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Symmetric brain-liver circuits mediate lateralized regulation of hepatic glucose output

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eLife Assessment

This manuscript proposes a lateralized, lobe-specific brain-liver sympathetic neurocircuit regulating hepatic glucose metabolism and presents anatomical evidence for sympathetic crossover at the porta hepatis using viral tracing and neuromodulation approaches. While the topic is of **important** significance and the methodologies are, in principle, state-of-the-art, significant concerns regarding experimental design, **incomplete** methodological reporting, sparse and ambiguous labeling, and over-interpretation of the data substantially weaken support for the study's central conclusions, thereby limiting the study's completeness. The work will be of interest to biologists, clinicians, and physiologists.

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Abstract

Hemispheric lateralization is well recognized in regulating contralateral somatic movement, yet its relevance to visceral organ regulation remains poorly understood. This study aims to investigate whether cerebral hemispheres differentially regulate hepatic glucose metabolism and localize the site of sympathetic crossover to the liver. Pseudorabies virus (PRV) tracing demonstrated symmetric projections from the paraventricular nucleus (PVN) with preferential innervation of contralateral hepatic lobes. Unilateral PVN activation elevated systemic glucose through enhanced glycogenolysis and gluconeogenesis in contralateral lobes, whereas bilateral activation produced additive effects. Following unilateral hepatic denervation, contralateral PVN activation induced metabolic compensation in the remaining innervated lobes, characterized by increased sympathetic release, glucose production, and glycogen depletion. Whole-mount clearing and dual tracing localized the sympathetic crossover to the porta hepatis, and developmental analysis showed lobar innervation along the vasculature emerging at postnatal week 2. These findings demonstrate that the brain exerts lobe-specific, lateralized control of hepatic glucose metabolism via symmetric brain-liver sympathetic pathways. Contralateral regulation arises from peripheral decussation at the porta hepatis, and compensatory activation following denervation reveals an intrinsic neuroadaptive mechanism that safeguards systemic glucose homeostasis.

Highlights

- Brain-liver sympathetic projections exhibit predominant contralateral innervation
- Unilateral LPGi activation drives glucose production in contralateral hepatic lobes
- Unilateral denervation augments contralateral LPGi-mediated metabolic compensation
- Sympathetic crossover to the liver localizes at the porta hepatis

Introduction

The cerebral hemispheres exhibit pronounced functional asymmetry,^(1,2) a phenomenon traditionally associated with cognitive and motor processes such as language, voluntary movement, and spatial navigation.^(3,4) Emerging evidence indicates that this lateralization extends beyond cortical functions to the autonomic regulation of visceral organs, thereby enhancing the efficiency of physiological responses.^(5–7) Such hemispheric specialization allows the central nervous system (CNS) to optimize resource allocation and prioritize functional outputs in a context-dependent manner. For instance, paired organs such as kidneys and adrenal glands receive predominantly contralateral neural inputs, enabling coordinated bilateral function.^(8,9) Previous studies have shown that the left hemisphere preferentially modulates sympathetic outflow to the right kidney and vice versa,^(8,10) and that cortical projections to the adrenal medulla are primarily contralateral, with the rostral cingulate motor area as a bilateral exception.⁽⁹⁾ However, the influence of hemispheric asymmetry on structurally asymmetric visceral organs remains incompletely understood and appears to be organ-specific. The heart, for instance, exhibits distinct lateralized autonomic innervation: right-sided fibers predominantly mediate chronotropic regulation via the sinoatrial node, whereas left-sided fibers exert greater inotropic and dromotropic functions.^(6,11) Additionally, cortical neurons regulating gastric sympathetic function are predominantly localized in the right hemisphere, whereas parasympathetic control displays only a modest right-sided predominance.^(7,12) These examples underscore the organ-specific nature of hemispheric asymmetry in autonomic neural projections.

The liver, as the largest asymmetrical visceral organ, plays a central role in systemic metabolic homeostasis,^(13,14) orchestrating physiological processes such as glucose production, lipid metabolism, protein synthesis, and detoxification.^(16,17) In mice, hepatic innervation first appears in extrahepatic bile ducts at embryonic day 17.5 and extends into lobes during the early postnatal period,⁽¹⁸⁾ yet the precise pattern of this innervation remains unknown. Previous anatomical and functional studies, including our own, have established that the liver receives dense sympathetic innervation from postganglionic neurons of the celiac-superior mesenteric ganglia (CG-SMG).^(19,20,21,22) Within this context, the brainstem lateral paragigantocellular nucleus (LPGi) has been identified as a key node in the regulation of hepatic sympathetic tone and glucose output. However, whether this regulation is lateralized and lobe-specific, and where sympathetic crossover occurs, has not been systematically defined.

To address these gaps, we applied retrograde trans-synaptic tracing with pseudorabies virus (PRV) to delineate LPGi projections to hepatic lobes and assess their lateralization.⁽²²⁾ Functional experiments employing chemogenetic and optogenetic activation of LPGi neurons clarified the physiological impact of this neural circuitry on hepatic glucose production (HGP). Additionally, we identified compensatory neural mechanisms that maintain systemic glucose homeostasis following unilateral cerebral disruption of hepatic innervation. Whole-mount clearing identified the porta hepatis as the site of sympathetic crossover and revealed developmental axon extension of hepatic fibers along the vasculature. Together, these findings reveal that lateralized neuronal circuits exert lobe-specific control over hepatic glucose output and recruit neuroadaptive responses to preserve metabolic homeostasis following unilateral disruption.

Results

1. Contralateral projections from the LPGi to distinct hepatic lobes

Previous studies of intrahepatic innervation mainly focused on individual lobes, such as the left or right lateral lobes in mice.^(14,15) We first asked whether sympathetic innervation is a uniform feature across all lobes. To address this, we examined the intact mouse liver using tissue clearing combined with immunofluorescence staining of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis. This analysis revealed that, although hepatic lobes differ in volume (Figure 1A [↗](#)), sympathetic fibers consistently branch into all lobes with comparable density (Figure 1A [↗](#)). To further investigate the anatomical basis of central neural regulation of the liver, we performed multi-synaptic retrograde tracing by injecting PRV into individual hepatic lobes, including the left lateral, median, right posterior, right anterior, and caudate lobes (Figures S1A [↗](#) and S1B [↗](#)). Five days post-injection, PRV-labeled neurons were consistently identified in the LPGi of the brainstem (Figure 1B [↗](#), Figures S1C [↗](#)-S1F [↗](#)). Quantitative analyses revealed a clear contralateral dominance: the injection into left lateral and median lobes yielded approximately twice as many labeled neurons in the right LPGi as in the left, whereas the injection into right posterior, right anterior, and caudate lobes preferentially labeled neurons in the left LPGi (Figure 1C [↗](#)). These findings provide direct anatomical evidence of a symmetric projection pattern in which each LPGi hemisphere preferentially innervates contralateral hepatic lobes (Figure 1D [↗](#)). Based on their robust lateralization, the right anterior and median lobes were selected as representative targets for subsequent analyses.

To validate these projection patterns, we performed dual labeling by co-injecting PRV-CAG-EGFP into the right anterior lobe and PRV-CAG-mRFP into the median lobe. In the left LPGi, 73.7% of PRV-positive neurons expressed EGFP, showing projections to the right anterior lobe, whereas in the right LPGi, 62.5% expressed mRFP, indicating projections to the median lobe (Figures 1E [↗](#) and 1F [↗](#)). A subset of neurons displayed co-labeling, suggesting that although the predominant projection pattern is contralateral, a population of LPGi neurons innervates both lobes bilaterally. These findings establish a contralaterally biased projection pattern from each LPGi to distinct hepatic lobes, providing the anatomical foundation for lateralized brain-liver circuits that regulate hepatic glucose metabolism.

2. Differential HGP by LPGi lateralization

To assess the functional implications of this anatomical laterality, we examined whether activation of the LPGi in each hemisphere modulates HGP in a lobe-specific manner. Building on our previous findings that liver-associated LPGi neurons are predominantly GABAergic, we selectively activated these neurons unilaterally or bilaterally using chemogenetics (Figure 2A [↗](#), Figure S2A [↗](#)). LPGi activation significantly increased blood glucose, with area under the curve (AUC) increases by 27%, 34%, and 52% following left-, right-, and bilateral stimulation, respectively (Figure 2A [↗](#)). Moreover, right-sided activation induced greater hyperglycemia than left-sided stimulation, possibly reflecting differences in the metabolic capacity or volume of the contralateral hepatic lobes (Figure 1A [↗](#)).

At the molecular level, one hour following stimulation, phosphorylation of glycogen phosphorylase (pPYGL) and glycogen synthase (pGS) increased in contralateral lobes, as assessed by immunofluorescence and western blot. Left LPGi activation preferentially enhanced pPYGL and pGS levels in the right anterior lobe compared with right-sided activation, whereas right LPGi activation exerted a greater effect in the median lobe relative to left-sided stimulation (Figures 2B [↗](#)-2D [↗](#)). Bilateral stimulation further amplified pPYGL and pGS expression in both lobes compared with unilateral activation. PAS staining confirmed glycogen depletion in metabolically engaged contralateral lobes (Figure S2B [↗](#)). In terms of gluconeogenesis, enzymatic activity assays revealed increased phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6PC) activity in hepatic lobes undergoing enhanced glycogenolysis, despite unchanged protein levels within the one-hour window (Figures S2C [↗](#)-S2F [↗](#)).

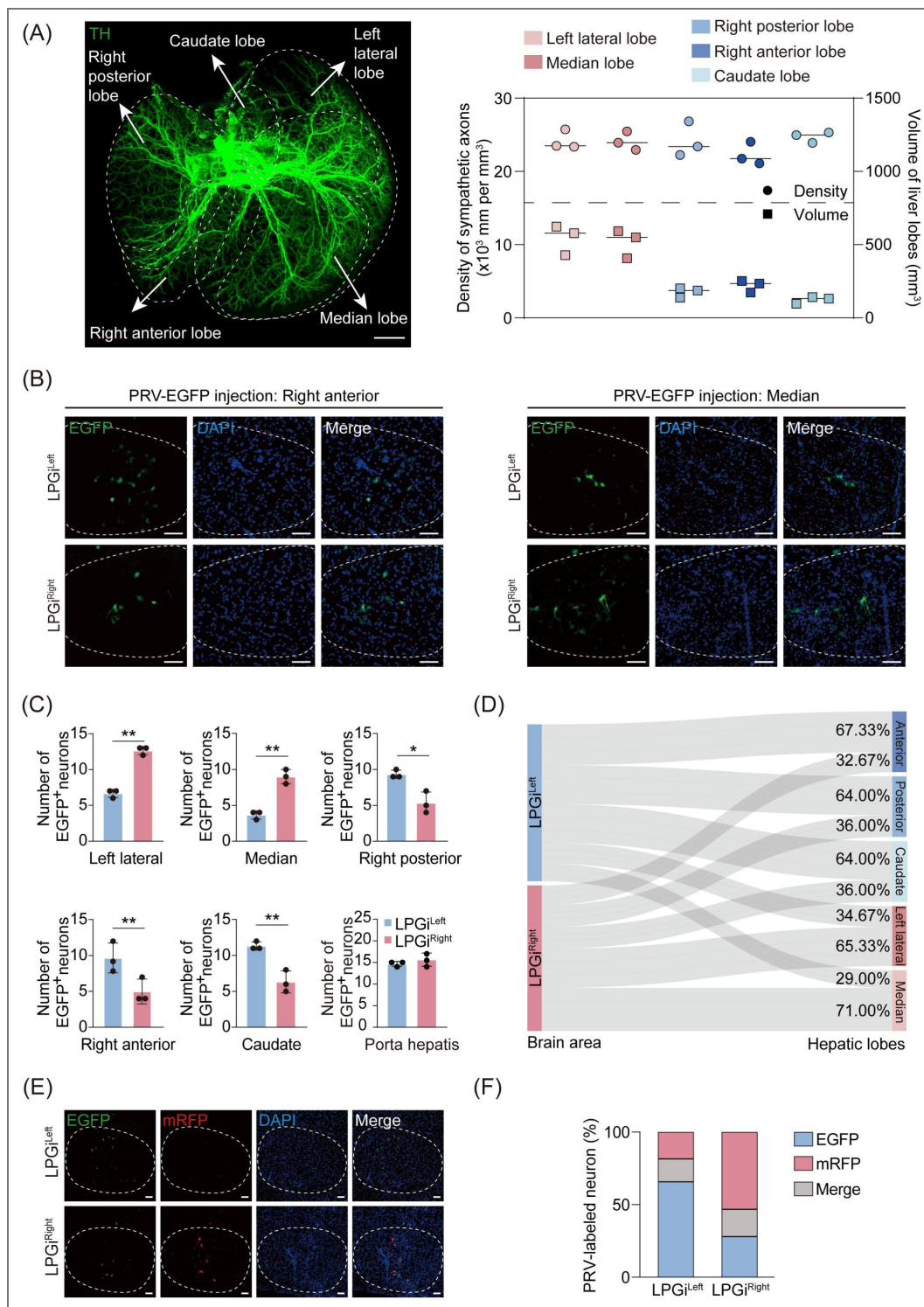


Figure 1. The brain contralaterally projects to the liver.

(A) Representative 3D images of TH-immunostained mouse liver (left), with quantification of sympathetic axon density and lobe volume (right). Scale bar, 3,000 μm . (B) Representative immunofluorescence images of PRV-labeled neurons (EGFP) in the left (top) and right (bottom) LPGi following PRV injections into the right anterior (left) and median (right) lobes. Scale bars, 100 μm . (C) Quantification of PRV-labeled neurons in left and right LPGi across different hepatic lobes: left lateral, median, right posterior, right anterior, caudate, and porta hepatis ($n = 3$). (D) Sankey diagram showing projection patterns from left and right LPGi to individual hepatic lobes. (E and F) Representative slices of EGFP⁺ and mRFP⁺ neurons in left (top) and right (bottom) LPGi following PRV-EGFP (right anterior lobe) and PRV-mRFP (median lobe) injections. Proportions of EGFP⁺, mRFP⁺, and co-labeled neurons in left and right LPG (F, $n = 3$). Scale bars, 100 μm . Data are expressed as means $\pm$ SEM, with individual values shown for all experiments. * $p < 0.05$, ** $p < 0.01$. Unpaired Student's t-test for (C).

