



# Arduino based intra-cerebral microinjector device for neuroscience research



Jorge Bravo-Martínez <sup>\*</sup>, Sonia Ortega-Tinoco, Julieta Garduño, Salvador Hernández-López

*Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), PO Box 70250, Ciudad de México 04510, Mexico*

## ARTICLE INFO

### Article history:

Received 28 June 2022

Received in revised form 8 June 2023

Accepted 26 June 2023

### Keywords:

Arduino

Intracerebral injection

Stereotaxic surgery

Neuroscience

Microinjection

Open hardware

## ABSTRACT

Stereotaxic surgery is a less invasive form of surgery that uses a three-dimensional coordinate system to place instruments at a specific location in the brain. Through this type of surgery, one can place needles among other tools within the structures of the brain. Therefore, injections can be given in order to deliver substances that cannot cross the blood–brain barrier. The two most important parameters of the microinjection to control are volume and speed. The volume should not be so large that it displaces the brain tissue and tears it. The injection speed must also be slow so that the liquid that comes out of the syringe can diffuse into the tissue without displacing it and damaging it. Thus, the objectives of the present work are: 1) To develop not a 3D printed prototype but an end-user device. 2) The device must be for animal research only. 3) It must have the same precision in volume and speed as commercial devices. 4) It must be adjustable for microsyringes from 0.5  $\mu$ l to 1 ml. 5) It must be possible to place it directly on the stereotaxic surgery apparatus and to use it separately. 6) The price must be substantially lower than that of the commercial devices.

© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Hardware in context

Stereotaxic surgery is a less invasive form of surgery that uses a three-dimensional coordinate system to place instruments at a specific location on the body. A mechanical framework of cartesian, polar, or mixed coordinates is used. The placement of this frame in the body requires fixed anatomical references. As the only place with these characteristics is the skull, this type of surgery is used only in neurosurgery. The stereotaxic surgery method was first published in 1908 by British researchers Victor Horsely, a neurosurgeon, and Robert H. Clark, a physiologist [1]. Although this system was initially used for neuroscience research, it was used in 1933 by the North American physician Martin Kirschner in humans to treat trigeminal neuralgia [2]. Through this type of surgery, one can place: probes, electrodes, needles, among other instruments, within the structures of the brain, making the following procedures possible: ablation, biopsy, injury, stimulation, electrophysiological recording, and injection [3,4]. Currently, intracerebral injection is widely used for the treatment of various diseases in humans [5] and neuroscience research in animals, mainly rodents [6,7,8]. It has been used in order to deliver toxins [9], neurotransmitters [10], antibiotics [11], stem cells [12], viral vectors [13], and nanomaterials [14], among others, to various brain structures. These substances could not otherwise cross the blood–brain barrier. The injection systems were initially manual and later automated. They consist of a body where the microsyringe is held and which contains in its interior a

<sup>\*</sup> Corresponding author.

E-mail address: [jbravo4@mac.com](mailto:jbravo4@mac.com) (J. Bravo-Martínez).

stepper motor. This motor is connected to a fine threaded rod that moves a platform which in turn pushes the syringe's plunger. The body can be attached to the stereotaxic surgery apparatus. The motor is controlled by a microprocessor which controls the two most important parameters of the microinjection: volume and speed. The volume should not be so large that it displaces the brain tissue and tears it, forming an abscess. The maximum volume tolerated by brain tissue in a single injection has been determined to be 1500 nL [15,16,17]. The injection speed must also be slow so that the liquid coming out of the syringe can diffuse into the tissue without displacing it and damaging it. Thus, the maximum injection rate should not exceed 250 nl/min [18–20]. Since the microsyringe's needle will move through the brain tissue [21], its characteristics are also important, so that minimum damage is caused to the nervous tissue. These characteristics are the diameter, the length, and the tip. The microinjection system does not control these characteristics, but the researcher must choose the indicated microsyringe (Hamilton, Reno NV, USA) [22], depending on the size of the brain to be injected. For example, Hamilton syringes are suitable for the human brain and larger animal species but not for rodents. In this circumstance, an insulin syringe needle (held by the stereotaxic surgery apparatus) connected to a Hamilton syringe by a tube with a smaller diameter and rigid walls is implemented. There are many models of microinjectors on the market, some of which are more specific for intracerebral injection, while others are for general purposes. Some of those specific for neuroscience can be mounted on the stereotaxic apparatus [23]; others cannot or are larger [24]. These, depending on the capacity of syringe used, can inject volumes from nanoliters to microliters with slow injection rates for intracerebral injection (less than 250 nl/min). General purpose syringe pumps have injection volumes from microliters to milliliters [25] and cannot be placed in the stereotaxic surgery apparatus. The latter are not suitable for intracerebral injection. Others are for clinical use in humans that cannot do this job, and we will not consider them in this work. Prices range from \$1,550.00 USD to \$6,000.00 USD. Thus, the objectives that we considered for the design presented in this work are as follows:

- o To develop a 3D printed device that is not a prototype but rather an end-user device.
- o It must be for animal research only.
- o It must have the same precision in volume and speed as specific commercial devices.
- o It must be adjustable for microsyringes with total volumes from 0.5 µl to 1 ml.
- o It must be possible to place it directly on the stereotaxic surgery apparatus and to use it separately
- o The price must be substantially lower than that of the commercial devices.

## Hardware description

The device was 3D printed with PLA plastic, and the goal was to achieve not a prototype but a final product that could be used immediately. It has the same components as commercial devices but it is cheaper. The central part of the device is made up of a 28BYJ-48 stepper motor which is quite inexpensive and can be found practically anywhere. This motor is connected to an M5 threaded rod with steps of 0.8 mm, which improves the precision of the microinjector. We measure the pitch of the rod and found that it was 0.795 instead of 0.8. We used 0.795 in the calculations. This motor is controlled by an Arduino Uno microprocessor using an EasyDriver V4.4 motor controller. Since the motor shaft is eccentric due to the gearbox it contains, to move the threaded rod, it uses a 1:1 motor and rod gear. Unlike commercial microinjectors, the part where the microsyringe is held is designed so that any microsyringe can be placed, as well as a 1 ml insulin syringe. Another difference with commercial microinjectors is that it can be mounted on the stereotaxic surgery device or on a scissor jack stand (included in the design) printed in PLA. The jack was included in the design as it is helpful in cases where the hose used to connect the microsyringe to the needle mounted on the stereotaxic surgery device must be very short.

- This intracerebral microinjection system is designed for animal neuroscience research animals (it is NOT suitable for human). It has the same precision as the commercial systems but it is cheaper.
- Since it can be placed on its platform, it can also be used to deliver substances directly to cells in culture.
- Another application that this device may have, is applying substances during electrophysiological experiments on isolated cells or brain slices.
- It can also be used in microfluidics where nano or micro-volumes are used.

## Design files summary

### Bill of materials summary

### Build instructions

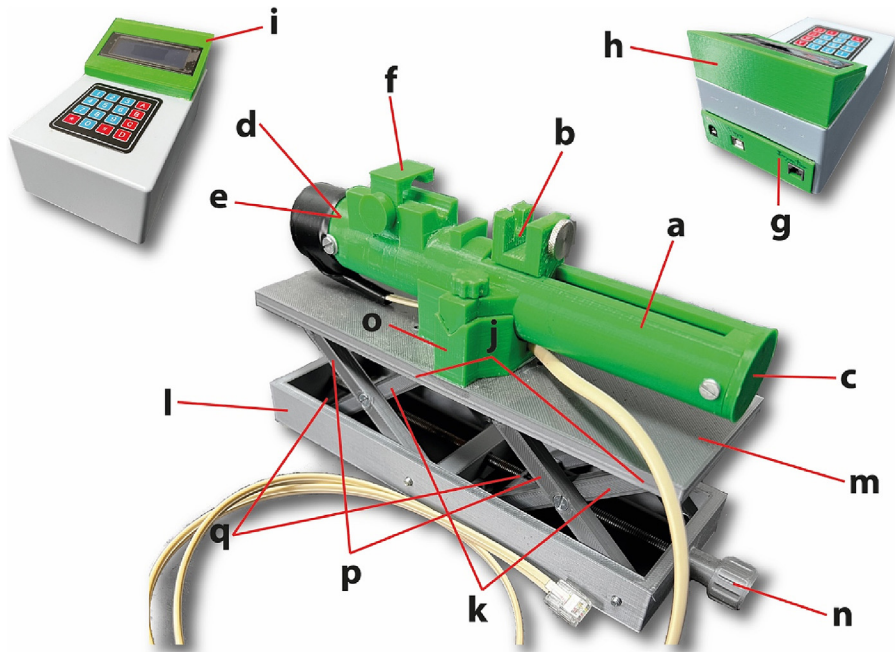
**3D print.** All STL files must be 3D printed, and those that appear in [Table 1](#) with an asterisk are printed as the indicated copies. They are printed in 1.75 mm PLA plastic, without rafts, without supports, with an infill of 30%.

