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Supplementary information

Scalable and modular wireless-network infrastructure for large-scale behavioural neuroscience

In the format provided by the
authors and unedited

Contents

Supplementary Notes

Fabrication of µ-ILED optical neural probes (Step-by-Step Process)

The WNBN can be used to control any type of neural probes (i.e., electrical, optical or microfluidic). Material selection, design construction, and fabrication process can be varied depending on the nature, requirements and scalable needs for a specific experiment. For ideally chronic studies, we suggest using our soft, ultra-thin and flexible optical (for photostimulation), microfluidic (for drug delivery), or optofluidic probes (for combined photostimulation and drug delivery), whose fabrication steps are extensively covered in our previous studies[19,24,40]. Here, we highlight another fabrication technique for implementation and deployment of low-cost and mass-manufacturable flexible optical probes that were used in this study.

A. Preparation of µ-ILED optical probes:

1. Design neural probes and their electrodes with desired configurations (e.g., unilateral, bilateral, etc.) using a PCB design software.
2. Based on the design, construct a probe substrate with copper electrodes on a 25 μm-thick polyimide (PI) film.
3. To eliminate the need for a temporary injection assist needle (38 μm-thick stainless steel needle[19,24,40]), deposit a thin copper layer (18 μm in thickness) on the bottom side of the PI layer to make the probe stiff enough for penetrating into brain tissue.
4. Laser-cut the PI substrate into a large array of individual neural probe needles.
5. Clean the probes with acetone, isopropyl alcohol (IPA), followed by deionized (DI) water, and dehydrate their surface with a nitrogen gun (N_2).
6. Mount the neural probes on a PDMS (10:1 or 5:1) coated glass slide in preparation for µ-ILED bonding.
7. Attach µ-ILEDs (e.g., blue (470 nm, TR2227, Cree Inc.), orange (589 nm, TCE10-589, Three Five Materials), etc.) on copper electrodes after applying ultra-fine solder paste (T5, SMDLTLFP10T5, Chip Quik Inc.).
8. Put the assembled probes in a reflow oven and cure them with the following recipe: a) Ramp up to 150 °C with a ramping speed of 1 °C/sec and preheat at 150 °C for 90 sec, b) Ramp up to 170 °C with a ramping speed of 1 °C/sec and heat at 170 °C for 105 sec, c) Solder for 90 sec with a peak temperature at 215 °C, d) Cool down for more than 15 min until the temperature drops to the room temperature.

B. Encapsulation and electrical connection

9. In order to avoid damaging of µ-ILED probes by biofluid in brain tissue, prepare neural probes for encapsulation by mounting them at the edge of a glass slide stack using a Kapton tape such that the thin injection part is completely hanging off in the air.
10. Coat the probes with 7 µm-thick Parylene C using a parylene deposition tool (PDS 2010 Labcoater 2, Speciality Coating Systems). This will protect the probes from biofluid in brain tissue.
11. Remove the Kapton tape to expose the copper electrode area to connect probes to electrical connectors.
12. After removing the probes from the glass slide stack carefully (without bending the probe structure), solder electrical connectors (M50-3140345, Harwin Inc.) on the copper electrodes in the wide base part of the neural probes using a solder paste (T5, SMDLTLFP10T5, Chip Quik Inc.).
13. Finally, test each individual µ-ILED probes in a water bath by supplying a 2.7–3 V power using a DC power generator (GPC-3030D, GW Instek).

C. 3D printing of probe holders

14. Model probe holders (plastic structures that provide additional strength the connector region of the probe) using a commercial CAD software (SolidWorks, Dassault Systèmes)
15. Setup a 3D printer (B9 Core 530, B9 Creations) with a photo curable resin (B9R-4-Yellow resin, B9 Creations).
16. Clean the build head with IPA and dry it with a soft microfiber cloth.
17. After loading the CAD design file into the 3D printer, double check all setup parameters (X, Y resolution: 30 µm).
18. Start the printing process. Putting multiple samples per run can allow for rapid prototyping through Digital Light Processing Stereolithography (DLP SLA) in the 3D printer since all horizontal layers in all samples are exposed simultaneously, thus reducing overall printing time.
19. Once printing is complete, wash components in IPA and water. Then make additional UV treatment (405 nm wavelength) for at least 1 minute inside a UV box (Post-curing: B9 Model Cure, B9 Creations).

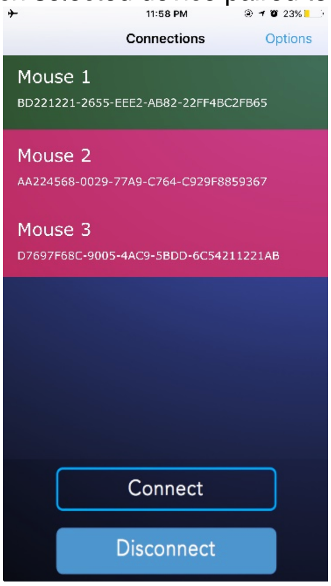
D. Mounting of neural probes to 3D printed probe holders

20. Mount and align a neural probe on the plastic holder (parallel with holder edges along the central axis). Make sure the probe is perpendicular in both vertical and horizontal coordinates with respect to holder edges.
21. Apply epoxy (5-min epoxy, Devcon) around the female connector and also on the edges of the 3D printed plastic holder. This will ensure strong hold of the wide connector region of the neural probe with the probe holder walls.
22. After curing the epoxy, test all the probes again before in vivo studies.

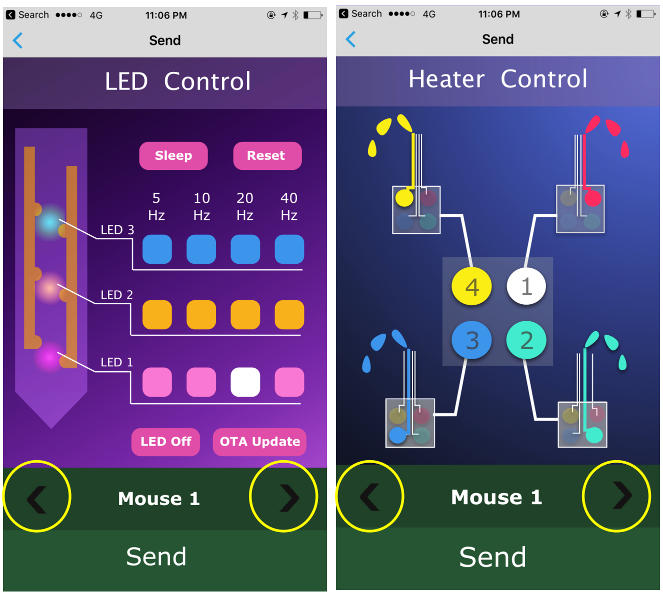
Remote-control centre (RCC) user guide for WNBN

A. Smartphone App for local piconet control consists of three main interfaces:

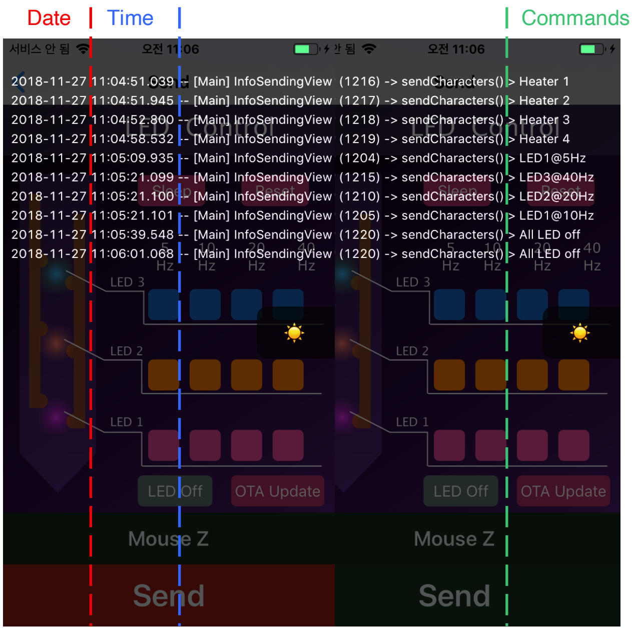
1. Device list page: It consists of all available advertising BLE devices in the vicinity of the smartphone. Select one or multiple devices (by holding for at least one second on each) before pressing the "Connect" button. This will make each selected device paired to the smartphone.