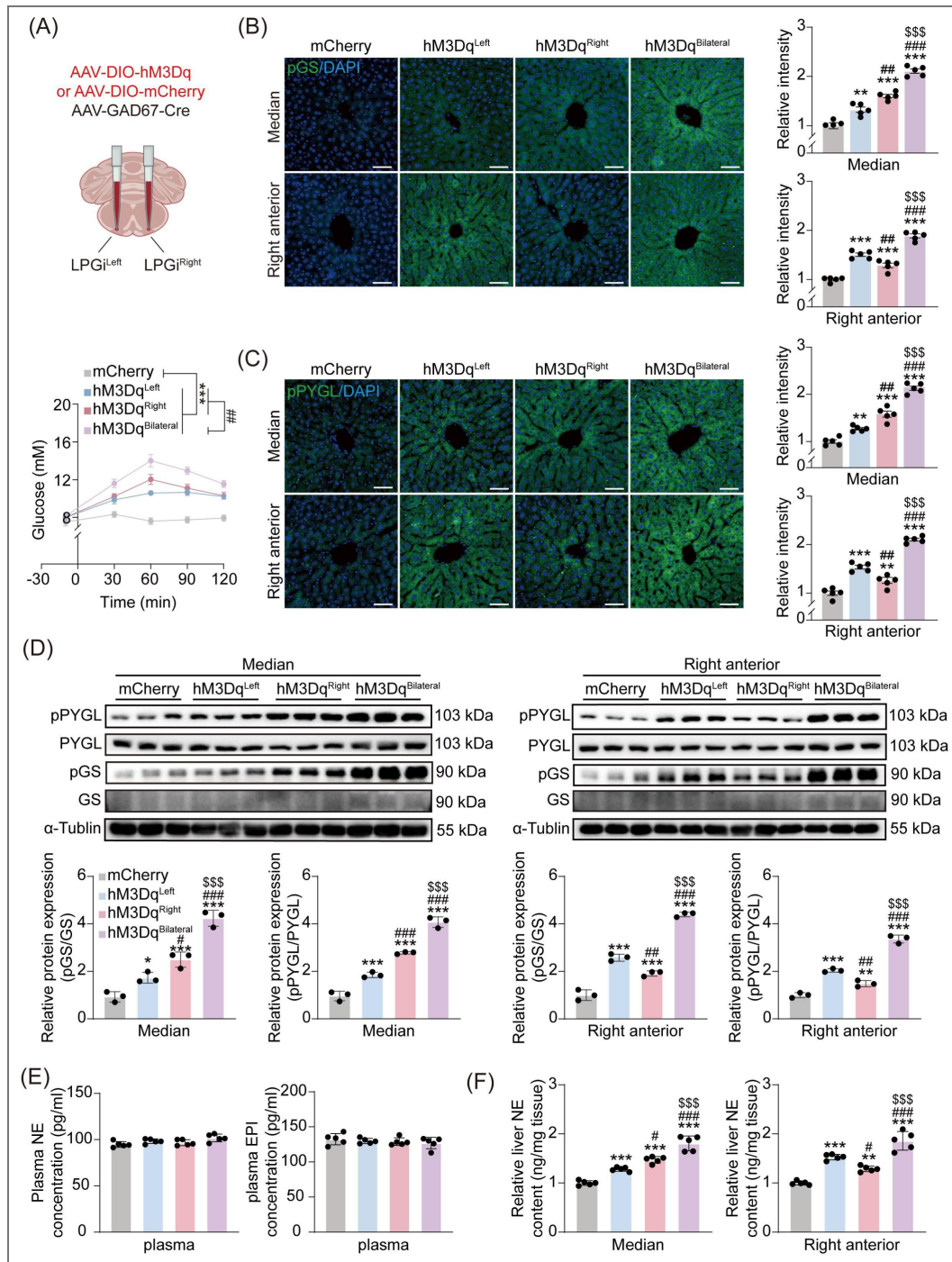


Figure 2. LPGi stimulation raises blood glucose by driving contralateral, lobe-specific hepatic glycogenolysis.

(A) Scheme for injecting AAV-DIO-hM3Dq or AAV-DIO-mCherry (top). Blood glucose levels following chemogenetic activation of the left, right, or bilateral LPGi, measured at -10, 30, 60, 90, and 120 min (bottom, $n = 6$). (B and C) Representative immunofluorescence images of pGS (B) and pPYGL (C) expression in the median (top) and right anterior (bottom) lobes after 1 h chemogenetic activation of left, right, or bilateral LPGi, with quantification of relative fluorescence intensity ($n = 5$). Scale bars, 100 μ m. (D) Western blot analysis of pPYGL, PYGL, pGS, and GS in median (left) and right anterior (right) lobes after 1 h activation, with densitometric quantification (bottom, $n = 3$). (E) Plasma concentrations of norepinephrine (NE) (left, $n = 5$) and epinephrine (EPI) (right, $n = 5$) following 1 h activation. (F) NE contents in median (left, $n = 5$) and right anterior (right, $n = 5$) lobes after 1 h activation. Data are expressed as means $\pm$ SEM, with individual values shown for all experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$; \$\$\$ $p < 0.001$. One-way ANOVA with the Tukey test for (A - F).

To exclude systemic endocrine contributions, particularly via the hypothalamic-pituitary-adrenal (HPA) axis, we measured plasma NE and EPI 1 hour after LPGi activation. Neither hormone changed significantly following unilateral nor bilateral stimulation (Figure 2E). In contrast, intrahepatic NE content rose specifically in contralateral lobes: by 1.49-fold in the right anterior lobe after left LPGi activation, 1.53-fold in the median lobe after right LPGi activation, and over 1.85-fold in both lobes after bilateral activation (Figure 2F). These findings support a mechanism whereby lobe-specific sympathetic activation drives hepatic glucose output in a lateralized manner.

To achieve higher temporal precision, we applied optogenetics by delivering AAV9-GAD67-Cre with AAV9-DIO-ChR2-mCherry viruses to the LPGi (Figure S3A, Figure S4A). Compared with chemogenetics, optogenetic activation elicited greater hyperglycemia, with AUC rising by 32%, 42%, and 62% for left-, right-, and bilateral stimulation, respectively (Figure S3A). Molecular and histological analyses paralleled those from chemogenetics, including contralateral and additive effects on pPYGL and pGS (Figures S3B–S3D), glycogen depletion (Figure S4B), elevated intrahepatic NE content (Figure S3F), and enhanced PEPCCK and G6PC activity (Figures S4C–S4F), without changes in plasma catecholamines (Figure S3E). Taken together, these findings demonstrate that each LPGi hemisphere preferentially drives glucose production in contralateral hepatic lobes, primarily through localized sympathetic activation and glycogenolysis in contralateral hepatic regions.

3. Compensatory neural responses following unilateral hepatic denervation

While compensatory adaptations following unilateral damage are well described in symmetrical organs, it remains unclear whether similar mechanisms operate in asymmetrical organs such as the liver.^(26–28) To test this, we chemically denervated the right- or left-sided lobes using 6-OHDA. Despite the absence of directly sympathetic input to denervated lobes, systemic blood glucose levels were unchanged compared with controls (Figures 3A–3C, Figure S5A), indicating functional compensation through the remaining intact liver. Further analyses revealed increased expression of pPYGL and pGS, as well as elevated intrahepatic NE levels and glycogen depletion in non-denervated hepatic lobes (Figures 3D–3H, Figures S5B–S5E).

To elucidate the underlying mechanism, we quantified c-FOS expression in the LPGi following unilateral hepatic denervation. Right-sided lobes denervation increased c-FOS in the left LPGi, and vice versa (Figure 3I), suggesting that contralateral LPGi upregulates its sympathetic output to intact ipsilateral hepatic lobes, thereby compensating for the loss of innervation in denervated lobes. Together, these findings identify a neuroadaptive mechanism in which the ipsilateral LPGi augments sympathetic output to intact hepatic lobes, thereby maintaining systemic glucose homeostasis despite partial intrahepatic neural disruption.

4. Peripheral decussation of sympathetic hepatic control at the porta hepatis

Contralateral neural circuitry typically arises from central decussations, such as corticospinal tracts that cross at the pyramids for limb motor control^(29,30) and visual pathways that decussate at the optic chiasm for hemispheric processing of visual fields.⁽³¹⁾ Whether the brain's contralateral regulation of the liver occurs centrally or peripherally, however, has remained unknown. Using whole-mount clearing, we reconstructed the brain-liver sympathetic pathway and found that central projections directly descend from the brainstem along the sympathetic chain (SyC) to the CG-SMG, which then sends postganglionic fibers to the liver (Figure 4A). This anatomical arrangement suggested that contralateral control might arise from a peripheral decussation from the CG-SMG to the liver. To test this hypothesis, we transparentized the mouse trunk and observed that sympathetic nerve bundles from the CG-SMG tracked along the hepatic portal vein deep into the liver (Figure 4B). We speculate that these nerve bundles decussate in the hepatic portal region before extending into the individual liver lobes.

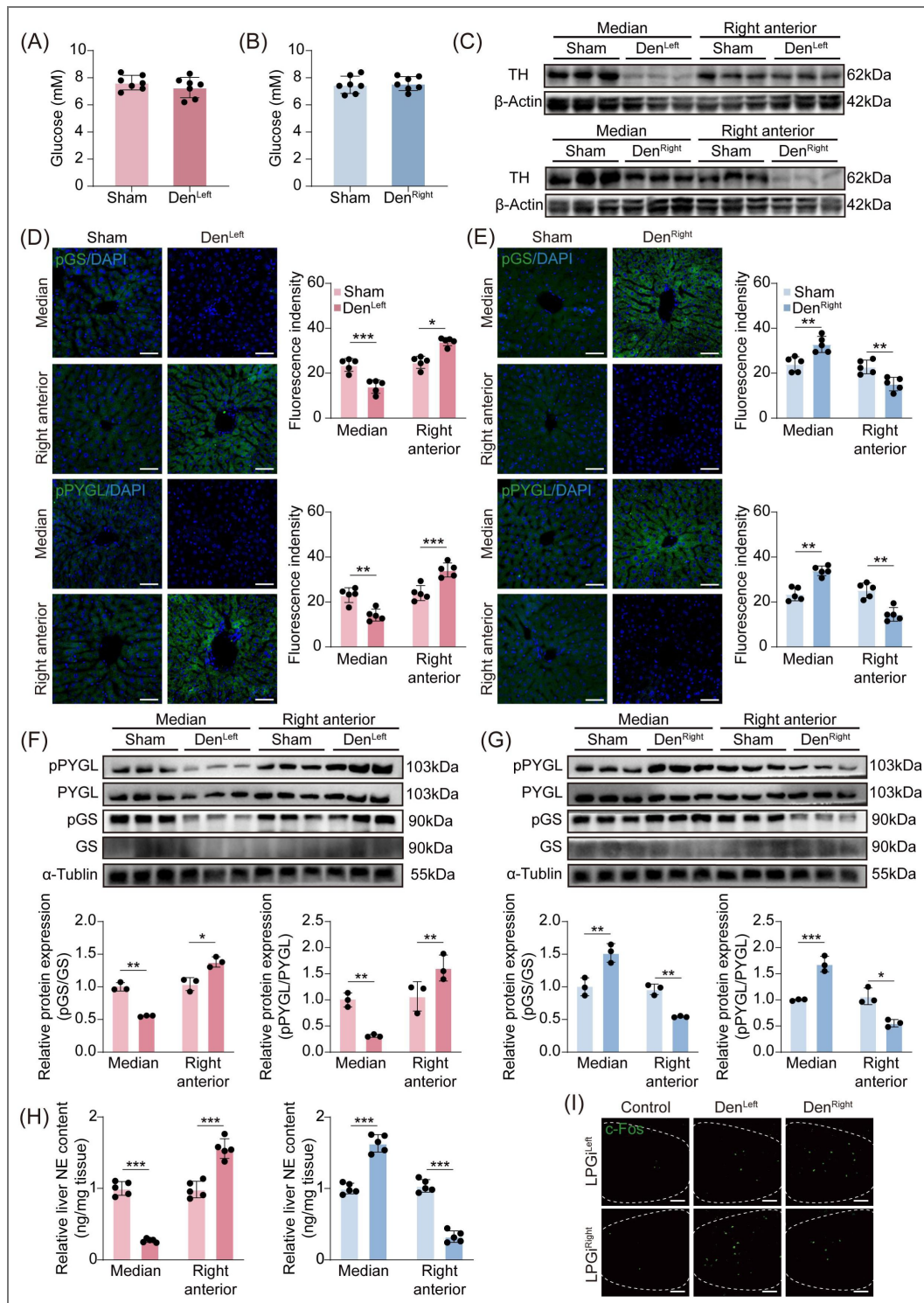


Figure 3. LPGi maintains glucose balance through compensatory use of ipsilateral hepatic lobes under contralateral lobe impairment.

(A and B) Blood glucose levels in the left- (A, $n = 7$) or right-sided (B, $n = 7$) lobes denervated mice. Western blot of TH protein in median and right anterior lobes after denervating left- (top) or right-sided (bottom) lobes. (D and E) Representative immunofluorescence images of pGS (top) and pPYGL (bottom) expression in right anterior and median lobes in left- (D) or right-sided (E) lobes denervated mice, with quantification of relative fluorescence intensity (right, $n = 5$). Scale bars, 100 μ m. (F and G) Western blot analysis of pPYGL, PYGL, pGS, and GS proteins in median and right anterior lobes (top) in left- or right-sided lobes denervated mice, with densitometric quantification (bottom, $n = 3$). (H) NE contents of median and right anterior lobes in left- or right-sided lobes denervated mice ($n = 6$). (I) Representative immunofluorescence images of c-FOS expression in left and right LPGi in left- or right-sided lobes denervated mice. Scale bars, 100 μ m. Data are presented as means $\pm$ SEM, with individual values shown for all experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Unpaired Student's t-test for (A-B, D-H).

