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Stress-free microinjections in conscious rats

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ABSTRACT

Microinjections are a major tool in modern neuroscience. Microinjection techniques in conscious animals typically involve four steps: (1) animal adapts to experimental setup; (2) injection system is filled and the microinjector is carefully inserted; (3) a drug solution is injected; (4) 1–2 min later the microinjector is carefully removed. Steps 2 and 4 are difficult to perform in rodents without disturbing the animal. This disruption can cause stress and accompanying tachycardia and hyperthermia – unwanted artifacts in physiological research. To reduce these effects, we altered the traditional approach. Our procedure of microinjection consisted of the following steps: (1) we filled the injection setup and fixed the microinjector in its guide cannula; (2) allowed an animal to adapt to the setup; (3) performed an experiment including microinjection(s); (4) removed the microinjector after the experiment was complete. The key change we incorporated was a 1 m long piece of tubing with a small internal diameter; it allowed us to inject nanoliter volumes through the injector which had been placed into the guide cannula in advance. This way we avoided the usual manipulations related to microinjection, and minimized extraneous disturbances to the rat. In this report we describe the details of this technique in conscious rats and provide examples of the effects and the reproducibility of a 100 nL drug injection on cardiovascular function.

1. Introduction

The topical application of drugs to specific brain areas in non-anaesthetized animals is an experimental technique dating back almost a hundred years. Since the initial study in 1915 by Hashimoto et al. in which 200 μL volumes were injected intracerebrally in rabbits, this approach has been refined and modified in many ways (Greenshaw, 1998). For instance, smaller animals such as rats, which are less expensive and easier to standardize, are now widely used. In addition, the use of small injectors sizes and volumes has allowed a greater degree of anatomical resolution. In this regard, microinjection with glass pipettes using air-pressure puffs to deliver accurate and reproducible volumes in the nanoliter range (Amaral and Price, 1983) constituted a technical revolution. Despite limited success (Azami et al., 1980), due to the extreme fragility of glass pipettes this approach is impractical in conscious animals. Microinjections using metal or polymer needles which target the brain area of interest through guide cannulas implanted in advance, and using injection volumes in the range of 50–100 nL are most common in current rat studies.

The current paradigm in microinjection generally includes four steps with variations. First, a guide cannula is cemented to the rat's skull. During the healing period, the rat is handled by testers and habituated to the conditions. Second, on the testing day after the animal is acclimated to experimental conditions, a microinjector is carefully inserted into a guide cannula with the intent of creating as little disturbance to the animal as possible. Third, immediately after the placement of the microinjector, the drug or solution of interest is injected, typically over 30–60 s. Fourth, approximately 1–2 min after the injections, the microinjector is carefully removed, bothering the animal again.

One of problems with microinjections in rodents is that the placement of the injectors typically arouses the animals and causes stress, shown by an increased heart rate (HR) and activity. These effects can mask changes from the injected drugs. Given this problem, there is a need for a reliable and reproducible method by which small volumes of drugs can be injected, which does not disturb conscious animals, so that physiological parameters can be measured shortly after the injection is made.

In our method we inserted the microinjectors, and then allowed the animals to acclimate for 1–2 h; this permitted injection of the drug or solution of interest without evoking stress. This eliminated

Abbreviations: ACTH, adrenocorticotropic hormone; BMI, bicuculline methiodide; DMH, dorsomedial hypothalamus; HR, heart rate; MBP, mean blood pressure; PBS, phosphate buffered saline; PVC, polyvinylchloride.

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often seen artifacts which can mask immediate effects of whatever is injected. In addition, we found that leaving the injector in place until the end of the experiment likewise reduced animal stress.

The purpose of this manuscript is to both describe in detail our microinjection technique, and to provide evidence supporting its reliability and reproducibility.

2. Materials

2.1. Hardware

The Dataquest telemetry system (Data Sciences Int., St. Paul, MN) was used for measurements of arterial blood pressure, HR, and locomotor activity. Telemetric probes were sterilized according to manufacturer's recommendations before implantation.

Guide cannulas for acute experiments (#C315GA; 26 ga; ID = 0.24 mm; OD = 0.46 mm; length 10 mm below pedestal; Plastics One, Roanoke, VA) were used with corresponding single internal cannulas (#C315A; Plastics One; 33 ga, ID = 0.1 mm; OD = 0.2 mm). The internal cannula had been made with long enough to allow the tip of the injector to protrude 1 mm below the tip of guide cannula. Dummy cannulas (#C315DC; OD = 0.2 mm; Plastics One) were cut to the exact length of the guide cannula. In experiments which involved chronic guide cannulas (#C315G, same specifications as #C315GA), we used the corresponding injectors (#C315I). Guide cannulas were fixed to the skull with metal screws (#0-80, Plastics One), veterinary glue (Vetbond, 3M, St. Paul, MN) and fast-curing cranioplastic cement (Jet Denture Repair, Lang Dental Manufacturing Co., Wheeling, IL).

