Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 639-642, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00191-X

New Technique for Cannulae Implantation Into the Dorsal Raphe Nucleus Using Layer 5 of Cerebellum Reference in Rat Stereotaxic Surgery

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Received 12 January 1994

DIB, B. *New technique for cannulae implantation into the dorsal raphe nucleus using layer 5 of cerebellum reference in rat stereotaxic surgery.* PHARMACOL BIOCHEM BEHAV 49(3) 639-642, 1994.—A new surgical method for needle or electrode implantation using layer 5 of the cerebellum (C5) as anatomical reference in rat stereotaxic surgery was developed. The coordinates of the new technique for electrode or needle implantation into dorsal raphe nucleus (DRN) are: PA: 3.20-340 mm, V: 6.00-6.40 mm, and L: 0.00 mm. The success of the new surgical procedure was confirmed by histological control of the lesion impact of the electrode into NRD. This lesion impact was well positioned in 83-86% of implanted rats. The use of this method can be extended to other nuclei than the DRN of the brain for cannula implantation. In addition, it can be used in rats on a larger scale, because it suppresses the limitations due to age and body weight.

Surgical procedure Cerebellum Layer 5 Security of implantation

MANY investigators have developed a stereotaxic coordinates atlas for reaching brain structures referring only to rats of a particular body size (1,3).

This is unfortunate because rats continue to grow throughout their lifetime and many experimental designs could well use rats of different age and size (2). The actual stereotaxic atlases coordinates of Paxinos and Watson (1982) (6) use bregma as the zero reference for small-sized rats (180-290 g). In their atlas introduction, Paxinos and Watson explain that no substantial stereotaxic error will occur when rats of different sex and strain are chosen, provided the rats are of a similar weight as those on which the atlas is based (290 g). In fact, there is a difference between juvenile and mature rats if we consider the anteroposterior distance between the interaural line and the bregma. For example, the craniometric data for the juvenile (180 g) and mature (435 g) rats differ substantially. The anteroposterior distance between the interaural line and bregma is 7.7 mm in the juvenile and 9.7 mm in the mature rats (9.0 mm in atlas rats). This difference between the two groups is due to continued growth throughout the rat's lifetime. However, at the present time, the Paxinos and Watson atlas is widly used in laboratories.

Previously, Wishaw et al. (7), had compared the accuracy of stereotaxic localization using the interaural line and the bregmoidal intersection as the zero referent in male rats weighing from 161 to 782 g (bregma and lambda were horizontal). These authors showed that the change in the interaural line to bregma distance is apparently related to the increase in the size of the rat's head with age. For example the interaural line to incisor distance in a 161 g rat was 27.8 mm; in a 665 g rat, the distance was 39.2 mm. As body weight increased, so did head length, so that the angle of the skull and the distance from intraural line to both the incisors and bregma varied as a linear function of body weight (7). In order to circumvent the error of stereotaxic coordinates between juvenile and mature rats, these authors adjusted their electrode emplacement using an appropriate regression formula.

The purpose of the present study was, thus, to develop a new simple surgery technique, but not a new atlas, to improve the access to different structures of the brain. It is proposed that the layer 5 of the cerebellum should be used instead of the bregma as zero referent for juvenile and mature rats.

METHOD

Before developing the new surgical technique to reach the dorsal raphe nucleus (DRN) to study the effect of electrical stimulation or of glutamate injection into the DRN on the