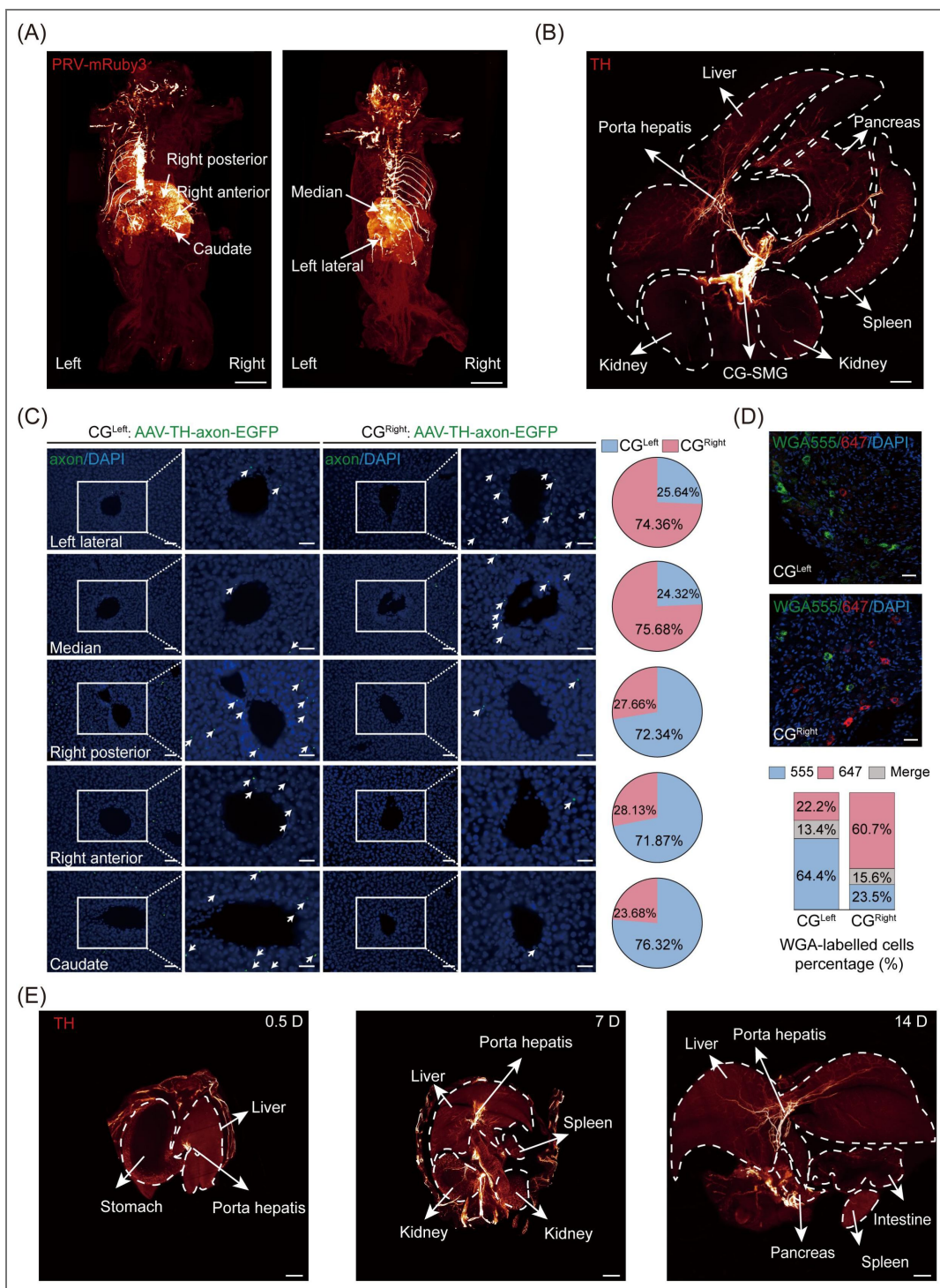


Figure 4. Sympathetic projections to the liver decussate at the porta hepatis, progressively formed during development.

(A) Representative 3D images of mice following PRV injection into right- (left) or left-sided (right) lobes. Scale bars, 3000 μ m. (B) Representative 3D images of CG-SMG projections to peripheral organs. Scale bar, 3,000 μ m. (C) Representative images of sympathetic axons after unilateral CG injection, with quantification of EGFP-labeled axon distribution across hepatic lobes. Scale bars, 100 μ m. (D) Representative images of WGA-labeled neurons in left and right CG, with percentages of WGA555⁺, WGA647⁺, and co-labeled neurons. Scale bars, 100 μ m. (E) Representative images showing the developmental progression of CG-SMG projections to the liver from postnatal week 0 to week 2. Scale bars, 3,000 μ m.

To test this, we performed unilateral anterograde tracing by injecting AAV-TH-DIO-EGFP into a single CG (Figure S5F). This revealed a strictly contralateral innervation pattern: the left CG mainly projected to the right posterior, right anterior, and caudate lobes, whereas the right CG prominently innervated the left lateral and median lobes (Figure 4C). To validate this pattern, we performed dual retrograde tracing by injecting Alexa Fluor 647- and 555-conjugated wheat germ agglutinin (WGA) into left- and right-sided lobes, respectively (Figure S5G). Consistent with the anterograde results, left CG neurons predominantly projected to the right-sided lobes, while right CG neurons targeted the left-sided lobes, confirming robust contralateral preference (Figure 4D). Thus, these findings demonstrate that central inputs descend via the SyC to the CG-SMG complex, where nerve bundles converge and extend along the portal vein, branching within the porta hepatis to innervate individual hepatic lobes.

To explore the development of the intrahepatic neural network in mice, we therefore performed tissue clearing on the torsos of mice from postnatal weeks 0 to 2. We observed that at week 0, the sympathetic nerve bundles were located at the porta hepatis but had not yet extended into the liver (Figure 4E, left). By week 1, the sympathetic nerves had begun to grow into the liver (Figure 4E, middle). It was not until week 2 that the sympathetic nerves fully infiltrated all liver lobes (Figure 4E, right). These findings indicate that, after the first postnatal week, sympathetic nerves grow from the porta hepatis into the liver lobes and form a well-developed sympathetic neural network by the second week.

Discussion

The brain exhibits marked functional asymmetry despite structural bilateral symmetry, a phenomenon well recognized in language, handedness, and spatial cognition.^(32,33) Recent advances extend this concept beyond cortical domains to central autonomic regulation of peripheral organs.^(34,35) Trans-neuronal tracing of symmetrical organs such as the kidneys and thymus has revealed contralateral control with bilateral coordination,^(8,33) whereas the lateralized regulation of asymmetrical organs like the liver, heart, and stomach remains poorly defined and conflicting.^(6,12,37) For instance, parasympathetic cardiac control has been reported to exhibit left-hemispheric dominance,⁽⁶⁾ yet clinical evidence revealed that arrhythmias may arise after strokes in either hemisphere.⁽³⁸⁾ These discrepancies highlight the complexity of lateralized autonomic control and the intrinsic bilaterality within the autonomic network. In this study, we systematically mapped and functionally interrogated the laterality of brain-liver circuitry, focusing on the LPGi. Our anatomical and functional experiments provide compelling evidence that although a subset of LPGi neurons project bilaterally, the predominant organization is contralateral and lobe-specific.

Recent advances in viral tracing and whole-tissue imaging have greatly accelerated our understanding of peripheral neuroanatomy.^(39–41) For instance, distinct gastrointestinal regions are differentially regulated by subdivisions within the CG-SMG,⁽¹⁹⁾ while the kidneys predominantly receive ipsilateral input from sympathetic postganglionic neurons residing in the renal sympathetic ganglia.⁽²⁰⁾ In contrast, components of the immune system, such as the thymus, exhibit lateralized central regulation, with each hemisphere preferentially modulating the contralateral thymic lobe.⁽³⁶⁾ Using PRV-mediated retrograde tracing with whole-mount clearing, we visualized a brain-liver axis originating from the LPGi and revealed a strikingly lateralized projection pattern: left LPGi neurons preferentially innervated right-sided lobes, whereas right LPGi neurons predominantly targeted left-sided lobes. A smaller subset projected bilaterally, suggesting a degree of cooperative regulation across hemispheres. This high-resolution anatomical mapping provides direct evidence for lateralized central projections to an asymmetrical organ, underscoring a previously unrecognized dimension of laterality in autonomic liver innervation.

Functionally, this organization translates into lobe-specific regulation of HGP. Sympathetic innervation of the liver is well established as a key driver of glucose output, with prior electrical stimulation studies demonstrating robust hepatic glucose release in response to sympathetic activation.^(42–44) Consistent with these observations, both chemogenetic and optogenetic activation of LPGi GABAergic neurons significantly elevated systemic glucose levels by promoting

glycogenolysis and gluconeogenesis in contralateral lobes. These metabolic effects were accompanied by localized NE release, increased phosphorylation of key metabolic enzymes (pPYGL and pGS), and glycogen depletion.^(45,46) Notably, bilateral LPGi activation produced additive effects, indicating that both hemispheres can cooperate to augment hepatic metabolism, but in a spatially segregated manner. However, whether such asymmetric input also extends to other hepatic functions, including lipid metabolism, protein synthesis, or detoxification, remains to be determined.⁽¹³⁾

Beyond identifying lateralized control, we also reported a robust compensatory mechanism following unilateral hepatic denervation. When neural input from one hemisphere was attenuated, compensatory neural plasticity emerged through increased sympathetic drive from contralateral LPGi, enhancing gluconeogenesis and glycogenolysis within the remaining innervated hepatic lobes. This response was supported by increased c-FOS expression in contralateral LPGi, higher intrahepatic NE content, and elevated pPYGL and pGS expression in unaffected lobes. Such plasticity parallels compensatory phenomena in other systems, including contralateral enhancement after renal dysfunction and cortical dominance shifts in patients with unilateral hearing loss.^(26,47) Our findings suggest that the brain-liver axis possesses a similar adaptive capacity, enabling preservation of systemic glucose homeostasis and potentially other hepatic functions (e.g., lipid metabolism, bile secretion) despite partial loss of hepatic innervation.

We further show that contralateral regulation of hepatic metabolism arises from a peripheral decussation, challenging the long-held view that such organization arises exclusively from central crossings. Unlike somatic and sensory systems, where contralateral control is mediated by pyramidal or optic decussations in CNS nuclei,^(2,26,27) hepatic sympathetic projections descend ipsilaterally from the brainstem to the CG-SMG before crossing to innervate contralateral lobes. This peripheral arrangement suggests that spatially organized control can be implemented downstream of peripheral ganglia, representing a potential evolutionary adaptation. More broadly, it calls for re-examination of neural laterality paradigms and identifies peripheral ganglia as decision nodes and therapeutic targets for organ-specific neuromodulation.

Despite these advances, several limitations warrant mention. First, the specific neuronal subtypes within the LPGi that mediate contralateral projections remain unidentified. Future research employing genetic labeling strategies and single-cell RNA sequencing will be necessary to characterize their molecular and functional properties. Second, technical constraints prevented simultaneous bilateral viral tracing and whole-mount mouse imaging in the same animal, restricting direct assessment of interhemispheric interactions. Finally, although our murine data provide new insights into lateralized autonomic control of hepatic metabolism, their translational relevance to humans remains to be established. Progress in noninvasive imaging and neural circuit mapping may ultimately enable analogous studies in clinical settings.

In conclusion, this study establishes that the brain exerts lateralized and lobe-specific control over hepatic glucose metabolism, with contralateral regulation arising from a peripheral decussation at the porta hepatis, and a compensatory mechanism maintaining glucose homeostasis after unilateral hepatic injury. Developmentally, sympathetic projections began extending into the liver during the first postnatal week, forming a mature network by week 2 that enables both baseline regulation and adaptive responses. Together, these findings extend the concept of lateralization beyond cognition, motor control, and symmetric organs, with potential implications for targeted neuromodulation strategies in organ-specific diseases.

Methods

Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University and adhered to the principles outlined in the Guide for the Care and Use of Laboratory Animals. Mice were housed under a 12-hour light-dark cycle at 22 °C with

ad libitum access to food and water, unless otherwise specified. The age and number of mice used in each experiment are provided in the figure legends. Littermates of the same sex were randomly assigned to either experimental or control groups.

Viral vectors

For retrograde tracing of LPGi projection to individual hepatic lobes, we injected PRV-CAG-EGFP (BrainVTA, Cat#P03001) and PRV-CAG-mRFP (BrainVTA, Cat#P03002) into distinct hepatic lobes, respectively. For chemogenetic manipulation, we used a combination of AAV9-GAD67-Cre (BrainVTA, Cat#PT-6600) with AAV9-DIO-hM3Dq (BrainVTA, Cat#PT-0019) or AAV9-DIO-mCherry (BrainVTA, Cat#PT-0115). For optogenetic manipulation, we used a combination of AAV9-GAD67-Cre with AAV9-DIO-ChR2-mCherry (BrainVTA, Cat#PT-0002) or AAV9-DIO-mCherry. For anterograde tracing from the CG-SMG to hepatic lobes, we injected AAV9-TH-Cre (BrainVTA, Cat#PT-2947) with AAV9-EF1 α -DIO-Axon-EGFP (BrainVTA, Cat#BC-0197) into the unilateral CG-SMG. All viral vectors were used at titers $>5 \times 10^{12}$ vg/mL.

Brain stereotactic injection

Mice were anesthetized with isoflurane (RWD Life Science Co., Ltd., Cat#68811). For brain injection, the coordinates of LPGi were determined using the Allen Mouse Brain Atlas (AP -6.75 mm, ML ± 0.95 mm, DV -5.85 mm). A total of 200 nL of viral vector was bilaterally delivered at a rate of 50 nL/min.

