Optogenetic activation of liver-innervating vagal sensory neurons increases anxiety-like behavior in mice.

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Running title: Role of the liver-brain axis in anxiety-like behavior

Abstract

The liver plays a central role in energy balance, glucose homeostasis, and lipid metabolism through neural and humoral pathways. Intriguingly, impaired hepatic lipid metabolism has been also associated with an increased risk of anxiety and depression in rodents and humans. However, the mechanisms by which it affects mood behaviors via neural pathways remain poorly understood. This study investigated whether activation of the liver-brain axis can modulate anxiety-like behavior in mice. Advillin (Avil)^{CreERT2}; channelrhodopsin-tdTomato mice and wireless optogenetics were used to selectively stimulate Avil-positive vagal sensory neurons that innervate the liver in freely moving mice. Acute optogenetic stimulation of their nerves in the liver activated neurons in the nodose ganglia and the dorsal motor nucleus of the vagus, and to a lesser extent, those in the nucleus of the solitary tract (NTS). Behavioral assessments revealed that acute optogenetic stimulation of these liver-innervating vagal sensory nerves increased anxiety-like behavior in male and female mice during open field, elevated plus maze, and light/dark box tests. Retrograde viral tracing revealed that neurons in the NTS sent projections to the locus coeruleus (LC), and optogenetic stimulation of liver-innervating vagal sensory nerves resulted in significant activation of norepinephrine-expressing neurons in the LC. Therefore, these findings reveal a novel liver–NTS–LC circuit that plays a role in the regulation of anxiety-like behavior through vagal sensory neurons. Unlike the traditional top-down neuronal circuits associated with the liver, this newly identified liver-brain axis is essential for regulating not only systemic energy homeostasis but also emotional behaviors.

Keywords: interoception, vagal, liver, mood, obesity, hepatic steatosis

Introduction

The brain continuously receives and responds to dynamic feedback from afferent (sensory) visceral signals, which influences its functions [1]. This process is known as interoception and involves bidirectional signal processing between the brain and internal organs to reflect the internal state of an organism [2]. Vagal sensory neurons in the vagus nerve ganglia innervate most visceral organs, including liver, lung, heart, and gut [3-6]. Interestingly, the influence of these interoceptive pathways, especially the gut-brain axis, on mental health has been the subject of extensive research, as the gut microbiome plays a significant role in neurodegenerative diseases, cognitive function, and emotion [7-9]. Additionally, our recent study has revealed that vagal sensory neurons that innervate the liver are also implicated in regulating mood behavior, particularly anxiety-like behavior, in mice fed a high-fat diet (HFD)[3]. Given that impaired lipid metabolism in the liver significantly affects psychiatric conditions in humans [10, 11], liver-innervating vagal sensory neurons that detect changes in hepatic interoceptive signals might contribute to the onset of mental disorders.

The liver is the largest metabolic organ that responds to the energy needs and surfeits. Namely, the liver plays a crucial role in the production and storage of energy in the form of lipids and carbohydrates. Metabolic interactions between the liver and other visceral organs, such as the gut, pancreas, and adipose tissue, are critical for maintaining the energy balance. Particularly, the hepatic portal vein transports blood rich in nutrients and hormones from the gastrointestinal tract and pancreas [12]. Our recent findings with anterograde and retrograde viral tracing indicate that Avil-positive vagal sensory neurons that innervate the liver send their nerve terminals predominantly to the portal vein [3]. This may suggest that these particular sensory neurons are capable of detecting and responding to interoceptive signal molecules, subsequently conveying this sensory information to the dorsal motor complex (DVC), which includes the area postrema (AP), nucleus tractus solitarius (NTS), and dorsal motor nucleus of the vagus (DMV). Importantly, all these DVC nuclei are also innervated by liver-innervating Avil-positive vagal sensory neurons [3]. The caudal NTS is a primary site that integrates visceral sensory information, which then relays to several other brain regions, including the amygdala, paraventricular hypothalamus, dorsal raphe, and locus coeruleus (LC) [13].

The LC contains norepinephrine (NE)-expressing neurons, and optogenetic activation of these NE neurons enhances anxiety-like behavior in mice [14]. Both acute and chronic stimulation of the vagus nerve increase the activity of neurons in the LC in rodents and humans [15-18]. The improvement in anxiety-like behavior observed in mice lacking liver-innervating vagal sensory neurons in mice fed HFD [3] might be attributed to alterations in the liver-NTS-LC pathway via liver-innervating vagal sensory neurons. To gain better insight into how vagal sensory neurons that innervate the liver affect anxiety-like behavior, we expressed channelrhodopsins in Avil-positive sensory neurons and optogenetically

stimulated their nerve fibers within the liver with a wireless implantable LED device in freely moving animals. Animal behavioral assessments revealed that optogenetic activation of the liver-innervating vagal sensory nerves enhanced anxiety-like behavior compared to the control group. Importantly, this anxiogenic effect was attributed, in part, to the activation of NE-expressing neurons in the LC.

Materials and methods

Animals

All mouse care and experimental procedures were approved by the Institutional Animal Care Research Advisory Committee of Albert Einstein College of Medicine.

