HardwareX 8 (2020) e00120

Contents lists available at ScienceDirect

HardwareX

journal homepage: www.elsevier.com/locate/ohx

A low-cost and customizable alternative for commercial implantable cannula for intracerebral administration in mice

Rogneda B. Kazanskaya [a], Alexander V. Lopachev [b], Tatiana N. Fedorova [b], Raul R. Gainetdinov [c], Anna B. Volnova [a,c]

[a] Biological Department, Saint Petersburg State University, Universitetskaya Emb. 7/9, 199034 St Petersburg, Russia
[b] Research Center of Neurology, Volokolamskoye shosse 80, 125367 Moscow, Russia
[c] Institute of Translational Biomedicine, Saint Petersburg State University, Universitetskaya Emb. 7/9, 199034 St Petersburg, Russia

ARTICLE INFO

Article history:
Received 8 April 2020
Received in revised form 2 June 2020
Accepted 22 June 2020

Keywords:
Stereotaxic surgery
Drug testing
Intracerebral cannula

ABSTRACT

Stereotaxic intracerebral cannula implantation for neuroactive agent administration is a wide-spread method for chronic experiments requiring bypassing the blood-brain barrier in rodents. However, commercially available cannula are bulky and may interfere with animal movement or lead to their dislodging during grooming. As the number of cannula needed in one experiment, and the accompanying costs can be high, it is in the interest of researchers to produce them on their own. Custom cannula manufacturing also offers the flexibility of different cannula lengths, which is required for agent delivery to various brain structures. In this article we present a protocol for making guide cannula along with the accompanying systems required for injection, which are small, cost-effective, light, easy to make, reusable, and can be made from easily procured materials.

Specifications table

Hardware name	Cannula for intracerebral administration
Subject area	Neuroscience
Hardware type	Intracerebral drug administration in mice
Open Source License	GPL-3.0
Cost of Hardware	$37 (USD) for a set including x10 guide cannula, x10 dummy cannula, x2 injection systems, and x1 Stereotaxic holder.
Source File Repository	All source files are available with the article and on Mendeley Data at https://doi.org/10.17632/zx7kd5pw64.1.

E-mail address: lopachev@neurology.ru (A.V. Lopachev)

https://doi.org/10.1016/j.ohx.2020.e00120
2468-0672/

1. Hardware in context

In neurological studies, site-specific agent delivery, whether via acute injection [1] or stereotaxic cannula implantation [2], enables administration of neuroactive agents which cannot bypass the blood–brain barrier. While methods of direct injection exist, implantation of cannula, in particular, is utilized when chronic treatments are necessary in non-anesthetized animals. There are 3646 papers indexed in pubmed in response to "intracerebroventricular mice ", implying that icv injections are widely utilized among the scientific community. However, there are few manufacturers which can provide guide, injection, and dummy cannula for the injection of substances into the brain of conscious animals. For example: P1 Technologies (USA) [3], Harvard Apparatus (USA) [4], Amuza (USA) [5], RWD + Life Science (China/USA) [6], and World Precision Instruments (USA) [7]. The lengthy shipment times and high prices, a consequence of a lack of distributors, decrease the availability of these cannula to researchers located in countries other than the US.

As such, we were confronted with the task of crafting cannula which would be cost-effective, easy and quick to make, allow high precision of positioning, and light and small enough to mount on the murine skull cap without using bolts. These cannula also had to cause little damage to the brain tissues, and not interfere with animal movement or grooming [8]. To accompany the guide cannula, dummy cannula, a stereotaxic implantation holder, and systems for agent delivery via the implanted guide cannula were developed. Here we present a protocol for making guide cannula, along with the accompanying systems, which are small, light, easy to make, and reusable. Specifications of the parts in question can be found in the Specifications Table.

While writing this article, we have found that prior methods of custom cannula manufacturing have been previously published [9–12]. However, we believe that our method has a number of advantages over those listed, and strikes a balance between the amount of time and effort spent on making the cannula, and a higher precision than the simpler of the suggested methods. Apart from being moderately easy to make, our cannula are lighter and smaller than those described previously, and are easy to reuse and clean.

2. Hardware description

Cannula length varies greatly depending on both the animal model and the specific site of agent delivery, and as such, requires a high level of customizability, which many commercial manufacturers are unable to provide, or charge extra for. It is also worth noting that the cannula produced by the above listed manufacturers are quite bulky, as shown in Fig. 1, and in the case of usage in mice may get in the animal's way, and potentially affect its behavior in experiments.

The components described in this article include the following: a stereotaxic cannula holder (see Fig. 2a), dummy cannula (see Fig. 2b), guide cannula (see Fig. 2c), and injection cannula systems (see Fig. 2d). The dummy and injection cannula are made from 33G needles, with medical tubing used for the connectors. The guide cannula are made from 26G needles, with 2.54 male breakable pin headers used for the plastic mount. The stereotaxic holder uses Vacutainer needles, a 33G needle, and a 26G needle. No specialized expertise is required for assembly. A dremel is necessary – any small hand-held drill, which will be needed for the cannula implantation, with a disk bit can be used. Pliers, forceps, clamps, brushes, and some other tools which can be easily procured are needed. Most of the parts are readily available from online suppliers, the most expensive being the tubing, which can last for a very long time, and is not used in large quantities.