Intrahepatic sympathetic nerve tracing

After laparotomy, the intestines were gently exteriorized and covered with a sterile saline-moistened gauze to expose the CG-SMG. For anterograde tracing of CG-SMG projections to hepatic lobes, AAV9-TH-Cre and AAV9-EF1 α -DIO-Axon-EGFP were injected into left CG or right CG, and tissues were collected 3 weeks post-injection. For retrograde tracing, 5,000 nL of 1 mg/ml Alexa Fluor 647-conjugated wheat germ agglutinin (WGA 647, Thermo, Cat#W32466) and Alexa Fluor 555-conjugated wheat germ agglutinin (WGA 555, Thermo, Cat#W32464) were injected into the left- and right-sided lobes, respectively, using an infusion rate of 100 nL/min. Tissues were harvested 3 days thereafter.

Intrahepatic sympathetic denervation

For liver injections, we calculated volumes based on the proportion of each lobe relative to the total liver weight. We injected a volume of 3,000 nL of 6-hydroxydopamine (6-OHDA, 12 mg/ml; Sigma, Cat#162957) solution into each lobe at a flow rate of 1,000 nL/min.

Whole-mount clearing and imaging

The liver and trunk staining procedure was performed according to established protocols as previously described.^(14,21) In brief, the liver tissues were fixed in 4% paraformaldehyde (PFA), followed by permeabilization in PBS containing 0.2% Triton X-100, 20% DMSO, and 0.3 M glycine. Immunostaining was performed using the primary antibody against TH (Merck, Cat #AB152, 1:500), followed by incubation with a secondary antibody (Alexa Fluor 568-conjugated donkey anti-rabbit IgG, Thermo Fisher, Cat#A-10042). After staining, tissues were embedded in 1% agarose, dehydrated, and cleared using dibenzyl ether (DBE; Sigma, Cat#422053).

The brain-liver neurocircuit visualization was performed as previously described.⁽²⁵⁾ At 5 days post-injection of PRV, the mice were anesthetized with isoflurane and perfused with PBS. Samples were permeabilized in PBS containing 1.5% goat serum, 0.5% Triton X-100, 0.5 mM methyl- β -cyclodextrin, and 0.2% trans-1-acetyl-4-hydroxy-L-proline. Following permeabilization, samples were stained with mRuby3 nanobody (NanoTag, Cat#N3302-AF647-L, 1:5,000). They were then dehydrated with upgraded tetrahydrofuran (THF; Sigma, Cat#401757), and subsequently incubated in dichloromethane (Sigma, Cat#270997). Finally, samples were cleared by benzyl alcohol and benzyl benzoate mixture (Sigma, Cats #24122 and #W213802).

The cleared samples were imaged using a light-sheet fluorescence microscope (Nuohai, LS18) at 1.25×, 3.2×, and 12.6× magnification. Finally, full-tissue image stacks were reconstructed and analyzed using Imaris ×64 software (version 9.5.0, Bitplane).

Chemogenetic and optogenetic manipulations

For chemogenetic manipulation, we stereotactically injected mice with 200 nL of AAV carrying the Cre-dependent activator DREADD (AAV9-DIO-hM3Dq-mCherry) and allowed them to recover for at least four weeks before conducting tests.

For optogenetic manipulation, mice received stereotaxic injections of 200 nL AAV9-DIO-ChR2-mCherry into the LPGi, followed by implantation of optical fibers (Inper, Cat#D3429). After a four-week recovery, mice were habituated to a fiber-optic patch cord in the home cage for 2 hours before the stimulation. Blue light (465 nm) stimulation, consisting of pulse trains (10 ms pulses of 20 Hz at approximately 10 mW; 1 s on, 1 s off), was delivered.

Biochemical assays

The concentrations of epinephrine (EPI) and norepinephrine (NE) were quantified using the EPI enzyme-linked immunosorbent assay (ELISA) kit (Kexing, Cat#Fankew F2351-A) and NE ELISA kit (Kexing, Cat#Fankew F2533-A). PEPCK activity assay kit (Kexing, Cat#Fankew F2533) and G6PC activity assay kit (Kexing, Cat#Fankew F2736-A) were used to quantify the activity of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6PC).

Western blot analysis

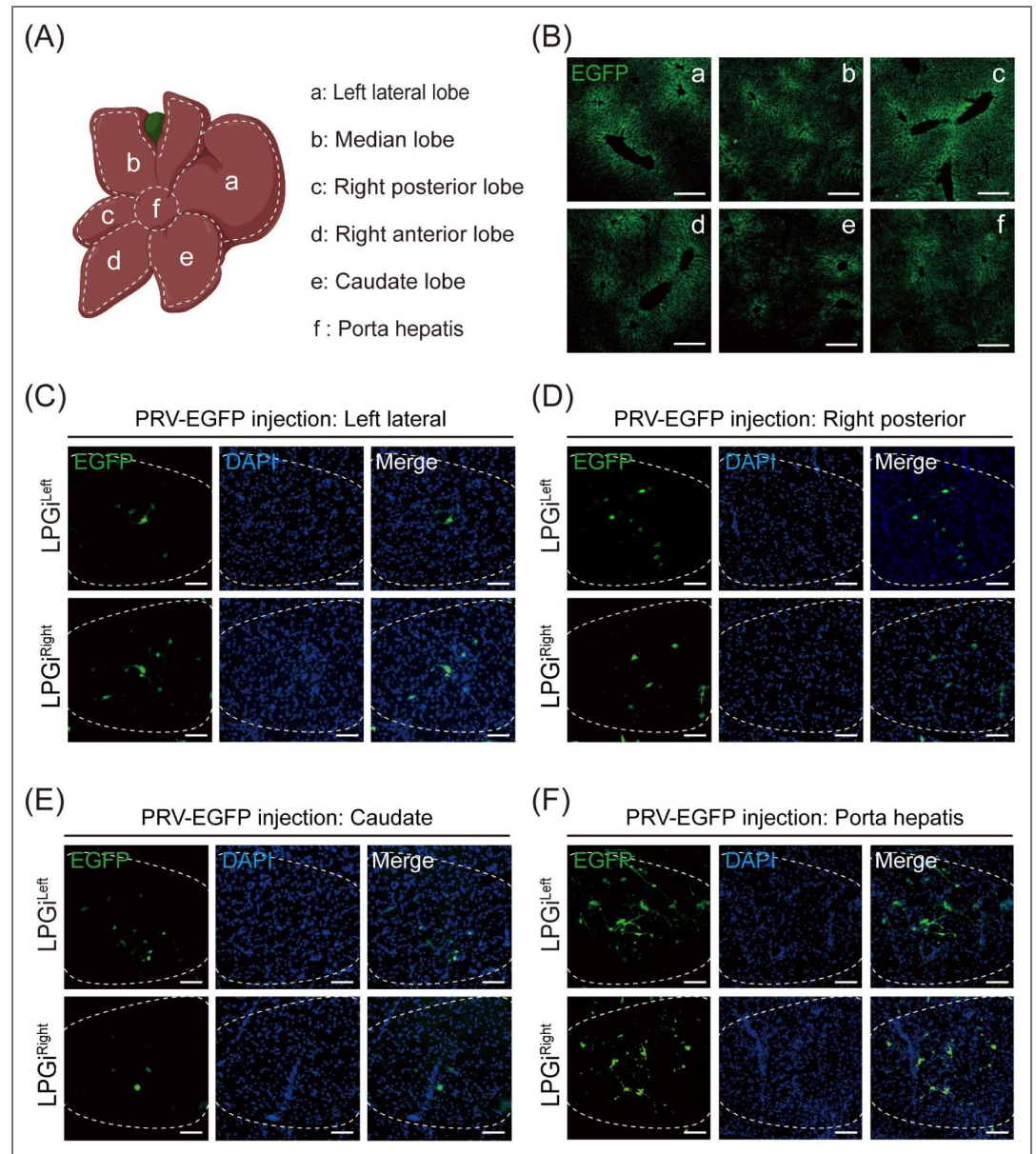
Tissue samples were homogenized in RIPA lysis buffer (Beyotime, Cat#P0013B) with 100 μM protease and phosphate inhibitors (Beyotime, Cat#P1045) using a homogenizer (JieChen Instrument, JCZYM-48R) at 4 °C. The following primary antibodies were used: PEPCK (Santa, Cat#sc-271029, 1:1,000), G6PC (Novus, Cat#NBP1-80533, 1:1,000), pPYGL (Ser15) (Abcam, Cat#ab227043, 1:1,000), PYGL (Proteintech, Cat#15851-1-AP, 1:1,000), pGS (Ser641) (Cell Signaling Technology, Cat#3891S, 1:1,000), GS (Santa, Cat#sc-81173, 1:1,000), TH (Merck, Cat#AB152, 1:1,000), and α-Tubulin (Huabio, Cat#ER130905, 1:10,000), followed by incubation with appropriate secondary antibodies. Imaging of membranes was performed with the iBright Imaging Systems (Thermo, iBright 1500). We quantified the signal intensities by performing densitometric analyses using Fiji (ImageJ, NIH).

Histology and immunofluorescence analyses

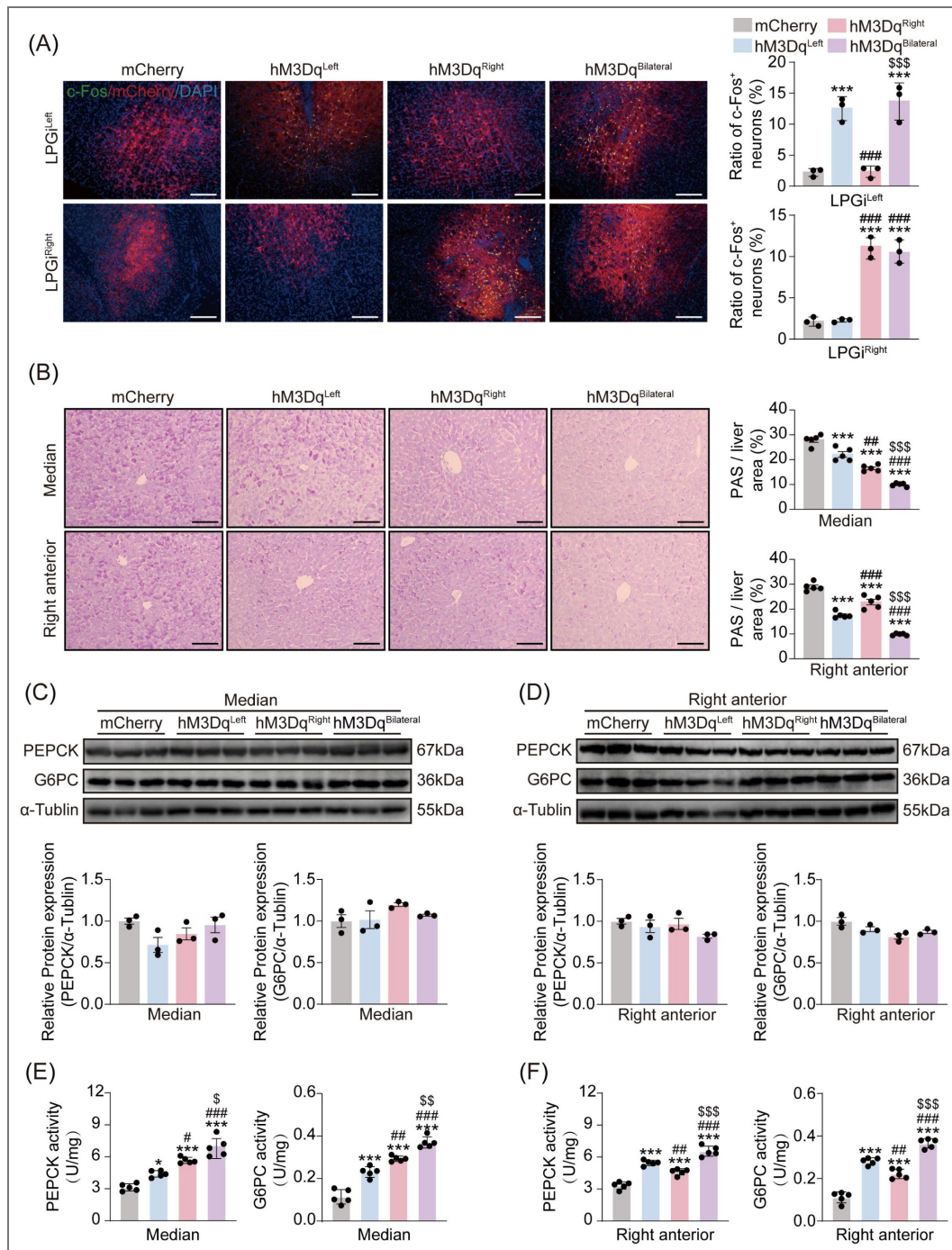
Mouse livers and brains were collected separately and fixed with 4% PFA overnight at 4 °C. For Periodic Acid-Schiff (PAS) staining, 25 μm paraffin sections were prepared and stained with a PAS staining kit (Beyotime, Cat#C0142S). For immunostaining, 25-μm-thick frozen sections were prepared by a freezing microtome (Leica, CM 1950) and stained with primary antibodies, including anti-c-FOS (Synaptic Systems, Cat#226-008, 1:1,000), anti-TH (Merck, Cat#AB152, 1:500), anti-pPYGL (Ser15) (Abcam, Cat#ab227043, 1:100), and anti-pGS (Ser641) (Cell Signaling Technology, Cat#3891S, 1:100), followed by incubation with secondary antibody (Alexa Fluor 488-conjugated goat anti-rabbit IgG, Yeasen, Cat#33116ES60). The sections were imaged using confocal microscopes (Zeiss, LSM 700). Images were quantified with ImageJ (v1.8.0).

Quantification and statistical analysis

An unpaired two-tailed Student's t-test was used to compare differences between two groups. Comparisons across multiple groups were conducted using one-way ANOVA with the Tukey test. All results, including graphs, were expressed as means ± SEM unless otherwise specified. Significance was defined as $p < 0.05$, with significance annotations of * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$; \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$. Statistical analyses were conducted using software such as GraphPad Prism (v.10.0) and ImageJ (v1.8.0).