2. Control page: The control page consists of panels with commands of different types (LED, drug, system commands, etc.) and each control page corresponds to only one of the paired devices. To access control page of another paired device, simply press the "right" and "left" arrow keys (highlighted with yellow circles in the pictures below) at the bottom the app besides the device name. In order to send commands simultaneously to multiple devices, first select all appropriate commands in each control page of each mice, before finally pressing the "Send" button. The smartphone will send the control commands to the devices with short latency (~20 ms).

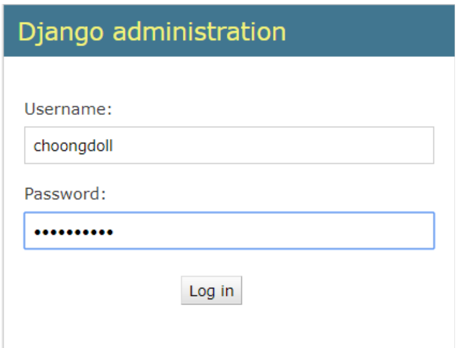


3. Log page: The log page displays the confirmation for a successfully communicated command with all RCMs. It also highlights the timestamp of each command with millisecond accuracy.

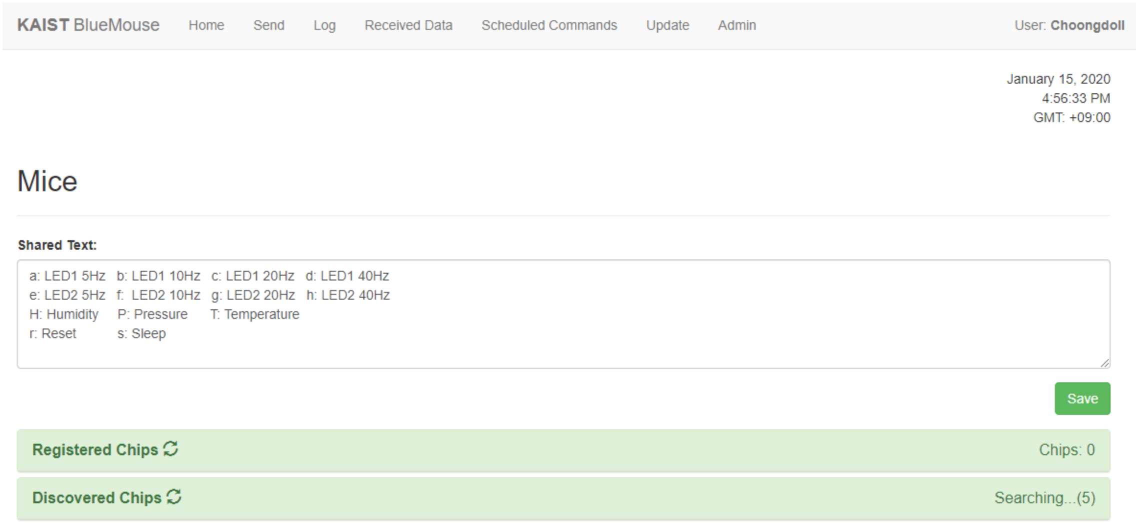


B. Webserver control website for global internet control

1. Log in page: A person, previously registered by the administrator and given access privileges, can log in securely to the website by inserting the unique username and password. This is the first page a new user will see after logging the appropriate WNBN Server IP address in any browser.

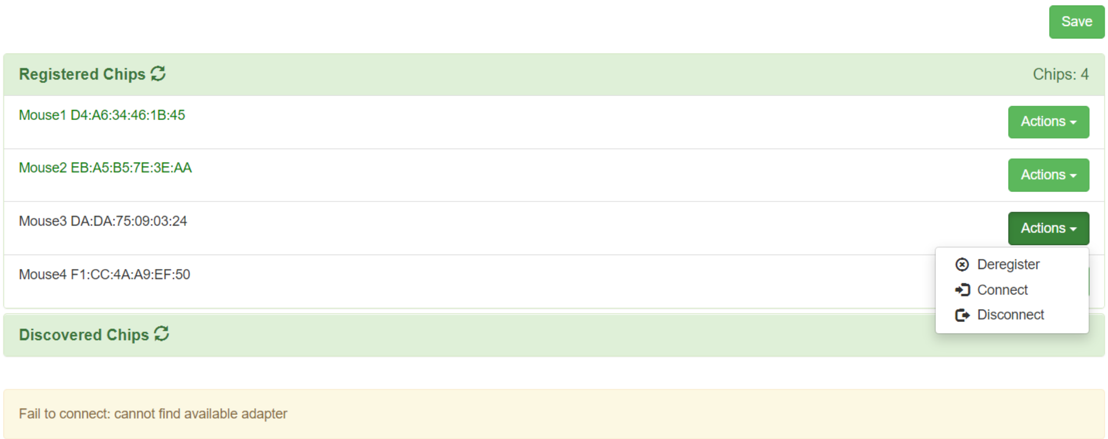


2. Device list page ('Home' tab):
This is the main page of the webserver. The 'Shared text' field can be used as a bulletin board which can be viewed by all registered users accessing the server. As an example (in the figure below), it is being used to highlight the various control commands for RCMs so researchers know how to create their own custom commands to control specific RCMs and/or their multimodal stimulation parameters.
a) BLE devices (RCMs) can be paired to the webserver (minicomputer) by registering them from the discovered devices. The lists of registered chips and discovered chips will appear when a corresponding button is pressed.

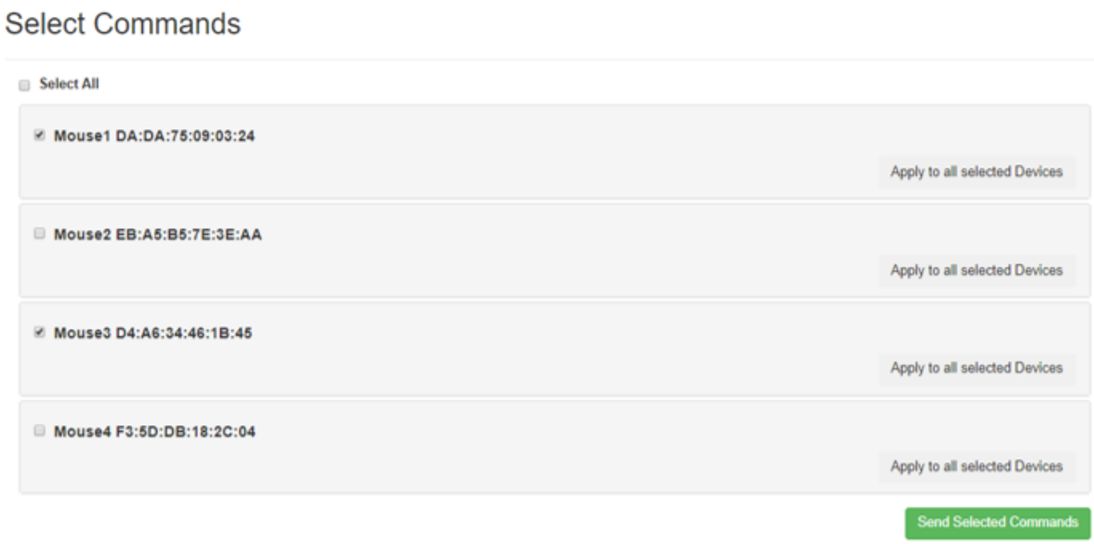


b) 'Discovered Chips' section shows all available advertising BLE devices placed near the minicomputer. BLE devices can be registered by pressing the "Register" button for connection.