Fig. 1. Visual comparison of mice with commercially available cannula (left), and with custom-made cannula (right) [8].

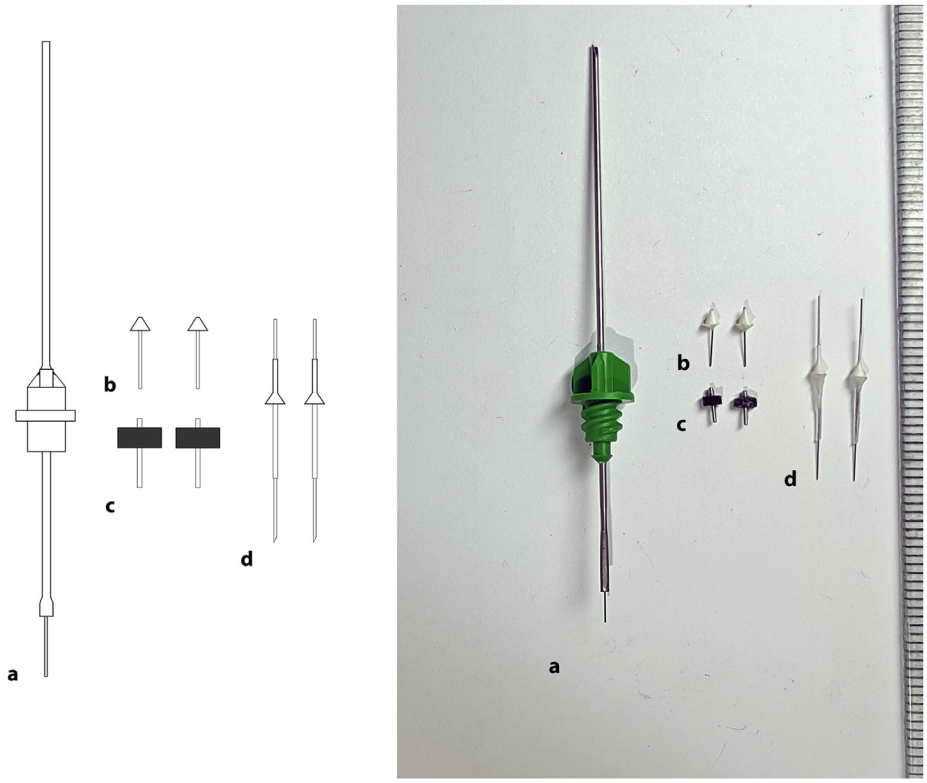

Fig. 2. Cannula set. (a) Stereotaxic holder (b) Dummy cannula (c) Guide cannula (d) Injection cannula.

Some advantages of using our cannula design:

- Chronic intracerebral injection experiments with less dependence on funding
- Rapid access to cheap, easy-to-make, and highly customizable cannula
- Possibility of injection into conscious animals
- All or most of the materials or their analogs can be easily procured around the lab
- Their small size and weight makes them interfere less with grooming

3. Design files

Design files summary

Design file name	File type	Open source license	Location of the file
Guide_cannula_design	Figure(TIF)	GPL-3.0	Available with the article (Fig. 3)
Dummy_cannula_design	Figure(TIF)	GPL-3.0	Available with the article (Fig. 6)
Injection_cannula_design.jpg	Figure(TIF)	GPL-3.0	Available with the article (Fig. 7)
Stereotaxic_cannula_holder_design	Figure(TIF)	GPL-3.0	Available with the article (Fig. 9)

Each of the above listed files (Design Files Summary) is a diagram showing the key steps in part assembly. They are accompanied by photos of the process as well.