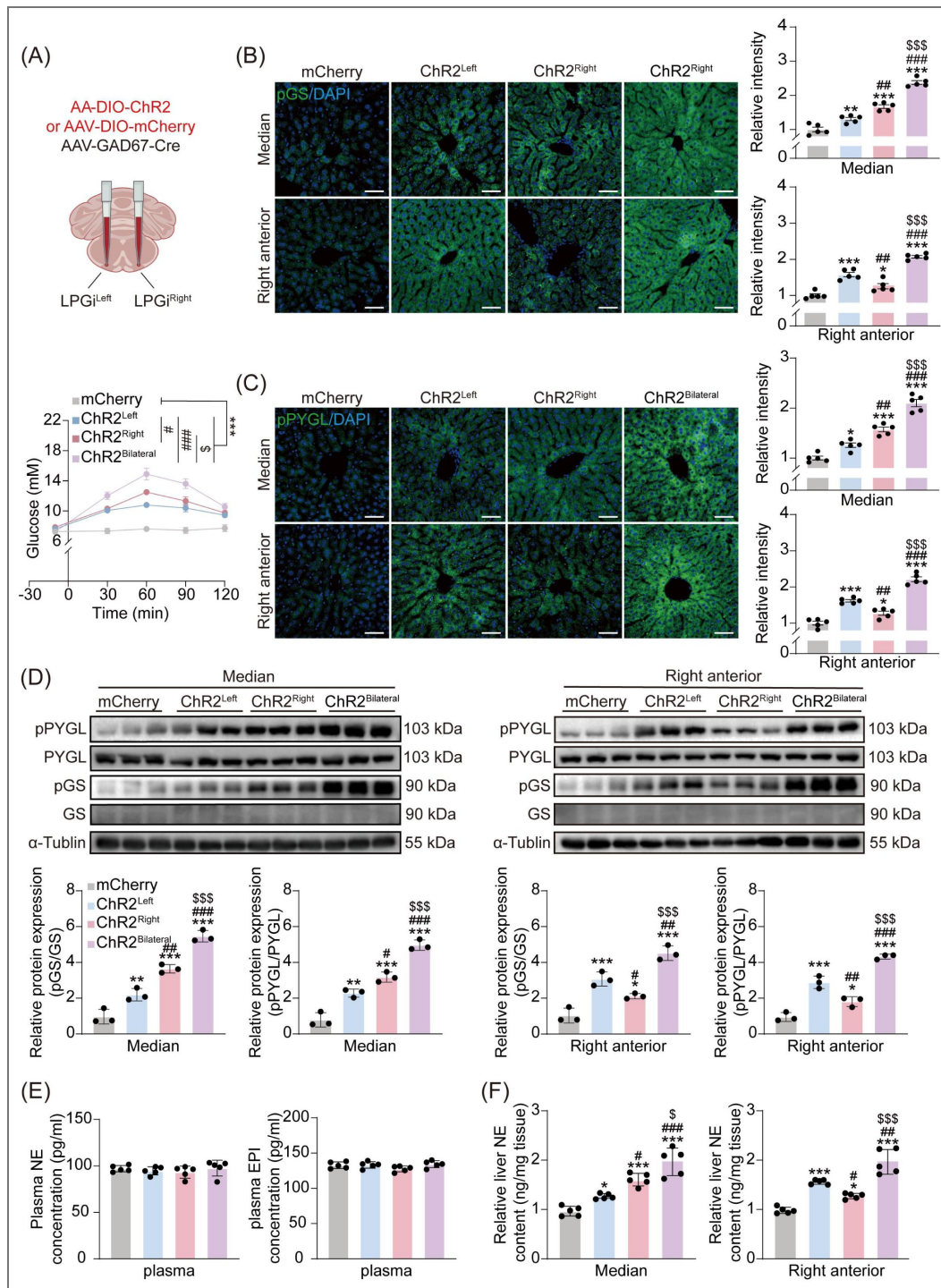


Supplementary figure 1. Supplementary evidence for contralateral projections from the brain to the liver, related to Figure 1. (A) Schematic illustration of hepatic lobes with anatomical labeling. (B) Representative images of hepatic lobes after PRV-EGFP injections. Scale bars, 150 μ m. (C-F) Representative immunofluorescence images of PRV-labeled neurons (EGFP) in the left (top) and right (bottom) LPGi following PRV injections into left lateral lobe (C), right posterior lobe (D), caudate lobe (E), and porta hepatis (F). Scale bars, 100 μ m.



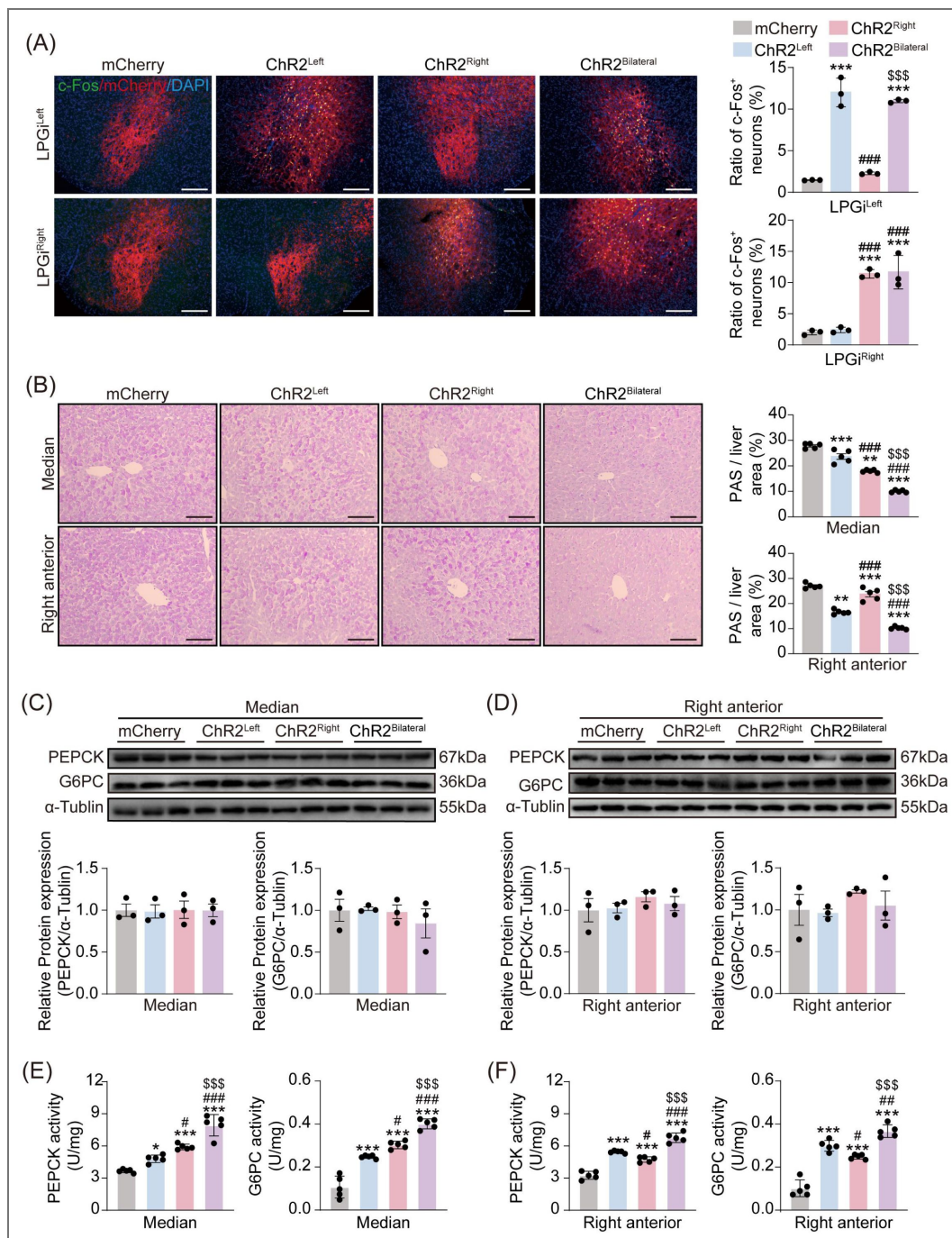
Supplementary figure 2. Chemogenetic activation of LPGi induces c-Fos expression, promotes hepatic glycogenolysis, and activates glycogenolytic enzymes, related to Figure 2.

(A) Representative immunofluorescence images of c-Fos⁺ LPGi neurons in hM3Dq mice following 1 h stimulation (Left), with quantification of c-Fos⁺ neuron percentage (right, $n = 3$). Scale bars, 100 μ m. (B) Representative PAS staining images of median (top) and right anterior (bottom) lobes in hM3Dq mice following 1 h stimulation, with semi-quantitative analysis of glycogen levels (right, $n = 5$). Scale bars, 100 μ m. (C and D) Western blot analysis of PEPCK and G6PC proteins in the median (C, top) and right anterior (D, top) lobes, with densitometric quantified (bottom, $n = 3$). (E and F) Enzyme activity of PEPCK and G6PC in the median (E) and right anterior (F) lobes ($n = 3$). Data are expressed as means $\pm$ SEM, with individual values shown for all experiments. * $p < 0.05$, *** $p < 0.001$; ## $p < 0.01$, ### $p < 0.001$; \$\$\$ $p < 0.001$. A one-way ANOVA with the Tukey test was used for (A-F).



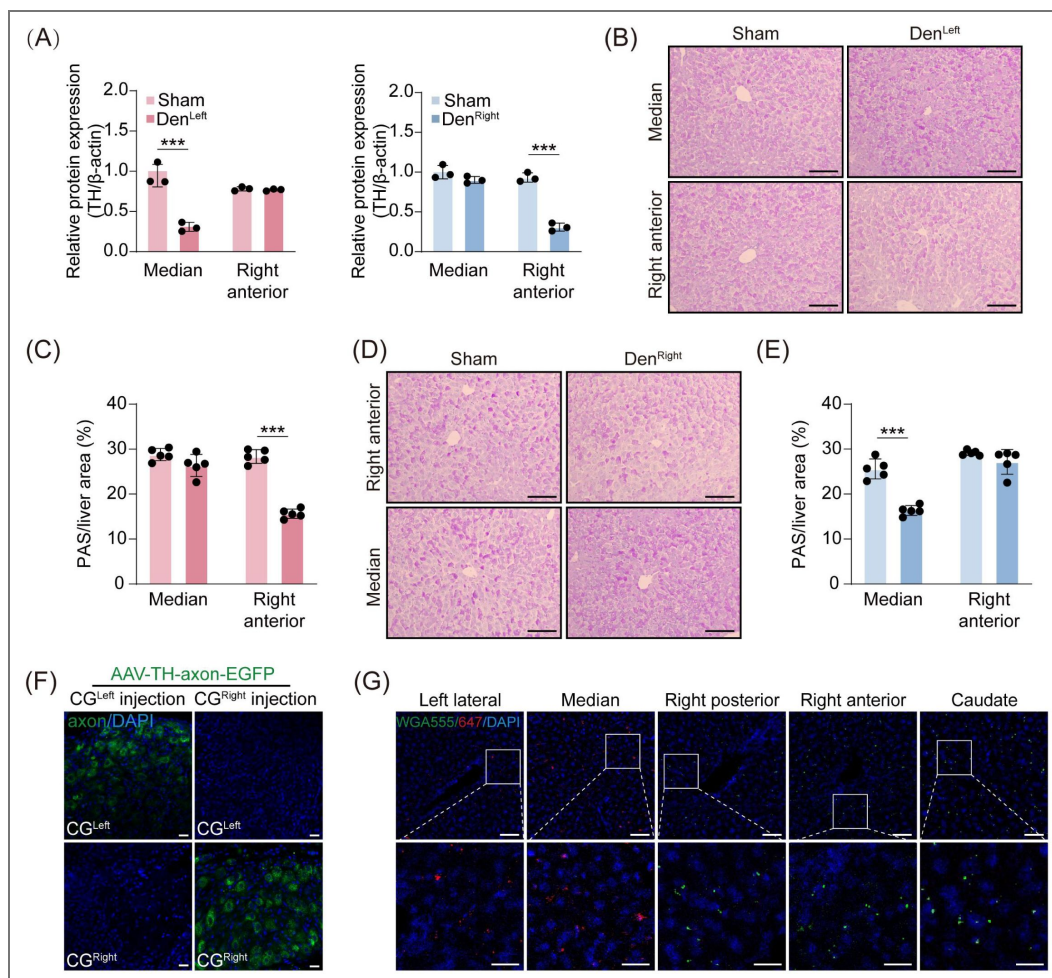
Supplementary figure 3. Optogenetic activation of LPGi confirms contralateral lobe-specific mobilization observed in Figure 2.

(A) Scheme for injecting AAV-DIO-ChR2 or AAV-DIO-mCherry (top). Blood glucose levels following optogenetic activation of left, right, or bilateral LPGi, measured at -10, 30, 60, 90, and 120 min (bottom, $n = 6$) (B and C) Representative immunofluorescence images of pGS (B) and pPYGL (C) expression in median (top) and right anterior (bottom) lobes after 1 h optogenetic activation of left, right, or bilateral LPGi, with quantification of relative fluorescence intensity ($n = 5$). Scale bars, 100 μm. (C) Western blot analysis of pPYGL, PYGL, pGS, and GS in median (left) and right anterior (right) lobes after 1 h activation, with densitometric quantification (bottom, $n = 3$). (D) Plasma concentrations of norepinephrine (NE) (left, $n = 5$) and epinephrine (EPI) (right, $n = 5$) following 1 h optogenetic activation. (E) NE contents in median (left, $n = 5$) and right anterior (right, $n = 5$) lobes after 1 h optogenetic activation. Data are expressed as means $\pm$ SEM, with individual values shown for all experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; $\#p < 0.05$, $##p < 0.01$, $###p < 0.001$; $\$p < 0.05$, $$$$p < 0.001$. A one-way ANOVA with the Tukey test was used for (A-F).



Supplementary figure 4. Optogenetic activation of LPGi recapitulates c-Fos expression, hepatic glycogenolysis, and activity of glycogenolysis-related enzymes observed in Figure S2.