Eight-nine-week-old Avil^{CreERT2} (stock # 032027) and Ai27 (RCL-hChR2(H134R)/tdTomato) mice (stock# 012567) were purchased from Jackson Laboratory. We crossbred Avil^{CreERT2} mice with Ai27 mice to generate Avil^{CreERT2};ChR2-tdTomato. Mice were housed in cages at a controlled temperature (22 °C) with a 12:12 h light-dark cycle and were fed a standard chow diet with water provided *ad libitum*. Mice were administered intraperitoneal injections of 75 mg tamoxifen/kg body weight on five consecutive days. The tamoxifen solution was prepared by dissolving it in a mixture of 90% corn oil and 10% ethanol

Wireless optogenetics

The wireless optogenetic system (Neurolux) consists of a power distribution control box, three autotuners, three modular boxes, peripheral implantable optogenetic devices with μ-LED of 470 nm, and a laptop with NeuroLux software [19]. In contrast to the traditional wired optogenetic method, this wireless optogenetic approach enables mice to move freely without physical restraints. This device operates without the use of batteries. To implant a peripheral implantable optogenetic device, the animals were anesthetized and the abdominal region was shaved. A 1 cm incision was made on the skin of the abdomen. Blunt forceps were used to form a subcutaneous pocket and the main body of the device was inserted into the pocket. A 0.5 cm incision was made in the abdominal muscle directly over the liver to position the μ-LED-containing head of the device. This device included two separate LEDs: one with blue emission (470 nm) adjacent to the targeted tissue to serve as the source for optogenetic simulation, and the other with red emission (650 nm) just under the skin next to the coil to provide an externally visible signal of system activation [19]. We optogenetically stimulated liver-innervating Avil-positive vagal sensory nerve fibers at a frequency of 10 Hz during the animal behavior tests. The stimulation parameters included a pulse duration of 5 s, with each step lasting 10 ms and a 1-second interval between pulses.

Immunostaining

To assess if optogenetic stimulation increases the neuronal activity, we optogenetically stimulated ChR2-expressing Avil-positive nerve fibers in the livers for five minutes and then sacrificed animals 1 hr post stimulation. The mice were anesthetized with isoflurane (3%) and transcardially perfused with 4% paraformaldehyde. Nodose ganglia and brain samples were post-fixed in 4% paraformaldehyde overnight in a cold room, and then in 30% sucrose the following day. Tissues were sectioned using a cryostat at 16-20 μm. The sections were incubated with 0.3% Triton X-100 at room temperature (RT) for 30min, and then blocked in PBS buffer containing 10% donkey serum and 0.1% Triton X-100 for 1hr at RT and then incubated with rabbit anti-pS6 (2215S, Cell signaling), goat anti-ChAT (AB144P, Millipore), rabbit anti-RFP (600-407-379, Rockland), and mouse anti-TH (MAB318, Millipore) antibodies for overnight at cold room, and then sections were washed three times in PBS and incubated with Alexa 594 anti-rabbit IgG for pS6 and RFP (711-585-152, Jackson immunoresearch), Alexa 488 anti-goat IgG for ChAT (705-545-147, Jackson immunoresearch), and Alexa 488 anti-mouse IgG for TH (ab150105, Abcam) for 3hr at RT. The sections were washed, dried, and mounted using VECTASHIELD medium containing DAPI. Images were acquired using a Leica SP8 confocal microscope. Using the cell count plugin in the Image J software (version FIJI), pS6-positive neurons were counted.

Animal behavior tests

Open-field exploration test: Mice were placed in the center of a chamber measuring 40 cm (length) × 40 cm (width) × 40 cm (height) (Maze Engineers) and allowed to explore the chamber for 5 min freely (750 lx throughout the chamber). The center region was designated as 20 x 20 cm^2. The EthoVision XT video tracking system (Noldus) was used to record the sessions and analyze the behavior, movement, and activity of the animals. The chamber was wiped with 95% ethanol before the subsequent tests to remove any scent clues [20]. The total distance traveled and time spent in the center and outer zones of the chamber were measured.

Elevated plus maze test: The maze consists of four arms (two open and two closed arms) in the shape of a plus sign (35 cm arm in length, 5 cm arm in width, and 20 cm wall in height) elevated 60 cm from the ground (Maze Engineers). The mice were placed in the center zone and allowed to explore the maze freely for 5 min (750 lx throughout the arms). The session was recorded, and the number of arm entries and amount of time spent in the open and closed arms were analyzed using the EthoVision XT video tracking system.

Light-dark box test: The box consisted of two chambers (one light chamber, 25 cm in length × 40 cm in depth and one dark, 17.5 cm in length × 40 cm in depth, Maze Engineers). Mice were placed in the center of the light chamber and allowed to freely explore the chamber for 5 min (750 lx in the light chamber). The time spent in each compartment and crossing from one compartment to the other were measured using the video tracking system.

Statistics

All statistical results are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism (v.10). Two-tailed *t*-tests were used to calculate the p-values for pairwise comparisons. Data were considered significantly different when the probability value was less than 0.05.