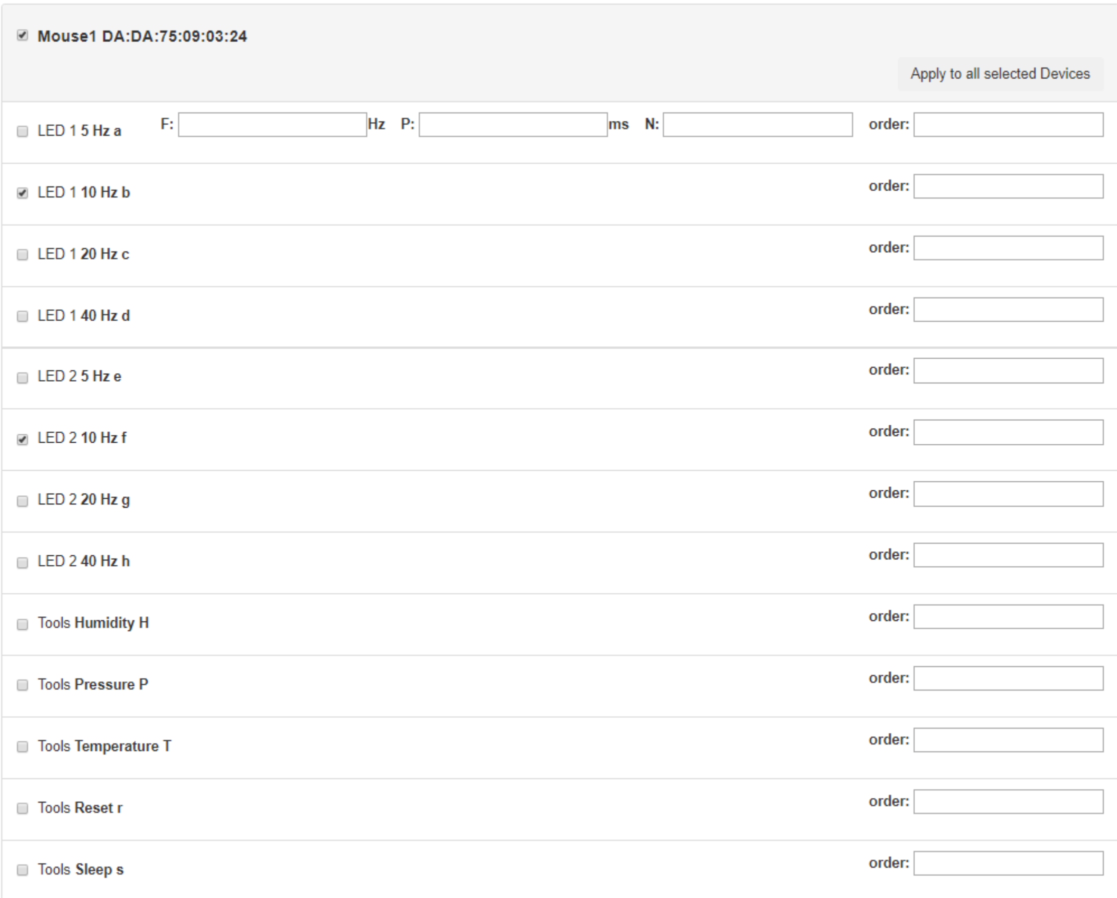
c) 'Registered Chips' section shows all registered BLE devices. BLE devices can be removed from the registered chips list by pressing the "Deregister" button, if necessary. As the registered BLE devices are connected to the webserver by pressing the "Connect" button, the device names turn into green color. If the registered BLE device is unavailable for connection, failure notice appears below the device list. BLE devices can be disconnected by pressing the "Disconnect" button.



3. Control page ('Send' tab):
   a) The control page manages control of connected RCMs. This page consists of options with selecting RCM devices (that were already paired in previous step) and appropriate commands for operation of each device. The list of BLE devices, which are connected at the previous device list page, will appear on this page. Select one or multiple devices, which are to be operated, by checking the checkbox next to the device name.

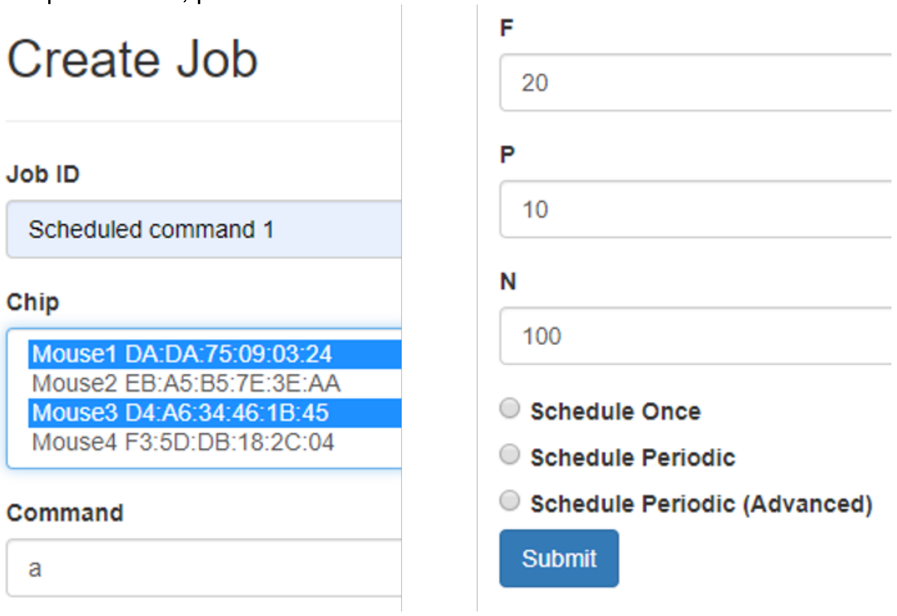


b) A list of commands will appear when each device name is pressed. Select appropriate commands for the corresponding device. In order to send the same commands simultaneously to multiple selected devices, after choosing a unique set of commands for one checked device, press “Apply to all selected Devices” button right next to it. All the selected (checked) devices will get the same commands activated (to save time for large scale experiments). When ready to send all commands to selected devices, press “Send Selected Commands” to operate the selected devices. The webserver will send the control commands to multiple devices with latency of less than 0.1 sec ((~55 + α) ms).

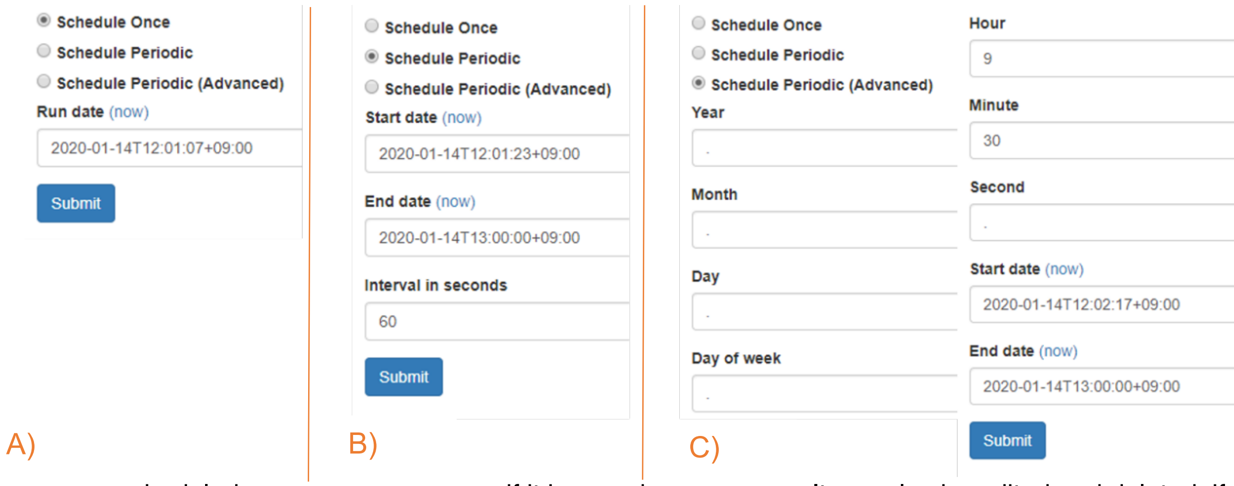


c) If the commands are sent to the selected BLE devices successfully, success notice will appear for a few seconds below the “Send Selected Commands” button.