### Microinjector.

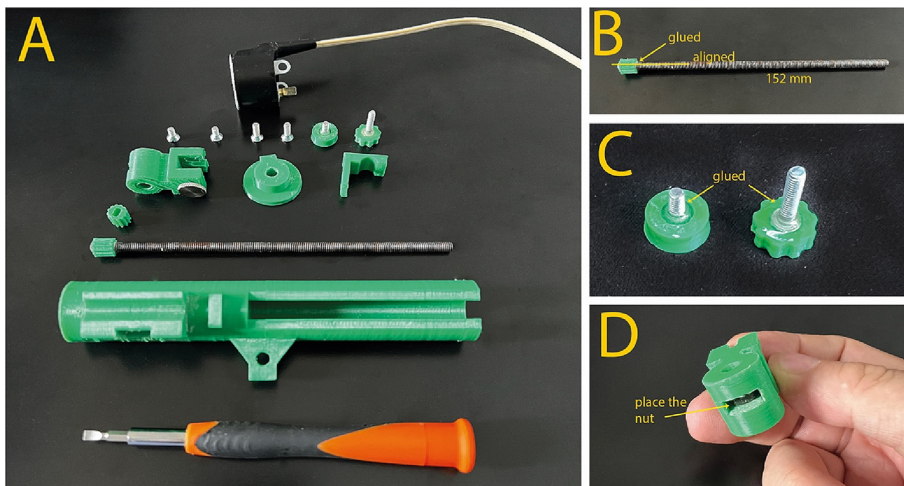
- Cut 152 mm of threaded rod and sand the edges of the ends ([Fig. 2B](#)).
- The rod gear must be screwed onto the rod and glued with cyanoacrylate-based glue ([Fig. 2B](#)). Care must be taken that they are aligned.

**Table 1**

Hardware name	Microinjector II
Subject area	Neuroscience
Hardware type	Biological sample handling and preparation
Closest commercial analog	UMP3 UltraMicroPump From World Precision Instruments Inc, USA.
Open source license	CC-BY 4.0
Cost of hardware	\$190.88 USD
Source file repository	Mendeley, <a href="https://doi.org/10.17632/m77h37dfj.1">https://doi.org/10.17632/m77h37dfj.1</a>



**Fig. 1.** The different parts of the microinjector and their location.



**Fig. 2.** Assembly of the microinjector. A parts list for the microinjector and the first steps of assembly.

- Two screws must be glued with their respective knobs with epoxy glue (Fig. 2C).
- A nut must be placed on the bottom of the plunger. It has to be well placed so that the threaded rod can pass through it without forcing anything (Fig. 2D).
- The motor gear has to be placed on the motor, and it only has one way to place it. It must not be glued (Fig. 3D).
- The threaded bar is placed in the body of the microinjector, as shown in the figure (Fig. 3A). Subsequently, the plunger is inserted from the back and the rod is screwed into the plunger nut (Fig. 3B, 3C).
- The motor is placed with its gear engaging it with the gear of the bar (Fig. 4A).
- The motor is screwed into position (Fig. 4B).
- The body cap is screwed into position (Fig. 4C, 4D).
- The syringe holder is placed in position and screwed on (Fig. 4E).

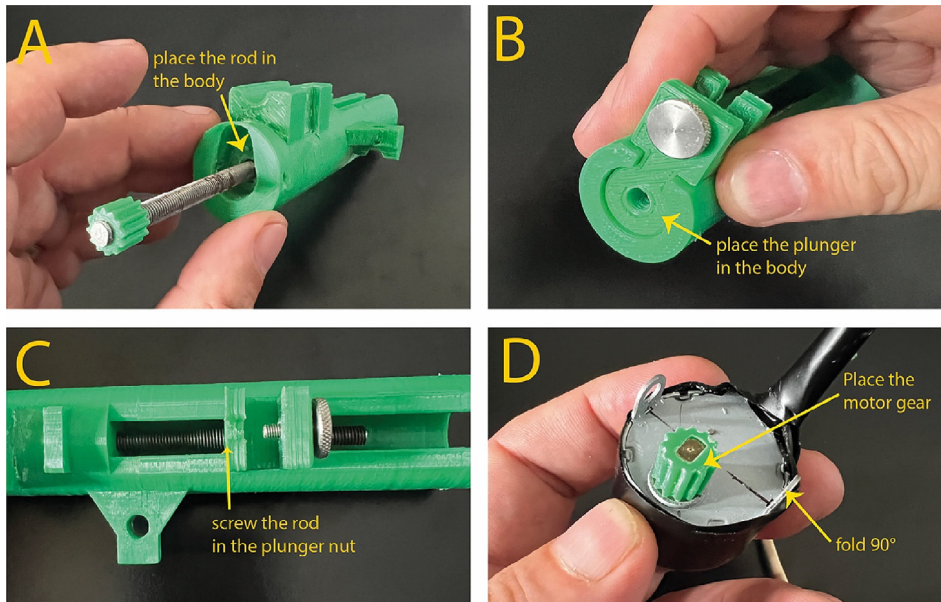


Fig. 3. Steps for assembly of the microinjector.

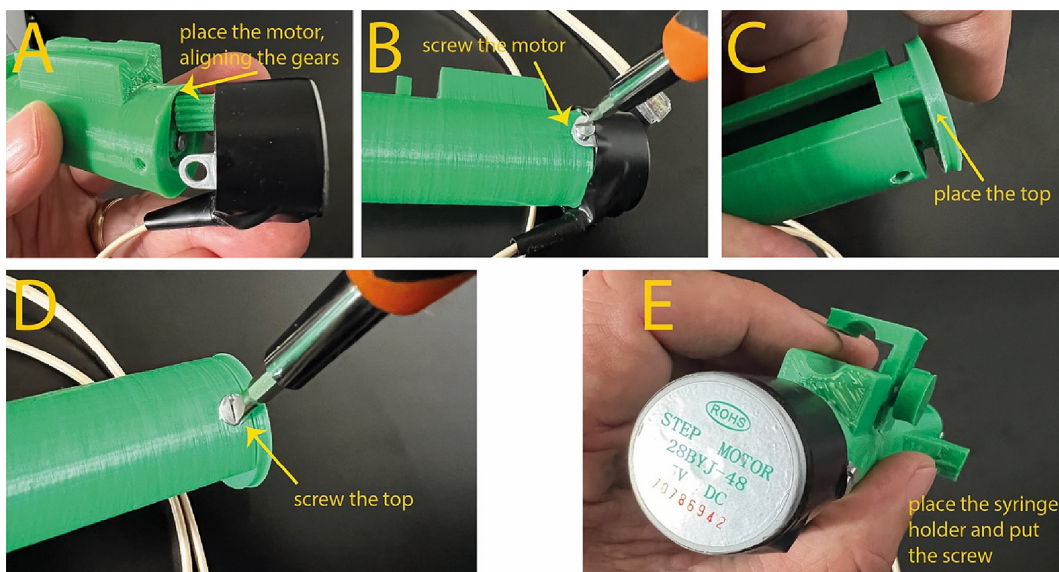


Fig. 4. Final steps for assembly of the microinjector.

**Housing.**

- Cut the back of the housing so that the circuit mount is in place (Fig. 5a) and glue it to the housing base (with epoxy or cyanoacrylate-based glue), as shown in Fig. 5b.
- Glue the display chassis at one end of the housing (Fig. 5c).
- Cut the housing to gain access to the display chassis from the back and be able to pass the display cables to the Arduino (Fig. 5d).
- Cut an access to the keyboard cables in the housing (Fig. 5e).
- Screw the display into the display chassis cover (Fig. 5f).
- Glue the cover to the display chassis (Fig. 5g).
- Pass the display and keyboard cables inside the housing (Fig. 5h).
- Glue the keyboard in place (Fig. 5i).
- Screw the Arduino and EasyDrive on the circuit mount (Fig. 5j).
- Glue the power jack and the RJ11 connector (Fig. 5k).

**Stand scissor jack.**

- Screw the internal arms with the external arms to form four scissors (Fig. 6a).
- Join two scissors by screwing the slider to the internal arms (Fig. 6b).
- Join the external arms by screwing the bar (Fig. 6c).
- Join the formed structure to the base by screwing it through the other ends of the external arms (Fig. 6d).
- Make a second structure and screw it to the base like the first (Fig. 6e).
- Place nuts in the slots of the sliders (Fig. 6f).
- Cut 19 mm of threaded rod, sand the edges of the ends and pass it through the holes in the base and the sliders, screwing it into the nuts found in the sliders, leaving it protruding at the ends (Fig. 6g).
- At one end, screw a nut and glue it with cyanoacrylate glue and screw the knob on the other side and glue it also (Fig. 6h). Screw loosely so the rod can rotate freely.
- Screw the other ends of the internal arms to the cover, as shown in Fig. 6i.
- Glue or screw the microinjector pedestal to the lid (Fig. 6j).