Results

Optogenetic stimulation of liver-innervating Avil-positive nerves activates neurons in the DMV and NTS.

We first assessed if optogenetic stimulation of liver-innervating Avil-positive nerve fibers using our wireless implantable LED device increases the activity of Avil-positive neurons in the nodose ganglia of Avil^{CreERT2};ChR2-tdTomato mice (Fig. 1A). A 10 Hz light stimulation of the nerve fibers in the liver with a pulse duration of 5 s, with each step lasting 10 ms and a 1-second interval between pulses for five min, effectively activated a subset of vagal sensory neurons (Fig. 1B). Quantification of the number of pS6-positive cells, a marker of neuronal activity, revealed a significant increase in pS6-positive cells in the nodose ganglia of animals exposed to stimulation compared to those without stimulation (Fig. 1C). This result validated the effectiveness of our wireless optogenetic stimulation method.

Given that liver-innervating vagal sensory neurons send their central nerve terminals to the DVC, we investigated whether the stimulation of these particular nerve fibers increases the activity of neurons in the DVC. Five min of optogenetic stimulation of the nerve fibers in the liver of Avil^{CreERT2};ChR2-tdTomato mice resulted in marked expression of pS6 in the DMV, whereas a small number of pS6-positive cells were activated in the NTS. Most pS6-positive cells in the DMV also co-expressed the choline acetyltransferase (ChAT), indicating that they are parasympathetic preganglionic cholinergic neurons. These findings suggest that the DMV cholinergic neurons are the primary neurons that are activated by vagal sensory input from the liver, which aligns with a previous study showing that vagal sensory neurons that project to the portal vein innervate predominantly the DMV in rats [21]. Intriguingly, the activation of vagal sensory neurons innervating the gastrointestinal tract via DREADDs resulted in increased neuronal activity within the NTS [6]. Hence, it appears that vagal sensory neurons innervating the liver may have distinct targets within the DVC compared to those innervating the gastrointestinal tract.

Optogenetic stimulation of liver-innervating Avil-positive nerve fibers enhances anxiety-like behavior in both male and female mice.

We then explored the physiological impact of the acute activation of liver-innervating Avil-positive neurons on anxiety-like behavior. A series of anxiety-like behavioral assessments was conducted

on male mice. First, we performed an open field test, a common assessment for evaluating anxiety-like behavior in rodents [20, 22]. We placed the animals at the center of the open field maze and measured the total distance traveled, along with the time spent and distance traveled in the inner zone. When Avil-positive nerves innervating the liver were optogenetically stimulated during the assessment, there was a significant decrease in the total distance traveled compared to that in the control group (Fig. 2A and B), which indicates a reduction in the exploratory locomotor activity during the optogenetic stimulation. Moreover, Avil^{CreERT2};ChR2-tdTomato mice with optogenetic stimulation exhibited a decrease in both the distance traveled and the time spent in the inner zone compared to those in the controls (Fig. 2A and B). The percentage of the time spent in the inner zone zone was also significantly different between the groups (Fig. 2B). These findings indicate that acute activation of Avil-positive nerves innervating the liver may enhance anxiety-related behavior.

We then performed an elevated plus maze (EPM) test to assess the natural exploratory behavior of mice, offering them a choice between the open and enclosed arms. The mice were placed at the center of the EPM. During acute optogenetic stimulation, mice traveled significantly less in the maze than the control group (Fig. 2C and D), further supporting the findings of the open-field test. More importantly, mice with optogenetic stimulation spent less time in the open arms and significantly more time in the closed arms than the controls. The percentage of time spent in the open arms was significantly lower in the mice with stimulation than in those without stimulation. In the light/dark (LD) box test, to evaluate changes in the willingness to explore the illuminated, unprotected area, Avil^{CreERT2};ChR2-tdTomato mice with optogenetic stimulation demonstrated a decrease in both the distance traveled and the duration spent in the light chamber compared to the control group (Fig. 2E and F). In addition, stimulation of liver-innervating Avil-positive nerves resulted in fewer transitions between compartments. Hence, both the EPM and LD box tests provide further evidence supporting the interpretation that the acute activation of vagal sensory neurons innervating the liver results in an increase in anxiety-like behavior.

We performed the same behavioral assessments on female mice. The results of the open field test revealed that, similar to male mice, Avil^{CreERT2};ChR2-tdTomato mice with acute stimulation exhibited a decrease in the total distance traveled as well as in the time spent and distance traveled within the center zone. During the assessment of mice on the EPM, acute optogenetic stimulation of their nerves in the liver resulted in a significant reduction in the overall distance traveled and an increase in the time they spent within the closed arms compared to mice that did not receive such stimulation. In the light-dark (LD) box assessment, female mice exhibited no significant differences in the distance traveled and number of transitions between compartments, regardless of the presence of optogenetic stimulation. Results from both the open field test and the elevated plus maze (EPM) test suggest that female mice also displayed an

increase in anxiety-like behavior following the acute stimulation of Avil-positive nerves innervating the liver.