(A) Representative immunofluorescence images of c-FOS⁺ neurons in LPGi after 1 h optogenetic stimulation, with quantification of ratio of c-FOS⁺ neurons (right, $n = 3$). Scale bars, 100 μ m. (B) Representative PAS staining images of the median (top) and right anterior lobes (bottom) lobes following 1 h optogenetic stimulation, with semi-quantitative analysis of glycogen levels ($n = 5$). Scale bars, 100 μ m. (C and D) Western blot analysis of PEPCK and G6PC proteins in median (C) and right anterior (D) lobes, with densitometric quantification ($n = 3$). (E and F) Enzyme activity of PEPCK and G6PC in median (E) and right anterior lobes (F) ($n = 3$). Data are expressed as means $\pm$ SEM, with individual values shown for all experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$; \$\$\$ $p < 0.001$. A one-way ANOVA with the Tukey test was used for (A-F).



Supplementary figure 5. Validation of sympathetic nerve ablation, enhanced glycogenolysis in non-denervated hepatic lobes, and accurate targeting of AAV and WGA injections, related to Figure 3 and 4.

(A) Densitometric quantification of TH protein in median and right anterior lobes after denervating left- (left) or right-sided (right) lobes. (B-E) Representative PAS staining images of median (top) and right anterior (bottom) lobes in left- (B) and right-sided (D) lobes denervated mice, with semi-quantitative analysis of glycogen levels (C and E, $n = 5$). Scale bars, 100 μ m. (A) Represent slices of the CG in unilateral AAV-TH-axon-EGFP injected mice. Scale bars, 20 μ m. (B) Represent slices of hepatic lobes after injecting WGA 647 and WGA 555 into left- and right-sided lobes. Scale bars, 50 μ m. Data are expressed as means $\pm$ SEM, with individual values shown for all experiments. *** $p < 0.001$; Unpaired Student's t-test for (A, C, and E).

Data availability

All data generated or analyzed during this study are included in the manuscript and supporting files. Source data files have been provided for all figures.

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Additional information

Author contribution

H.X., H.H., and X.Z. conceived, designed, and supervised the project. Z.W., M.R., Y.C., and Y.L. performed glucose level monitoring and data analysis. X.G., K.W., Y.C., and L.J. performed chemogenetic and optogenetic experiments. X.S., S.W., and J.W. performed whole-mount immunolabeling. H.X. and H.H. wrote the manuscript with input from H.W. and X.C. All authors validated and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University and adhered to the principles outlined in the Guide for the Care and Use of Laboratory Animals.

Abbreviations

AUC: area under the curve
 CG: celiac ganglion
 CNS: central nervous system
 ChR2: channelrhodopsin-2
 ELISA: enzyme-linked immunosorbent assay;
 EPI: epinephrine
 G6PC: glucose-6-phosphatase
 HPA: hypothalamic-pituitary-adrenal
 LPGi: lateral paragigantocellular nucleus
 NE: norepinephrine
 6-OHDA: 6-hydroxydopamine
 PAS: periodic acid-Schiff
 PEPCK: phosphoenolpyruvate carboxykinase
 PRV: pseudorabies virus
 GS: glycogen synthase
 PYGL: glycogen phosphorylase
 WGA: wheat germ agglutinin.

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Peer reviews

Reviewer #1 (Public review):

Summary:

This manuscript by Wang et al. reports the potential involvement of an asymmetric neurocircuit in the sympathetic control of liver glucose metabolism.

Strengths:

The concept that the contralateral brain-liver neurocircuit preferentially regulates each liver lobe may be interesting.

Weaknesses:

However, the experimental evidence presented did not support the study's central conclusion.

(1) Pseudorabies virus (PRV) tracing experiment:

The liver not only possesses sympathetic innervations but also vagal sensory innervations. The experimental setup failed to distinguish whether the PRV-labeling of LPGi (Lateral Paraganglionic Nucleus) is derived from sympathetic or vagal sensory inputs to the liver.

(2) Impact on pancreas:

The celiac ganglia not only provide sympathetic innervations to the liver but also to the pancreas, the central endocrine organ for glucose metabolism. The chemogenetic manipulation of LPGi failed to consider a direct impact on the secretion of insulin and glucagon from the pancreas.

(3) Neuroanatomy of the brain-liver neurocircuit:

The current study and its conclusion are based on a speculative brain-liver sympathetic circuit without the necessary anatomical information downstream of LPGi.

(4) Local manipulation of the celiac ganglia:

The left and right ganglia of mice are not separate from each other but rather anatomically connected. The claim that the local injection of AAV in the left or right ganglion without affecting the other side is against this basic anatomical feature.

<https://doi.org/10.7554/eLife.109599.1.sa4>

Reviewer #2 (Public review):

Summary:

The manuscript by Wang and colleagues aims to determine whether the left and right LPGi differentially regulate hepatic glucose metabolism and to reveal decussation of hepatic sympathetic nerves.

The authors used tissue clearing to identify sympathetic fibers in the liver lobes, then injected PRV into the hepatic lobes. Five days post-injection, PRV-labeled neurons in the LPGi were identified. The results indicated contralateral dominance of premotor neurons and partial innervation of more than one lobe. Then the authors activated each side of the LPGi, resulting in a greater increase in blood glucose levels after right-sided activation than after left-sided

activation, as well as changes in protein expression in the liver lobes. These data suggested modulation of HGP (hepatic glucose production) in a lobe-specific manner. Chemical denervation of a particular lobe did not affect glucose levels due to compensation by the other lobes. In addition, nerve bundles decussate in the hepatic portal region.

Strengths:

The manuscript is timely and relevant. It is important to understand the sympathetic regulation of the liver and the contribution of each lobe to hepatic glucose production. The authors use state-of-the-art methodology.

Weaknesses:

(1) The wording/terminology used in the manuscript is misleading, and it is not used in the proper context. For instance, the goal of the study is "to investigate whether cerebral hemispheres differentially regulate hepatic glucose metabolism..." (see abstract); however, the authors focus on the brainstem (a single structure without hemispheres). Similarly, symmetric is not the best word for the projections.

(2) Sparse labeling of liver-related neurons was shown in the LPGi (Figure 1). It would be ideal to have lower magnification images to show the area. Higher quality images would be necessary, as it is difficult to identify brainstem areas. The low number of labeled neurons in the LPGi after five days of inoculation is surprising. Previous findings showed extensive labeling in the ventral brainstem at four days post-inoculation (Desmoulins et al., 2025). Unfortunately, it is not possible to compare the injection paradigm/methods because the PRV inoculation is missing from the methods section. If the PRV is different from the previously published viral tracers, time-dependent studies to determine the order of neurons and the time course of infection would be necessary.

(3) Not all LPGi cells are liver-related. Was the entire LPGi population stimulated, or was it done in a cell-type-specific manner? What was the strain, sex, and age of the mice? What was the rationale for using the particular viral constructs?

(4) The authors should consider the effect of stimulation of double-labeled neurons (innervating more than one lobe) and potential confounding effects regarding other physiological functions.

(5) The authors state that "central projections directly descend along the sympathetic chain to the celiac-superior mesenteric ganglia". What they mean is unclear. Do the authors refer to pre-ganglionic neurons or premotor neurons? How does it fit with the previous literature?

(6) How was the chemical denervation completed for the individual lobes?

(7) The Western Blot images look like they are from different blots, but there are no details provided regarding protein amount (loading) or housekeeping. What was the reason to switch beta-actin and alpha-tubulin? In Figures 3F -G, the GS expression is not a good representative image. Were chemiluminescence or fluorescence antibodies used? Were the membranes reused?

(8) Key references using PRV for liver innervation studies are missing (Stanley et al, 2010 [PMID: 20351287]; Torres et al., 2021 [PMID: 34231420]; Desmoulins et al., 2025 [PMID: 39647176]).

<https://doi.org/10.7554/eLife.109599.1.sa3>

Reviewer #3 (Public review):

Summary:

This study found a lobe-specific, lateralized control of hepatic glucose metabolism by the brain and provides anatomical evidence for sympathetic crossover at the porta hepatis. The findings are particularly insightful to the researchers in the field of liver metabolism, regeneration, and tumors.

Strengths:

Increasing evidence suggests spatial heterogeneity of the liver across many aspects of metabolism and regenerative capacity. The current study has provided interesting findings: neuronal innervation of the liver also shows anatomical differences across lobes. The findings could be particularly useful for understanding liver pathophysiology and treatment, such as metabolic interventions or transplantation.

Weaknesses:

Inclusion of detailed method and Discussion:

- (1) The quantitative results of PRV-labeled neurons are presented, and please include the specific quantitative methods.
- (2) The Discussion can be expanded to include potential biological advantages of this complex lateralized innervation pattern.

<https://doi.org/10.7554/eLife.109599.1.sa2>

Reviewer #4 (Public review):

Summary:

The studies here are highly informative in terms of anatomical tracing and sympathetic nerve function in the liver related to glucose levels, but given that they are performed in a single species, it is challenging to translated them to humans, or to determine whether these neural circuits are evolutionarily conserved. Dual-labeling anatomical studies are elegant, and the addition of chemogenetic and optogenetic studies is mechanistically informative. Denervation studies lack appropriate controls, and the role of sensory innervation in the liver is overlooked.

Specific Weaknesses - Major:

- (1) The species name should be included in the title.
- (2) Tyrosine hydroxylase was used to mark sympathetic fibers in the liver, but this marker also hits a portion of sensory fibers that need to be ruled out in whole-mount imaging data
- (3) Chemogenetic and optogenetic data demonstrating hyperglycemia should be described in the context of prior work demonstrating liver nerve involvement in these processes. There is only a brief mention in the Discussion currently, but comparing methods and observations would be helpful.
- (4) Sympathetic denervation with 6-OHDA can drive compensatory increases to tissue sensory innervation, and this should be measured in the liver denervation studies to implicate potential crosstalk, especially given the increase in LPGi cFOS that may be due to afferent nerve activity. Compensatory sympathetic drive may not be the only culprit, though it is clearly assumed to be. The sensory or parasympathetic/vagal innervation of the liver is altogether ignored in this paper and could be better described in general.

<https://doi.org/10.7554/eLife.109599.1.sa1>

Author response:

Public Reviews:

Reviewer #1 (Public review):

Summary:

This manuscript by Wang et al. reports the potential involvement of an asymmetric neurocircuit in the sympathetic control of liver glucose metabolism.

Strengths:

The concept that the contralateral brain-liver neurocircuit preferentially regulates each liver lobe may be interesting.

Weaknesses:

However, the experimental evidence presented did not support the study's central conclusion.

We sincerely thank the reviewer for recognizing the conceptual novelty of our work and for constructive comments aimed at enhancing its rigor and clarity. In response, we will carry out targeted experiments to address the points raised, including: (i) further characterization of LPGi projections to vagal and sympathetic circuits; (ii) evaluation of potential pancreatic involvement; and (ii) validation of the specificity of chemogenetic activation within the proposed circuit. We anticipate completing the revised version within 8 weeks.

(1) Pseudorabies virus (PRV) tracing experiment:

The liver not only possesses sympathetic innervations but also vagal sensory innervations. The experimental setup failed to distinguish whether the PRV-labeling of LPGi (Lateral Paragigantocellular Nucleus) is derived from sympathetic or vagal sensory inputs to the liver.

Thank you for raising this important point. We fully agree that the liver receives both sympathetic and vagal sensory innervation, and we acknowledge that PRV-based tracing alone does not definitively distinguish between these two pathways. This represents a limitation of the original experimental design.

Based on established anatomical literature as well as our experimental observations, vagal sensory neuron cell bodies reside in the nodose ganglion (NG), and their central projections terminate predominantly in the nucleus of the solitary tract (NTS) (Nature. 2023;623(7986):387-396; Curr Biol. 2020;30(20):3986-3998.e5.), which is located in the dorsomedial medulla. In contrast, the LPGi, together with other sympathetic-related nuclei, is predominantly distributed in the ventral medulla (Cell Metab. 2025;37(11):2264-2279.e10; Nat Commun. 2022;13(1):5079.).

To directly assess the contribution of vagal sensory pathways, we will perform an additional PRV tracing experiment using two groups of mice: one with bilateral nodose ganglion (NG) removal and a sham-operated control group. Identical PRV injections will be delivered to the liver in both groups, and PRV labeling in the LPGi will be quantitatively compared. Preservation of LPGi labeling following NG ablation would indicate that PRV transmission occurs primarily via sympathetic, rather than vagal sensory, pathways. These data will be incorporated into the revised manuscript and are expected to be completed within 3 weeks.

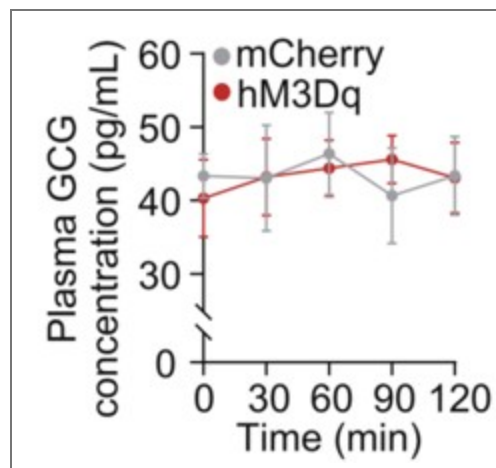
(2) Impact on pancreas:

The celiac ganglia not only provide sympathetic innervations to the liver but also to the pancreas, the central endocrine organ for glucose metabolism. The chemogenetic manipulation of LPGi failed to consider a direct impact on the secretion of insulin and glucagon from the pancreas.

Thank you for this important comment. We agree that the celiac ganglia (CG) provide sympathetic innervation not only to the liver but also to the pancreas, which plays a central role in glucose homeostasis through the secretion of both insulin and glucagon. Therefore, the potential pancreatic implications associated with LPGi chemogenetic manipulation worth careful consideration.

To address this concern, we examined circulating glucagon levels following chemogenetic manipulation of the LPGi. As shown in the Supplementary Figure below, plasma glucagon (GCG) concentrations were not significantly altered at 30, 60, 90, or 120 minutes compared with control mice (n = 6), indicating that LPGi manipulation does not measurably affect glucagon secretion under our experimental conditions.