4. Bill of Materials

Bill of materials

Designator	Component	Number	Cost per unit - currency	Total cost - currency	Source of materials	Material type
Dummy cannula and injection cannula	a) Mesoram 33G/0,20 × 12 mm needles b) Meso-relle® 33G × 12 mm needles c) TSK Regular hub needles are available in size up to 33G. In a length of 13 mm, 9 mm, 6 mm and 4 mm.	a) x1 pack (100 units) b) x1 pack (100 units) c) x1 pack (100 units)	a) $0.67 b) €0.5 c) $0.63	a) $67.00 b) $55 c) $63.00	a) https://www.mesoram.com/needles/33g/33g-0-20-x-12mm b) https://www.biotekne.it/en/portfolio-articoli/aghi-per-mesoterapia/ c) https://tsklab.com/pre-regular-hub/	Metal
Plastic mount	2.54 male breakable pin headers	x1 pack (400 units)	$0.00165	$0.66	http://www.connfly.com/productshow.aspx?id=818	Polymer
Implantable guide cannula	26G needles	x1 pack (100 units)	$0.195	$19.50	https://www.keelerusa.com/ax10618.html	Metal
Injection systems and injection cannula	BD Intramedic™ PE Tubing	x1 pack (10 feet)	$12	$52.00	https://www.fishersci.com/shop/products/bd-intramedia-polyethylene-tubing-0–011-in-0–28mm-i-d-0-02-in-0-61mm-o-d-10-ft-long/22204008	Polymer
---	Super glue	x1 tube	$0.004	$3.99	https://www.amazon.com/Gorilla-Super-Glue-Gram-Clear/dp/B001IY82FM	Polymer
Stereotaxic cannula holder	BD Vacutainer™ PrecisonGlide™ Multi Sample Needle 22G × 1.5″	x1 pack (100 units)	$0.0845	$8.45	https://www.fishersci.ie/shop/products/vacutainer-multi-sample-blood-collection-needles/p-8576001	Metal, Polymer, Silicone
Injection systems and injection cannula	BD Needle, 30G X1″, Hypodermic	x1 pack (100 units)	$0.1995	$19.95	https://www.shopmedvet.com/product/b-d-needle-30-x-1–100-bx/syringes-and-needles	Metal, Polymer

Most, if not all, of these materials are already present in a standard lab or can be found in a neighboring one, making the cost of cannula even lower, since few of the components need to be bought. The full list of materials and prices can be found in the Bill of Materials.

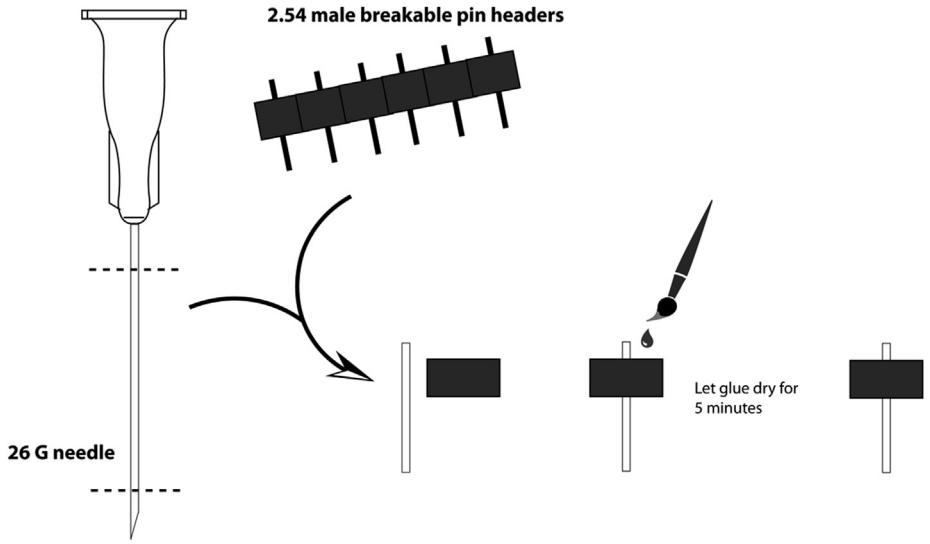

Fig. 3. Guide cannula design and assembly diagram.

5. Build Instructions

General safety concerns: Construction of the cannula requires cutting, handling, and sanding metal needles. It is advisable to wear a respirator to prevent inhalation of metal dust, and protective goggles to shield the eyes from flying scraps. Caution should be taken to avoid getting dust or superglue on clothing and skin. The work surface should be wiped down with a damp disposable cloth or paper towel after work is concluded.

5.1. Cannula description

Guide cannula were made of 26G needles and 1 × 2 mm fixed plastic holders, dummy and injection cannulas were made of 33G needles. The plastic holders were made out of 2.54 male breakable pin headers.

5.2. Cannula preparation

5.2.1. Guide Cannula

5.2.1.1. Making the implantable barrel portion. The bevel of a 26G needle is cut off (Fig. 4a), after which the remaining barrel is cut to the required length, and both ends are polished with sandpaper to ensure smooth edges and avoid additional tissue damage during implantation (Fig. 4b–c). The resulting barrel segment is cleaned by inserting a 33G needle into the lumen, and pistoning it until it can move through the larger needle smoothly (Fig. 4d). Twisting motions can be used when the blockage inside of the needle segment is too impacted to be dislodged easily. Care must be taken in order to avoid bending the thinner needle.