Optogenetic stimulation of liver-innervating Avil-positive nerve fibers activates NE-expressing neurons in the LC.

Given that stimulating liver-innervating Avil-positive nerve fibers increased anxiety-like behavior, we further explored to understand how this neural pathway triggers such a behavioral response. The LC-NE system plays a crucial role in anxiety-like behavior [23], and activating the vagus nerve enhances neuronal activity in the LC in both rodents and humans [15-18]. We assessed whether NTS neurons project to LC-NE neurons by injecting retrograde adeno-associated virus (AAV)-tdTomato into the LC of C57BL6/J mice (Fig. 4A). Immunostaining revealed tdTomato-positive cells in the LC, confirming successful viral infection (Fig. 4B). In the same animal, we found that tdTomato-positive cells were primarily located in the NTS (Fig. 4B). The results, along with the presence of pS6-positive cells in the NTS, indicate that LC-NE neurons might be activated by the stimulation of liver-innervating Avil-positive nerves via a subset of NTS neurons. Double immunostaining with anti-tyrosine hydroxylase (TH), a marker for catecholaminergic neurons, and anti-pS6 antibodies showed the absence of pS6-positive cells in the LC of mice that were not stimulated (Fig. 4D). Conversely, in stimulated mice, the LC exhibited that most TH-positive cells co-expressed pS6 (without stimulation, n= 9 out of 436 TH-positive cells, 2 ± 0.1 %, n= 3 mice; with stimulation, n= 382 out of 505 TH-positive cells, 75 ± 1.4 %, n = 5 mice; Fig. 4E). These results support the interpretation that the activation of the liver-> NTS->LC-NE pathway via liver-innervating vagal sensory neurons enhances anxiety-like behavior in mice.

Discussion

Communication between the visceral organs, particularly the gut and brain has been extensively investigated due to its critical importance in regulating not only energy metabolism such as food intake [6, 24], energy expenditure [25], glucose metabolism [3, 26] but also emotional and mood-related behaviors [27]. As gut-innervating vagal sensory neurons express receptors for nutrients [28] as well as microbiota-derived metabolites [24], these interoceptive signal molecules exert influence on these various functions through the gut-brain axis. In addition, the gut-derived interoceptive signal molecules are transported to the liver via the hepatic portal vein that carries blood from other visceral organs, including the gastrointestinal tract, pancreas, and spleen to the liver [12]. Prior studies, including ours, have demonstrated that the vagal afferent nerves sent axonal projections predominantly to the hepatic portal vein [3, 29]. It is plausible that certain functions of the gut-brain axis are also mediated through the liver-brain pathway. In fact, we demonstrated that the loss of function of vagal sensory neurons innervating the liver prevented the onset of diet-induced obesity (DIO) in mice fed a high-fat diet (HFD), partly due to

increased energy expenditure [3]. Interestingly, mice without hepatic vagal sensory innervation displayed less anxiety-like behavior during HFD feeding, although their depression-like behavior remained unchanged [3]. These findings imply that disruptions in interoceptive signal detection of liver-innervating vagal sensory neurons may alter mood behaviors, especially anxiety-like behavior.

In our current study, we expanded upon our prior study [3] to further investigate the critical role of vagal sensory neurons innervating the liver in anxiety-like behavior. We employed a novel optogenetic stimulation technique that enabled mice to move freely without any physical constraints during the behavioral assessments. This method effectively stimulated the neurons within the nodose ganglia. Despite the relatively small number of pS6-positive neurons observed in the NTS following acute stimulation (5 min) of liver-innervating Avil-positive nerves, this limited neuronal population appears to be sufficient to activate NE neurons in the LC, which is consistent with prior studies [15-18]. In fact, our retrograde viral tracing indeed revealed that LC-projecting neurons were located predominately in the NTS. These findings indicate the existence of a liver -NTS-LC NE pathway via vagal sensory neurons. It is well established that directly activating LC NE neurons using optogenetics resulted in enhanced anxiety-like behavior [14]. Hence, our finding of increased anxiety-like behavior following the stimulation of liver-innervating Avil-positive neurons would be due to the activation of these LC NE neurons. When hepatic vagal sensory innervation was disrupted, the animals exhibited the opposite behavioral phenotype during HFD feeding [3]. Hence, it is plausible that the liver-brain axis is crucial not only for regulating liver functions but also for influencing emotional behavior.

Although our results support the importance of the liver-brain neural circuit in the control of anxiety-like behavior, it is plausible that the humoral pathway could also play a role in emotional behaviors. In fact, the liver acts as an endocrine organ because it produces and secretes various hepatokines, cytokines, bile acids, and hormones. They are implicated in the regulation of overall energy metabolism and liver functions [30, 31]. A recent study demonstrated that stress enhanced anxiety-like behavior in rodents and humans through secreting the cytokine lipocalin 2 [32]. Interestingly, restraint stress activated primarily DMV cholinergic neurons in mice. Moreover, optogenetic stimulation of liver-innervating DMV neurons increased lipocalin 2 levels in the liver and the serum. This enhanced anxiety-like behavior [32]. Much surprise to us, acute stimulation of liver-innervating vagal sensory neurons led to the expression of pS6 primarily in cholinergic neurons in the DMV rather than in neurons in the NTS. In fact, a study by Watts et al. using anterograde H129 viruses demonstrated that the DMV cholinergic neurons were the primary neurons that received vagal sensory input from the hepatic portal and superior mesenteric veins without engaging NTS neurons in rats [21].

