetic nerve fibres; however, the innervation that hepatocytes receive varies among species. Thus, in cats, rabbits, guinea pig liver, and primate liver, nerve endings appear to be connected to all hepatocytes, unlike rats and mice, in which only hepatic cells in the portal region appear to be in contact with intrahepatic nerve endings [22]. Based on the view that intrahepatic nerves generally do not extend into the hepatic lobules but end up on the vasculature (tubular structures) in the triads (Glisson's sheath) [4], we

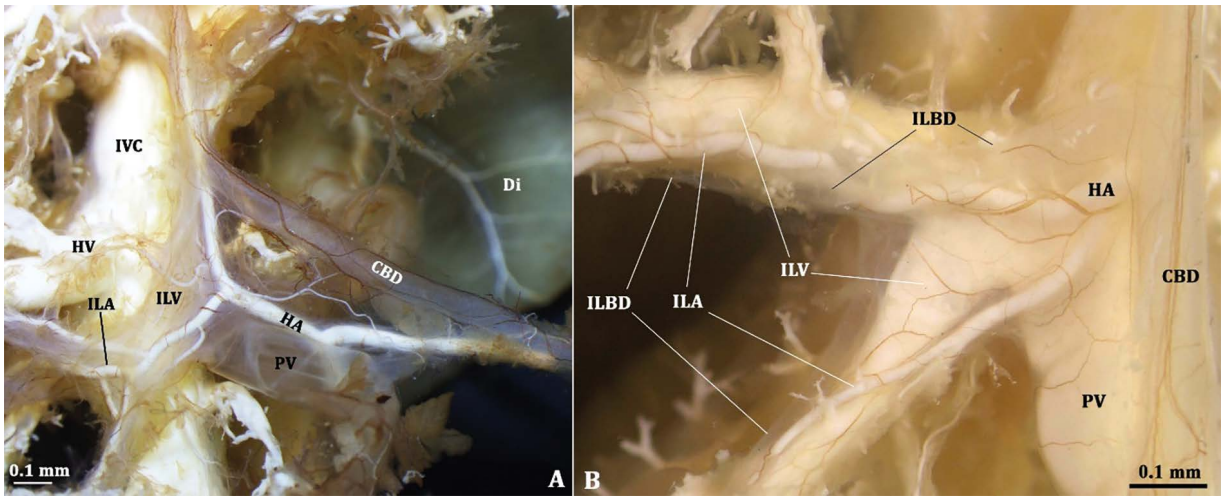

Figure 3. Innervation of the hepatic hilum (between extrahepatic and intrahepatic). CBD — common bile duct; Di — diaphragm; HA — hepatic artery; ILA — interlobular artery; ILBD — interlobular bile duct; ILV — interlobular vein; IVC — inferior vena cava; PV — portal vein.

