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Successful intracerebroventricular cannulation of a eusocial mammal

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HIGHLIGHTS

- We performed intracerebroventricular cannulations in the naked mole-rat.
- Cannulated animals were successfully reintegrated into a colony setting.
- Exposure of cannulation head-caps was minimized and none were lost.
- Cyanoacrylate gel provides good stability for anchoring guide cannulae in small rodents.

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ABSTRACT

Background: Manipulating neural activity in live animals within a colony would allow researchers to more fully explore the neurobiology of complex social behaviors. However, some colony-living animals like the naked mole-rat (*Heterocephalus glaber*) cannot be reintroduced to a colony after the extended recovery time required following cranial surgery. Furthermore, the colony setting creates increased risk of infection and interruption of cranial surgical sites.
New method: A protocol for intracerebroventricular cannulations was developed for securing and minimizing exposure of the intracranial apparatus. We tested whether animals could be reintroduced to the colony immediately following surgery and whether they showed full recovery and expression of normal behavior a week later, after intracerebroventricular infusion of saline.
Results: Animals were successfully reincorporated into their home colony and showed normal behavior. No animals lost guide cannulae within their colony and loss of dummy cannulae was minimized. Any loss of animals was due to surgical complications or multiple intracerebroventricular infusions of saline rather than recovery in the colony, per se.
Comparison with existing methods: Standard cranial cannulation methods for small rodents were used with the addition of implanting a shortened guide cannula under the skin for limited exposure of cannulae to the external environment. Furthermore, dummy cannulae were sealed to guides to avoid loss in-colony.
Conclusion: The use of intracranial cannulations is a viable option for colony-living rodents when the proper care is taken to minimize cannula exposure and when animals are carefully and promptly reintroduced to the colony setting after surgery.

1. Introduction

Understanding the underlying neurobiology of social behavior in colony-living mammals may offer insights into complex sociality. However, challenges may arise when attempting in vivo manipulation of relevant neural systems in these animals. One such challenge is diffusion into the central nervous system as the blood-brain-barrier is impermeable to many molecules that might be of interest. For example, oxytocin is a peptide hormone that is produced primarily in the hypothalamus and acts as a neuromodulator or neurotransmitter (Landgraf and Neumann, 2004). While this neuropeptide has effects on a multitude of social behaviors across a large variety of species (Insel, 2010), it demonstrates low blood-brain-barrier permeability (Ermisch et al., 1985). Thus, although examining oxytocin-related neural systems might appear an attractive line of study, peripheral manipulation of oxytocin is not a practical option. Traditionally, problems like this would be overcome using stereotaxic surgical methods such as ventricle-directed cannulation for the general manipulation of neurochemical activity or nuclei-directed cannulation for more region-specific manipulation.

While stereotaxic techniques work effectively for rodents that can remain in solitary recovery such as rats, mice, or voles (Ferguson

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et al., 2001; Pedersen et al., 1994; Williams et al., 1994), they present problems for animals that need to be immediately reintroduced to a colony setting. For example, in our lab we use the naked mole-rat (*Heterocephalus glaber*) to assess the neurobiology of social behavior. Cannulation surgery would be an ideal method for directly manipulating the brain and assessing effects on in-colony social behavior. However, these surgeries often require recovery times that last 3–7 days (Jho et al., 2003). Members of a naked mole-rat colony that are removed for a period of at least 12 h are treated as unfamiliar intruders upon reintroduction and are subject to aggression by other members of the natal colony (O'Riain and Jarvis, 1997).

Even if surgery and reintroduction are possible, laboratory-housed naked mole-rats are maintained in multi-chambered structures connected with tubes, along with numerous conspecifics, in order to mimic the natural subterranean ecology of the species. Therefore, an additional concern is that the structural environment or conspecifics would cause unintentional removal of the dummy or guide cannulae. We developed an approach allowing intracerebroventricular cannulations to be performed and maintained reliably in this species and tested whether we could safely reintroduce animals back into the colony within the 12-h window.

2. Methods

2.1. Animals

103 adult naked mole-rats weighing between 22 and 60 g were used. Naked mole-rat colonies were housed in large (45.75 cm L × 24 cm W × 15.25 cm H) and small polycarbonate cages (30 cm L × 18 cm W × 13 cm H) connected by plastic tubes (25 cm L × 5 cm D). The number and sizes of cages for each colony are determined by the number of members. Rooms were kept on a 12:12 light/dark cycle at 28–30 °C. Animals had ad libitum access to a diet of sweet potato and wet 19% protein mash (Harlan Laboratories, Inc.). All procedures adhered to federal and institutional guidelines and were approved by the University Animal Care Committee.