Although there were differences between their study and ours [3], possibly due to the short survival time (less than 120h) of the animals [21], the use of different viral vectors (anterograde vs.

retrograde), and viral injection sites (portal vein vs. liver parenchyma), both studies clearly demonstrated that the DMV received vagal sensory input that project to the portal vein. This may imply that the increased activity of liver-innervating Avil-positive nerves directly activated DMV cholinergic neurons, which subsequently influenced liver function. For example, we demonstrated that the DMV cholinergic neurons innervated the liver and deleting these DMV cholinergic neurons prevented the development of hepatic steatosis during HFD feeding through releasing bile acids [33]. In parallel, this activation could also trigger the release of lipocalin 2, thereby altering anxiety-like behavior via the humoral pathway. Hence, a potential feedforward circuit between the liver and brainstem via vagal sensory neurons and DMV cholinergic neurons might also play a role in regulating hepatic energy metabolism as well as anxiety-like behavior.

In contrast to the conventional top-down neuronal circuits associated with the liver, this novel liver-brain axis is crucial in regulating not only systemic energy homeostasis but also emotional behaviors. Given that hepatic fatty liver disease elevates the risk of anxiety and depression in both rodents and humans [10, 34, 35], elucidating the function of the liver-brain axis may offer alternative therapeutic approaches for mental health issues associated with chronic stress and liver diseases.

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Figure legends

Figure 1. Optogenetic stimulation of liver-innervating Avil-positive neurons

(A) Schematic illustration of the experimental condition. A wireless implantable LED device was placed on top of the livers of Avil^{CreERT2};ChR2-tdTomato mice.

(B and C) Images of confocal fluorescence microscopy showing the expression of pS6-positive neurons in the nodose ganglia of Avil^{CreERT2};ChR2-tdTomato mice, with (right) and without (left) optogenetic stimulation. Scale bar, 40um. Quantitative analysis of pS6-positive neurons in the nodose ganglia (n = 4 mice per group) revealed a significant increase in their number in Avil^{CreERT2};ChR2-tdTomato mice with optogenetic stimulation compared to those without it. Unpaired t-test, *p=0.01. Data are presented as mean ± SEM.

(D) Images of confocal fluorescence microscopy showing the expression of pS6 in the DVC of Avil^{CreERT2};ChR2-tdTomato mice with (bottom) and without (top) optogenetic stimulation. White arrowheads indicate the pS6-positive neurons in the NTS. Scale bar, 40um.

Figure 2. Optogenetic stimulation of liver-innervating Avil-positive nerves enhances anxiety-like behavior in male mice.

(A and B) Representative examples of the travel paths of mice without (left) and with stimulation (right) during the open field test (A). The time spent in the center zone (*p=0.02) and the percentage of time spent in the center zone (*p=0.02) were significantly different between the groups (n = 8 mice vs. 14 mice). Two-tailed *t*-test

(C and D) Examples of the travel paths of mice without (left) and with stimulation (right) during the EPM test (C). Graphs showing significant differences in the total distance traveled (***p=0.0001), time spent in closed arms (***p=0.0008), and percentage of time spent in open arms (*p=0.05) between the groups (n = 9 mice vs. 13 mice). Two-tailed *t*-test

(E and F) Examples of the travel paths of mice without (left) and with stimulation (right) during the LD box test (E). There were significant differences in the distance traveled in the light chamber (*p=0.02), time spent in the light chamber (*p=0.02), and number of transitions (*p=0.01) between the groups (n = 8 mice vs. 13 mice). Two-tailed *t*-test

Figure 3. Optogenetic stimulation of liver-innervating Avil-positive nerves increases anxiety-like behavior in female mice.

(A and B) Representative examples of the travel paths of mice without (left) and with stimulation (right) during the open field test (A). Graphs showing significant differences in the total distance traveled (**p=0.001), distance traveled in the center area (*p=0.02), time spent in the center area (*p=0.04), and

percentage of time spent in the center area (*p=0.04) between the groups (n = 6 mice vs. 6 mice). Two-tailed *t*-test

(C and D) Examples of the travel paths of mice without (left) and with stimulation (right) during the EPM test (C). Significant differences were observed in the total distance traveled (**p=0.002) and time spent in the closed arms (*p=0.04) between the groups (n = 6 mice vs. 7 mice). Two-tailed *t*-test

(E and F) Examples of the travel paths of mice without (left) and with stimulation (right) during the LD box test (E). Although there was a trend toward spending less time in the light chamber, the behavioral parameters did not show any significant differences between the groups. (n = 6 vs. 6 mice).