5.2.1.2. Plastic holder/mount. 2.54 breakable pin headers are separated, and the pins are removed from the plastic (Fig. 5a–b). The hole is widened slightly in order to allow easy insertion of the guide portion – the diameter of the hole should be small enough to ensure a snug fit when the 26G barrel section is inserted (Fig. 5c). The plastic holder is then moved along the shaft

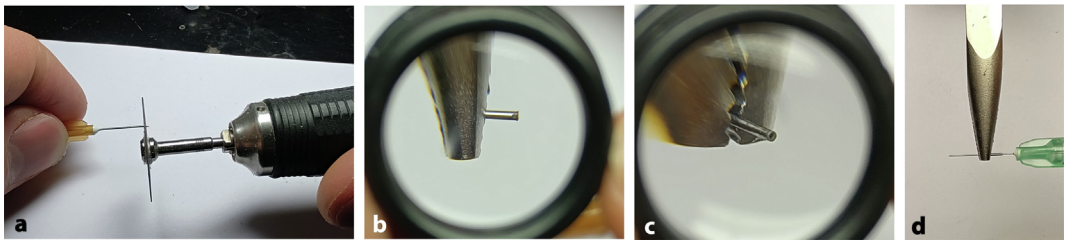

Fig. 4. Implantable barrel preparation. (a) Cutting off the bevel with a rotary disk cutter, (b) unsanded cannula end, (c) sanded cannula end, (d) cleaning the lumen with a 33G needle.

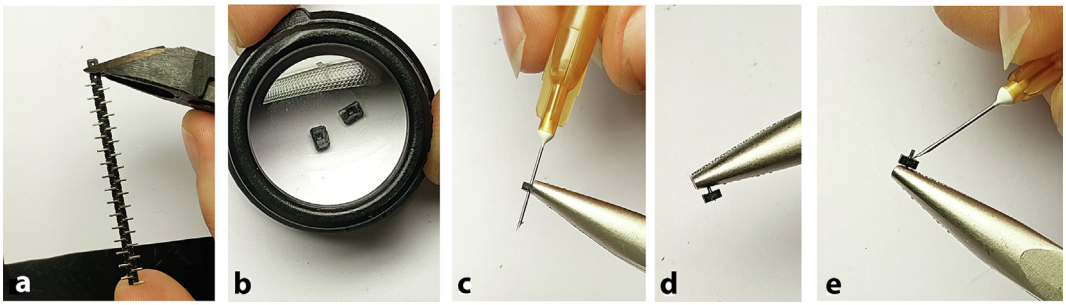

Fig. 5. Plastic holder preparation and Guide cannula assembly. (a) Separation of 2.54 pin headers, (b) separated pin headers, (c) widening the diameter of the plastic mount, (d) assembling and (e) gluing the guide cannula.

of the previously prepared barrel until it is at the correct distance from the leading end (Fig. 5d). After this, the top of the holder is glued to the shaft – a thin needle or paintbrush can be used for the purpose of delivering glue to the area (Fig. 5e). After glue application, the cannula needs to be placed leading-side down on a holder, for example, a clamp, and left to dry.

5.2.2. Dummy Cannula

As seen in Fig. 6, pliers are used to deform the needle adapter of a 33G, 12 mm needle, and the entire plastic funnel is removed. The white cement is what will be the "cap" of the dummy cannula. The needle shaft is cut to a length approximately 0.2 mm greater than the length of the guide cannula and the tip lightly sanded. When viewed from below after insertion, the dummy cannula tip should be visible inside the lumen.

5.2.3. Injection cannula and systems

The sequence of key steps can be seen in Fig. 7 and Fig. 8. Pliers are used to deform the needle adapter of a 33G needle, and the entire "root" of the needle is removed (Fig. 8a–c). Care must be taken not to damage the portion of the needle which is located inside of the adapter. A fragment of tubing is cut and attached to the needle in a way which will leave the required length revealed, and glued (Fig. 8d). The length of the uncovered barrel should be 0.2 mm longer than the guide cannula. The rear end of the needle is then inserted into a section of BD Intramedic™ PE Tubing. The length of the tubing should be long enough to allow easy manipulation (approximately 20–30 cm for mice). A 30G needle is inserted into the other end of the tubing.

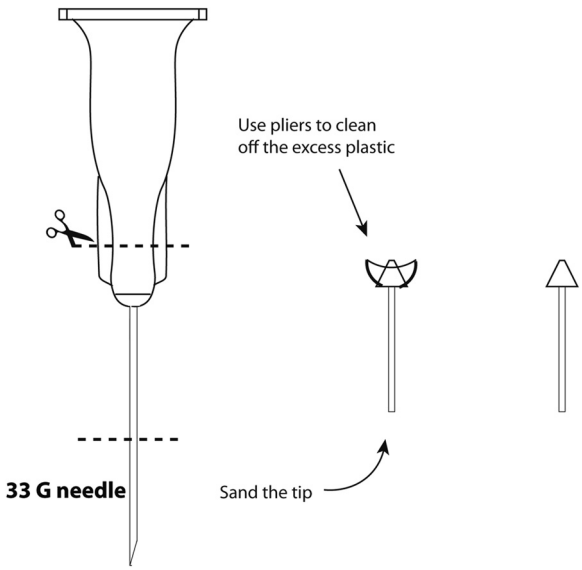

Fig. 6. Dummy cannula design and assembly diagram.