Arterial and venous lines were prepared from microbore polyvinylchloride (PVC, Tygon) tubing (Y-TGY-020; ID = 0.5 mm; OD = 1.5 mm; Small Parts Inc., Miami Lakes, FL) and Teflon tubing (Y-SWTT-28; 28 ga; ID = 0.38 mm; OD = 0.83 mm; Small Parts). One end of a 22 cm piece of Tygon tubing was soaked in acetone for 10 min, and then a segment of Teflon tubing was inserted 5 mm into the temporarily expanded end of the Tygon tubing. This joint was allowed to dry for at least 30–60 min and cut with a sharp blade to leave 5.5 cm long Teflon leader which was later inserted into the blood vessel. No glue was required. Prepared catheters were sterilized for at least 2 h by UV light before being placed in the animal (Section 3.2).

2.2. Microinjection setup

Microinjections were performed through commercially available microinjectors (Plastics One). The microinjector was connected to a 10 μL syringe using thick-wall, low-internal volume Teflon tubing (FEB tubing; ID = 0.12 mm; OD = 0.6 mm; internal volume = 12 μL/m) from CMA Microdialysis (North Chelmsford, MA). Connectors were cut from the same microbore PVC (Tygon) tubing, which was used to manufacture intravascular catheters. Polyetheretherketone (PEEK) tubing (ID = 0.12 mm; OD = 0.5 mm; Upchurch Scientific, Oak Harbor, WA) could have been used for solution delivery, but due to smaller outer diameter we found it difficult to provide tight connections between components.

To affix the injector to the guide cannula, a custom cap with a 0.5–1 mm hole at the top was fashioned from a dust cap (#C303DC/1) or from a used dummy cannula (#C315DC, wire was removed during drilling). A bigger dust cap (#C303DC) could also be used.

Syringes (10 μL, 1800 series, Hamilton Company, Reno, Nevada) with a 22S needle (point style No. 3) were mounted on a syringe pump appropriate for reliable delivery of 100 nL (KD Scientific, Model 200, Holliston, MA).

3. Methods

3.1. Animals

Male Sprague–Dawley rats (280–300 g) (Harlan, Indianapolis, IN) were maintained under standard animal housing conditions, including a 12-h light (lights on 07:00 am) and 12-h dark illumination cycle. Animals were housed singly. Care and use of rats was in accordance with protocols approved by the Indiana University Animal Care and Use Committee and was carried out under the supervision of veterinarians. Rats were housed in the Indiana University Laboratory Animal Resource Center, an Association for Assessment and Accreditation of Laboratory Animal Care-approved facility, following IACUC guidelines. Animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). All procedures were performed using antiseptic and aseptic techniques. For surgical procedures rats were anesthetized with Nembutal (50 mg/kg, i.p.; Abbott Laboratories, North Chicago, IL) or a Ketamine/Xylazine mixture (80 mg/kg Ketamine, Hospira Inc., Lake Forest, IL and 11.5 mg/kg Xylazine, Lloyd Inc., Shenandoah, IA, i.p.). After surgery, animals received injections of buprenorphine (15 μg/kg s.c., Hospira) and were monitored until they recovered from anesthesia. All animals for which data was reported in this manuscript remained in good health throughout the surgical procedures and experimental protocols as assessed by appearance, behavior, and maintenance of body weight.

3.2. Telemetric probe implantation

Telemetric probe for recording of HR, mean blood pressure (MBP) and locomotor activity were implanted intraabdominally using manufacturer's instructions.

3.3. Guide cannula implantation

At least seven days after implantation of the telemetric transmitter, the rats underwent cranial guide cannula placement. Rats were anesthetized as above and placed in a stereotaxic apparatus with the incisor bar set 5 mm above the interaural line. Skin overlying the dorsal surface of the skull was infiltrated with Lidocaine HCl/Epinephrine (2%/1:100,000; Hospira), then cut and retracted. Soft tissue was removed to expose the surface of the skull. This area was then washed using cotton-tipped applicators saturated with a 30% hydrogen peroxide solution. In addition to reducing bleeding and aiding in the maintenance of sterility, hydrogen peroxide enhances the visibility of sutures used as stereotaxic landmarks.

Stainless steel guide cannulas were placed unilaterally to allow microinjections targeting the dorsomedial hypothalamus (DMH) as described previously (Bailey and DiMicco, 2001). Disinhibition of the DMH produces dramatic cardiovascular, neurohumoral and behavioral responses (Bailey and DiMicco, 2001; Zaretskaia et al., 2002). As neurons in this region are extremely sensitive to the placement of microinjectors and to injections of solutions, it serves as an excellent model to validate our technique. Stereotaxic coordinates for cannula placement, using bregma as the reference point and a 10° angle from the sagittal plane were 1.2 mm posterior, 2.1 mm lateral and 9.1 mm ventral.

A small hole in the skull to insert a cannula was made with a rotary tool (MiniMite Cordless 4.8V, Dremel, Racine, WI) equipped with a surgical carbide burr (DHP557, Miltex, Plainsboro, NJ). Three jeweler's screws (size 80) were placed into the skull near the hole to guarantee the reliable attachment of cement cap. After positioning of the guide cannula, the exposed skull and most of the wound surface was covered with veterinary cyanoacrylate glue, followed