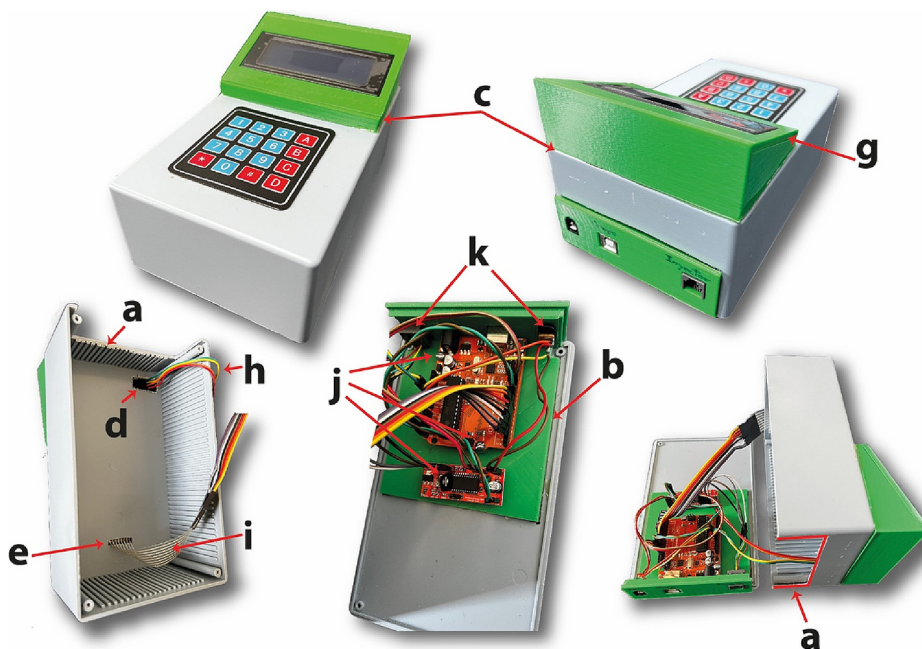


Fig. 5. Steps for assembly of the microinjector controller.

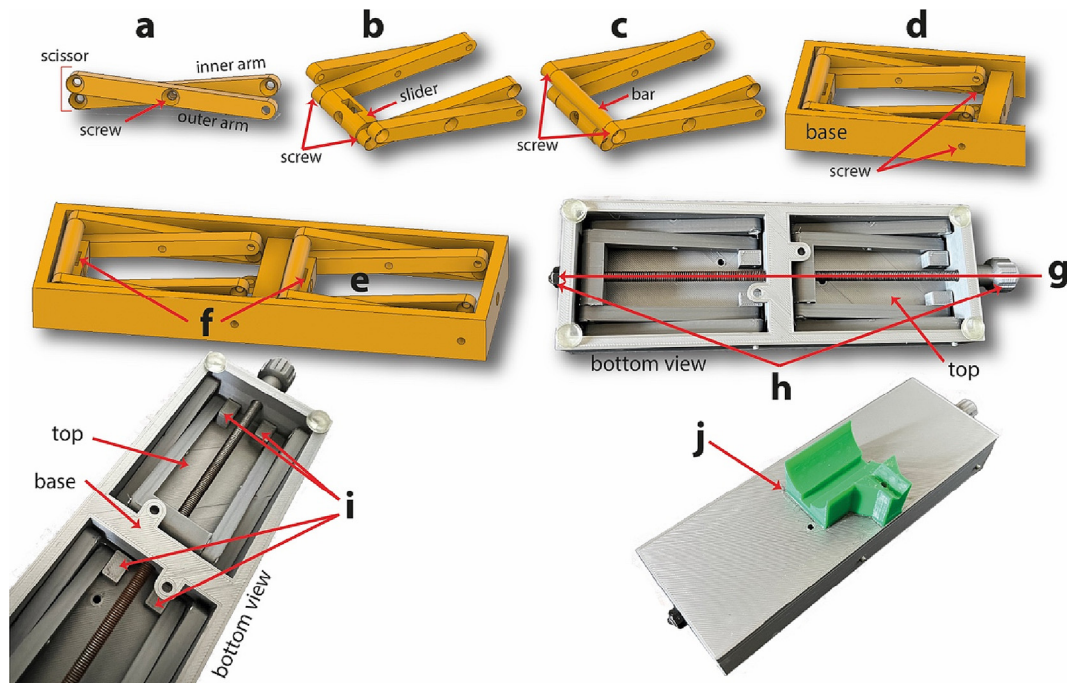


Fig. 6. Steps for assembly of the scissor jack.

#### Electronics.

- 28byj-48 motor
  - The stepper motor is unipolar and we have to convert it to bipolar [26,27].
  - The red wire is cut or desoldered, and it is no longer used.
  - The BJ11 telephone cable is cut off the connector on one side, and the cables are uncovered.
  - The cables can be soldered directly to the motor PCB (unsoldering those with the motor) or to the motor cables. **ALWAYS BE CAREFUL THAT THE COLOR OF THE TELEPHONE CABLE MATCHES THE COLOR OF THE CORRECT MOTOR CABLE, AS ILLUSTRATED IN Fig. 7.**
- Easy Drive motor controller
  - All cables connected to the EasyDrive are soldered, so the female connectors of the Dupont cable are cut. The male end must be connected directly to the Arduino in the places indicated in Fig. 7. **Take care not to connect it elsewhere.**
  - The BJ11 connector cables (telephone) are also soldered to the EasyDriver, **taking care that the color of the cable is in the correct position** in the EasyDriver Fig. 7.
  - Power supply cables are soldered in the EasyDriver and the power jack with those from the Arduino.
- LCD
  - Four male–female Dupont cables have the female connector cut off and soldered to the LCD. The SCL and SDA cables are connected to the Arduino with the male connectors in the positions indicated in Fig. 7.
  - The power cables go directly to the Arduino, also soldered on one side and connected to the Arduino.
- Keypad
  - The keypad has a female connector where the Dupont ribbon can be directly connected. Eight male–male leads of the Dupont ribbon cable are connected directly to the keypad and Arduino. **ALSO, BE CAREFUL TO CONNECT THEM IN THE POSITION INDICATED IN Fig. 7.**
- Power supply
  - The entire circuit can be powered with 7 V, except the display that is powered by 5 V taken from the arduino. The circuit draws 200 milliamps with the engine running. This means that the power supply can be 7 V, 500 or more milliamps.

#### Firmware.

- Installation of the Arduino IDE 2.0.0 or newer
  - Visit <https://www.arduino.cc>, download, and run the installer.
  - Follow the instructions.
  - Detailed instructions [28]

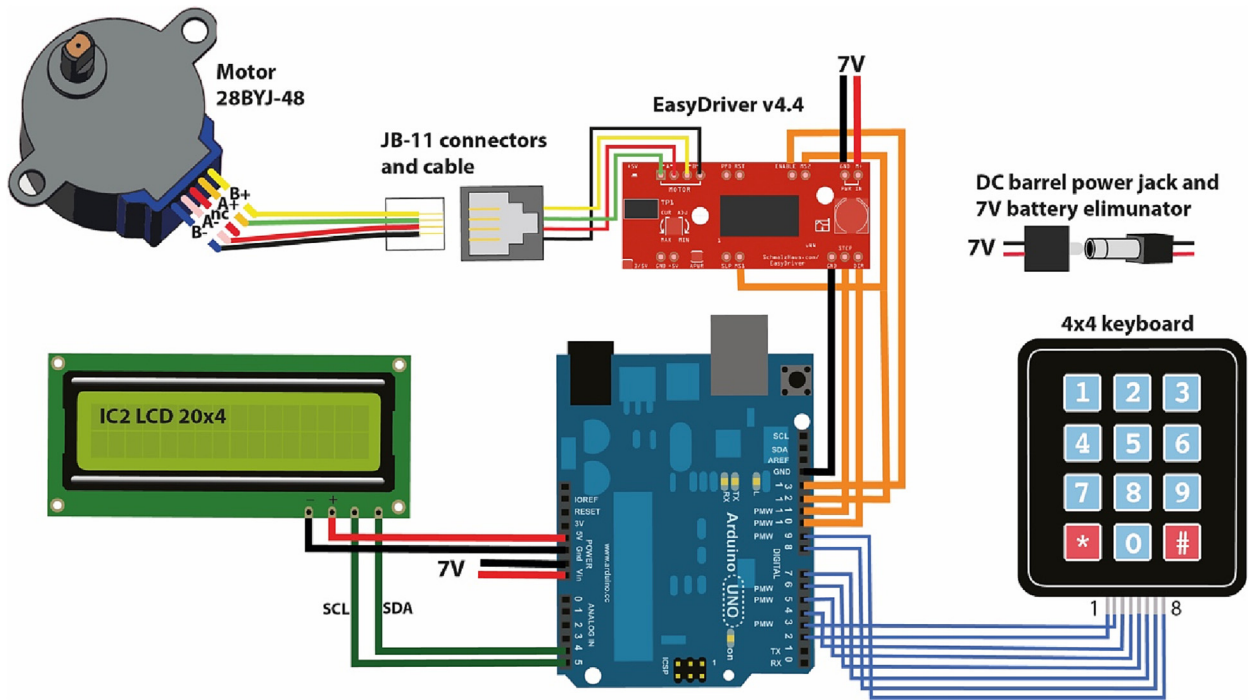


Fig. 7. Electronic circuit connections.