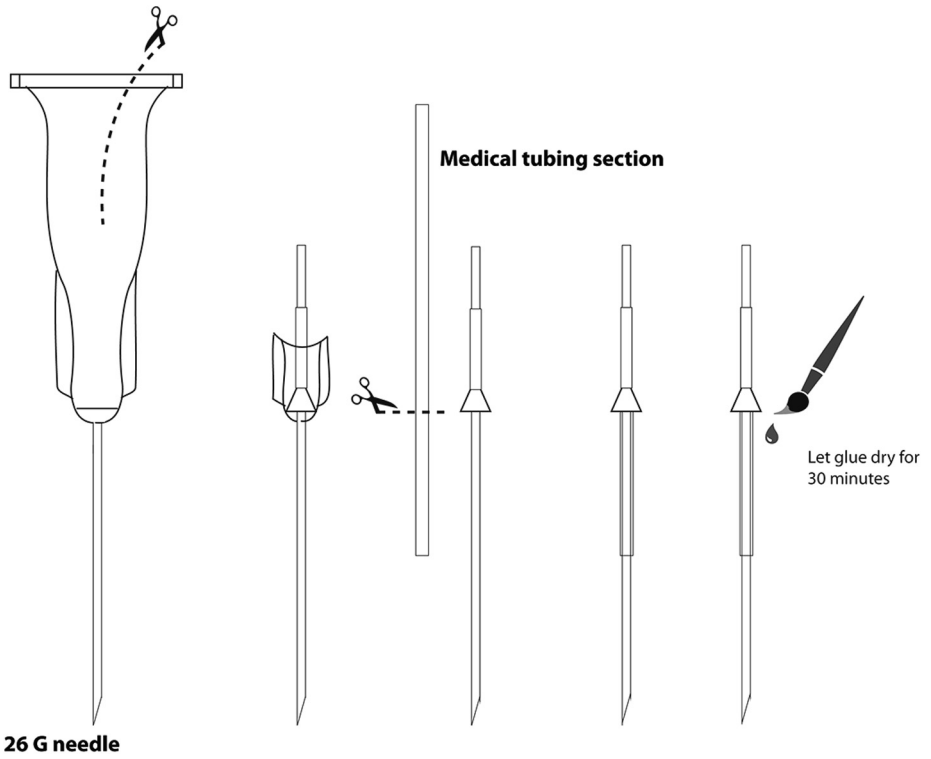

Fig. 7. Injection cannula design and assembly diagram.

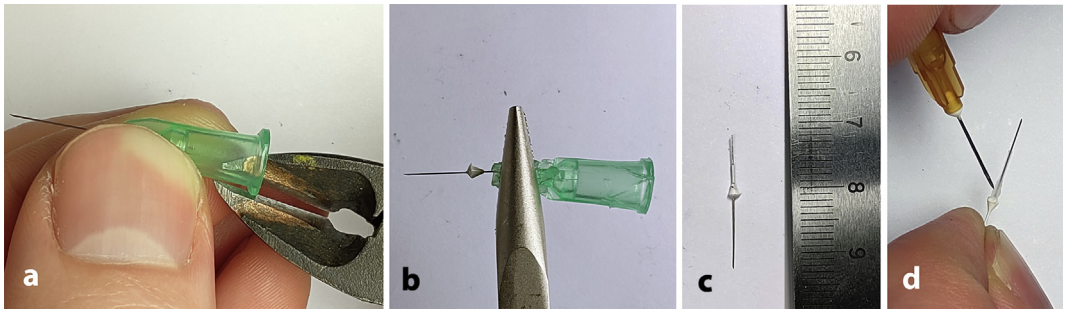

Fig. 8. Injection cannula assembly. (a, b) Extraction of the needle from the plastic holder, (c) the extracted needle, (d) gluing the tubing to the needle "root".

5.2.4. Guide cannula stereotaxic holder

The sequence of photographs of the key steps can be seen on Fig. 9 and Fig. 10. A Vacutainer multisample needle is opened. The silicone cap is removed from the shorter needle – this is the end which will be used to create the guide cannula holder (Fig. 10a). The bevels are cut off of both needles to prevent potential harm to the handler. A segment is cut from a 26G needle. A 33G needle is cut, the bevel of it trimmed, and is inserted into the 26G needle segment in a way that will leave a length of needle equivalent to the length of the guide cannula exposed (Fig. 10b). The resulting double needle segment is inserted into the short side of the Vacutainer needle (Fig. 10c). The barrel of the Vacutainer needle is then flattened with pliers to ensure immobility of the 33G needle, which should be exactly parallel to the Vacutainer needle (Fig. 10d).