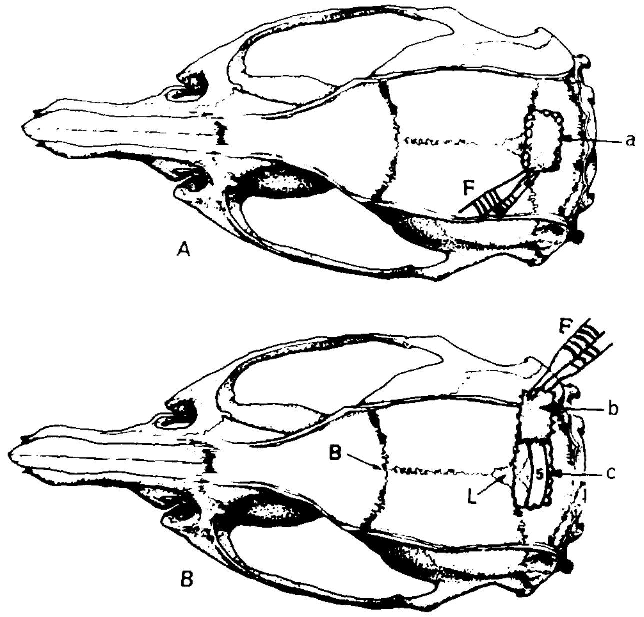

FIG. 1. (A) a—Drilled occipital bone (4 × 3 mm); F—forceps. (B) b—The occipital bone drilled above was removed using the forceps; c—posterior limit of the layer 5 of cerebellum. In this figure, the posterior limit of the layer 5 is taken as zero reference coordinate for electrode or needle implantation into dorsal raphe nucleus (DRN).

activation of brown adipose tissue (BAT) in rats weighing between 190-350 g, we first used the following stereotaxic coordinates from Paxinos and Watson (6): AP 7.30, 7.80 mm; V: 6.00, 6.40 mm; and L: 0.00 mm. The bregma was used as the reference (bregma and lambda were horizontal). The results showed that only 7 out of the 26 instrumented rats manifested an increased thermogenesis after glutamate injection (1 M in 0.50 μl). The absence of the expected thermogenetic response in a majority of rats led us to verify histologically the syringe insertion, using the atlas of Paxinos and Watson. In each rat, it appeared that the lesion practiced at the end of the experiments was more posterior or more anterior with respect to the DRN. However, at the start of each experiment, when the rats were mounted in the stereotaxic apparatus, we often verified the horizontal position of bregma and lambda. It was, therefore, surprising to find that in 75% of the cases the needle insertion was out of the DRN.

In order to circumvent such difficulties in the needle (or electrode) implantation into the DRN, we, thus, have developed a simple surgical technique that appeared to be much more successful.

Animals

For this purpose, semisterile instruments, electrodes, or needles were used. Forty fed male Wistar Canadian rats weighing between 220-440 g were anesthetized with urethane (1.4 g/kg) intraperitoneally (IP), and then mounted on a Kopf stereotaxic apparatus. After the animals had been prepared for electrode or needle insertion into DRN through a hole in the skull, a small opening (4 × 3 mm) 1-2 mm back of the bregma was drilled without harming the dura. When the parcel of the occipital bone was removed, the layers 5-6 of cerebellum (C5-C6) were visible. In this region, the venous sinus is just anterior to C5 and between bregma and C5 or just down to bregma. The posterior limit of C5 (between C5 and C6) was used as zero referent. The time necessary to drill and visualize the C5 was very small (5-10 min) from the moment where the occipital bone was drilled until the visualization of the limits of C5 (Fig. 1). The final coordinates of the new technique for electrode or needle implantation into NRD were—PA: 3.20 mm for small rats; PA: 3.40 mm for adult rats; V: 6.00, 6.40, and 6.80 mm; L: 0.00 mm.

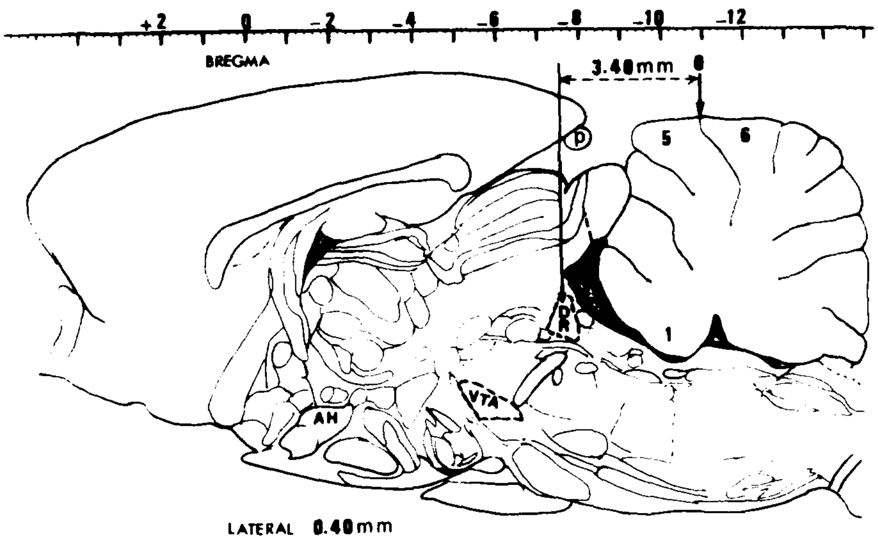

FIG. 2. This figure shows the lateral section of the brain and the cerebellum from Paxinos and Watson. This figure shows the zero (0) coordinates taken from the posterior limit of the layer 5. The stereotaxic coordinates (PA) to reach the DRN and VTA were, respectively: 3.20–3.40 mm; 5.00–5.20 mm.