We acknowledge that insulin secretion was not assessed in the study, which represents an important limitation given the pancreatic innervation of the CG. To further strengthen our interpretation, we are performing additional experiments in newly prepared mice to measure circulating insulin levels following LPGi manipulation. These data together with Author response image 1 below will be included in the revised manuscript upon completion.



Author response image 1. Plasma concentrations of GCG in mice following LPGi GABAergic neurons activation.

(3) Neuroanatomy of the brain-liver neurocircuit:

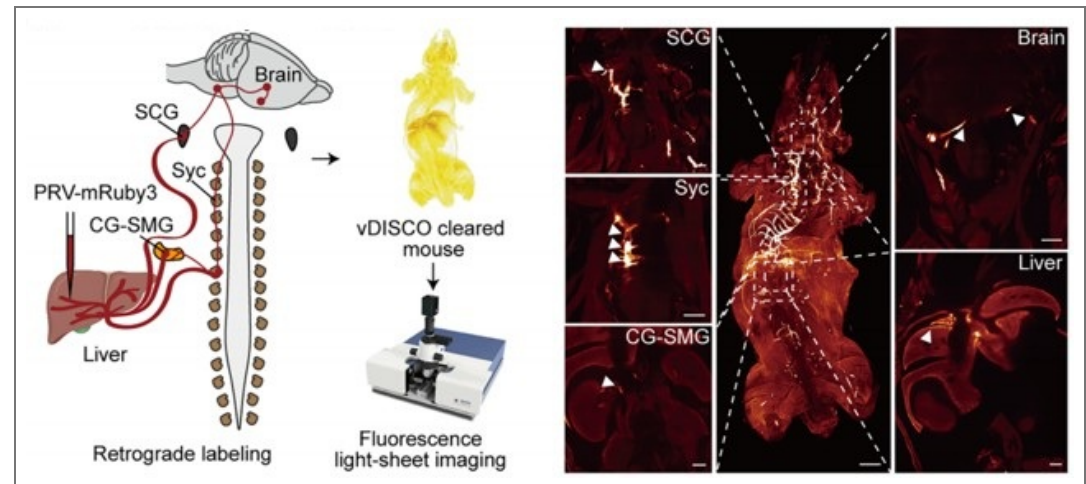
The current study and its conclusion are based on a speculative brain-liver sympathetic circuit without the necessary anatomical information downstream of LPGi.

Thank you for raising this important point. A clear anatomical definition of the downstream pathways linking the brain to the liver is essential for interpreting the proposed brain-liver sympathetic circuit.

However, the present study (Figure 4A) provides direct anatomical evidence supporting the organization of the brain-liver sympathetic neurocircuit. These observations are consistent with our recent detailed characterization of the brain-liver sympathetic circuit published in Cell Metabolism (Cell Metab. 2025;37(11):2264–2279), LPGi GABAergic neurons inhibit GABAergic neurons in the caudal ventrolateral medulla (CVLM). Disinhibition of CVLM

reduces GABAergic suppression of rostral ventrolateral medulla (RVLM) neurons, which are key excitatory drivers of sympathetic tone. RVLM neurons project to sympathetic preganglionic neurons in the sympathetic chain (Syc). These neurons synapse with postganglionic sympathetic neurons in ganglia such as the celiac-superior mesenteric ganglion (CG-SMG). Postganglionic sympathetic fibers then innervate the liver, releasing NE to activate hepatic β_2 -adrenergic receptors and stimulate HGP.

Together, these data establish a coherent anatomical basis for the proposed brain-liver sympathetic pathway and clarify the downstream organization relevant to the functional experiments presented here.



Author response image 2. Tracing scheme (Left) and whole-mount imaging (Right) of PRV-labeled brain-liver neurocircuit. Scale bars, 3,000 (whole mount) or 1,000 (optical sections) μm .

(4) Local manipulation of the celiac ganglia:

The left and right ganglia of mice are not separate from each other but rather anatomically connected. The claim that the local injection of AAV in the left or right ganglion without affecting the other side is against this basic anatomical feature.

Thank you for raising this important anatomical point. We fully acknowledge that the left and right celiac ganglia (CG) in mice are interconnected, and that unilateral viral injection could theoretically affect the contralateral side. The celiac-superior mesenteric ganglion (CG-SMG) complex serves as a major sympathetic hub that regulates visceral organ functions. Recent transcriptomic, anatomical, and functional studies have revealed that the CG-SMG is not a homogeneous structure but is composed of molecularly and functionally distinct neuronal populations. These populations exhibit specialized projection patterns and regulate different aspects of gastrointestinal physiology, supporting a model of modular sympathetic control. (Nature. 2025 Jan;637(8047):895-902). Therefore, we were aware of this phenomenon during the initial stages of these experiments.

To minimize unintended spread to the contralateral CG, we took two complementary approaches.

First, we optimized the injection strategy by using an extremely small injection volume (100 nL per site), with a very slow infusion rate (50 nL/min), and fine glass micropipettes. With these refinements, contralateral viral spread was rarely observed.

Second, and importantly, all animals included in the final analyses were subjected to post hoc anatomical verification. After completion of the experiments, CG were collected, sectioned,

and examined for viral expression. As shown in Supplementary Figure 5F, only mice in which viral expression was strictly confined to the targeted CG, with no detectable infection in the contralateral ganglion, were included in the presented data.

Together, these measures ensure that the reported effects are attributable to local manipulation of the intended CG. We will ensure that the Methods section more explicitly details these technical precautions and that the legend for Figure S5F clearly states its role in validating injection specificity.

Reviewer #2 (Public review):

Summary:

The manuscript by Wang and colleagues aims to determine whether the left and right LPGi differentially regulate hepatic glucose metabolism and to reveal decussation of hepatic sympathetic nerves.

The authors used tissue clearing to identify sympathetic fibers in the liver lobes, then injected PRV into the hepatic lobes. Five days post-injection, PRV-labeled neurons in the LPGi were identified. The results indicated contralateral dominance of premotor neurons and partial innervation of more than one lobe. Then the authors activated each side of the LPGi, resulting in a greater increase in blood glucose levels after right-sided activation than after left-sided activation, as well as changes in protein expression in the liver lobes. These data suggested modulation of HGP (hepatic glucose production) in a lobe-specific manner. Chemical denervation of a particular lobe did not affect glucose levels due to compensation by the other lobes. In addition, nerve bundles decussate in the hepatic portal region.

We thank the reviewer for the thorough and constructive evaluation of our manuscript. In direct response, we will undertake comprehensive revisions to enhance the rigor and clarity of the study, including: (i) correcting ambiguous or misleading terminology pertaining to anatomical resolution and sympathetic circuit organization; (ii) expanding the Methods section with complete experimental details, improved image presentation, and explicit justification of our viral and genetic approaches; and (iii) strengthening data interpretation by addressing issues related to sparse PRV labeling, projection heterogeneity, and the functional implications of double-labeled neurons. All revisions are expected to be completed within 8 weeks.

Strengths:

The manuscript is timely and relevant. It is important to understand the sympathetic regulation of the liver and the contribution of each lobe to hepatic glucose production. The authors use state-of-the-art methodology.

Weaknesses:

(1) The wording/terminology used in the manuscript is misleading, and it is not used in the proper context. For instance, the goal of the study is "to investigate whether cerebral hemispheres differentially regulate hepatic glucose metabolism..." (see abstract); however, the authors focus on the brainstem (a single structure without hemispheres). Similarly, symmetric is not the best word for the projections.

We thank the reviewer for raising these critical points regarding terminology and conceptual framing. We acknowledge that certain phrases in our original manuscript may have been overly broad or ambiguous, particularly in describing the scope of sympathetic heterogeneity and the specificity of neural projections. Due to practical constraints and the scope of our study, our investigation is focused on the brainstem, which represents the final common

pathway for these lateralized commands. We acknowledge that terms referring to the cerebral hemispheres do not accurately describe our study.

We are revising the manuscript to ensure accurate and consistent terminology and will submit the revised version with these corrections.

(2) Sparse labeling of liver-related neurons was shown in the LPGi (Figure 1). It would be ideal to have lower magnification images to show the area. Higher quality images would be necessary, as it is difficult to identify brainstem areas. The low number of labeled neurons in the LPGi after five days of inoculation is surprising. Previous findings showed extensive labeling in the ventral brainstem at four days post-inoculation (Desmoulins et al., 2025). Unfortunately, it is not possible to compare the injection paradigm/methods because the PRV inoculation is missing from the methods section. If the PRV is different from the previously published viral tracers, time-dependent studies to determine the order of neurons and the time course of infection would be necessary.

We sincerely thank the reviewer for these detailed and constructive comments regarding the PRV tracing experiments. We fully agree that careful presentation and interpretation of the anatomical data are essential for ensuring rigor and transparency. We address each point in detail below.

(1) Image magnification and anatomical context of LPGi labeling

We agree that the original images did not sufficiently convey the broader anatomical context of the LPGi. In the revised manuscript, we will replace the original panels in Figure 1 with new images that include lower-magnification overviews of the brainstem, alongside higher-magnification views of the LPGi. These images clearly delineate the LPGi with respect to established anatomical landmarks and atlas boundaries. Image contrast and resolution will also be optimized to allow unambiguous identification of PRV-labeled neurons and surrounding structures.

(2) Sparse LPGi labeling at 5 days post-injection and methodological details

We apologize for the omission of the detailed PRV injection protocol in the original Methods section. We deliberately used small-volume, focal injections (1 μ L per liver lobe) to minimize viral spread and to restrict labeling to circuits specifically connected to the targeted hepatic region. Under these conditions, early-stage or intermediate-order upstream nuclei such as the LPGi are expected to exhibit relatively sparse labeling compared to more proximal autonomic nuclei. This information will add, including the PRV strain, viral titer, injection volume, precise injection coordinates, and surgical procedures.

(3) Not all LPGi cells are liver-related. Was the entire LPGi population stimulated, or was it done in a cell-type-specific manner? What was the strain, sex, and age of the mice? What was the rationale for using the particular viral constructs?

We thank the reviewer for this insightful and important question. We agree that not all neurons within the LPGi are liver-related, and we apologize that our rationale was not clearly articulated in the original manuscript.

(1) Our decision to target GABAergic neurons in the LPGi using Gad1-Cre mice was based on prior experimental evidence rather than an assumption about the entire LPGi population. In our previous study (Cell Metab. 2025;37(11):2264-2279.e10), we performed single-cell RNA sequencing on retrogradely labeled LPGi neurons following liver tracing. These analyses revealed that the majority of liver-projecting LPGi neurons are GABAergic in nature. Based on these findings, we chose to selectively manipulate GABAergic neurons in the LPGi rather than the entire LPGi neuronal population, in order to achieve greater cellular specificity and to minimize potential confounding effects arising from heterogeneous neuron types within

this region. We regret that this rationale was not clearly described in the original submission and have now revised the manuscript to explicitly state this reasoning.

(2) In addition, we apologize for the omission of mouse strain, sex, and age information in the Methods section. These details will be fully added.

(3) We selected AAV-based viral vectors, specifically the AAV9 serotype, due to their well-established efficiency in transducing neurons in the brainstem, relatively low toxicity, and widespread use in circuit-level chemogenetic and optogenetic studies. When combined with Cre-dependent viral constructs in Gad1-Cre mice, this approach enabled selective and reliable manipulation of LPGi GABAergic neurons.

(4) The authors should consider the effect of stimulation of double-labeled neurons (innervating more than one lobe) and potential confounding effects regarding other physiological functions.

We thank the reviewer for raising this important point. We agree that neurons innervating more than one liver lobe could, in principle, introduce potential confounding effects and may reflect higher-order integrative autonomic neurons.

This consideration is consistent with a key finding of the cited study: the celiac-superior mesenteric ganglion (CG-SMG) contains molecularly distinct sympathetic neuron populations (e.g., RXFP1⁺ vs. SHOX2⁺) that exhibit complementary organ projections and separate, non-overlapping functions. Specifically, RXFP1⁺ neurons innervate secretory organs (pancreas, bile duct) to regulate secretion, while SHOX2⁺ neurons innervate the gastrointestinal tract to control motility. This functional segregation supports the concept of specialized autonomic modules rather than a uniform, “fight or flight” response, reinforcing the need for careful interpretation of circuit-specific manipulations. (Nature. 2025;637(8047):895-902; Neuron. Published online December 10, 2025).

In our PRV tracing experiments, the proportion of double-labeled neurons was relatively small, suggesting that the majority of labeled LPGi neurons preferentially associate with individual hepatic lobes. Nevertheless, we recognize that activation of this minority population could contribute to broader physiological effects beyond strictly lobe-specific regulation. We acknowledge that the absence of single-cell-level resolution in the current study limits our ability to further dissect the functional heterogeneity of these projection-defined neurons, and we will explicitly state this as a limitation in the revised manuscript. We will explicitly acknowledge this possibility in the revised manuscript and included it as a limitation of the current study. We thank the reviewer for highlighting this important conceptual consideration.

(5) The authors state that "central projections directly descend along the sympathetic chain to the celiac-superior mesenteric ganglia". What they mean is unclear. Do the authors refer to pre-ganglionic neurons or premotor neurons? How does it fit with the previous literature?

We thank the reviewer for pointing out this imprecise wording. We agree that the original phrasing was anatomically inaccurate and potentially confusing. The pathways we intended to describe involve brainstem premotor neurons that project to sympathetic preganglionic neurons in the spinal cord. These preganglionic neurons then innervate neurons in the celiac-superior mesenteric ganglia, which in turn provide postganglionic input to the liver.

We are revising the manuscript to clearly distinguish premotor from preganglionic neurons and to describe this pathway in a manner consistent with the established organization of sympathetic autonomic circuits reported in the previous literature. The revised wording will explicitly reflect this hierarchical relay structure.

(6) *How was the chemical denervation completed for the individual lobes?*

We thank the reviewer for raising this important methodological concern. We agree that potential diffusion of 6-OHDA is a critical issue when performing lobe-specific chemical denervation, and we apologize that our original description did not sufficiently clarify how this was controlled.