5.3. Cannula reusability

All of the parts described in this article are reusable and easy to store (Fig. 11). The guide cannula can be salvaged after the experiment. The cement "caps" taken from decapitated mice need to be soaked in 95% ethanol until the dental cement softens. The cannula can then be extracted from the cement using forceps and pliers. The remaining cement particles can be removed from the mount using a scalpel. The dummy cannula can be salvaged from the cages after the experiment, either

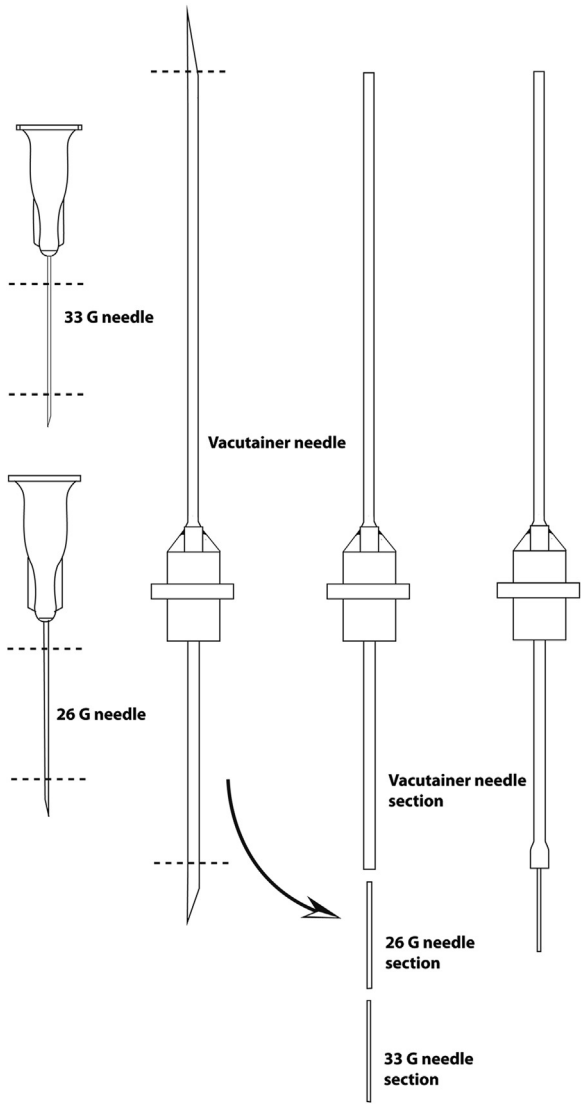

Fig. 9. Stereotaxic holder design and assembly diagram.

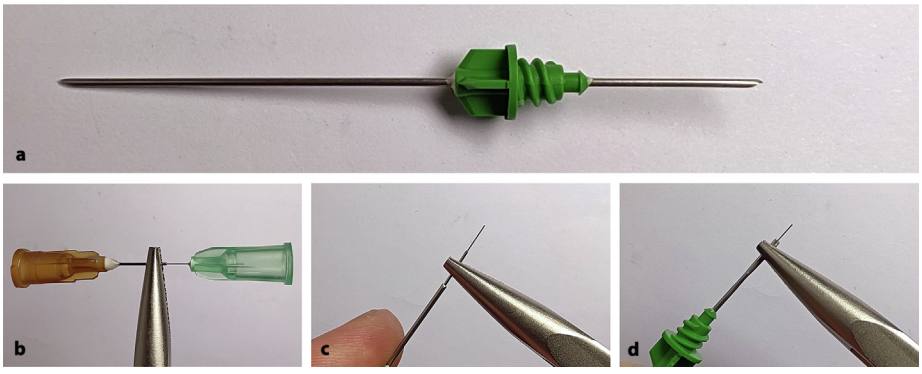

Fig. 10. Stereotaxic holder assembly. (a) Vacutainer needle with the caps removed, (b-c) assembly of the guide portion, (d) insertion of the guide portion into the vacutainer needle and fixation of the vacutainer needle.

Fig. 11. Cannula storage and caps soaked in ethanol.

by hand or using a strong magnet. The injection systems and stereotaxic holder are reusable unless the needles become bent from misuse.

6. Operation Instructions

The long end of the vacutainer needle is pressed into the ridges of the stereotaxic needle holder, and the screw of the holder tightened to hold the needle firmly in place. After the holes are drilled in the skull, a guide cannula is placed onto the tip of the stereotaxic cannula holder, and pushed up until it touches the Vacutainer needle. Fig. 12a–c illustrates the process. The cannula can then be lowered into the brain. When a cannula is implanted, it is affixed to the skull cap with dental cement (Fig. 12d), the stereotaxic holder lifted by twisting the microdrive screw for dorsoventral adjustment. As the cement is still not fully dry at this point, the cannula should be held in place lightly with forceps during holder removal to prevent accidental dislodging.

A full description of stereotaxic surgery falls outside the scope of this article, but is available online [13].