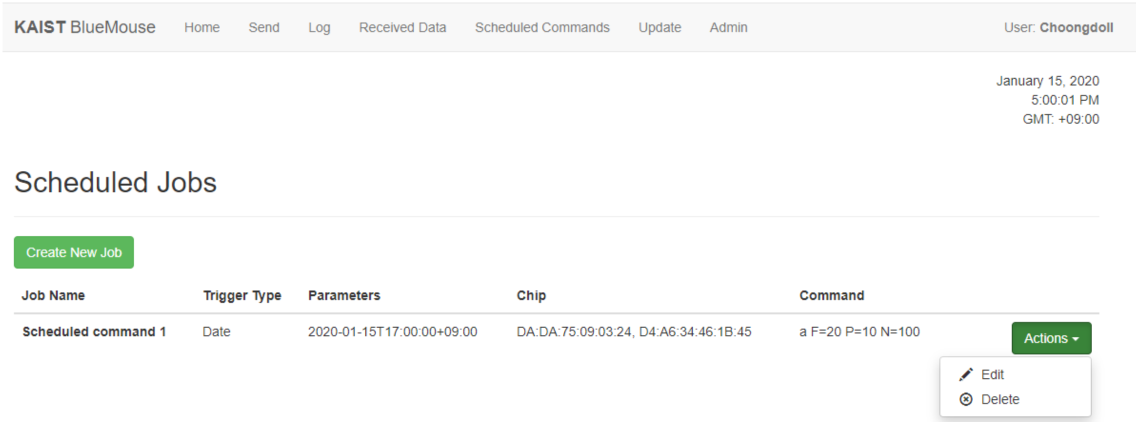
4. Command scheduling page ('Scheduled Commands' tab):
a) Scheduled commands for the registered chips can be added and set on the command scheduling page. Various options need to be set when adding the scheduled command. These include target device, trigger date/time, frequency (F), pulse width (P), and a number of cycles (N), etc. A special firmware code is required in BLE devices (RCM) to be able to control F/P/N on-the-fly (for optogenetic control). In this case the commands are sent in the form of a string like aF20fP10pN100n (a → LED1, F = 20 Hz, P = 10 ms, N = 100 cycles). This string can be further extended to control multiple parameters of variety of actuators and sensors in a laboratory in real-time by integration control signals within the command string that is being sent to an RCM with adjusted firmware (that can decode that string). If these parameters are not required to be controlled on-the-fly, select values 0, 0, and -1 for F, P and N fields, respectively. In this case, the target µ-ILED and operation frequency will depend on the 'Command' (a - h), and the µ-ILED will be operated with a fixed pulse width of 10 ms for infinite cycles. After setting the parameters, press the "Submit" button to add the scheduled command.



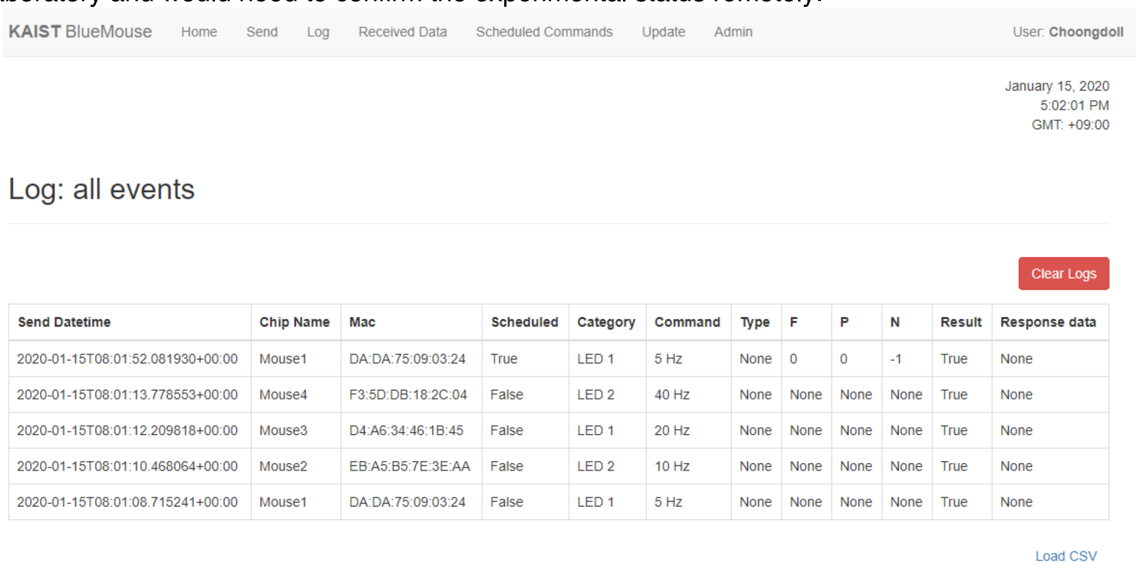
The date and time for triggering the desired operation can be scheduled in three different options - A) Schedule Once: will schedule the task once at a specific date and time in the future. B) Schedule Periodic: will allow repeated scheduling of the same task (from a specific start date and time) periodically over and over again until the end date and time. C) Schedule Periodic (Advanced): enables repeated execution of a task at a specific year/month/date/time in the future.



2) The scheduled command will appear if it is saved successfully. It can also be edited and deleted, if necessary. The BLE device will be operated at the start time until the end time after it is successfully scheduled.



5. Log page ('Log' tab):
   The log page displays the confirmation for a successfully communicated command, highlighting the timestamp of each command with millisecond accuracy. This page becomes a critical component of WNBN global internet control since the researcher is not expected to be physically present in the laboratory and would need to confirm the experimental status remotely.



Send Datetime	Chip Name	Mac	Scheduled	Category	Command	Type	F	P	N	Result	Response data
2020-01-15T08:01:52.081930+00:00	Mouse1	DA:DA:75:09:03:24	True	LED 1	5 Hz	None	0	0	-1	True	None
2020-01-15T08:01:13.778553+00:00	Mouse4	F3:5D:DB:18:2C:04	False	LED 2	40 Hz	None	None	None	None	True	None
2020-01-15T08:01:12.209818+00:00	Mouse3	D4:A6:34:46:1B:45	False	LED 1	20 Hz	None	None	None	None	True	None
2020-01-15T08:01:10.468064+00:00	Mouse2	EB:A5:B5:7E:3E:AA	False	LED 2	10 Hz	None	None	None	None	True	None
2020-01-15T08:01:08.715241+00:00	Mouse1	DA:DA:75:09:03:24	False	LED 1	5 Hz	None	None	None	None	True	None

6. Received data page ('Receive' tab):
   The received data page displays the log for successfully received data. The 'Response data' column shows the collected data for Temperature (T), Pressure (P), and Humidity (H), expressed in the units of °C, hPa, and %, respectively.