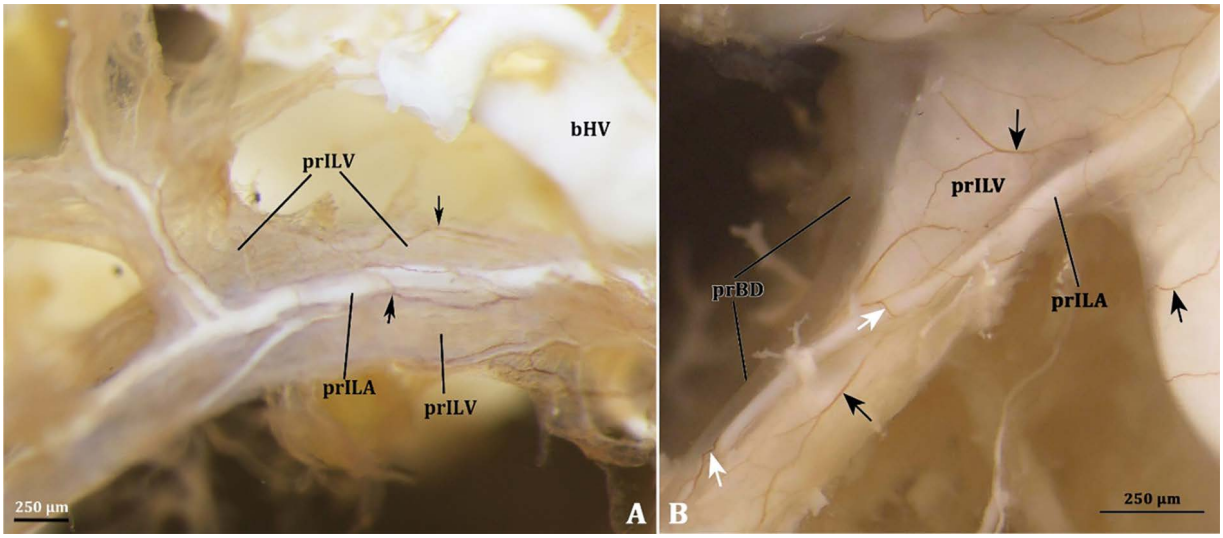

Figure 4. Proximal region of the intrahepatic system slightly closer to the porta hepatis. prBD — proximal bile duct; prILA — proximal interlobular vein; prILV — proximal interlobular artery; bHV — branch of hepatic vein. White arrows — nerves along arteries; black arrows — nerves along veins.

adopted a method of removing liver cell parenchyma as much as possible while keeping the intrahepatic blood vessels intact to prepare whole-mount immunohistochemistry specimens. We believe that the specimens obtained using this method can completely preserve the in situ existence of NFP-immunoreactive positive nerves and achieve three-dimensional visualisation of the intrahepatic nerves.

Autonomic nerves that innervate the liver are principally associated with the portal vein, hepatic artery, and bile duct and enter the hilum. Postganglionic sympathetic nerves are derived from splanchnic nerves originating in the celiac and superior mesenteric ganglia, while postganglionic parasympathetic nerves are derived from ganglia located at the hepatic hilum and within the portal spaces, and preganglionic parasympathetic fibres are branches of the vagus nerve [11]. Our previous studies have reported that, in humans and *Suncus murinus*, there are 2 communicating extrahepatic nerve plexuses: the anterior hepatic plexus and the posterior hepatic plexus. The anterior hepatic plexus runs along the common hepatic artery and the proper hepatic artery, and the posterior hepatic plexus runs along the wall of the common bile duct and behind the portal vein and then enters the liver [19, 20, 31, 33]. Furthermore,

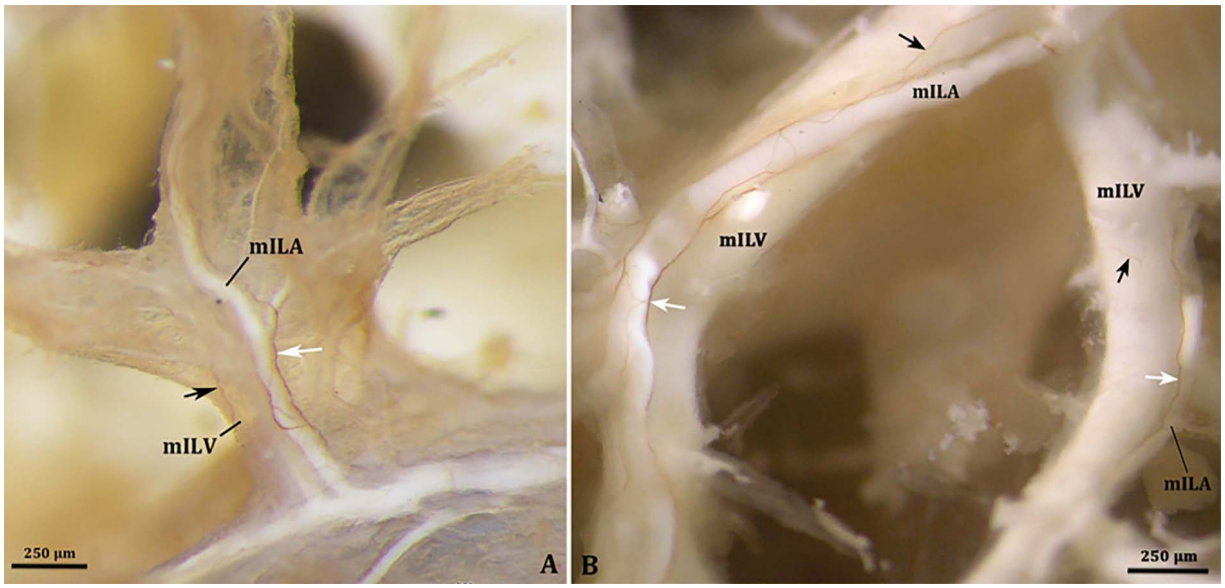

Figure 5. Middle region of the intrahepatic system. mILA — middle interlobular vein; mILV — middle interlobular artery. White arrows, nerves along arteries; black arrows, nerves along veins.