- Install libraries
  - o The libraries that need to be installed are Keypad.h v3.1.1, Wire.h v2.3.7, LiquidCrystal\_I2C.h v1.1.2, and AccelStepper.h v1.0.4
  - o To install a new library, use the Library Manager of the IDE.
  - o Open the IDE.
  - o Click on the “Sketch” menu and then on “Include Library > Manage libraries”.
  - o A new menu appears. Put the library’s name in the “Filter your search” field.
  - o It appears below and click on “INSTALL” or “UPDATE”.
  - o Detailed instructions [29]
- Upload the sketch to the IDE and the Arduino Uno
  - o Run the Arduino IDE. Go to “File”, then to “Open”.
  - o Locate the sketch folder and upload it.
  - o To upload it to the Arduino Uno, click on the arrow pointing to the right in the IDE menu bar.
  - o Detailed instructions [30]

*Operation instructions.*

- Main Menu
  - o In this menu, there are two options: A, to go to the setup menu and prepare the injection; B, to go to the liquid loading and injection menu.
  - o The desired letter is typed (Fig. 8).
- A Setup
  - o First decide in which units the volumes are going to be written: 1 = nanoliters; 2 = microliters; 3 = milliliters. Key in the desired number (Fig. 8a).
  - o VJ = The maximum volume of the syringe (in the previously chosen units). Type the number, the key “\*” is the decimal point (Fig. 8B). When you finish entering the number, type the “#” symbol, which is the “Enter” key, and go to the next one (Fig. 8).
  - o SJ = The distance between zero and the maximum volume of the syringe scale in millimeters (Fig. 8c). It is measured with a ruler from zero to the maximum volume (in mm) of the syringe scale and keyed in (Fig. 8j). Type “Enter” for the next one.
  - o VI = The volume to be injected, also in the previously chosen units. Type and press “Enter” (Fig. 8d).

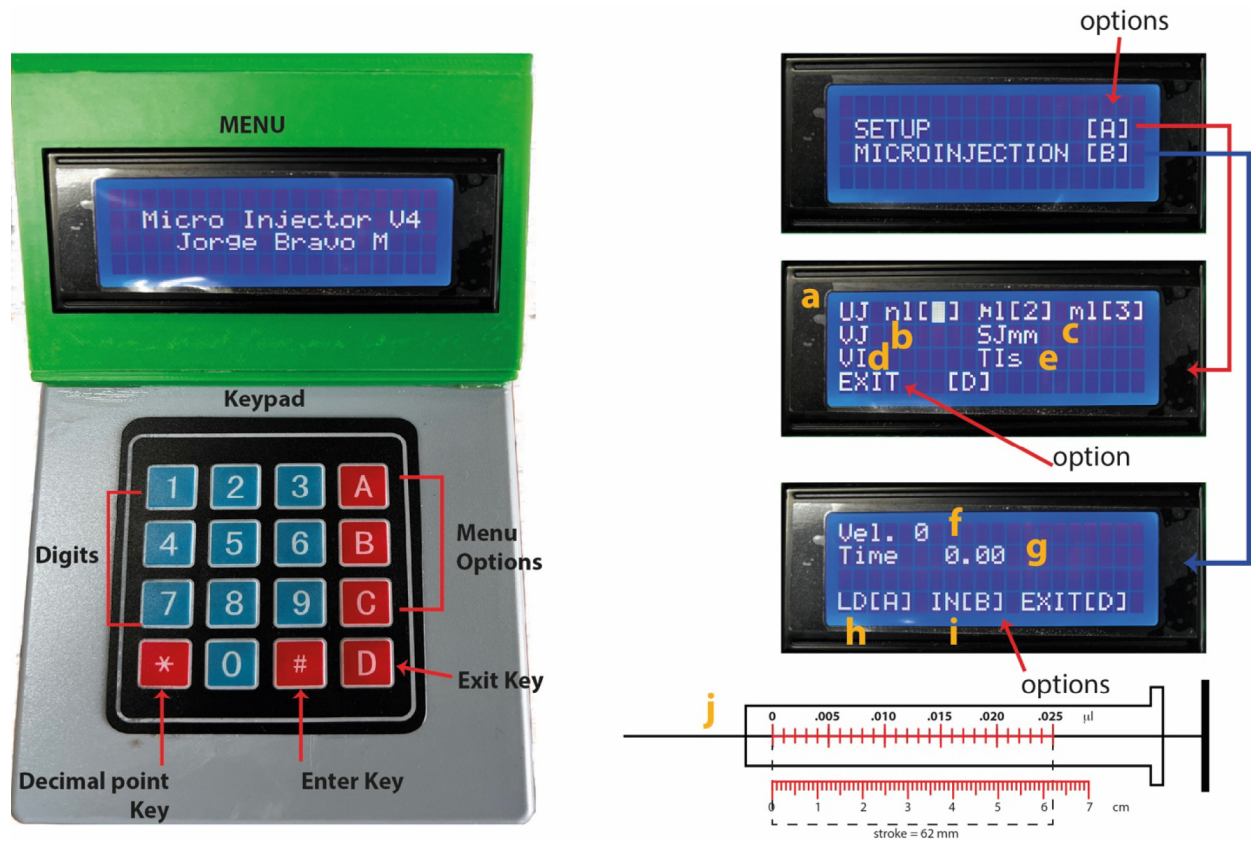


Fig. 8. Software menu screens. The stroke of the syringe is the distance measured in mm from zero volume to the maximum of the scale.

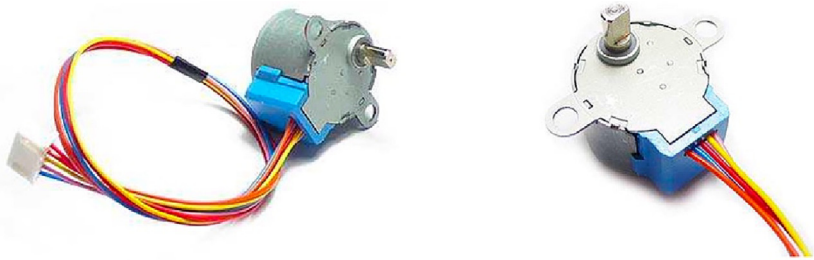
- o TI = Injection time in seconds. Type and press “Enter” (Fig. 8e) to save the parameters in the memory. If another microinjection is going to be done with the same parameters, it is unnecessary to retype them just go directly to the “Microinjection” menu.
- o If only one parameter will be changed, the others can be skipped with the “D” key, but always make sure that “Enter” is pressed after the new parameter.
- o Option “D” is to exit to the previous menu.
- B Microinjection
  - o Vel = gives us the injection speed in motor steps per second (Fig. 8f).
  - o Time = indicates the injection time in seconds (Fig. 8g).
  - o Option “A” starts loading the selected volume into the syringe at maximum speed. A status bar appears (filled circles), indicating what remains to be loaded.
  - o Option “B” starts the intracerebral microinjection. A status bar appears (filled squares), indicating the elapsed injection time.
  - o Option “D” is to abort and exit to the previous menu.