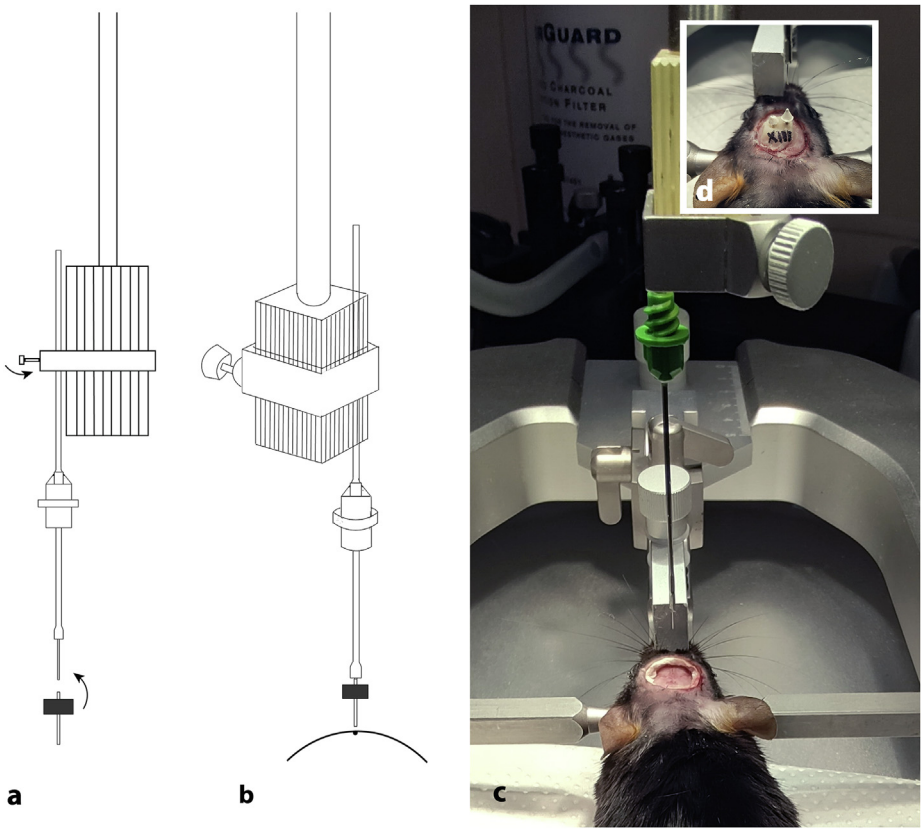

Fig. 12. Using the stereotaxic holder and guide cannula. (a) Mounting the stereotaxic holder, (b) assembled stereotaxic holder with attached cannula, (c) photograph of the stereotaxic holder mounted on the stereotaxic arm, (d) mouse with implanted guide cannula and inserted dummy cannula.

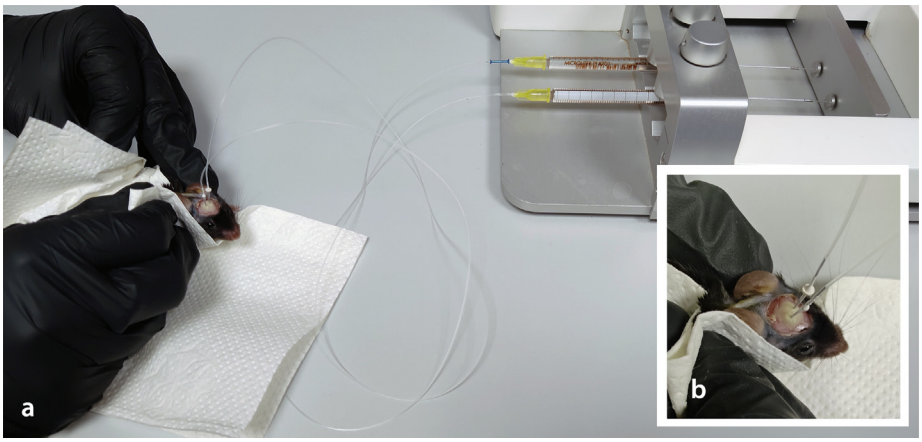

Fig. 13. Injections into conscious mice using injection cannula. (a) overview, (b) close up of the inserted injection cannula.

The injection cannula can be used on conscious mice. The systems are connected to two syringes for bilateral administration. To ensure even injection, a syringe pump can be used. The systems and syringes are filled up with the solution which will be injected, ensuring that no air bubbles are present within the tubing or needle adapters. Two people are needed – one to hold the animal in place, while the other removes the dummy cannula and inserts the injection cannula. An overview of the process can be found in Fig. 13.

7. Validation and characterization

Validation and demonstration of usage has been previously published by the authors [8].

To verify that the cannula function as desired, trypan blue was administered into the lateral ventricles of a mouse (Fig. 14b) using 4 mm long guide cannula, which was decapitated 2 min post administration. The brain was removed and fixed in 4% PFA overnight. The brain was then sliced with a razor to demonstrate the dispersion of the staining agent through the tissue. As can be seen in Fig. 14a, blue staining has appeared along the entire surface area of the ventricles, evidencing that both injection cannula had reached the ventricles.