(A)

(B)

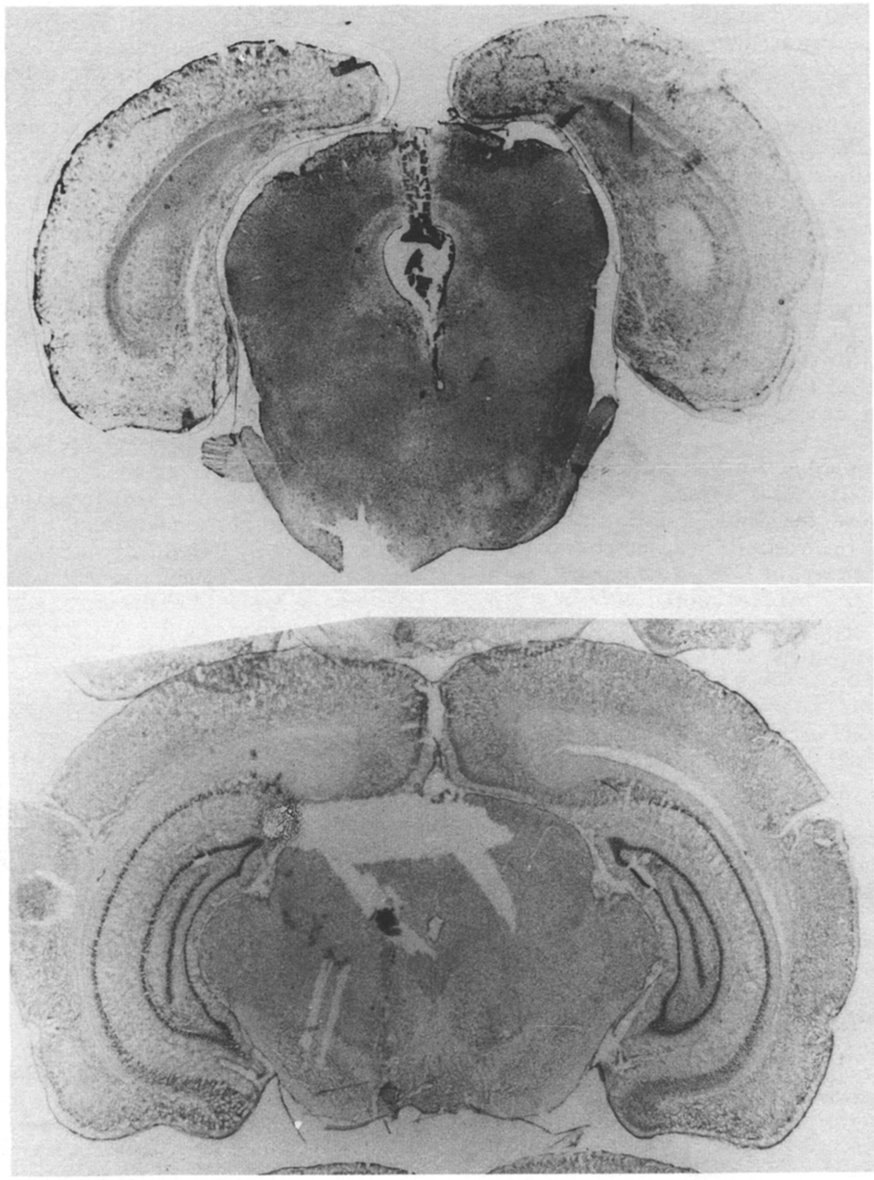

FIG. 3. (A) Photomicrograph of a rat weighing 315 g showing the location of electrode insertion and electrolytic lesion of the DRN. (B) Photomicrograph of a rat weighing 280 g showing the location of electrode insertion and electrolytic lesion of the VTA. In both cases, the electrode was well positioned as shown in these figures.

These coordinates cited above were calculated from the lateral section of the brain and the cerebellum according to Paxinos and Watson (Fig. 2). This figure shows the zero coordinates taken from the posterior limit of the layer 5 of cerebellum. These were the new coordinates used to reach the DRN.