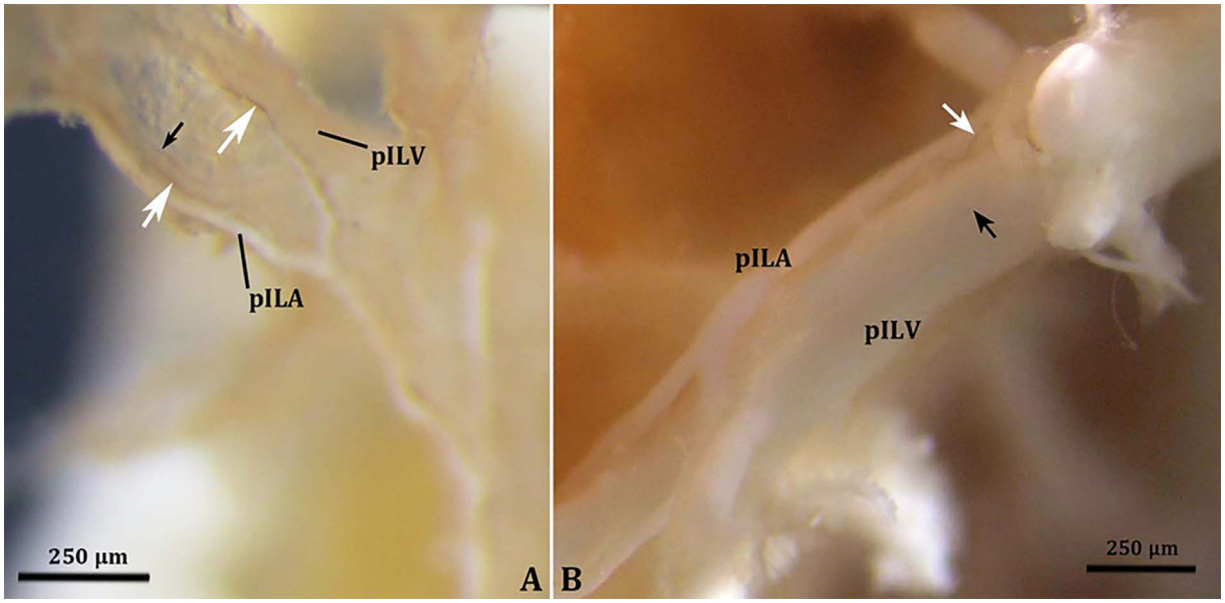

Figure 6. Peripheral region of the intrahepatic system. pILA — peripheral interlobular artery; pILV — peripheral interlobular vein. White arrows — nerves along artery; black arrows — nerves along veins.

the present study showed that the abundance of nerve fibres in these 3 extrahepatic nerve bundles was relatively similar.

Very little connective tissue is distributed in the liver parenchyma, and intrahepatic innervation is restricted to large branches of blood vessels [2]. According to the results of our study, the nerves running along the proper hepatic artery were divided out of the nerves in proportion to the cystic artery, while the rest entered the liver. After entering the liver, these nerves continue to extend along the intrahepatic interlobular arteries until they reach the end of the arteries. This group of nerves is closely related to the intrahepatic artery function. Carlei et al. [6] indicated the presence of a rich, selective peptidergic plexus surrounding afferent hepatic blood vessels. This plexus may play an important role in the regulation of the hepatic blood flow. We also believe that these nerves are related to the function of nutrient blood vessels like general visceral nerves, i.e. they regulate

the blood supply in the liver, and most of them belong to the aminergic, cholinergic, peptidergic, and nitrergic nerves [14].

In *Suncus murinus*, the nerves that coursed behind the portal vein essentially continued along the interlobular veins after entering the liver. The number of nerves distributed was significantly reduced compared with the extrahepatic system, and the deeper into the liver, the more rapidly the nerve distribution associated with the intrahepatic vein decreased. This suggests that smaller intrahepatic interlobular veins do not have a high demand for nerves, especially near the terminal interlobular veins, which appear to be uninnervated.

