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Preparation of Durable Cannula for Intracranial micro-infusion of NeuroactiveSubstances in Small Animals

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Abstract: Intracranial infusion of neuroactive chemicals is routine method in neurophysiology research. We devised a simple, durable and reusable internal cannula for this purpose. The guide cannula, placed securely on the skull using Dental acrylic and securing screw, was fabricated from a needle of 22 gauge hypodermic needle. The internal cannula was fabricated from 30 gauge Dental needle, which has a white coloured hub. The length of this internal cannula will be enough to reach the nucleus when inserted through the guide cannula at the time of infusion process. We infused Orexin A into BLA by using this cannula assembly in overnight fasted rats, which increased the food intake significantly. On histological examination we found that the location of injection was as desired in the BLA. The guide cannula and the internal cannula fabricated by us appeared to be highly dependable. The dental needle has high temper and it does not change the shape like insulin needle with limited reusability. We conclude that this method of fabrication of internal cannula will be a better alternative to those fabricated from hypodermic needles.

Keywords:Internal cannula, guide cannula, Orexin A,Basolateral amygdala

I. INTRODUCTION

In order to study the effects of neuroactive substances, it is necessary to administer them into specific sites by stereotaxic method using intracranial cannulation technique in animal models. For over half century, intracranial drug infusion studies have been in vogue. [1,2] They used hypodermic needle inserted through guide cannula of 20 gauge needle to study the analgesic effects of Morphine injected into ventricles. Perspex boxes were embedded to secure the guide cannula. Robert MG (1980) et al [3] used Guide cannula of 18 gauge hypodermic needle, whose 'Leur Lock' has been removed and cut to a length of 1.5 mm. They minimised the length of guide cannula to minimise the cortical damage caused during implantation of guide cannula. They reportedly used micro syringe needle for infusion of drugs by fitting it with a depth collar. This methodology could be good enough for intra ventricular injections. Thirty gauge stainless hypodermic needle has been used by other workers also. [4,5,6] For the purpose of studying effect of specific neurotransmitter on focal regions in the brain more precision was required. In our study we implanted cannula into the BasolateralAmygdlaoid Nucleus (BLA). It required injection of Orexin-A [7] into BLA in the basal brain. For this precision injection, longer guide cannula was devised from a 20 gauge needle. We used a 30 gauge dental needle with a hub for internal cannula. The methodology of preparation of guide cannula, internal cannula and the results obtained using the device is discussed here.

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II.MATERIALS AND METHODS

Preparation of guide cannula: Guide cannula was prepared using 22 gauge stainless steel injection needles (0.60×25mm, Hindustan Syringes & Medical Devices Ltd, India India) (Fig 1-1). Remove the funnel, hub of hypodermic needle by melting (Fig. 1-2) and clean the needle by alcohol. Then cut the stainless steel barrel to required length by using a polisher, clear its bore by 27gauge needle or wire, flatten the tip of guide cannula by polisher. (Fig. 1-3). The required length depends on the vertical coordinate from the surface of the skull, described for the rat brain.[8] Measure the length of the guide cannula using a vernier scale. This guide Cannula is attached to 24gauze stainless steel electrode (Fig.1-4) by adhesive Araldite (Huntsman advanced materials, India Pvt. Ltd) and allowed for drying overnight. This is necessary to handle the guide cannula. The guide cannula is ready for implantation. A stillet of 28 gauge stainless steel wire, which was bent at upper end for preventing it from slipping into guide cannula, was placed to block lumen of the guide cannula and only to be removed for the injections.

*Preparation of Infusion cannula:*Infusion cannula was fabricated from Septoject Sterile dental needle of 30 gauge stainless steel needle (Septodont. Saint-Maur-des-Fosses Cedex, Fracia). This needle is siliconized and it ensures smooth insertion of cannula (Fig 2-A) . It has a white coloured hub, convenient for handling insertion into brain nuclei. Cut the needle with the help of scissors, keeping the length of about 3mm more than required length (Vertical co-ordinatein Stereotaxic Atlas)[8]. Polish off the tip to make the standard length of internal cannula (Fig. 2-B) such a way that its tip extends 1 mm beyond the respective guide cannula. Clear the bore of internal cannula, check for blockade by injecting normal saline or double distilled water using syringe. Opposite end of this internal cannula is attached to polyethylene microbore Tube (Tycon flexible plastic tubing S-54-HL) secured by adhesive Araldite. After air dry, internal cannula is ready for use (Fig.2.C).