KAIST BlueMouse Home Send Log Received Data Scheduled Commands Update Admin User: **Choongdoll**

January 15, 2020
5:04:24 PM
GMT: +09:00

Log: events with received data

Clear Logs

Send Datetime	Chip Name	Mac	Scheduled	Category	Command	Type	F	P	N	Result	Response data
2020-01-15T08:04:15.289609+00:00	TPH Sensor	F1:25:68:BC:41:01	True	Tools	Temperature	None	0	0	-1	True	27.639
2020-01-15T08:03:37.789876+00:00	TPH Sensor	F1:25:68:BC:41:01	False	Tools	Humidity	None	None	None	None	True	20.8
2020-01-15T08:03:34.189557+00:00	TPH Sensor	F1:25:68:BC:41:01	False	Tools	Pressure	None	None	None	None	True	1020.8
2020-01-15T08:03:31.265272+00:00	TPH Sensor	F1:25:68:BC:41:01	False	Tools	Temperature	None	None	None	None	True	27.159

Load CSV

Supplementary Tables

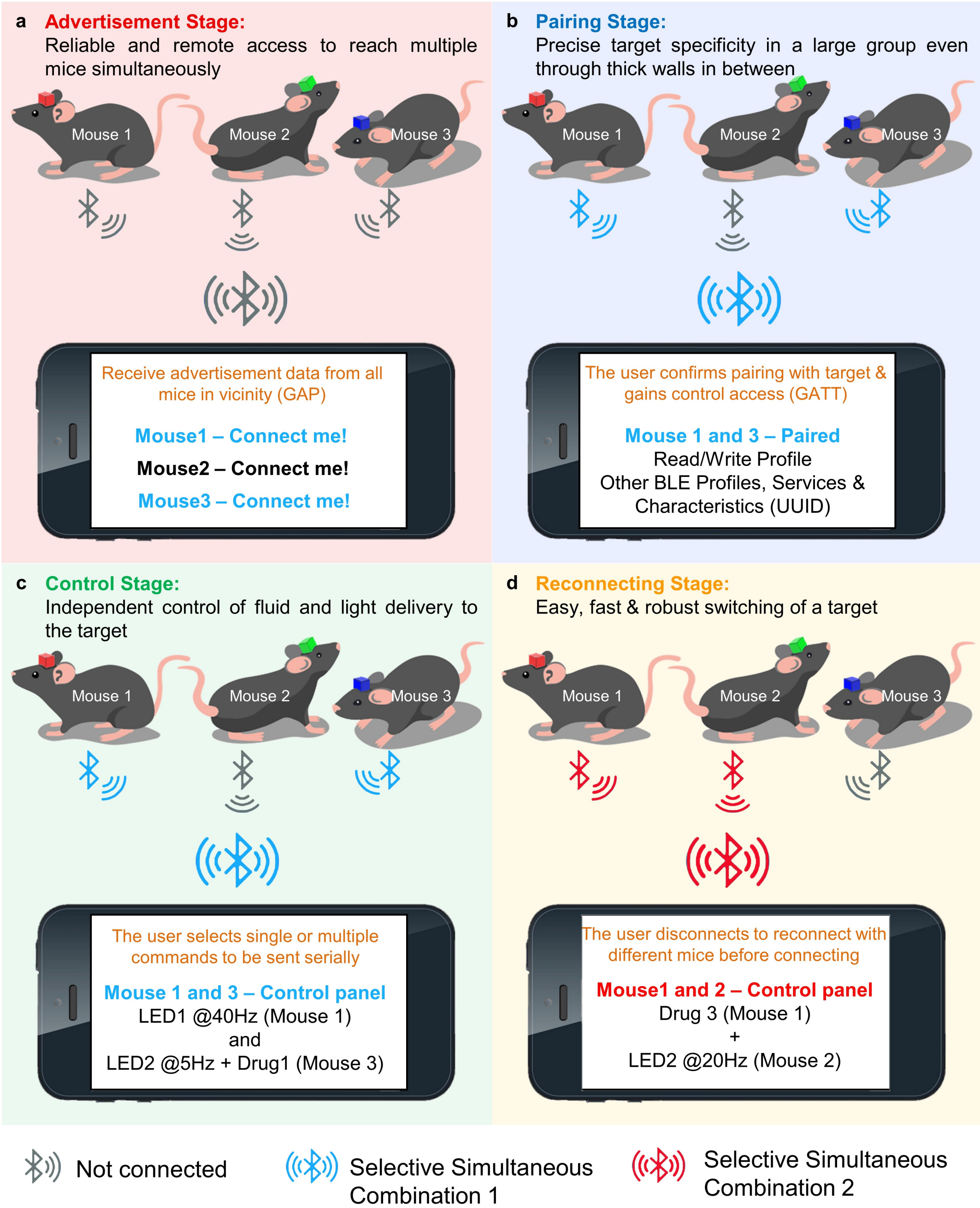
Supplementary Table 1 | Comparison of high throughput technologies in neuroscience.

	WNBN (This paper)	**Kim et al., 2021**	**Shao et al., 2017**	**Reinert et al., 2019**	**Parkison et al., (2012)**	**Beraldo et al., (2019)**	**PhenoMaster (Commercial; TSE Systems)**	**PhenoTyper (Commercial; Noldus Information Technology)**	**Promethion Line (Commercial; Sable Systems International)**
Application	Automated Large-scale and bi-directional global IoT ecosystem (GENERIC)	Wireless RF based peripheral optogenetic implants (SPECIFIC)	Semi-Automated Smartphone Control of Optogenetic Cells in Diabetic Mice (SPECIFIC)	Automated Olfactory phenotyping of group-housed mice (SPECIFIC)	Behavioral Phenotyping in Rodent Home Cages (SPECIFIC)	Automated touchscreen based cognitive assessment (SPECIFIC)	Multimodal Home Cage Monitoring (SPECIFIC)	Behavioral Observational Cage with Optical stimuli and sensors (SPECIFIC)	Multiplexed metabolic and behavioral sensing of up to 8 cages (SPECIFIC)
Type	Optofluidic stimulation + Ambient sensing	Optogenetic stimulation	Optogenetic stimulation	Olfactory sensing	Movement, Food, and water sensing	Touch sensing	Metabolic, behavioral & physiological sensing	Infrared LED and optical sensing	Phenotype, behavioral, calorimetry, respirometry or gas sensing
Wireless	Internet + BLE	RF	Internet + RF	RFID	X	X	X	X	X
Range	Global	<1 m	Global	<1 m	X	X	X	X	X
Modular Customization	Very High	None	None	Low	Low	Low	Low	Low	X
Simultaneous Selectivity and Scheduling	Yes (100+ devices)	No (<8 devices)	No (<6 devices)	No	No	No	No	No	No
Cost	Very Low	Very High	High						

Supplementary Table 2 | Comparison of the state-of-the art internet-based control schemes.

Wireless control technology	**WNBN technology**	**Shao *et al.,* 2017**
User Interface	Webpage based system	Android based system
Access point	Accessed through any laptop, smartphone or any system with a browser	Accessed only through an “Android” smartphone.
Number of users	Multiple User Control and Access	Single User Control
Device and temporal specificity	Scheduled Single and Multiple Control	No Scheduled Control, no device specificity
Limitation of device access	Better IoT for large diverse stimulations in large populations	Stimulation limited by space inside the coil
Range of device access	Longer ‘stimulation end’ range (<100m)	Very short ‘stimulation end’ range (within Antenna)
Output capability	Can trigger different commands and combinations in different populations	Can only trigger same stimulation on different populations
Output compatibility	Can give both AC and DC triggering to any type of implant or behavioral device at any frequency or specified parameter	Can only give AC triggering of LED blinking at a certain frequency
Input capability	Can receive triggers from behavioral equipment and/or video capture software	N/A
Wireless access scheme	Internet → BLE	Internet → RF
Wireless information transfer scheme	Low Energy BLE based end interface	High energy required for RF transmission
Relative costs	Affordable - No need to bulky electronics, antennas or special setup, but does require one or more Raspberry PIs	Most expensive - Needs large RF sources, antennas and special coil setup
Throughput (~number of animals/per control unit)	Scalable to hundreds of implants and other devices	As many as can fit inside coil – likely 1 cage of mice
Directionality limitations	No limitation of directionality on efficient stimulation	Efficiency is dependent on directionality and has a line-of-sight handicap