Developmental studies of the liver have shown that interlobular bile ducts play an important role in intrahepatic neurogenesis. The interlobular bile ducts guide the extension of nerve fibres by secreting NGF during nerve fibre development and regeneration [26]. In immunohistochemical identification of nerve fibres in formalin-fixed and paraffin-embedded sections using antibodies against S-100 protein (S-100) and neuron-specific enolase (NSE), S-100- and NSE-immunoreactive nerve fibres were present in the walls of the intrahepatic large, medium-sized, and septal bile ducts as well as in the peribiliary glands. Some nerve fibres were in close contact with the epithelia of the bile ducts and peribiliary glands. Serial section observations showed that the nerve fibres arising from nerve bundles approached and came into close contact with the epithelia of the bile ducts and peribiliary glands. Nerve fibres are sparse around the interlobular bile ducts and bile ductules [27]. Our findings therefore suggest that intrahepatic bile ducts and peribiliary glands are innervated, and biliary functions are regulated in part by these nerve fibres. Of note, increased nerve fibres may alter the biliary function in hepatolithiasis [27].

The innervation of the extrahepatic bile duct system mainly originates from the posterior hepatic nerve plexus and is quite abundant [33]. These nerves extend to the gallbladder in large numbers before entering the porta hepatis and branch to the other end of the common bile duct, the major duodenal papilla (Vater's papilla), where they form a direct neural connection between the gallbladder and Vater's papilla [35]. The present study found that the interlobular bile duct nerves distributed in the liver decreased sharply after entering the porta hepatis, and no NFP-immunoreactive nerve fibres were found from the liver parenchyma to the terminals. The results of our study are inconsistent with the above findings for NGF-immunoreactive nerve fibres and S-100/NSE-immunoreactive nerve fibres. This seems to imply a limitation of NFP in detecting intrahepatic nerve fibre distribution, which has not yet been verified.

Using neurohistochemical and electron microscopic methods in rats, Frank et al. [10] reported that the biliary system was sparsely innervated. Although nerves were interposed between vessels and bile ducts, they tended to be associated more closely with the vasculature. This result is consistent with those of our study.

CONCLUSIONS

In conclusion, whole-mount immunohistochemical analyses with an anti-NFP antibody showed that intrahepatic innervation mainly accompanied the hepatic interlobular arteries and extended to their terminal ends. Neuronal regulation is very important in the functional regulation of intrahepatic nutritional vessels. However, there were very few NFP-immunoreactive nerves accompanying the intrahepatic bile duct system, possibly suggesting that the functional regulation of the intrahepatic biliary system mainly relies on hormones and neuropeptides.

ARTICLE INFORMATION AND DECLARATIONS

Ethics statement

All experimental procedures were approved by the Tokyo Metropolitan University Institutional Animal Care and Use Committee (no. A3-23, A4-20, A5-15, A6-15). Animals were housed and handled in accordance with the Guide for the Care and Use of Laboratory Animals and the Guide for the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

Author contributions

SY acquired funding and designed and conceived the study. KR, XC, and TY participated in experiments. KR, XC, RL, and SY analysed the data. KR and XC wrote the article. SY revised the manuscript accordingly. All authors have contributed to the final version of the manuscript. All the authors have read and approved the final manuscript.

Acknowledgments

We would like to thank Dr. Yidan Dai for her help with the specific experiments in this study when she was a graduate student. She currently works at Tokyo Medical University.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