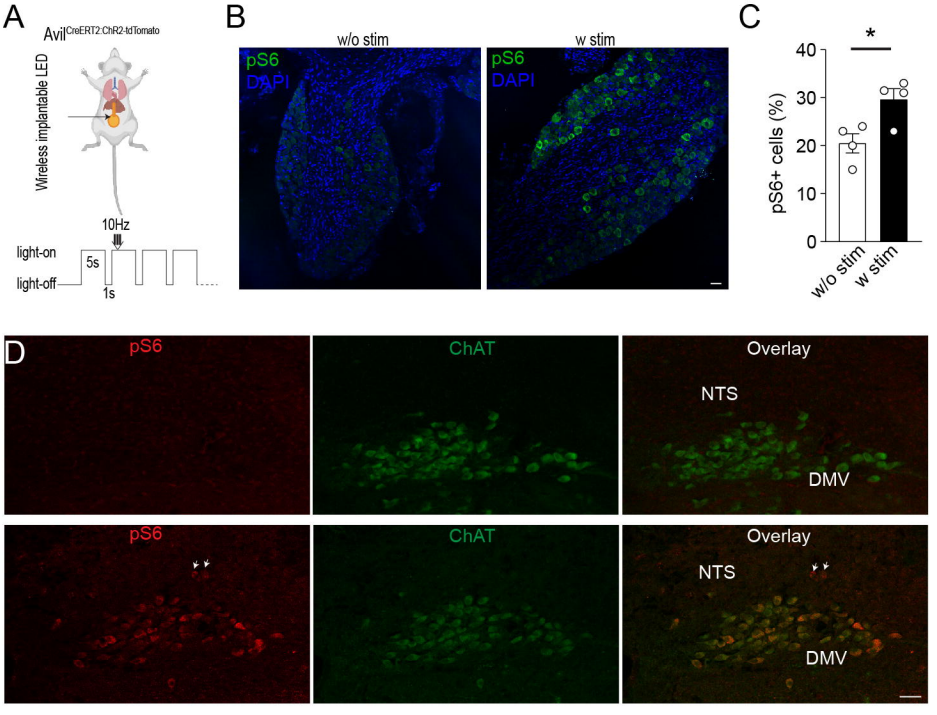
Figure 4. Optogenetic stimulation of liver-innervating Avil-positive nerves activates neurons in the LC.

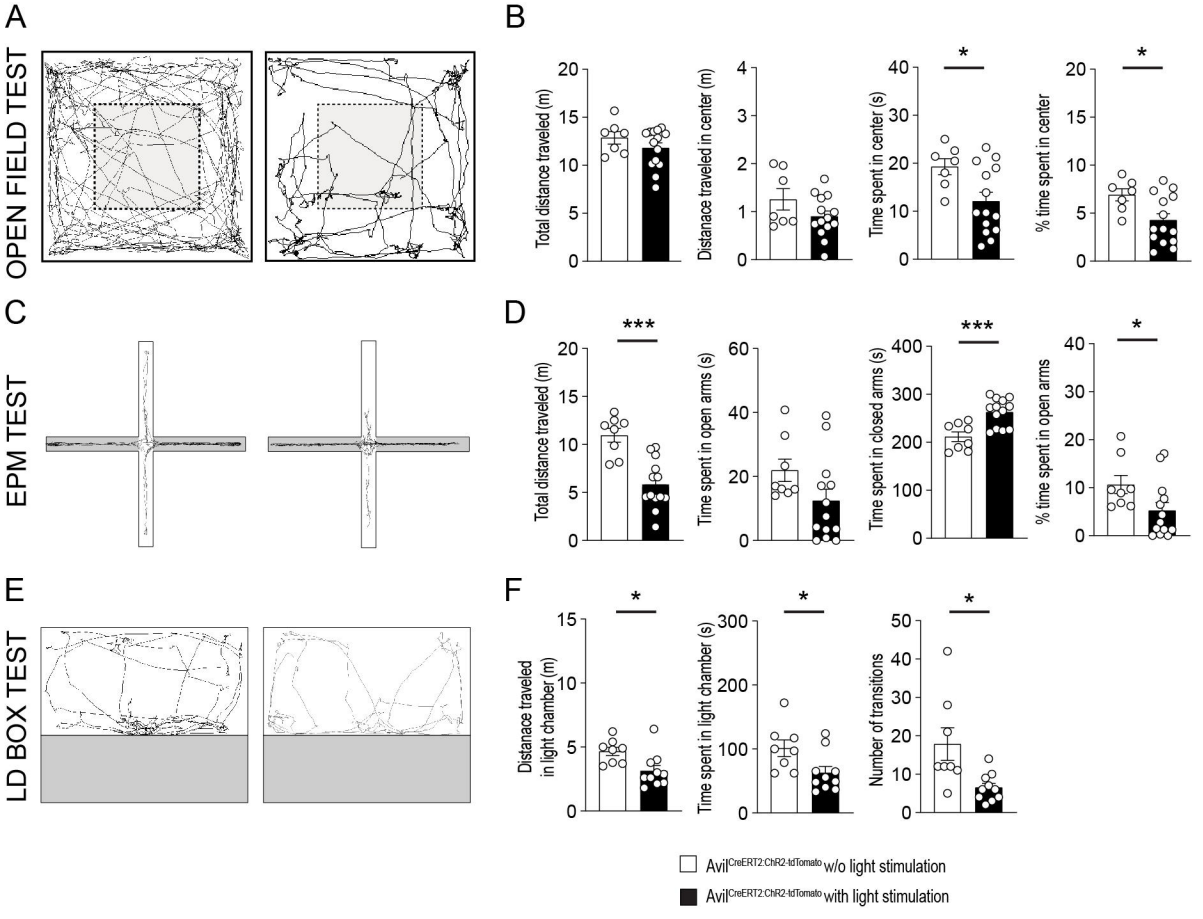
(A) Schematic illustration of the experimental condition. C57BL6/J mice were stereotaxically injected with AAVrg-tdTomato into the LC (A).