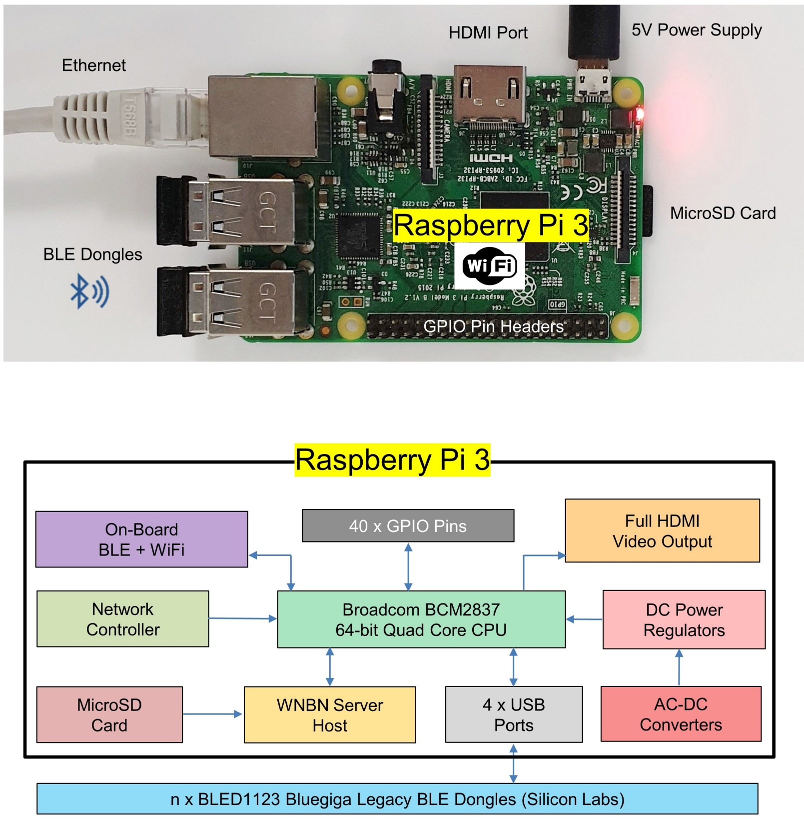
Supplementary Figures



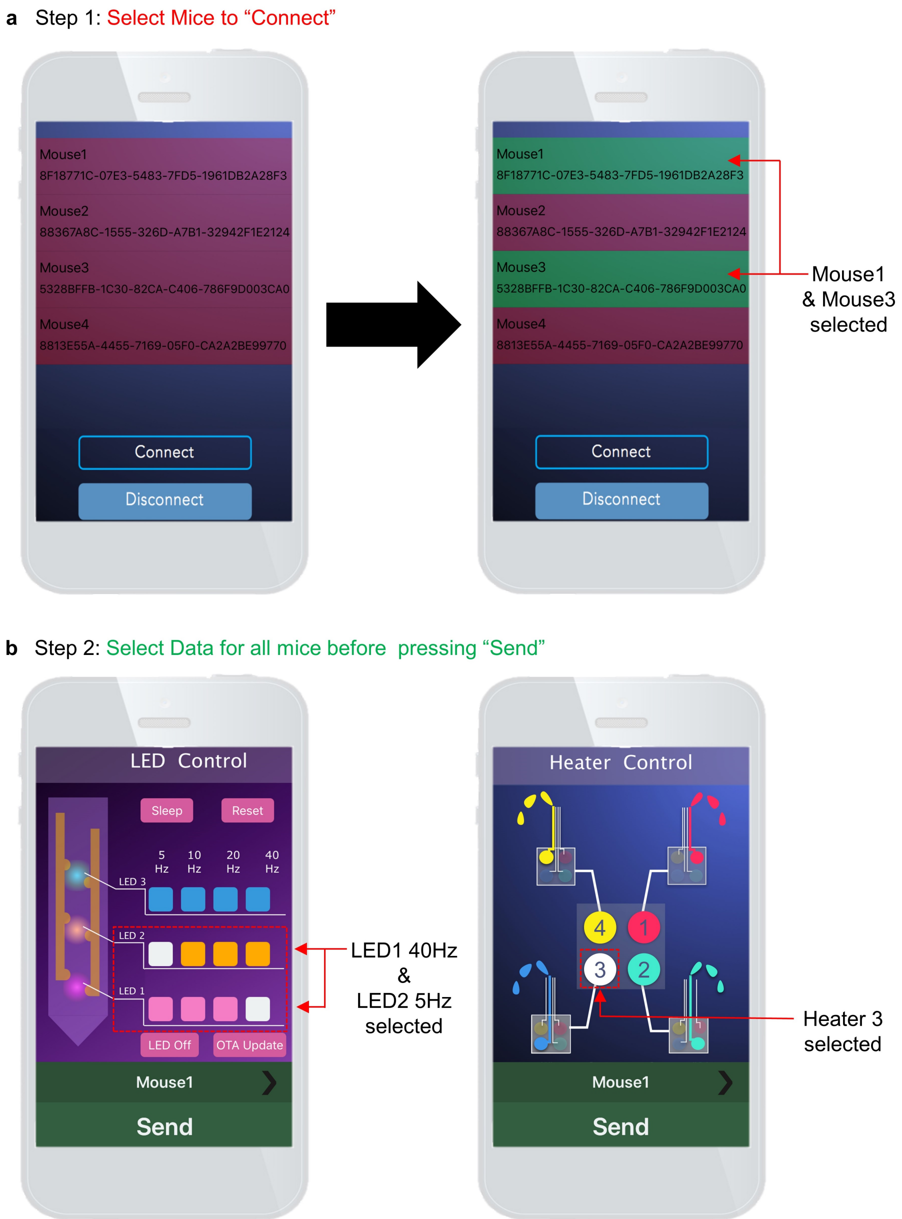
Supplementary Fig. 1 I Schematic illustration of selective simultaneous control in a WNBN. a, Advertisement Stage: The advertisement information of all neural devices (red, green, blue) in the vicinity of the Remote Control Centre (RCC) is collected by the RCC (smartphone or webserver), which is then displayed on the its graphical user interface (GUI). This information is handled by the Generic Access Profile (GAP) part of the Bluetooth Protocol Stack. Each mouse will have a unique advertisement name and such identification can help researcher select a specific group of mice within a large cohort with 100% accuracy. **b**, Pairing Stage: After the user selects a custom group of target mice (red and blue here) through the GUI of the RCC and clicks on the "Connect" button, all target devices stop advertising and enter into "connection mode" or "paired state", while the other remaining devices keep advertising (available to other users in the WNBN). The RCC then can access the various services and characteristics of the devices. This information is handled by the Generic Attribute Profile (GATT) of the Bluetooth Protocol Stack. **c**, Control Stage: After the target devices are paired, the user has the ability to selectively and simultaneously trigger one or multiple output modalities in each target device (independent of each other) within the paired subgroup. **d**, Reconnecting Stage: After finishing controlling a specific group of devices (red and blue), they can be easily disconnected and then will start advertising again. Meanwhile, a new subgroup of mice can be paired with the network, following the same communication protocol (steps **a** to **d**).