5. Akiyoshi H, Gonda T, Terada T. A comparative histochemical and immunohistochemical study of aminergic, cholinergic and peptidergic innervation in rat, hamster, guinea pig, dog and human livers. Liver. 1998; 18(5): 352–359, doi: 10.1111/j.1600-0676.1998.tb00817.x, indexed in Pubmed: 9831365.
6. Azanza MJ, Aisa J, Junquera C, et al. The autonomic innervation of the liver and gallbladder of Rana ridibunda. Histol Histopathol. 1989; 4(4): 405–410, indexed in Pubmed: 2520474.
7. Balemba OB, Salter MJ, Mawe GM. Innervation of the extrahepatic biliary tract. Anat Rec A Discov Mol Cell Evol Biol. 2004; 280(1): 836–847, doi: 10.1002/ar.a.20089, indexed in Pubmed: 15382017.
8. Berthoud HR. Anatomy and function of sensory hepatic nerves. Anat Rec A Discov Mol Cell Evol Biol. 2004; 280(1): 827–835, doi: 10.1002/ar.a.20088, indexed in Pubmed: 15382018.
9. Berthoud HR, Kressel M, Neuhuber WL. An anterograde tracing study of the vagal innervation of rat liver, portal vein and biliary system. Anat Embryol (Berl). 1992; 186(5): 431–442, doi: 10.1007/BF00185458, indexed in Pubmed: 1280009.
10. Carlei F, Lygidakis NJ, Speranza V, et al. Neuroendocrine innervation of the hepatic vessels in the rat and in man. J Surg Res. 1988; 45(5): 417–426, doi: 10.1016/0022-4804(88)90191-6, indexed in Pubmed: 3184922.
11. Colle I, Van Vlierberghe H, Troisi R, et al. Transplanted liver: consequences of denervation for liver functions. Anat Rec A Discov Mol Cell Evol Biol. 2004; 280(1): 924–931, doi: 10.1002/ar.a.20097, indexed in Pubmed: 15382009.
12. Delalande JM, Milla PJ, Burns AJ. Hepatic nervous system development. Anat Rec A Discov Mol Cell Evol Biol. 2004; 280(1): 848–853, doi: 10.1002/ar.a.20090, indexed in Pubmed: 15382016.
13. Esteban FJ, Jiménez A, Fernández AP, et al. Neuronal nitric oxide synthase immunoreactivity in the guinea-pig liver: distribution and colocalization with neuropeptide Y and calcitonin gene-related peptide. Liver. 2001; 21(6): 374–379, doi: 10.1034/j.1600-0676.2001.210602.x, indexed in Pubmed: 11903880.
14. Frank J, Goldfarb W, Eisenberg D, et al. Reconstruction of glutamine synthetase using computer averaging. Ultramicroscopy. 1978; 3(3): 283–290, doi: 10.1016/s0304-3991(78)80038-2, indexed in Pubmed: 32653.
15. Friedman MI. Hepatic nerve function. In: Arias I, Popper H, Jacoby WB. ed. The liver: biology and pathobiology. Raven Press, New York 1988: p949–959.
16. Fukuda Y, Imoto M, Koyama Y, et al. Demonstration of noradrenaline-immunoreactive nerve fibres in the liver. J Int Med Res. 1996; 24(6): 466–472, doi: 10.1177/030006059602400603, indexed in Pubmed: 8959530.
17. Magni F, Carobi C. The afferent and preganglionic parasympathetic innervation of the rat liver, demonstrated by the retrograde transport of horseradish peroxidase. J Auton Nerv Syst. 1983; 8(3): 237–260, doi: 10.1016/0165-1838(83)90108-x, indexed in Pubmed: 6668387.
18. McCuskey RS. Anatomy of efferent hepatic nerves. Anat Rec A Discov Mol Cell Evol Biol. 2004; 280(1): 821–826, doi: 10.1002/ar.a.20087, indexed in Pubmed: 15382019.
19. Mizuno K, Haga H, Okumoto K, et al. Intrahepatic distribution of nerve fibers and alterations due to fibrosis in diseased liver. PLoS One. 2021; 16(4): e0249556, doi: 10.1371/journal.pone.0249556, indexed in Pubmed: 33852613.
20. Nobin A, Baumgarten HG, Falck B, et al. Organization of the sympathetic innervation in liver tissue from monkey and man. Cell Tissue Res. 1978; 195(3): 371–380, doi: 10.1007/BF00233883, indexed in Pubmed: 103622.
21. Oda S, Kondo K. [Usefulness of wild insectivores as laboratory animals (author's transl)]. Jikken Dobutsu. 1977; 26(3): 273–280, doi: 10.1538/expanim1957.26.3_273, indexed in Pubmed: 561695.
22. Reilly FD, McCuskey PA, McCuskey RS. Intrahepatic distribution of nerves in the rat. Anat Rec. 1978; 191(1): 55–67, doi: 10.1002/ar.1091910106, indexed in Pubmed: 646138.
23. Ren K, Kinoshita M, Dai Y, et al. et al. A new model for localized pancreatitis, ventral pancreatic duct ligation in the adult house musk shrew, *Suncus murinus*. Academia J Biotechnol. 2016; 4(2): 055–061, doi: 10.15413/ajb.2015.0242.
24. Ren Ke, Dai Y, Yi K, et al. Using a Whole-mount Immunohistochemical Method to Study the Innervation of the Biliary Tract in *Suncus murinus*. J Vis Exp. 2017(124), doi: 10.3791/55483, indexed in Pubmed: 28654062.
25. Seseke FG, Gardemann A, Jungermann K. Signal propagation via gap junctions, a key step in the regulation of liver metabolism by the sympathetic hepatic nerves. FEBS Lett. 1992; 301(3): 265–270, doi: 10.1016/0014-5793(92)80254-e, indexed in Pubmed: 1577164.
26. Shimazu T. Progress and perspective of neuro-hepatology. In: Shimazu T. ed. Liver innervation and neural control of hepatic function. John Libbey & Company Ltd, Eastleigh 1996: 3–13.
27. Streba LA, Vere CC, Ionescu AG, et al. Role of intrahepatic innervation in regulating the activity of liver cells. World J Hepatol. 2014; 6(3): 137–143, doi: 10.4254/wjh.v6.i3.137, indexed in Pubmed: 24672643.
28. Stoyanova II, Gulubova MV. Peptidergic nerve fibres in the human liver. Acta Histochem. 1998; 100(3): 245––256, doi: 10.1016/s0065-1281(98)80011-1, indexed in Pubmed: 9717562.
29. Tadokoro F. Immunohistochemical study on the regeneration of nerve fibers of rat liver after surgical severance. Kanzo. 1994; 35(1): 29–38, doi: 10.2957/kanzo.35.29.
30. Tanimizu N, Ichinohe N, Mitaka T. Intrahepatic bile ducts guide establishment of the intrahepatic nerve network in developing and regenerating mouse liver. Development. 2018; 145(9), doi: 10.1242/dev.159095, indexed in Pubmed: 29615468.