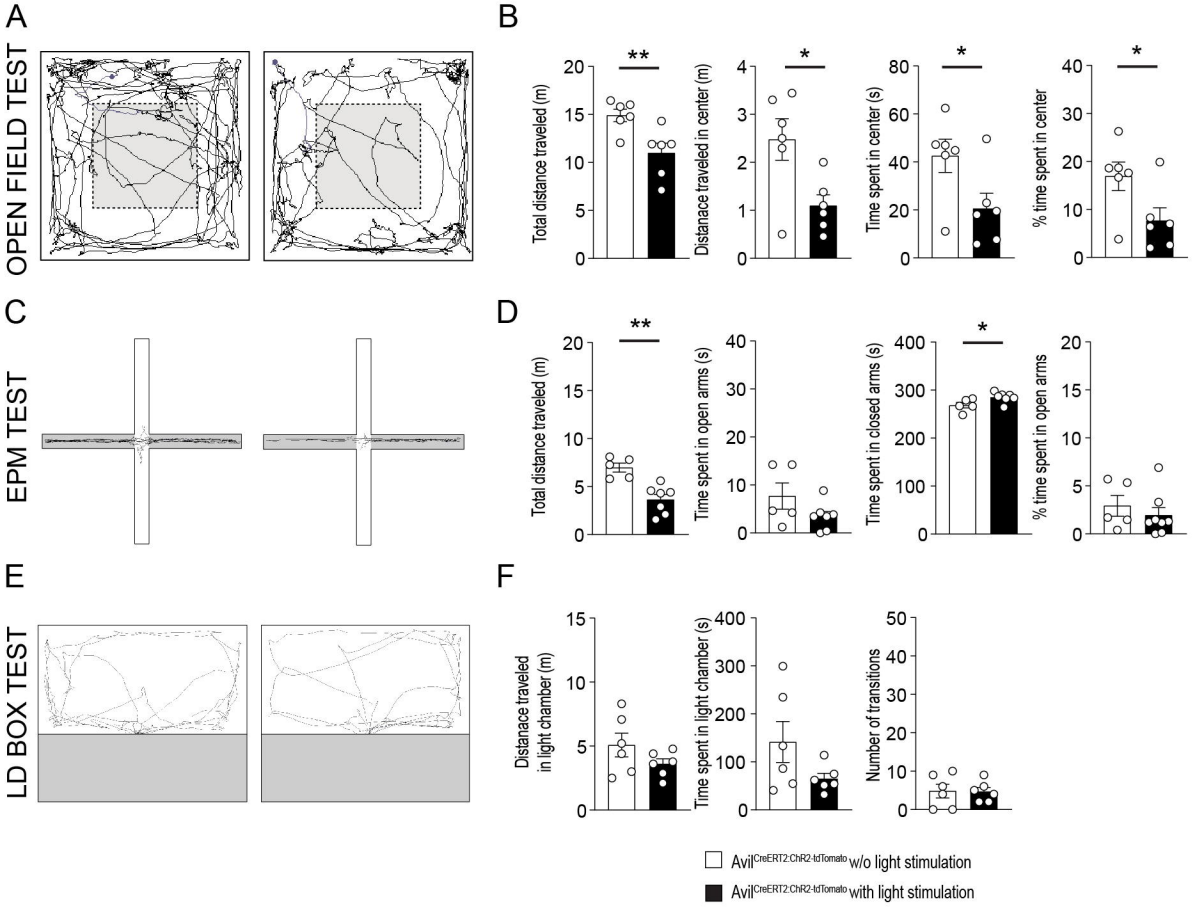
(B) Images of confocal fluorescence microscopy showing tdTomato-positive cells in the LC of C57BL6/J mice injected with AAVrg-tdTomato, validating our viral infection. (left) More importantly, tdTomato-positive cells were also detected in the NTS (right), indicating that these were LC-projecting NTS neurons. Scale bar, 40um.