In the revised Methods section, we will provide a detailed description of the denervation procedure, including the injection volume and concentration of 6-OHDA, as well as the physical separation and isolation of individual hepatic lobes during application to minimize diffusion to adjacent tissue.

To directly assess the specificity of the chemical denervation, we included immunofluorescence and Western blot analyses demonstrating a selective reduction of sympathetic markers in the targeted lobe, with minimal effects on non-targeted lobes. These results support the effectiveness and relative spatial confinement of the 6-OHDA treatment under our experimental conditions.

We thank the reviewer for highlighting this point, which has helped us improve both the clarity and rigor of the manuscript.

(7) *The Western Blot images look like they are from different blots, but there are no details provided regarding protein amount (loading) or housekeeping. What was the reason to switch beta-actin and alpha-tubulin? In Figures 3F-G, the GS expression is not a good representative image. Were chemiluminescence or fluorescence antibodies used? Were the membranes reused?*

We thank the reviewer for this careful and detailed evaluation of the Western blot data. We apologize that insufficient methodological detail was provided in the original submission.

(1) We would like to clarify that the protein bands shown within each panel were derived from the same membrane. To improve transparency, we will provide full, uncropped images of the corresponding membranes in the supplementary materials. In addition, detailed information regarding protein loading amounts, gel conditions, and housekeeping controls will be added to the Methods section.

(2) The use of different loading controls (β -actin or α -tubulin) reflects a technical consideration rather than an experimental inconsistency. In our experiments, the molecular weight of the TH (62kDa) was too close to α -tubulin (55kDa), and β -actin (42kDa) was therefore used to avoid band overlap and to ensure accurate quantification.

(3) Regarding the GS signal shown in Figures 3F–G, we agree that the original representative image was suboptimal. This appears to be related to antibody performance rather than sample quality. To address this, we are repeating the GS Western blot using a newly validated antibody. The original tissue samples had been aliquoted and stored at -80°C , allowing reliable re-analysis. This work will be done in 8 weeks.

(4) All Western blot experiments were detected using chemiluminescence, and membrane stripping and reprobing procedures are now explicitly described in the Methods section.

We thank the reviewer for highlighting these issues, which significantly improve the rigor and clarity of our data presentation.

(8) *Key references using PRV for liver innervation studies are missing (Stanley et al., 2010 [PMID: 20351287]; Torres et al., 2021 [PMID: 34231420]; Desmoulins et al., 2025 [PMID: 39647176]).*

We thank the reviewer for pointing out these important and highly relevant references that were inadvertently omitted in our initial submission. The studies by Stanley et al. (Proc Natl Acad Sci U S A, 2010), Torres et al. (Am J Physiol Regul Integr Comp Physiol, 2021), and Desmoulins et al. (Auton Neurosci, 2025) represent key PRV-based retrograde tracing work that has mapped central neural circuits innervating the liver and thus provide essential context for our anatomical analyses.

We agree that inclusion of these studies is necessary to properly situate our findings within the existing literature. Accordingly, we will incorporate citations to these references in the revised manuscript and discuss their relationship to our results.

Reviewer #3 (Public review):

Summary:

This study found a lobe-specific, lateralized control of hepatic glucose metabolism by the brain and provides anatomical evidence for sympathetic crossover at the porta hepatis. The findings are particularly insightful to the researchers in the field of liver metabolism, regeneration, and tumors.

Strengths:

Increasing evidence suggests spatial heterogeneity of the liver across many aspects of metabolism and regenerative capacity. The current study has provided interesting findings: neuronal innervation of the liver also shows anatomical differences across lobes. The findings could be particularly useful for understanding liver pathophysiology and treatment, such as metabolic interventions or transplantation.

Weaknesses:

Inclusion of detailed method and Discussion:

We sincerely thank the reviewer for the positive and constructive feedback, which will significantly enhance both the methodological rigor and the broader biological interpretation of our study. In direct response, we will revise the Discussion to elaborate on the potential physiological advantages of a lateralized and lobe-specific pattern of liver innervation. Furthermore, we will expand the Methods section to include a comprehensive description of the quantitative analysis applied to PRV-labeled neurons. Together, these revisions will strengthen the manuscript's clarity, depth, and relevance to researchers in hepatic metabolism, regeneration, and disease. We expect to complete all updates within 8 weeks.

(1) The quantitative results of PRV-labeled neurons are presented, and please include the specific quantitative methods.

We thank the reviewer for this helpful suggestion. We will add a detailed description of the quantitative methods used to analyze PRV-labeled neurons in the revised Methods section. This includes information on the counting criteria, the brain regions analyzed, how the regions of interest were delineated, and the normalization procedures applied to obtain the reported neuron counts.

(2) The Discussion can be expanded to include potential biological advantages of this complex lateralized innervation pattern.

We appreciate the reviewer's suggestion. We will expand the Discussion to include a paragraph addressing the potential biological significance of lateralized liver innervation. We highlight that this asymmetric organization could allow for more precise, lobe-specific regulation of hepatic metabolism, enable integration of distinct physiological signals, and

potentially provide robustness against perturbations. These points will discuss in the revised manuscript.

Reviewer #4 (Public review):

Summary:

The studies here are highly informative in terms of anatomical tracing and sympathetic nerve function in the liver related to glucose levels, but given that they are performed in a single species, it is challenging to translated them to humans, or to determine whether these neural circuits are evolutionarily conserved. Dual-labeling anatomical studies are elegant, and the addition of chemogenetic and optogenetic studies is mechanistically informative. Denervation studies lack appropriate controls, and the role of sensory innervation in the liver is overlooked.

We sincerely appreciate the reviewer's thoughtful evaluation and fully agree that findings derived from a single-species model must be interpreted with caution in relation to human physiology. In direct response, we will revise the manuscript to explicitly clarify that all experimental data were obtained in mice and to provide a discussion of the limitations regarding direct extrapolation to humans. Concurrently, we will expand the Discussion section by integrating our findings with recent human and translational studies, including a multicenter clinical trial demonstrating that catheter-based endovascular denervation of the celiac and hepatic arteries significantly improved glycemic control in patients with poorly controlled type 2 diabetes, without major adverse events (Signal Transduct Target Ther. 2025;10(1):371). While our current work focuses on defining the anatomical organization and functional asymmetry of this circuit in mice, the clinical findings suggest that the core principles, sympathetic control of hepatic glucose metabolism via CG-liver pathways, may be conserved and of translational relevance. Additionally, we will clarify the interpretation of tyrosine hydroxylase labeling and expand the discussion of hepatic sensory and parasympathetic innervation, acknowledging their important roles in liver–brain communication and identifying them as key directions for future research. Collectively, these revisions will provide a more balanced, clinically informed, and rigorous framework for interpreting our findings, and we aim to complete all updates within 8 weeks.

Specific Weaknesses - Major:

(1) The species name should be included in the title.

We thank the reviewer for this suggestion. We agree that the species should be clearly indicated. The findings presented in this study were obtained in mice using tissue clearing and whole-organ imaging approaches. Due to technical limitations, these observations are currently limited to the mouse strain. We will update the title and clarified the species used throughout the manuscript.

(2) Tyrosine hydroxylase was used to mark sympathetic fibers in the liver, but this marker also hits a portion of sensory fibers that need to be ruled out in whole-mount imaging data

We thank the reviewer for pointing this out. We acknowledge that tyrosine hydroxylase (TH) labels not only sympathetic fibers but also a subset of sensory fibers. We will add a limitation of this point in the revised manuscript. In addition, ongoing experiments using retrograde PRV labeling from the liver, combined with sectioning, are being used to distinguish sympathetic fibers from vagal and dorsal root ganglion–derived sensory fibers. These data will be included in a forthcoming update of the manuscript and are expected to be completed in approximately 6 weeks.

(3) Chemogenetic and optogenetic data demonstrating hyperglycemia should be described in the context of prior work demonstrating liver nerve involvement in these processes. There is only a brief mention in the Discussion currently, but comparing methods and observations would be helpful.

We thank the reviewer for this suggestion. Previous studies largely relied on electrical stimulation to modulate liver innervation, which provides relatively coarse control of neural activity (Eur J Biochem. 1992;207(2):399-411). By contrast, our use of chemogenetic and optogenetic approaches allows selective, cell-type-specific manipulation of LPGi neurons. We will revise the Discussion to place our functional data in the context of prior work, highlighting how these more precise approaches improve understanding of the contribution of liver-innervating neurons to hyperglycemia.

(4) Sympathetic denervation with 6-OHDA can drive compensatory increases to tissue sensory innervation, and this should be measured in the liver denervation studies to implicate potential crosstalk, especially given the increase in LPGi cFOS that may be due to afferent nerve activity. Compensatory sympathetic drive may not be the only culprit, though it is clearly assumed to be. The sensory or parasympathetic/vagal innervation of the liver is altogether ignored in this paper and could be better described in general.

We thank the reviewer for this insightful comment and agree that chemical sympathetic denervation with 6-OHDA may induce compensatory changes in non-sympathetic hepatic inputs, including sensory and parasympathetic (vagal) innervation. As the reviewer correctly points out, increased LPGi cFOS activity may reflect afferent nerve engagement rather than solely compensatory sympathetic drive.

More broadly, we agree that the central nervous system functions as an integrated homeostatic network that continuously processes diverse afferent signals, including hepatic sensory and vagal inputs, as well as other interoceptive cues. From this perspective, the LPGi cFOS changes observed in our study likely represent one component of a complex integrative response rather than evidence for a single dominant pathway.

We acknowledge that the present study did not directly assess hepatic sensory or parasympathetic innervation, which represents a limitation in scope. In the revised manuscript, we will expand the Discussion to explicitly note this limitation and provide a more balanced consideration of potential crosstalk among sympathetic, sensory, and parasympathetic pathways in shaping LPGi activity following hepatic denervation.

Recommendations for the authors:

Reviewer #2 (Recommendations for the authors):

Although the findings are interesting, this reviewer has major concerns about the experimental design, methodology, results, and interpretation of the data. Experimental details are lacking, including basic information (age, sex, strain of mice, procedures, magnification, etc.).

We thank the reviewer for this important recommendation. We agree that comprehensive reporting of experimental details is essential for rigor and reproducibility.

In the revised manuscript, we will add complete information regarding mouse strain, sex, age, and sample size for each experiment. In addition, detailed descriptions of surgical procedures, viral constructs, injection parameters, imaging magnification, and analysis methods have been incorporated into the Methods section.

These revisions ensure that all experiments are described with sufficient technical detail and clarity to allow accurate interpretation and replication of our findings.

Reviewer #3 (Recommendations for the authors):

Addressing a few questions might help:

(1) The study found that liver-associated LPGi neurons are predominantly GABAergic. It would be informative to molecularly characterize the PRV-traced, liver-projecting LPGi neurons to determine their neurochemical phenotypes.

We thank the reviewer for this insightful suggestion. We agree that molecular characterization of liver-projecting LPGi neurons is important for understanding their functional identity.

This issue has been addressed in detail in our recent study (Cell Metab. 2025;37(11):2264-2279.e10), in which we performed single-cell RNA sequencing on retrogradely traced LPGi neurons connected to the liver. These analyses demonstrated that the majority of liver-projecting LPGi neurons are GABAergic, with a defined transcriptional profile distinct from neighboring non-liver-related populations.

Based on these findings, the current study selectively targets GABAergic LPGi neurons using Gad1-Cre mice. We are now explicitly referencing and summarizing these molecular results in the revised manuscript to clarify the neurochemical identity of the PRV-traced LPGi neurons.

(2) Is it possible to do a local microinjection of a sodium channel blocker (e.g., lidocaine) or an adrenergic receptor antagonist into the porta hepatis? That would potentially provide additional evidence for the porta hepatis as the functional crossover point.

We appreciate the reviewer's thoughtful suggestion. While pharmacological blockade at the porta hepatis could modulate local neural activity, the proposed approach may not fully capture the distinction between ipsilateral and contralateral inputs, and may not conclusively establish neural crossover at this particular site.

In our view, the anatomical evidence provided by whole-mount tissue clearing, dual-labeled tracing, and direct visualization of decussating nerve bundles at the porta hepatis offers a more definitive demonstration of sympathetic crossover. Pharmacological blockade would affect both crossed and uncrossed fibers simultaneously and therefore would not specifically resolve the anatomical organization of this decussation.

Nevertheless, we agree that functional interrogation of the porta hepatis represents an interesting direction for future work, and we will now acknowledge this possibility in the Discussion.

(3) It is possible to investigate the effects of unilateral LPGi manipulation or ablation of one side of CG/SMG on liver metabolism, such as hyperglycemia?

We thank the reviewer for this important suggestion. We agree that unilateral ablation or silencing of the CG-SMG could provide additional insight into lateralized sympathetic control of liver metabolism.

However, precise and selective ablation of one side of the CG-SMG through 6-OHDA without affecting the contralateral ganglion or adjacent autonomic structures remains technically challenging, particularly given the anatomical connectivity between the two sides. We are currently optimizing approaches to achieve reliable unilateral manipulation.

If successful within the revision timeframe, we will include these experiments and corresponding metabolic analyses in the revised manuscript. If not, we will explicitly discuss

this experimental limitation and the predicted metabolic consequences of unilateral CG-SMG ablation as an important direction for future studies. This work will be done in 6 weeks.

Reviewer #4 (Recommendations for the authors):

In the abstract and elsewhere, the use of the term 'sympathetic release' is unclear - do you mean release of nerve products, such as the neurotransmitter norepinephrine? This should be more clearly defined.

We thank the reviewer for pointing out this ambiguity. We agree that the term “sympathetic release” was imprecise. In the revised manuscript, we will explicitly refer to the release of sympathetic neurotransmitters, primarily norepinephrine, from postganglionic sympathetic fibers.

We will revise the wording throughout the manuscript to ensure accurate and consistent terminology and to avoid potential confusion regarding the underlying neurobiological mechanisms.

<https://doi.org/10.7554/eLife.109599.1.sa0>