## Validation and characterization

### Performance characterization

This design is based on the 28BYJ-48 motor; it is a 64-step motor with a 1:64 gear box (Fig. 9) that makes the motor capable of taking (in complete steps) 4090.9 steps per revolution. Since the motor is coupled to an M5 threaded rod with 0.795 mm steps, the motor advances that distance per revolution. The rod gives more precision to the engine. To control the motor, we use the EasyDrive because it is less bulky than the others and has an excellent library. Thus, when we tested this motor with the EasyDrive in the lab, we found that it makes 2045.5 steps per revolution, which is the data we used for the firmware. Since each motor is different, you can do the same procedure of measuring the angle per step multiplying it by 360. This value is substituted in the firmware “Driver\_Microinjector\_V4.3, on line 103, which says “const int steps = 2045;”. To measure the step angle, a paper gauge is attached to the motor rotor. On a blank sheet, draw a circle of approximately





Rated voltage :	5VDC
Number of Phase	4
Speed Variation Ratio	1/64
Stride Angle	5.625°/64
Frequency	100Hz
DC resistance	50Ω±7%(25°C)
Idle In-traction Frequency	> 600Hz
Idle Out-traction Frequency	> 1000Hz
In-traction Torque	>34.3mN.m(120Hz)
Self-positioning Torque	>34.3mN.m
Friction torque	600-1200 gf.cm
Pull in torque	300 gf.cm
Insulated resistance	>10MΩ(500V)
Insulated electricity power	600VAC/1mA/1s
Insulation grade	A
Rise in Temperature	<40K(120Hz)
Noise	<35dB(120Hz, No load, 10cm)
Model	28BYJ-48 – 5V

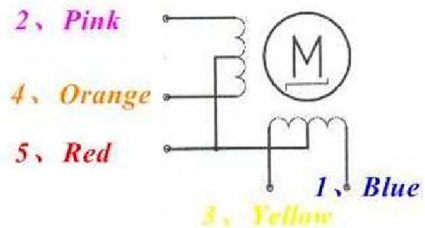
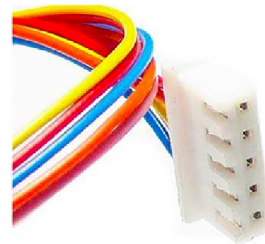


Fig. 9. 28byj-48 motor specifications.

15 cm with a compass and a straight line through the center. Place the motor in the center of the circle and align the indicator on the rotor with the straight line. Activate the motor at very low speed, count 10 steps and make a mark in the circle. Make a straight line between the mark and the center of the circle and measure the angle between both lines. Divide the angle by 10 and then divide 360° by the angle, this gives us the number of steps per revolution. See (Table 2 and Table 3).

This motor is unipolar and needs to be changed to bipolar in order to be used with the EasyDrive, but the method is straightforward. To relate the advance of the syringe plunger with the injected volume, the internal diameter of the syringe would have to be measured and the volume calculated. This method is impractical as we would have to ask the user to make this measurement with a caliper. A more practical method is to measure with a ruler the scale of the syringe from zero to maximum volume, which tells us how many millimeters the plunger must move to push the maximum volume of the syringe. We call this variable the “SJ” syringe stroke. The software also asks the user:

- o the maximum volume of the “VJ” syringe.
- o the volume to inject “VI.”
- o the injection time “TI”.

The software calculates:

- o the millimeters that the plunger has to advance in order to move the injection volume

$$DI = (VI * SJ) / VJ \tag{1}$$

- o the steps that the motor has to take to move the volume VI.

$$DI = abs((ID/0.795) * 2045) \tag{2}$$

Table 2

Design file name	Fig. 1**	File type	Open source license	Location of the file
<b>Microinjector</b>				
Microinjector body	a	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> "Available with the article".
Microinjector plunger	b	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Microinjector top	c	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Motor gear	d	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Rod gear	e	STL	CC-BY4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Syringe holder	f	STL	CC-BY4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Knob x2*		STL	CC-BY4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
<b>Housing for electronics</b>				
Circuit mount	g	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Display housing	h	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Display housing top	i	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
<b>Stand scissor jack</b>				
Bar x2*	j	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Inner arm x4*	k	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Jack base	l	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Jack top	m	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Knob	n	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Microinjector pedestal	o	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Outer arm x4*	p	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
Slider x2*	q	STL	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
<b>Electronic circuit</b>				
Microinjector circuit		jpg	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article
<b>Firmware</b>				
Driver_Microinjector_V4.3		ino	CC-BY 4.0	<a href="https://doi.org/10.17632/m77h37dfjf.1">https://doi.org/10.17632/m77h37dfjf.1</a> Available with the article

\* Number of parts to print.

\*\* Position of component in Fig. 1.

o the speed that the engine must reach so that in the desired amount of seconds "TI", it injects the desired volume "VI".

$$Vel = abs(DI/TI) \quad (3)$$

### Validation

Method: In the laboratory we tested the precision of the volume of displaced deionized water, with volumes equal to (6 µl) and greater than one drop (so that it can be detached from the microsyringe). All the validation was carried out with a 25 µl Hamilton micro-syringe. Then we measured the weight of the ejected volume with an analytical balance. To carry out the gravimetric measurements, a site with stable humidity and temperature conditions was selected. A recently calibrated Sartorius Practorus analytical balance (Sartorius Instruments, Goettingen, Germany) was used, which has a sensitivity of 10<sup>-6</sup> g; a Durac digital thermometer, model SP BEL\_ART (HB Instruments, NJ, USA) with a maximum uncertainty of 0.02 °C; an Extech model SD700 digital barometer (Extech Instruments, NH, USA) with an uncertainty equal to or less than 0.1 hPa.

Evaporation loss calculation. As a first step, the evaporation loss must be estimated to accurately convert mg to ml. To do this, deionized water was placed in a glass container and placed on the scale. 10 µl was loaded into the syringe with the

Table 3

Component	Number	Cost per unit - currency	Total cost - currency	Source of materials
28BY-48 stepper motor.	1	\$60 USD	\$60 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
EasyDriver V4.4	1	\$120 USD	\$120 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
Arduino Uno R3	1	\$160 USD	\$160 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
DuPont Wire Ribbon (assortment)	1	\$1899 USD	\$1899 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
4x4 membrane keypad	1	\$5 USD	\$5 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
20x4 LCD R2	1	\$100 USD	\$100 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
RPI1 phone jack	1	\$2.20 USD	\$2.20 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
RPI1 phone 6ft cable	1	\$1.13 USD	\$1.13 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
Plastic housing for electronics C37-A3.27-C29	1	\$899 USD	\$899 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
Female 3Pin DC Power Connector Socket Jack Plug 1	1	\$1.99 USD	\$1.99 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
Universal AC/DC Adapter Multi-Voltage Regulated Switching Power Supply	1	\$15.98 USD	\$15.98 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
Stainless Steel M5-0.8 8ply All Threaded Rod	2	\$5.49 USD	\$10.98 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
M3 X 0.8MM nuts	3	\$0.13 USD	\$0.39 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
M3, 15 mm long flat head screws	20	\$0.29 USD	\$5.80 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>
PKA1.75 mm filament for 3D printing	1	\$21.90 USD	\$21.90 USD	Amazon.com <a href="https://www.amazon.com/dp/B073K18849">https://www.amazon.com/dp/B073K18849</a>

**Table 4**

Expected volume ( $\mu\text{l}$ )	Measured volume			
	Mean ( $\mu\text{l}$ )	SD ( $\mu\text{l}$ )	Accuracy (%)	CV (%)
6	6.1	0.18708	+1.6	3.06
7	6.93	0.13038	-1	1.88
8	8.06	0.26315	+0.75	3.26
9	8.822	0.20055	-1.9	2.27
10	9.964	0.11104	-0.36	1.11

microinjector and a 25  $\mu\text{l}$  Hamilton micro-syringe, at a maximum speed of 500 steps/sec. The balance was tared with the water container inside, and then the water container was removed. The water was expelled from the microinjector at a rate of 12 sec/ $\mu\text{l}$ , producing a total time of 120 sec. The water container was placed back on the scale, and the weight was measured. Four measurements were made and averaged, showing an evaporation loss of 0.007 ml for each injection cycle.

Gravimetric test. Five different volumes (6, 7, 8, 9, and 10  $\mu\text{l}$ ) of deionized water were loaded into the microinjector at maximum speed. Five measurements were made for each volume. They were expelled in 120 sec. into a container placed on the analytical balance. Temperature and barometric pressure were recorded for each measurement. Subsequently, mg of water was converted to ml with the following formula:

$$V_i = Z(mi + \text{mean } e) \quad (4)$$

$V_i$  = individual volumes (ml).

$m_i$  = individual masses (mg).

mean  $e$  = evaporation loss (mg).

$Z$  = Z-factor (ml/mg) see in published tables [31].

The mean, standard deviation (random error, precision), systematic error (accuracy in %), and coefficient of variation (CV in %) of each group of volumes were obtained with Origin Pro 2021 software (OriginLab, MA, USA) (Table 4).

Mean and standard deviation were obtained with the well-known formulas included in the software. The systemic error (accuracy) was calculated with the following equation:

$$e_s = (\text{mean}V - V_s)/V_s * 100 \quad (5)$$

mean $V$  = mean of each group of volumes.

$V_s$  = selected volume.