a

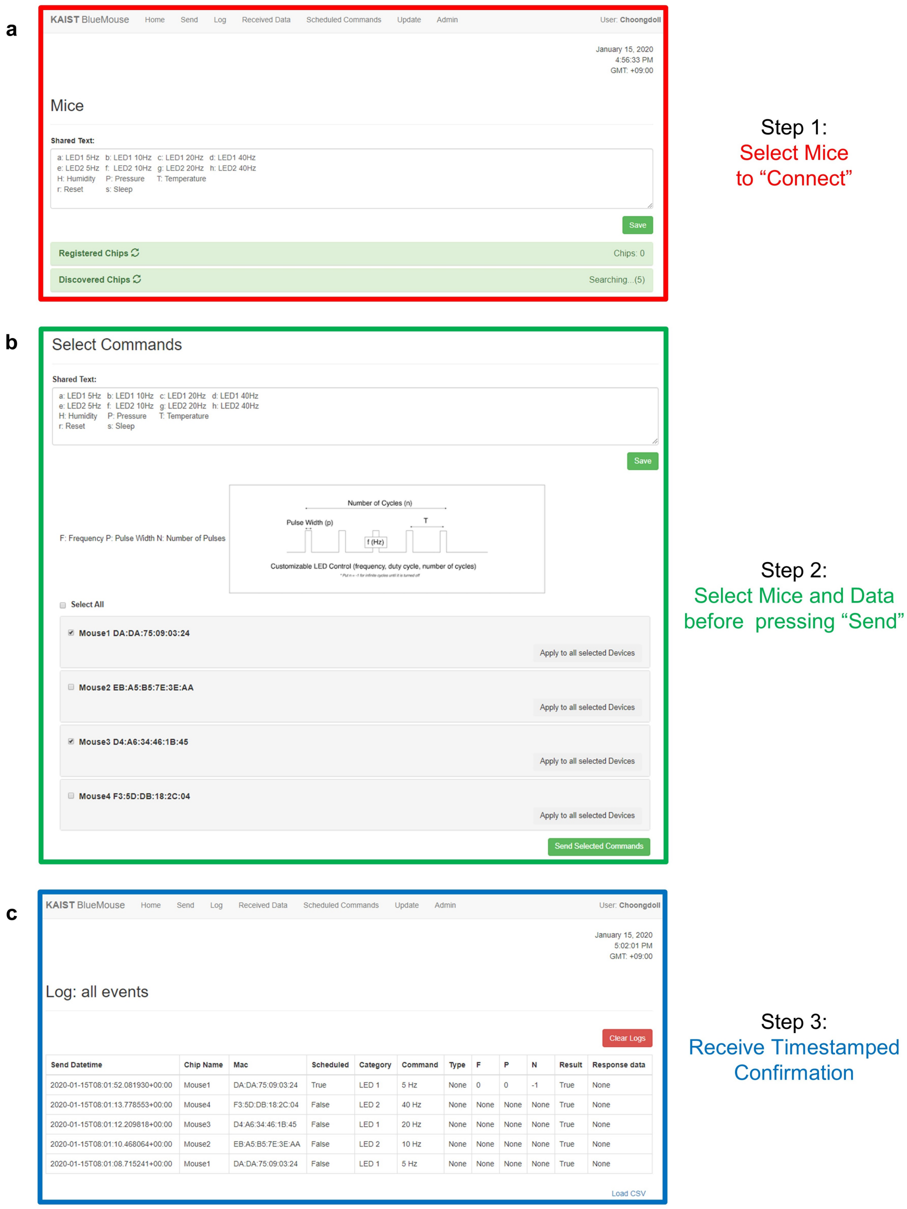
b



Supplementary Fig. 2 | WNBN minicomputer setup for global control mode. a-b, Optical image (**a**) and schematic component diagram (**b**) of a minicomputer (Raspberry Pi 3 Model B). To enable global WNBN control mode, a minicomputer is used to host a custom WNBN webserver (loaded inside its microSD card) over the Internet. Furthermore, multiple BLE dongles (BLED112, Silicon Labs) allow it to communicate seamlessly with numerous wireless RCMs in the vicinity (<100 m) once the command is received and decoded from the remote user. 40 GPIO pins enable access to the external equipment while the input power supply helps power various electronic components on the minicomputer. The ethernet port helps provide fast internet access. The HDMI port can be used to debug the minicomputer.

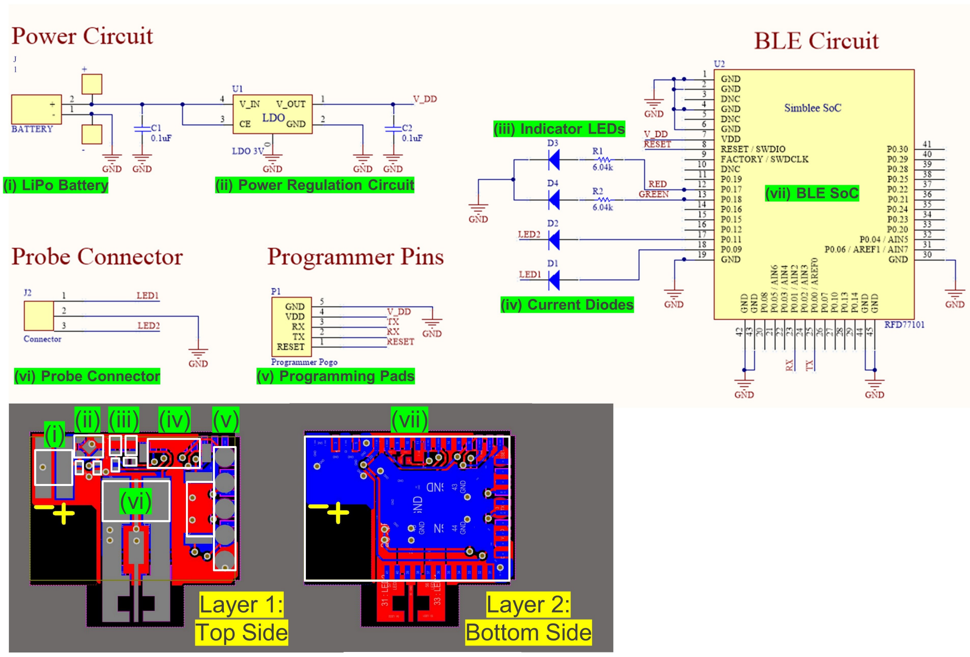


Supplementary Fig. 3 l Smartphone user interface for local piconet control, showing control of multimodal RCMs in two simple steps. a, Connect multiple target mice and/or WNBN enabled conventional equipment. **b**, Select multiple multimodal commands for each connected mice (by switching between devices using arrows on right and left of the device name) before finally pressing "Send" button. The smartphone app also has ability to provide timestamped confirmations which can be seen upon shaking the phone while the app is running.

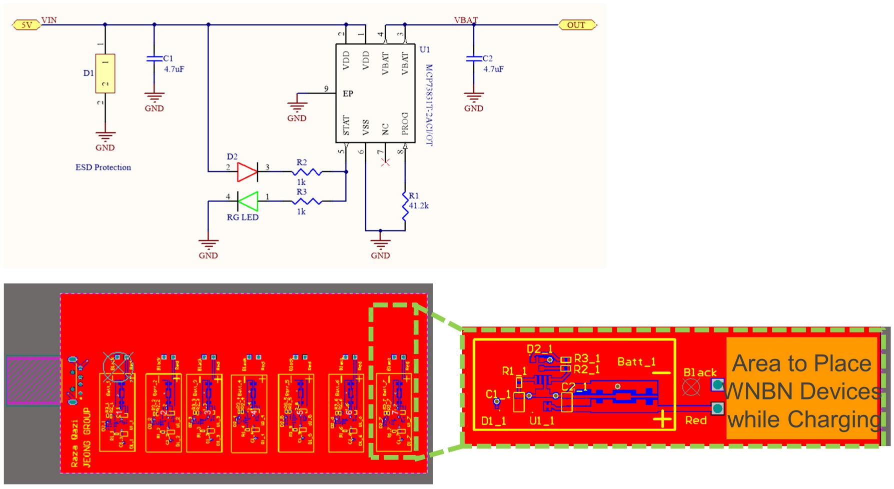


Supplementary Fig. 4 I Webserver user interface for global internet control in three steps. a, In the “Home” tab, connect multiple target mice and/or WNBN enabled conventional equipment. **b**, In the “Send” tab, select which of the connected mice you want to send data (defined in webserver settings) and press “Send” button. **c**, Verify the timestamped confirmations of the sent signals in the “Log” tab. The last step is highly recommended here since the researcher is not expected to be present in the laboratory to verify the wireless communication unlike the case of local piconet control.