Institutional ethical committee clearance was obtained before the commencement of the experiment.

Surgical Procedure: Male Wistar albino rats weighing about 250±10 g were selected and aneasthtised by injecting a mixture of ketamine HCl (60 mg/kg) and xylazineHCl (6 mg/kg). A 2 cm long incision is made on the scalp, thoroughly disinfected with surgical spirit. The area is cleaned up with cotton and Hydrogen Peroxide. Points are marked on the skull in corresponding areas to reach Basolateral Amygdala. Burr hole was made using a dental drill. Guide cannula was lowered by stereotaxic technique and secured to the surface with the help of support screw and dental acrylic cement (DPI-RR Cold Cure, acrylic powder, Dental Product of India, Mumbai). For Basolateral amygdala (BLA), co-ordinates are from Bregma: anteroposterior -2.6mm, Lateral ±4.9 and vertical 8.5mm) (Fig.3). A guide cannula of 8.5 mm is prepared for the purpose of implantation in BLA. The external cannula was implanted on to the skull surface through carefully made burr holes at points corresponding to the BLA, either on left side or right side, depending up on the choice (for Unilateral infusion). The guide cannula was inserted into the burr hole, for 7.4 mm, by handling the steel wire attached to it (with 'araldite' adhesive gum). Once the cannula is in place, then it is secured with the help of screw and dental acrylic. This is left for setting for a day. Then the steel wire attached to guide cannula was carefully removed and the guide cannula with the stillet inside it was ready for use. The rats were left for recuperation for 2-3 days.

Using internal cannula: The internal cannula fabricated using 30 gauge dental needle with plastic hub is connected to the polypropylene tubing. The cannula was held with the hub and inserted into guide cannula after removing the stillet. Because the length of internal cannula from the tip of plastic hub is carefully measured, such a way that when it is inserted, it will reach the exact point in the brain, in this case, the BLA. Cannula will fit into the guide cannula and it is ready to be used for infusion of chemical agents.

In the present experiment, bilateral injection Orexin A was tested. Two groups of adult male rats were selected and divided into control group (n=6) and experimental group (n=6). In the experimental group, 3 nmol/rat of Orexin A (Sigma Chemicals, USA) was infused over a period of 2 minutes using Harvard Pico Plus (USA) infusion pump connected to the tube with cannula placed on right and left BLA one after the other as the case may be (Fig 3). The infusion solution was taken in the cannulated polyethylene tube up 10 microliters with the help of Hamilton syringe. This syringe was fitted into the pump. Then the stillet placed in the guide cannula was removed. The internal cannula was inserted into the guide cannula and secured. Then the pump was started to deliver the solution into the BLA nucleus. Normal saline was infused in the control group, but for this all the experimental procedures are same for both groups. At the end of infusion, the cannula was removed and stillet was placed back in position. Time was noted. The food intake, water intake were measured, by providing pre weighed quantities to each animal, at intervals of 1, 2, 4 and 24 hours in both the groups. The procedure was repeated two times.

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III. RESULTS

Histological confirmation: At the end of this experiment, the rats were sacrificed by lethal dose of Ether anaesthesia and the transcardially perfused with buffered formal saline. The cannula was carefully removed. The brains tissue was processed and paraffin blocks were made. Sections of 7 microns were sliced and stained with crysyl violet and observed under light microscope (Fig.4), to confirm the site of injection.

Single dose Orexin A infusion increased the food intake and water intake in rats, compared to the control (Table 1). The increase was highly significant in the first hour (Table 1; $p<0.01$). Food intake showed significant increase in the fourth hour also ($p<0.05$). Though the intake was increased in all the time periods viz. 1, 2 and fourth hour, but total consumption for 24 hours did not show statistically significant increase in intake.

IV. DISCUSSION

We described here a simple method of fabrication of cannula assembly for intracranial administration of chemical agents. Two characteristics of dental needle used here for the internal cannula made it very useful. One is the plastic hub. Plastic hub offered a good grip to handle to internal cannula. Often the cannula required is very short (more or less One cm). The plastic hub can be firmly held in the hand to insert into the guide cannula without any difficulty or irritation to the moving animal. Second advantage of this needle is its temper. Dental needles are stiff and do not bend easily, unlike needle in insulin syringe or other stainless steel needles. Due to this property, this cannula can be used repeatedly, without bending or damaging it. The previously reported articles have used different varieties of stainless steel needles. When we use very thin needles, it is necessary to take great care so that the needle is not bent, without which there will be error in the delivery of the drug.