31. Terada T, Nakanuma Y. Innervation of intrahepatic bile ducts and peribiliary glands in normal human livers, extrahepatic biliary obstruction and hepatolithiasis. An immunohistochemical study. J Hepatol. 1989; 9(2): 141–148, doi: 10.1016/0168-8278(89)90044-5, indexed in Pubmed: 2809154.
32. Thoene M, Godlewski J, Rytel L, et al. Alterations in porcine intrahepatic sympathetic nerves after bisphenol A administration. Folia Histochem Cytobiol. 2018; 1(2): 113–121, doi: 10.5603/FHC.a2018.0012, indexed in Pubmed: 29888781.
33. Tsutsui C, Kajihara K, Yanaka T, et al. House musk shrew (*Suncus murinus*, order: Insectivora) as a new model animal for motilin study. Peptides. 2009; 30(2): 318––329, doi: 10.1016/j.peptides.2008.10.006, indexed in Pubmed: 18996160.
34. Yang J, Chen X, Wang W, et al. Role of cholinergic innervation in biliary remnants of patients with biliary atresia. Front Pediatr. 2023; 11: 1278978, doi: 10.3389/fped.2023.1278978, indexed in Pubmed: 38259596.
35. Yi SQ, Nagakawa Y, Ren Ke, et al. The mesopancreas and pancreatic head plexus: morphological, developmental, and clinical perspectives. Surg Radiol Anat. 2020; 42(12): 1501–1508, doi: 10.1007/s00276-020-02547-y, indexed in Pubmed: 32797265.
36. Yi SQ, Ohta T, Miwa K, et al. Surgical anatomy of the innervation of the major duodenal papilla in human and *Suncus murinus*, from the perspective of preserving innervation in organ-saving procedures. Pancreas. 2005; 30(3): 211–217, doi: 10.1097/01.mpa.0000158027.38548.34, indexed in Pubmed: 15782096.
37. Yi SQ, Ohta T, Tsuchida A, et al. Surgical anatomy of innervation of the gallbladder in humans and *Suncus murinus* with special reference to morphological understanding of gallstone formation after gastrectomy. World J Gastroenterol. 2007; 13(14): 2066–2071, doi: 10.3748/wjg.v13.i14.2066, indexed in Pubmed: 17465449.
38. Yi SQ, Ru F, Ohta T, et al. Surgical anatomy of the innervation of pylorus in human and *Suncus murinus*, in relation to surgical technique for pylorus-preserving pancreaticoduodenectomy. World J Gastroenterol. 2006; 12(14): 2209–2216, doi: 10.3748/wjg.v12.i14.2209, indexed in Pubmed: 16610023.
39. Yi SQ, Ren K, Kinoshita M, et al. Innervation of extrahepatic biliary tract, with special reference to the direct bidirectional neural connections of the gall bladder, sphincter of oddi and duodenum in *Suncus murinus*, in whole-mount immunohistochemical study. Anat Histol Embryol. 2016; 45(3): 184–188, doi: 10.1111/ahe.12186, indexed in Pubmed: 26179953.
40. Yi SQ, Shimokawa T, Akita K, et al. Anatomical study of the pancreas in the house musk shrew (*Suncus murinus*), with special reference to the blood supply and innervation. Anat Rec A Discov Mol Cell Evol Biol. 2003; 273(1): 630–635, doi: 10.1002/ar.a.10075, indexed in Pubmed: 12808647.