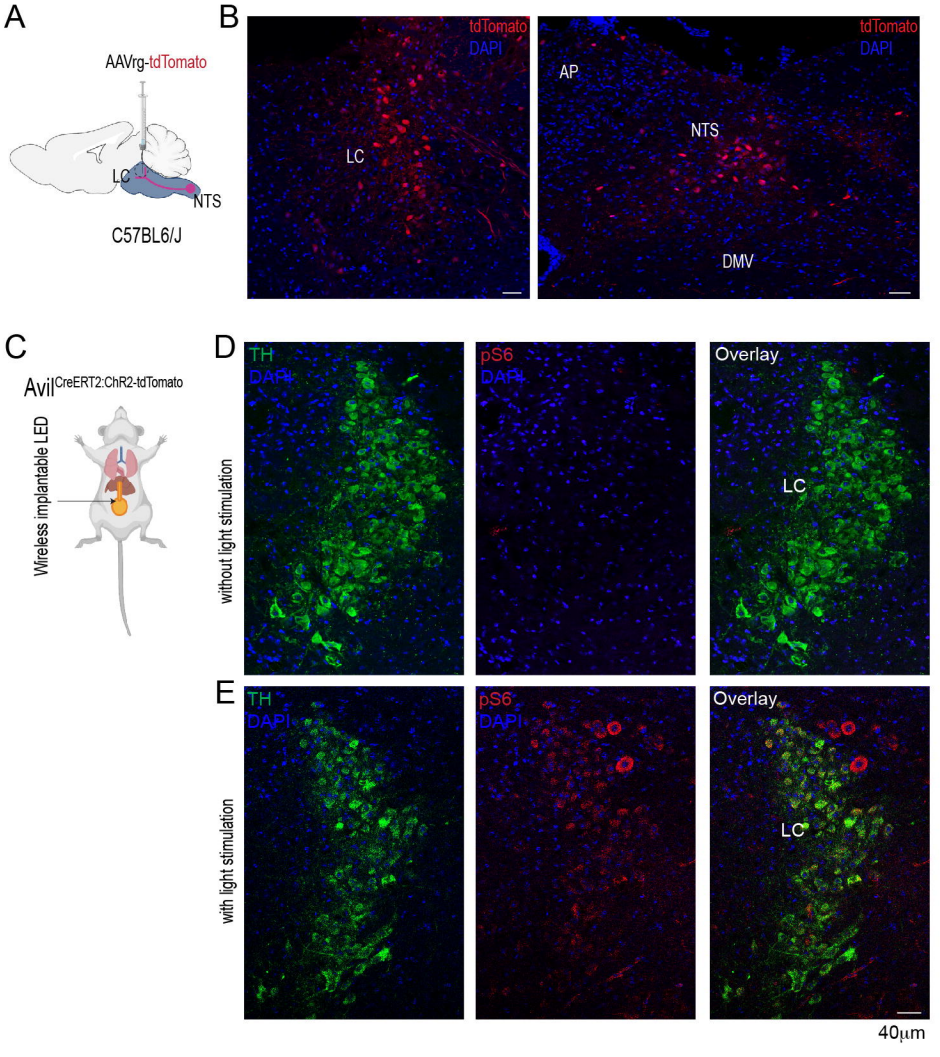
(C) Schematic illustration of the experimental condition.

(D and E). Images from confocal fluorescence microscopy showing that none of the TH-positive neurons in the LC were co-expressed with pS6 in Avil^{CreERT2};ChR2-tdTomato mice without optogenetic stimulation (D). In contrast, optogenetic stimulation of liver-innervating Avil-positive nerves robustly increased the neuronal activity in the LC. Scale bar, 40um


A
Avil CreERT2:ChR2-tdTomato
Wireless implantable LED
10Hz
light-on
light-off
5s
1s
B
w/o stim
w stim
pS6
DAPI
C
pS6+ cells (%)
40
30
20
10
0
*
w/o stim
w stim
D
pS6
ChAT
Overlay
NTS
DMV


A
OPEN FIELD TEST
B
Total distance traveled (m)
Distanace traveled in center (m)
Time spent in center (s)
% time spent in center
C
EPM TEST
D
Total distance traveled (m)
Time spent in open arms (s)
Time spent in closed arms (s)
% time spent in open arms
E
LD BOX TEST
F
Distanace traveled in light chamber (m)
Time spent in light chamber (s)
Number of transitions
AvilCreERT2:ChR2-tdTomato w/o light stimulation
AvilCreERT2:ChR2-tdTomato with light stimulation


A
OPEN FIELD TEST
B
Total distance traveled (m)
Distanace traveled in center (m)
Time spent in center (s)
% time spent in center
C
EPM TEST
D
Total distance traveled (m)
Time spent in open arms (s)
Time spent in closed arms (s)
% time spent in open arms
E
LD BOX TEST
F
Distanace traveled in light chamber (m)
Time spent in light chamber (s)
Number of transitions
Avil CreERT2:ChR2-tdTomato w/o light stimulation
Avil CreERT2:ChR2-tdTomato with light stimulation


A
AAVrg-tdTomato
LC
NTS
C57BL6/J
B
tdTomato
DAPI
LC
tdTomato
DAPI
AP
NTS
DMV
C
Avil CreERT2:ChR2-tdTomato
Wireless implantable LED
D
without light stimulation
TH
DAPI
pS6
DAPI
Overlay
LC
E
with light stimulation
TH
DAPI
pS6
DAPI
Overlay
LC
40μm