In the present study, we implanted BLA bilaterally with the cannula, fabricated in our laboratory and infused the rats with Orexin A. 3 nmol/rat for 2 min bilateral infusion of Orexin produced the data shown in the table. (Table 1) Two trials were carried out and the mean of two trials are represented in the table. Analysis of data suggested that a single dose of Orexin A infused for 2 minutes led to increase in the food and water intake in the study group of rats after 1st hour as well as the intake in 24th hours. The vales were significantly higher when compared to the placebo infused controls. Food intake in the second and fourth hour ($p<0.05$) in treated rats showed a decline. The Orexin has a half-life period of about 27- 40 minutes in blood. [9] Small dose injected to the BLA caused short term increase in the food and water intake. This effect was reduced in the second hour and fourth hour. These results proved that the efficacy of the newly fabricated internal cannula in infusing study drug, Orexin A, on target was as desired. This was also confirmed by the histological examination of the rat brain at the end of experimental procedure (Fig. 4).

Therefore, we here present economical method to prepare a dependable cannula assembly, for the purpose of neurophysiological manipulation of brain areas in animals.

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Table 1. Food intake (g), water intake (ml) in Control and Orexin treated rats (values Mean of two trials & Group mean±SD) in first hour, 2nd hrs, 4th hrs and 24 hrs.

Parameter	Rat. no	Control 1 hour	Treated 1 hour	Control 2 hours	Treated 2 hours	Control 4 hours	Treated 4 hours	Control 24 hours	Treated 24 hours
food	1	1.4	4.75	1.35	0.45	0.65	0.5	10.1	10.85
	2	4.25	6.85	0.2	0.1	2.7	0.5	12.05	13.9
	3	2.65	3.7	0.6	0.6	2.7	1.45	13.8	15.35
	4	3.55	6.4	2.1	0.35	1.05	0.4	14.7	15.3
	5	2.65	6.55	1.45	0.85	1.8	0.5	11.4	8.8
	6	2.05	4.2	0.45	1.05	1.55	0.8539	14.2	15.35
	Mean±SD	2.75±1.02	5.40±1.35**	1.02±0.72	0.56±0.34	1.74±0.84	0.7±0.39†	12.70±1.80	13.25±2.79
water	1	2	3	3.5	4	4	1.5	19	27
	2	6	5	4.5	3	4	2.5	28.5	24.5
	3	1.5	4.5	3	2	2.5	3	21.5	22
	4	4.5	4	2	3	5.5	2	28.5	29
	5	1.5	3.5	0.5	1	4	1	18.5	14
	6	1.5	4	1	8	6	5	32.5	29
	Mean±SD	2.83±1.94	4±0.70**	2.411±1.53	3.5±2.42	4.33±1.25	2.5±1.41†	24.75±5.84	24.25±5.70

** $p<0.01$ Control Vs Treated group; † $p<0.05$ Control Vs Treated group

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Fig 1: Cannula Preparation: 1. Needle for Guide Cannula; 2. Needle of the Syringe isolated; 3. Gide cannula of required length; 4. Guide Cannula fixed to steel wire ready for implantation.

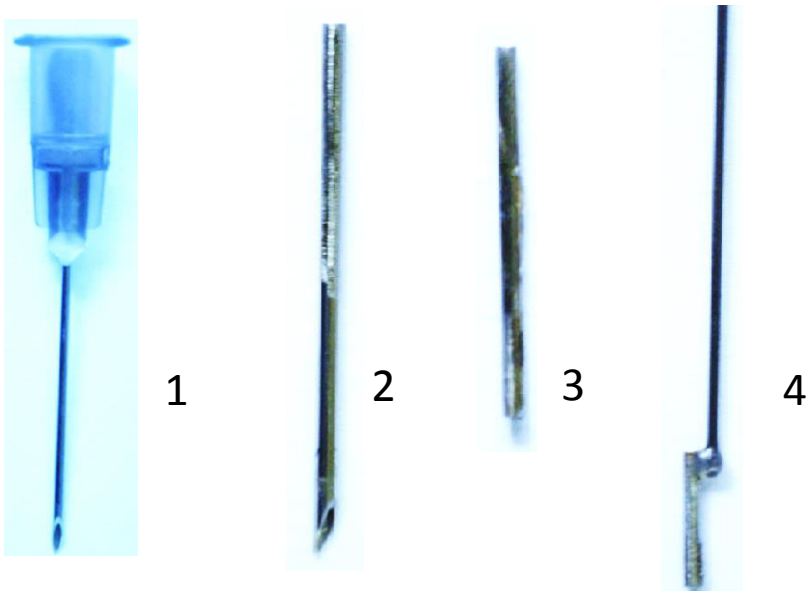

Fig 2. The Internal cannula prepared using Dental needle with polyethylene infusion tube. A – The fresh needle; B – Needle cut into required size to be used as cannula; C – Cannula fitted to the tube ready to be used.