The coefficient of variation was calculated with the equation:

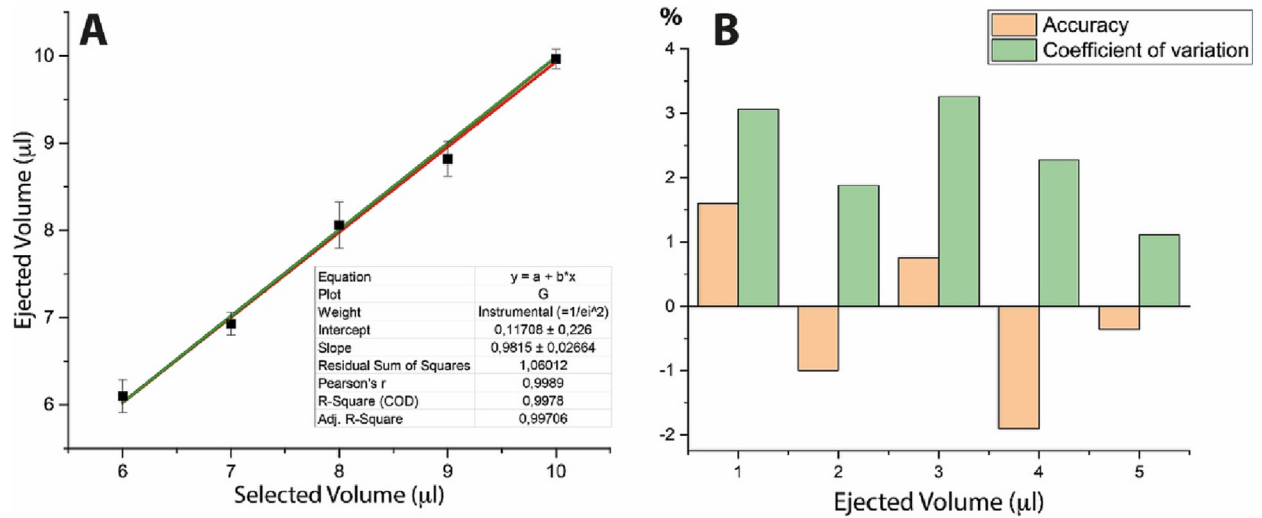
$$CV = 100 * s / \text{mean}V \quad (6)$$

$S$  = standard deviation.

## Results and discussion

Accuracy. Accuracy is defined as the repeatability of the measurements and it is calculated by taking the standard deviation. Table 4 shows the mean volume for each group of volumes, and judging by the standard deviation of each group, it can be seen that the dispersion is small. The same was verified by plotting the ejected volume means with their standard deviation as a function of the selected volume. The fit to a straight line is good, judging by  $r = 0.9989$  (Fig. 10A, red line). Regarding the variability coefficients (Table 4 and Fig. 10B), we can see that they are below 3.26%, which is quite acceptable for this range of volumes. When comparing a 25  $\mu\text{l}$  Hamilton micro-syringe with another brand (ILS, Germany) of the same volume, we see that its percentage of variability changes very little (data not shown). This variability leads us to suppose that the microinjector is involved in the performance of the device, but the syringe brand used is also a strong component. However, they are volumes that brain tissue tolerates very well. Unfortunately, the experimental data on the commercial microinjectors are not public, so we cannot compare them with the proposed design. Nevertheless, the theoretical precision values calculated as the minimum volume per step of the motor with a 10  $\mu\text{l}$  syringe. In the case of this design, it is 62 pl/step, and in the case of WPI's UMPE UltraMicroPump microinjector [22], it has to take 20,000 steps to advance 63 mm, which corresponds to 10  $\mu\text{l}$ . Therefore, it has a precision of 0.5 nl/step [22]. The difference between the two lies in the threaded rod, which in the case of the present design has a feed rate of 0.00039 mm/step, while that of the commercial design is 0.00315 mm/step.

Accuracy. Is related to the systematic error or also called offset of the mean. It tells us how far the mean is from the actual value. Even though the accuracy for a volume of 9  $\mu\text{l}$  was  $\pm 1.9$ , a volume of  $\pm 0.17 \mu\text{l}$  does not represent a problem for the nervous tissue Table 4 and Fig. 10B. If we compare the expected values (green line) with the ejected ones (red line, Fig. 10A), we see that the offset is minimal at small volumes and grows very little as the selected volume increases. Accuracy values



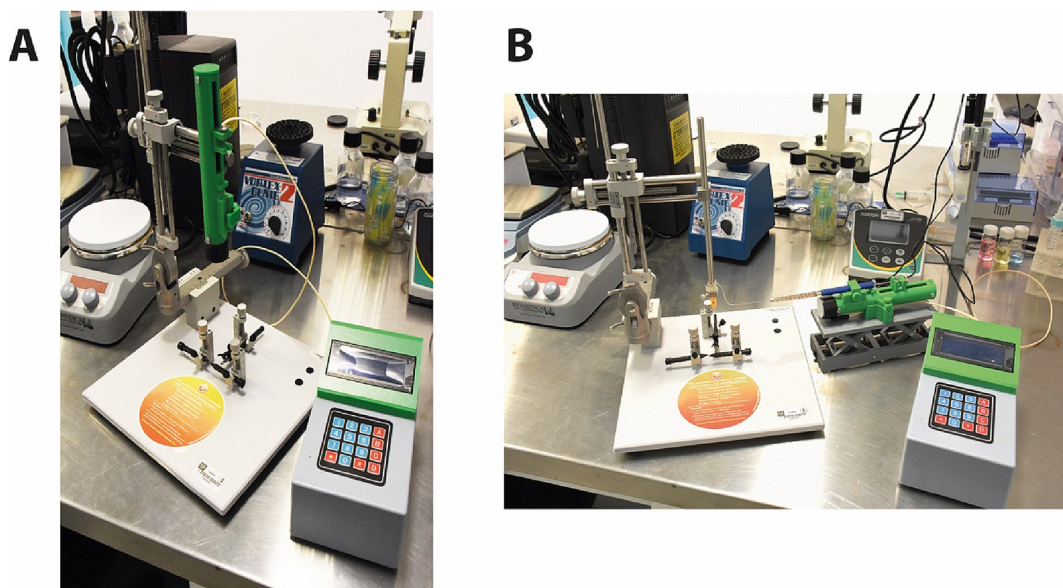
**Fig. 10.** Validation. A graph of ejected volume as a function of the selected volume. B graph of accuracy and variation coefficient.

reported for commercial microinjectors are merely indicated as being below  $\pm 0.5\%$ ; in the case of this design, it is below  $\pm 1.9\%$ .

The results of the validation experiments have only limited significance for the accuracy in the nanoliter range. But in the microliter range, this microinjector has enough precision for the work. In addition, as a result of 3D printing and electronics, "Open Access" is an order of magnitude cheaper than commercial systems. This microinjector can be mounted on the stereotaxic surgery apparatus or its platform, as shown in Fig. 11A and B.

*Scientific application*

**Method:** The experiments were conducted in accordance with the official Mexican standard for technical specializations for laboratory animal production, care, and use (NOM-062-ZOO-1999), and under the supervision of the Internal Committee for Animal Care Provisions and Use of Laboratory Animals (CUICAL) of the National Autonomous University of Mexico.

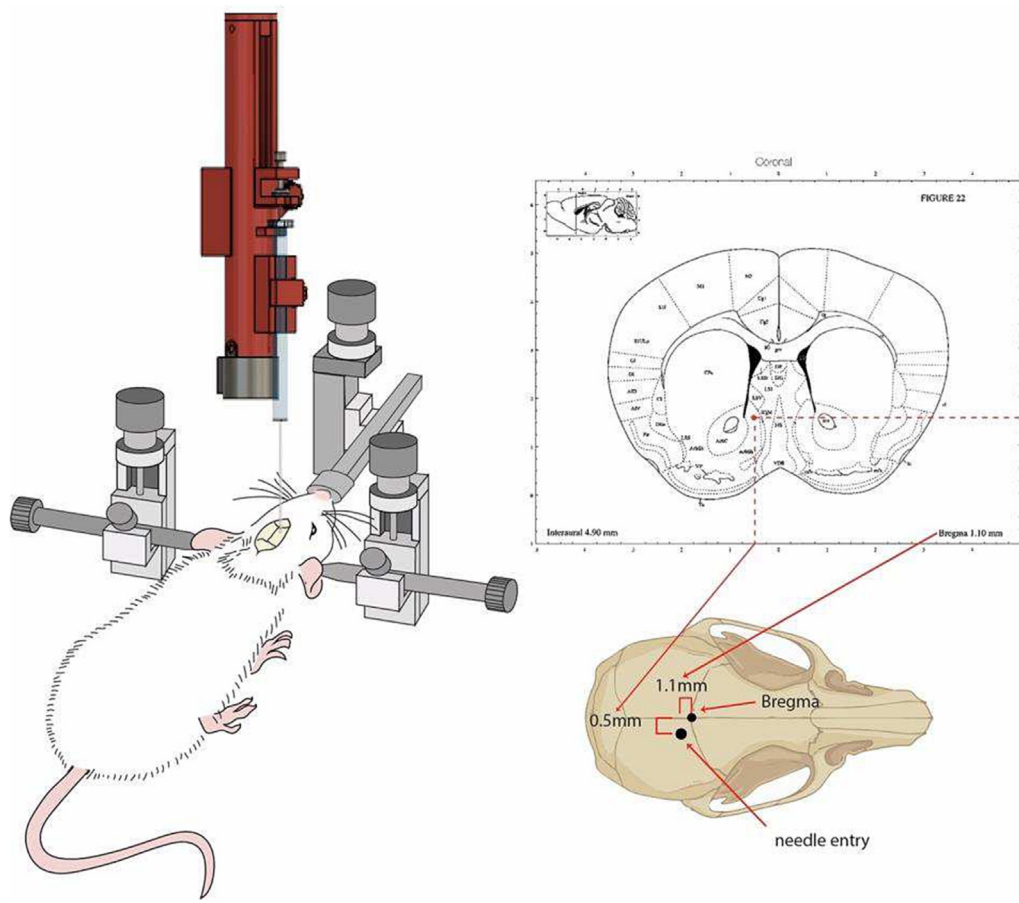


**Fig. 11.** Design. A microinjector mounted on the stereotaxic surgery apparatus. B microinjector mounted on its scissor jack outside the stereotaxic surgery apparatus.