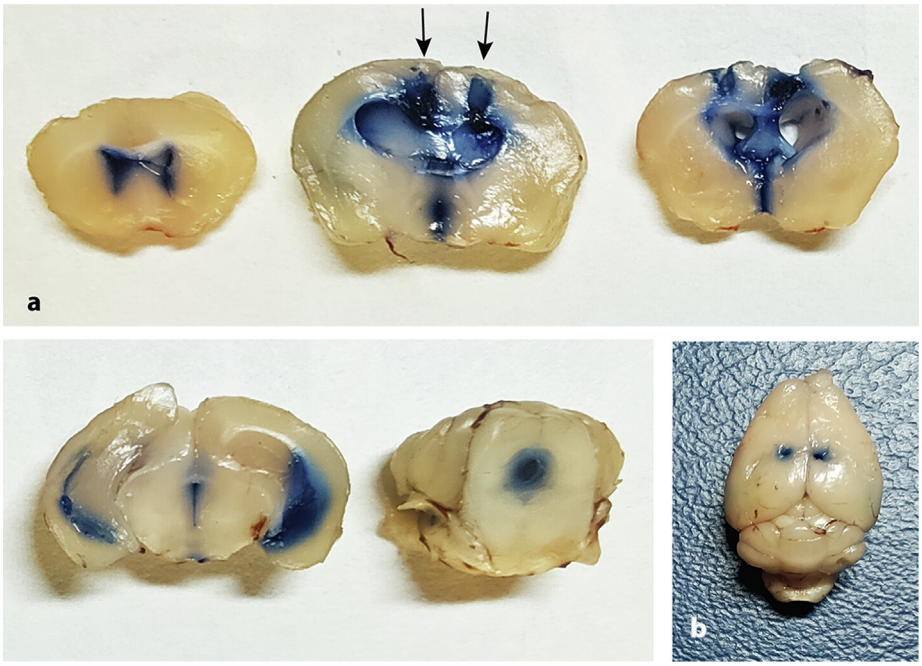

Fig. 14. Mouse brain slices post injection of trypan blue. (a) Slices of the brain with the arrows indicating cannula passing through the brain tissue and into the ventricles, (b) the extracted brain showing cannula placement.

The experimental procedures complied with the ARRIVE guidelines, and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The experiments were approved by the Ethical Committee for Animal Experiments of Saint Petersburg State University, permission #131-03-1.

Custom-made cannula possess the following characteristics:

- High precision due to separate cutting of the barrel and holder
- The length of the cannula is limited only by the length of the 26G needle
- Minimal damage to surrounding tissue if ends are sanded properly
- When fixed to the skull cap with dental cement, cannula can remain firmly attached for up to three months without observed clogging of the guide cannula (unpublished observations)
- Implantation of cannula allows injection of substances into conscious animals, with subsequent behavioral testing.

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.

Acknowledgements

This study was supported by the project ID: 51143531 of the St. Petersburg State University, St. Petersburg, Russia.

References

[1] M. Nizzardo, M. Rizzuti, Intracerebroventricular delivery in mice for motor neuron diseases, Meth. Mol. Biol. (2017), T. 1565. – C. 229-239.
[2] E.I. Poole, J.J. McGavin, N.L. Cochkanoff, K.M. Crosby, Stereotaxic surgery for implantation of guide cannulas for microinjection into the dorsomedial hypothalamus in young rats, MethodsX 6 (2019) 1652–1659, https://doi.org/10.1016/j.mex.2019.07.005.
[3] http://www.invivo1.com/Canula-Infusion-Systems.php.
[4] https://www.harvardapparatus.com/cma-guide-cannula.html.
[5] https://amuzainc.com/shop/microdialysis/probes-microinjection/cxmi-series/guide-cannula-cxg-x/.
[6] http://www.rwdstco.com/single-cannula.html.
[7] https://www.wpiinc.com/animal-physiology/neuroscience-cannulas.
[8] A. Lopachev, A. Volnova, A. Evdokimenko, D. Abaimov, Y. Timoshina, R. Kazanskaya, O. Lopacheva, A. Deal, E. Budygin, T. Fedorova, R. Gainetdinov, Intracerebroventricular injection of ouabain causes mania-like behavior in mice through D2 receptor activation, Sci. Rep. 9 (1) (2019), https://doi.org/10.1038/s41598-019-52058-z.
[9] L.A. Crane, S.D. Glick, Simple cannula for repeated intracerebral drug administration in rats, Pharmacol. Biochem. Behav. 10 (5) (1979) 799–800, https://doi.org/10.1016/0091-3057(79)90336-8.
[10] G.G. Dougherty Jr., E.H. Ellinwood Jr., A simple multiple-cannula headpiece for the rat, Physiol. Behav. 26 (5) (1981) 897–900, https://doi.org/10.1016/0031-9384(81)90115-3.
[11] K. Packard, L.A. Pohorecky, J. Brick, A simple cannula for intraventricular drug administration in rodents, J. Neurosci. Meth. 10 (2) (1984) 139–143, https://doi.org/10.1016/0165-0270(84)90067-0.
[12] D.M. Kokare, G.P. Shelkar, C.D. Borkar, K.T. Nakhate, N.K. Subhedar, A simple and inexpensive method to fabricate a cannula system for intracranial injections in rats and mice, J. Pharmacol. Toxicol. Meth. 64 (3) (2011) 246–250, https://doi.org/10.1016/j.vascn.2011.08.002.
[13] Rodent Stereotaxic Surgery, https://www.jove.com/science-education/5205/rodent-stereotaxic-surgery, 2020.