At the end of each experiment, a small electrolytic lesion was made to mark the tissue surrounding the needle or electrode tip according to the following procedure: a direct anodal current of 80–100 μA for 15–20 s duration was passed through the electrode or the needle.

At the end of each experiment, a small electrolytic lesion was made to mark the tissue surrounding the electrode tip by passing a direct anodal current. The animals were then perfused transcardially with physiological saline and 10% buffered formalin. Brains were removed and immediately placed for 16–24 h in a solution of potassium ferricyanide (3%), potassium ferrocyanide (3%), and trichloroacetic acid (0.5%), so as to stain the iron deposited by the lesioning current. Brains were then immersed in 10% formalin for 10 days followed by 20% sucrose for 24 h. Coronal sections (30 μm) were cut using a Leitz freezing microtome and stained with thionin.

Histological analysis for all rats indicated that the implantation of electrode or needle insertion for glutamate injection was well positioned in 33 out of 37 rats into NRD at AP 7.30, 7.80, 8.00, and 8.30 mm.

Figure 3 (A) shows the place of the electrolytic lesion in one rat that weighed 315 g. In this rat, the histological control revealed that the impact of the lesion of the needle insertion was into the DRN. In this rat and others the glutamate injection into NRD or the electrical stimulation of NRD produced an increase in heat production (>0.8°C) by the brown adipose tissue (BAT) (4,5).

The success obtained when emplacing a needle or electrode into DNR obtained following the coordinates (AP: 3.20–3.40 mm) from the C5 of cerebellum led us to extend this new technique to reach other nuclei, such as, for instance, the tegmental ventral area (VTA). The coordinates to reach VTA were—PA: 5.00–5.20 mm; V: 8.80–8.20 mm; L: 0.40–0.50 mm.

Figure 3 (B) shows the place of electrolytic lesions of VTA in one rat weighing 280 g. The photomicrograph of VTA shows that the tip of the electrode was well positioned into the VTA. Histological analysis for 12 rats indicated that the placement of the electrode was well positioned in 10 out of 12 rats.

The new surgical procedure for stereotaxic coordinates in rats appeared to give very positive results, because in 83.0–86.00% of rats implanted, the histological control proved, by the lesion impact, that the electrode was well positioned into the DRN or VTA. However, in 14% of the animals, the impact lesion of the electrode was behind the DRN. If we compare our new stereotaxic coordinates with those of Paxinos and Watson (6), it clearly appears that our stereotaxic coordinates from C5 (when C5 was taken as zero referent) are more valid than those from bregma (when bregma was taken as zero referent). From that point of view, the coordinates of Paxinos and Watson for electrode or needle implantation into DRN were not satisfactory, because only 7 out 26 rats were well implanted.

However, in the present work, we wish to emphasize that the atlas of Paxinos and Watson provided the anatomical and histological basis necessary to accomplish the new surgical procedure. Our technique is mainly a new surgical procedure with new stereotaxic coordinates.

It may, therefore, be hypothetized that the correct implantation of needles into DRN or VTA in juvenile or mature rats was made possible either by the simultaneous growth of both cerebellum and brain in the same direction (anteroposterior or posteroanterior) or, on the contrary, by little variation of growth between cerebellum and the brain in the juvenile and the mature rats. These hypotheses are capital to understand the success of the new surgical electrode or needle implantation.

Our hypotheses may be indirectly in agreement with these of Whishaw et al., because these authors took into consideration the growth variation of the skull in their stereotaxic coordinates mesures between juvenile and adult rats.

It may, therefore, be concluded that the stereotaxic coordinates reference from the layer 5 (C5) following our method is valid in both juvenile and adult rats.

In the present work, the surgical procedure is very simple and the correct acute implantation of the electrode into dorsal raphe nucleus (DRN) could be used for future chronic cannula or electrode implantation. In addition, the surgical procedure can be extended to the use of animals on a larger scale, because it suppresses the limitations due to age and body weight.

ACKNOWLEDGEMENTS

This study was developed at the Center for study of Behavioral Neurobiology (Concordia University), and supported by a grant (ERO 124) to Peter Shizgal, Pierre-Paul Rompré, and Shimon Amir from the "Fonds pour la formation de Chercheurs et l'aide à la Recherche du Québec."

I wish to thank Françoise Delorme for kindly typing the manuscript and Ghislaine Geelen for the English revision.

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