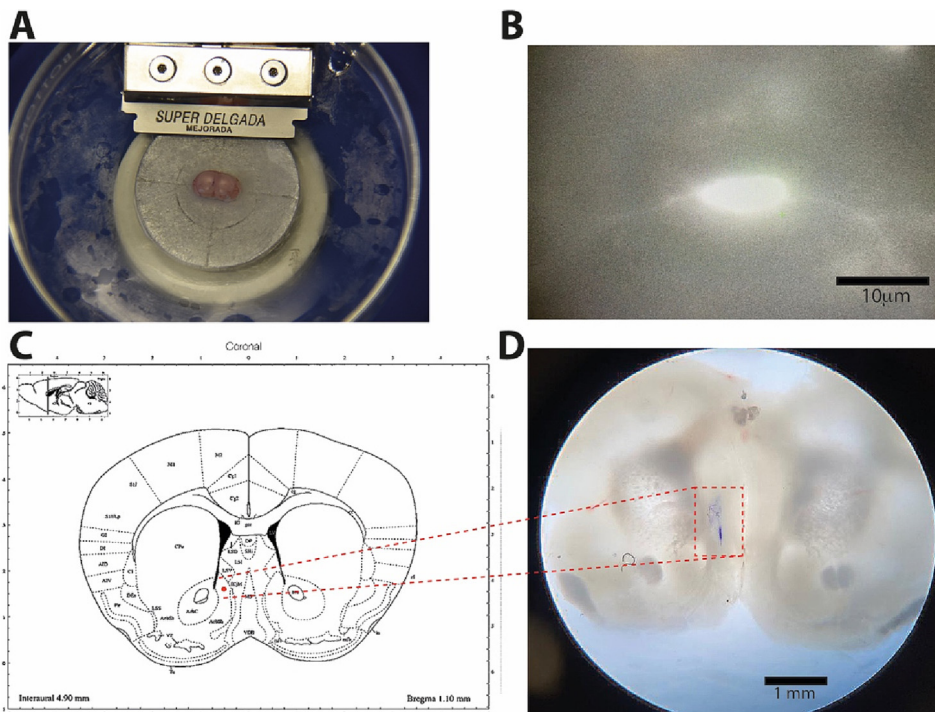
Three BALBC male mice and one Cre-D1 30–35 day old male with an approximate weight of 20–23 g were used. The subjects were kept in home boxes under controlled environmental conditions: 12–12 h light–dark cycle, temperature  $21 \pm 2$  °C, and humidity  $70 \pm 10\%$ . Subjects had ad libitum access to food and water until the day of sacrifice. The mice were obtained from the School of Medicine animal facility on the same day of the experiment.

**Stereotaxic Surgery.** Mice were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg, i.p.) (ANESKET®) and placed in a stereotaxic apparatus (Stoelting Co™). Sedation was monitored with the patellar reflex, and subjects were kept at a temperature of 35 °C using a thermoregulator. BALBC mice were microinjected with 0.6  $\mu$ l of methyl violet (BIOPACK) and Cre-D1 mice with 0.6  $\mu$ l of a viral vector containing the plasmid pAAV.Syn.Flex.GCaMP6f.WPRE.SV40 (Addgene), which contains the sensor for calcium GCaMP6f. The microinjections were in the coordinates for the nucleus accumbens from bregma: Anteroposterior + 1.1 mm, medial–lateral  $\pm$  0.5, and dorsoventral  $-4.2$  according to the Allen Institute Mouse Brain Atlas [16] (Fig. 12). The methyl violet microinjection time was 120 s, and it was allowed to diffuse in 120 s; for the viral vector, the time was 480 s, and it was allowed to diffuse in 300 s. The microinjector described in this work mounted on its platform was used. A 5  $\mu$ l Hamilton micro-syringe was utilized, to which a hose with an internal caliber of 0.01 in and rigid walls were adapted. This hose was connected to a 30G  $\times$  21 mm needle placed in the stereotaxic apparatus.

**Obtaining tissue.** After performing the surgeries, the mice were decapitated. The brain was removed and placed in a cold Krebs solution (4 °C) containing (in mM): 123.9 NaCl, 2.6 KCl, 1.2  $\text{KH}_2\text{PO}_4$ , 3.5  $\text{MgSO}_4\text{-H}_2\text{O}$ , 26  $\text{NaHCO}_3$ , 6.5  $\text{C}_6\text{H}_{12}\text{O}_6$  and 2.2  $\text{CaCl}_2\text{-H}_2\text{O}$  with a pH = 7.35, an osmolarity of 300 mOsm/L and balanced with carbogen (95%  $\text{O}_2$  and 5%  $\text{CO}_2$ ). The slices were kept in Krebs solution at room temperature 20–25 °C and with constant bubbling of carbogen. Subsequently, using a vibratome (Pelco 102, TedPella, INC), coronal slices of 300  $\mu$ m thickness were obtained, which contained the nucleus accumbens (Fig. 13C). For brains injected with methyl violet, the state of brain tissue was assessed under stereoscopic microscopy. For the mouse injected with the viral vector, after one hour of stabilization of the tissue in Krebs solution at room temperature, each slice was placed in the recording chamber (plexiglass) and perfused with Krebs solution at a rate of 4–5 ml/min at room temperature, using a gravity perfusion system. An upright microscope (Nikon FN1 Eclipse) with a 16X water immersion objective was used to visualize the cells. The tissue was stimulated with light pulses (488 nm wavelength) using an LED lamp



**Fig. 12.** Stereotaxic surgery. The animal is mounted on the apparatus and the lambda reference point is located, which is the point between the crossing of the sagittal and coronal suture. From this point on, the atlas of stereotaxic surgery maps all the structures of the brain in the three XYZ planes.



**Fig. 13.** Scientific Application of the microinjector. A The brain of the mouse during the slice. You can see the mark left by the injection of methyl violet without tissue damage. B cell transfected with viral vector containing a protein that fluoresces in the presence of intracellular calcium. C location of the mark left by methyl violet in the atlas of stereotaxic surgery. D Micrograph of the brain slice showing the methyl violet mark. No tissue damage noted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Lambda HpX L5, Sutter Instruments) connected by fiber optics to the microscope to observe changes in fluorescence. Exposure time was 15–30 ms. A photograph was obtained using a CCD digital camera (Cold SNAP MYO, Photometrics) controlled by custom software programmed in LabView (National Instruments).

## Results

When cutting the brain slices, the mark left by the methyl violet dye and the path left by the needle was appreciated (Fig. 13A). The injury left by the needle was minimal, considering its caliber. Below this path, the dye presence could be seen; however, there was no tissue tearing due to displacement caused by the liquid. The injection speed allowed the liquid to perfuse the tissue and not displace it. When reviewing the tissue under the stereoscopic microscope, we observed the same result: a minimal injury left by the needle and the diffusion of the dye without damaging the tissue (Fig. 13D).

The microinjection of the viral vector containing a plasmid that codes for a protein that fluoresces in the presence of calcium was successful. The viral vector introduced the plasmid into the cells of the nucleus accumbens, and it was not seen to spread to other nearby structures. Most importantly, many live cells can be seen, 90% of them with the fluorescent protein (Fig. 13B).

This microinjection device proved to be a 3D printed device that is an end-user device that has the same precision in volume and speed as specific commercial devices, that can be adjusted to microsyringes with total volumes from 0.5  $\mu$ l to 1 ml, and that can be placed directly on the stereotaxic surgery apparatus or used separately. The price is substantially lower than that of the commercial systems.