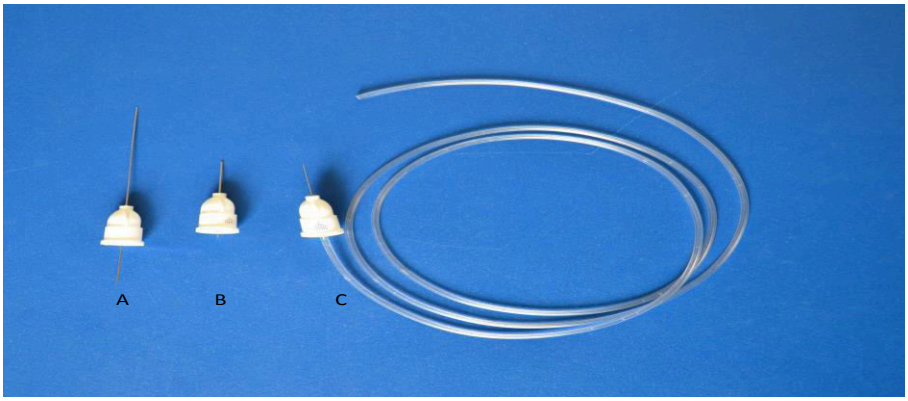

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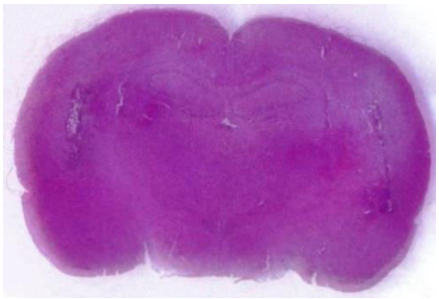
Fig 3: Rat being infused Orexin A

Fig 4: The photomicrograph showing the site of implantation of cannula. Magnification 2x.



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REFERENCES

[1] Hayden, J.F., Johnson, L.R. &Maickel, R.P. "Construction and implantation of a permanent cannula for making injections into the lateral ventricle of the rat brain". Life Sci., 5;1509-1515: 1966.
[2] Sparkes C.G & Spencer P.S.J. "Antinociceptive activity of morphine after injection of biogenic amines in the cerebral ventricles of the conscious rat", Br. J. Pharinac., 42;, 230-241: 1971.
[3] Gleeson R.M, Dragunow M.G, Kirton N.F, Villiger J.W, Chute D.L. "Intracranial cannulation of small animals Behavior Research Methods &Instrumentation", Vol. 12(3);346-348:1980.
[4] Dadasaheb M. Kokare, Gajanan P. Shelkar, Chandrashekhar D. Borkar, Kartik T. Nakhate, Nishikant K. Subhedar. "A simple and inexpensive method to fabricate a cannula system for intracranial injections in rats and mice". Journal of Pharmacological and Toxicological Methods, 64; 246–250: 2011.

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[5] Packard K, Pohorecky L.A, Brick J."A simple cannula for intraventricular drug administration in rodents". J Neurosci Methods.10(2);139-43:1984.
[6] Williams L.R, Vahlsing H.L, Lindamood T, Varon S, Gage F.H, Manthorpe M"A small-gauge cannula device for continuous infusion of exogenous agents into the brain". Exp Neurol. 95(3);743-54: 1987.
[7] Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli R.M, Tanaka H, et al., "Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour". Cell,92 (4); 573–85: 1998.
[8] Paxinos, G. and Watson, C.: "The rat brain in stereotaxic coordinates", 2nd Ed. New South Wales, Australia, Academic Press, 1986; Pp 54-56.
[9] Ehrström M, Näslund E, Levin F, Kaur R, Kirchgessner A.L, Theodorsson E, Hellström PM. "Pharmacokinetic profile of orexin A and effects on plasma insulin and glucagon in the rat". RegulPept.119(3);209-12: 2004.

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