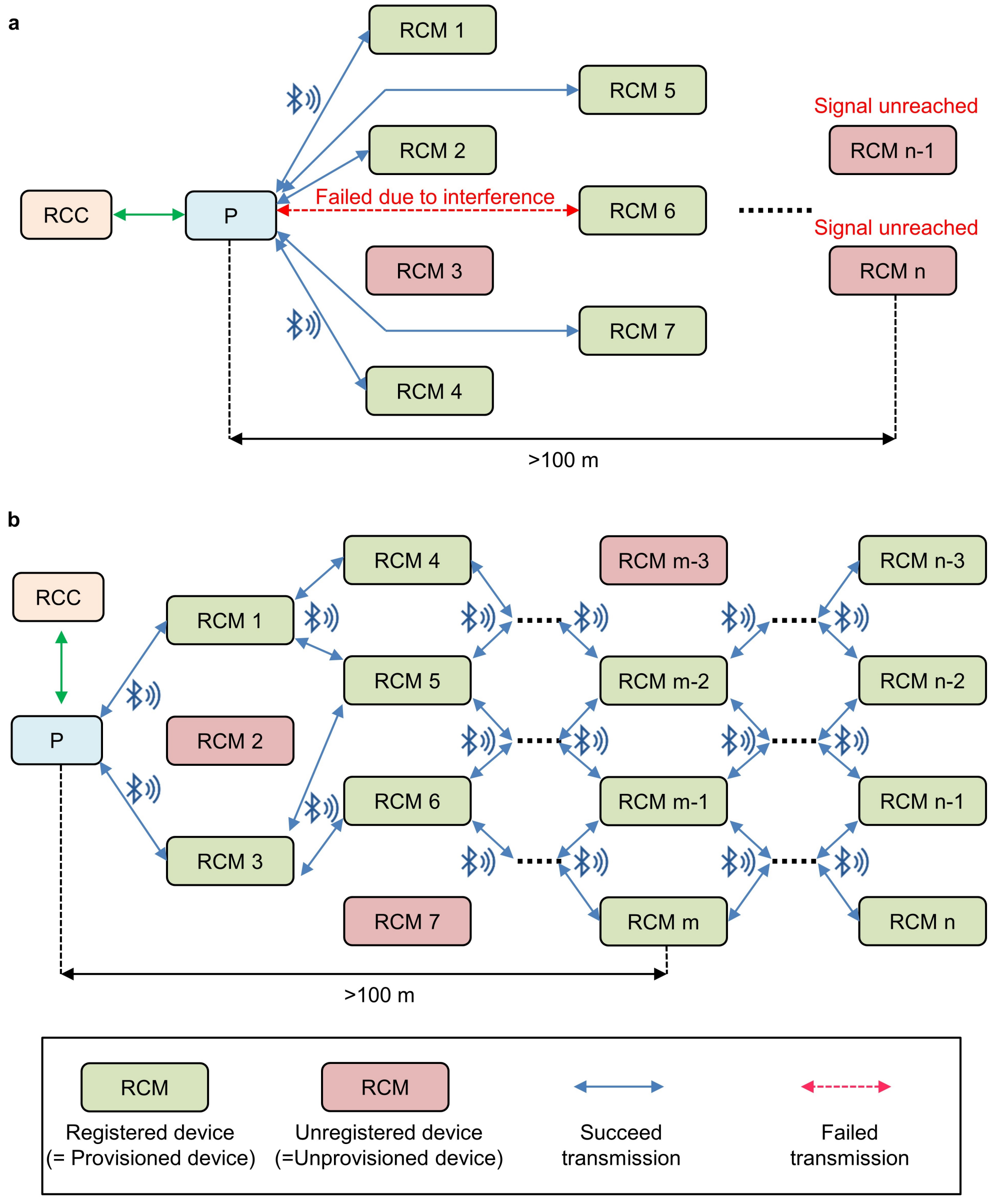
a



b



Supplementary Fig. 5 | Wireless BLE RCM and charger design. a, (Top) A schematic circuit diagram highlighting various connections and components that form the RCM – (i) Rechargeable Lithium Polymer (LiPo) battery (J1) for power supply, (ii) Power regulation circuit (U1, C1, C2) to improve efficiency, (iii) Indicator LEDs (D3 - red, D4 - green) with serially connected 6.04 kΩ resistors (R1, R2) for visual cues, (iv) Current limiting diodes (D1, D2) to limit current flowing to the µ-ILEDs, (v) UART custom port (P1) for programming and debugging, and (vi) Probe connector (J2) for modular assembly with the neural probe. (Bottom) PCB layout design of the circuit. The connector electrodes (vi) are designed to fit long (3.4 mm high M50-3140345, Harwin Inc.), medium (2 mm high M50-3130345 series, Harwin Inc.), or low (0.6 mm high BM29B-2DP/2-0.35V(53), Hirose Connector) profile connectors with unique electrode pitches for easy customizability in assembly orientation (straight vs. right angled connectors) and design (to adjust to custom probe electrodes as well as with conventional tools and equipment connectors). For experiments requiring more structural strength than offered by a single connector, multiple connectors can be accommodated in series. The PCB design can be easily redesigned to adjust additional control signals and data acquisition lines by using any of the Simblee BLE module's analog and digital GPIO ports that can communicate with the neural probes through multi-pin connectors with additional pins (1.27mm Archer Series, Harwin Inc.). **b**, (Top) A schematic circuit diagram highlighting various connections and components that form the RCM charger. (Bottom) PCB layout design of the circuit. The layout consists of 7 individual charger circuits, which allow each charger to charge seven RCMs simultaneously to support large scale in-vivo testing.



Supplementary Fig. 6 I Schematic diagram comparing the signal flow of the conventional Bluetooth communication and Bluetooth Mesh network. a, Block diagram showing one-to-many topology of the conventional Bluetooth network. A single piconet network (or RCC, in the case of Local Piconet) transmits signals to multiple registered RCMs. Failure in transmission can occur due to interference among RCMs (e.g., RCM 6), and RCMs located at a far distance (i.e., >100 m) from a piconet network are unable to be registered (e.g., RCMs n-1 and n). **b**, Block diagram showing many-to-many topology of Bluetooth Mesh network to highlight its reliability and scalability. Every node (i.e., RCC, piconet network, and registered RCMs) form a mesh network and act as both transmitter and receiver. By delivering the received signals to nearby registered RCMs, the number of RCMs and the distance between RCC and RCMs barely affect the performance of the system.

Captions for Supplementary Videos 1–5

Supplementary Video 1 | WNBN graphical user interface for Local Piconet control.
Supplementary Video 2 | WNBN graphical user interface for remote Internet control.
Supplementary Video 3 | Global Internet remote control using WNBN technology.
Supplementary Video 4 | WNBN *In vivo* study using Local Piconet control.
Supplementary Video 5 | Wireless control of multiple devices using Bluetooth mesh technology.

Captions for Supplementary codes 1–3

Supplementary Code 1 l Code in Simblee microcontroller for wireless control of a μ-ILED optical probe.
Supplementary Code 2 l Code in Simblee microcontroller for bidirectional wireless sensing of temperature, pressure, and humidity.
Supplementary Code 3 l Code in Simblee microcontroller (RCM) for parsing custom data strings for real-time customization of experimental parameters (such as frequency (f), pulse width (p) and number of cycles (n) for each individual optogenetic experiment).