## Ethics statements

The experiments were carried out following the official Mexican standard of technical specializations for the production, care, and use of laboratory animals (NOM-062-ZOO-1999) and under the Internal Committee for the Care and Use of Laboratory Animals (CUICAL) of the National Autonomous University of Mexico. It complies with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines; EU Directive 2010/63/EU for animal experiments; or the National Institutes of Health guide for laboratory animals' care and use (NIH Publications 8023, revised 1978). The animals used in this study were male, therefore there was no influence of sex on the results.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ohx.2023.e00446>.

## References

- [1] M. Rahman, G.J.A. Murad, J. Mocco, Early history of the stereotactic apparatus in neurosurgery, *Neurosurg. Focus* 27 (2009) E12, <https://doi.org/10.3171/2009.7.focus09118>. PMID 19722814.
- [2] M. Kirschner, Die Punktions- und die Elektrokoagulation des Ganglion Gasserii, *Arch Klin Chir.* 176 (1933) 581–620, ISSN 0365–3706.
- [3] K. Shimizu, A. Gupta, P.K. Brastianos, H. Wakimoto, Anatomy-oriented stereotactic approach to cerebrospinal fluid collection in mice. *Brain Res.*, 1, (2022)1774:147706. 10.1016/j.brainres.2021.147706. Epub 2021 Oct 31. PMID: 34732332.
- [4] J.L. Hect, A.A. Alattar, E.E. Harford, H. Reeher, D.T. Fernandes, N. Esplin, M. McDowell, T.J. Abel, Stereotactic laser interstitial thermal therapy for the treatment of pediatric drug-resistant epilepsy: indications, techniques, and safety, *Childs Nerv. Syst.* 38 (5) (2022) 961–970, <https://doi.org/10.1007/s00381-022-05491-x>, Epub 2022 Mar 11 PMID: 35274185.
- [5] C.R. Bjarkam, A.N. Glud, L. Margolin, K. Reinhart, R. Franklin, D. Deding, Safety and function of a new clinical intracerebral microinjection instrument for stem cells and therapeutics examined in the Göttingen Minipig, *Stereotact. Funct. Neurosurg.* 88 (2010) 56–63, <https://doi.org/10.1159/000268743>.
- [6] T.L. Yaksh, T.A. Rudy, Narcotic analgesics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques, *Pain* 4 (4) (1978) 299–359, [https://doi.org/10.1016/0304-3959\(77\)90145-2](https://doi.org/10.1016/0304-3959(77)90145-2), PMID: 25403.
- [7] J.A. Barcia, J.M. Gallego, Intraventricular and intracerebral delivery of anti-epileptic drugs in the kindling model, *Neurotherapeutics* 6 (2) (2009) 337–343, <https://doi.org/10.1016/j.nurt.2009.01.015>. PMID: 19332328; PMCID: PMC5084212.
- [8] K. Geletneký, A.L. Leoni, G. Pohlmeier-Esch, S. Loebhard, B. Leuchs, C. Hoefer, K. Jochims, M. Dahm, B. Huber, J. Rommelaere, O. Krebs, J. Hajda, Bioavailability, biodistribution, and CNS toxicity of clinical-grade parvovirus H1 after intravenous and intracerebral injection in rats, *Comp Med.* 65 (1) (2015) 36–45, PMID: 25730755; PMCID: PMC4396927.
- [9] A. Hawlitschka, V. Antipova, O. Schmitt, M. Witt, R. Benecke, E. Mix, A. Wree, Intracerebrally applied botulinum neurotoxin in experimental neuroscience, *Curr. Pharm. Biotechnol.* 14 (1) (2013) 124–130, PMID: 23092264.
- [10] E. Bromek, W.A. Daniel, The regulation of liver cytochrome P450 expression and activity by the brain serotonergic system in different experimental models, *Expert. Opin. Drug. Metab. Toxicol.* 17 (4) (2021) 413–424, <https://doi.org/10.1080/17425255.2021.1872543>.
- [11] S. Yamazaki, Y. Mochizuki, T. Terai, M. Sugimoto, I. Uchida, N. Matsuoka, S. Mutoh, Intracerebroventricular injection of the antibiotic cefoselis produces convulsion in mice via inhibition of GABA receptors, *Pharmacol. Biochem. Behav.* 74 (1) (2002) 53–59, [https://doi.org/10.1016/S0091-3057\(02\)00947-4](https://doi.org/10.1016/S0091-3057(02)00947-4), PMID: 12376152.
- [12] O. Detante, C. Rome, J. Papassin, How to use stem cells for repair in stroke patients, *Rev. Neurol. (Paris)* 173 (9) (2017) 572–576, <https://doi.org/10.1016/j.neurol.2017.09.003>, Epub 2017 Oct 21 PMID: 29033030.
- [13] A. Cetin, S. Komai, M. Eliava, P.H. Seeburg, P. Osten, Stereotaxic gene delivery in the rodent brain, *Nat. Protoc.* 1 (6) (2006) 3166–3173.
- [14] C. Portioli, C. Bussy, M. Mazza, N. Lozano, D.A. Jasim, M. Prato, A. Bianco, M. Bentivoglio, Intracerebral injection of graphene oxide nanosheets mitigates microglial activation without inducing acute neurotoxicity: a pilot comparison to other nanomaterials, *Small* 16 (48) (2020) 2004029.
- [15] R.D. Myers, Injection of solutions into cerebral tissue: relation between volume and diffusion, *Physiol. Behav.* 1 (2) (1966) 171–174.
- [16] K. Tomiyama, N. Koshikawa, M. Kobayashi, Methods for intracerebral injection. *Nihon Yakurigaku Zasshi.*, 104(1), (1994) 1-5. Japanese. 10.1254/fpj.104.1. PMID: 8076887.
- [17] M.A. Parada, M. Puig de Parada, B.G. Hoebel, A remote insertion technique for intracerebral microinjections in freely moving animals, *J. Neurosci. Methods.* 50 (2) (1993) 237–241, [https://doi.org/10.1016/0165-0270\(93\)90012-g](https://doi.org/10.1016/0165-0270(93)90012-g), PMID: 8107503.
- [18] M. Bertrand, N. Mérie, J. Simonnet, C. Le Duigou, S. Clemenceau, Increasing the effectiveness of intracerebral injections in adult and neonatal mice: a neurosurgical point of view, *Neuroscience Bulletin, Springer Verlag* 31 (6) (2015) 685–696, <https://doi.org/10.1007/s12264-015-1558-0>. hal-01196282.
- [19] G.A. Needham, P.D. Soden, M.A. Sambrook, A.R. Crossman, A remotely operated pump for intracerebral micro-injection in the primate, *J. Neurosci. Methods.* 7 (3) (1983) 281–288, [https://doi.org/10.1016/0165-0270\(83\)90011-0](https://doi.org/10.1016/0165-0270(83)90011-0), PMID: 6843169.
- [20] D.P. Sinha, Technique for intracerebral injection in the calf, *Am. J. Vet. Res.* 29 (4) (1968 Apr) 919–920, PMID: 5642364.
- [21] [www.hamiltoncompany.com](http://www.hamiltoncompany.com).
- [22] <https://www.wpiinc.com/var-3735-ump3-ultramicro-pump>.
- [23] <https://leadfluid.com/microinjection-lead-fluid-split-syringe-pump/>.
- [24] <https://www.fishersci.es/shop/products/syringe-pump-numeric-1-canal/11939747#?keyword=bomba%20de%20jeringa>.
- [25] Allen Institute for Brain Science, Allen Mouse Brain Atlas. mouse.brain-map.org. (2023).
- [26] <https://www.youtube.com/watch?v=FLJGCZslOcm>.
- [27] <https://www.youtube.com/watch?v=kq04-ReW1xY>.
- [28] <https://www.arduino.cc/en/software>.
- [29] <https://docs.arduino.cc/software/ide-v1/tutorials/installing-libraries>.
- [30] <https://www.youtube.com/watch?v=-g5sWQyROg>.
- [31] [https://www.integra-biosciences.com/sites/default/files/Z-factors-calibration\\_2017\\_01\\_26.pdf](https://www.integra-biosciences.com/sites/default/files/Z-factors-calibration_2017_01_26.pdf).





**Jorge Bravo-Martínez** is a researcher in the Membrane Biophysics Laboratory of the Department of Physiology, in the Faculty of Medicine, of the National Autonomous University of Mexico. His academic degrees are Master's and Ph.D. in Biomedical Sciences from the same university. The lines of research in which he works are the regulation of membrane proteins such as ion channels, ATPase, and lipid exchangers of the plasma membrane. Changes in excitability and neuronal synaptic efficiency due to deregulation of these same proteins are caused by changes in the lipid composition of the plasma membrane, giving rise to diseases such as epilepsy. These same themes led him to address the development of low-cost and customized equipment using "Open Access" and "Do it yourself" technologies such as Arduino microprocessors and 3D printing.