2.2. ICV cannulation

Mole-rats were deeply anesthetized using isoflurane (3%, delivered at a rate of 1 L/min inhalation) and the surgical site was cleaned and sterilized with 70% EtOH and then 10% iodine solution (Betadine; repeated twice). Animals were positioned in a stereotaxic instrument (Benchmark™, MyNeurolab.com, St. Louis, MO) so that the top of the skull was parallel to the base of the equipment. While in the stereotaxic apparatus, anesthesia was maintained by isoflurane (2%, delivered at a rate of 1 L/min) via a nose cone. A 1.5 cm incision was made on the cranium and clamps were put in place to reveal the muscle that sits atop the frontal and parietal bones. An incision was made along the intersection between the muscle and the midline of the skull on the right side of midline. The incision was continued along the intersection between the muscle and lambda. The muscle was then peeled away from midline until approximately 5 mm of bare skull was revealed. The muscle and the skin were clamped to keep them away from the implantation site. Droplets of sterile saline were useful in keeping muscle hydrated during the procedure. The skull was then cleaned with sterile saline and dried. A 22-gauge stainless-steel guide cannula with a 2 mm pedestal (Plastics One, Roanoke, VA) was directed to a spot 0.9 mm lateral and 1 mm anterior to bregma. The area was marked and a small hole was drilled through the skull. The cannula was then lowered to 3 mm below the top of the skull. Stereotaxic coordinates were determined by an atlas of the naked mole-rat brain developed by Xiao et al. (2006). Cyanoacrylate gel was applied to the base of the pedestal to secure the guide cannula to the top of the skull. A dummy cannula was inserted in the guide cannula to prevent exposure, infection or occlusion. After the gel had dried, a small incision was made in the muscle and it was laid back on the skull around the cannula. The skin was then sutured together over the pedestal of the guide cannula exposing only the cap of the dummy cannula. A small dab of cyanoacrylate gel was placed on the outside of the dummy cannula at the juncture where the pedestal of the guide cannula and the cap of the dummy cannula meet. This extra gel should be small enough that it does not fully fuse the cap and guide together. 10 min prior to the completion of surgery, animals were injected with ketoprofen (Anafen®, Merial; 5 mg/kg BW). This injection was also administered once a day for 3 days post-surgery.

2.3. Recovery and reintroduction

Following surgery, animals were placed in a cage with clean bedding that sat on a heating pad. 8 h after an animal had recovered from anesthesia, it was returned to its home colony. Animals were placed in the toilet chamber of the colony in order to make sure that they were first reintroduced to the colony's scent. Animals were then closely monitored to ensure that they were not the recipients of agonistic behavior by their colony mates.

2.4. Evaluation of infusion

2.4.1. Single infusion

7 days post-surgery, 53 animals were lightly anesthetized using isoflurane (2%, delivered at a rate of 1 L/min inhalation) and the cyanoacrylate gel seal maintaining the dummy cannula was broken by a slight twist. The dummy cannula was removed and replaced with an internal infusion cannula that sat 0.1 mm below the base of the guide cannula. 1 μl sterile saline was infused via 500-μl Bas gastight syringes (MD-0050; Bio Analytical Systems) connected to the internal cannula with PE50 tubing. Infusions were automated at a rate of 1 μl/min with a Harvard infusion pump (Harvard Apparatus Inc. 22, Natick, MA). Following infusions, animals were allowed to recover from anesthesia and behavior was monitored for 20 min. 100 min later, 8 animals were re-anesthetized and 1 μl of India ink (10%, v/v) was infused at a rate of 1 μl/min for examination of dye diffusion throughout the ventricles. All animals were then euthanized using avertin (400 mg/kg i.p.) and brains were collected. Brain tissue was postfixed for 4 h in 4% paraformaldehyde and then immersed in 30% sucrose for at least 24 h. Afterwards, tissue was sliced coronally at 30 μm on a freezing microtome. A 1-in-4 series was mounted on gelatin covered slides, dehydrated, and stained with thionin for examination.

2.4.2. Multiple infusions

Multiple infusions were attempted on 35 of the animals 7 days post-surgery. Infusions were carried out in the same manner as described above. However, infusions were carried out once a day on 2 consecutive days. These animals were returned to their home colony after each infusion.

3. Results

Approximately 83% of animals that underwent intracerebroventricular cannulation surgery recovered fully from anesthesia and were reintroduced to the home colony (Fig. 1). No guide cannulae were lost while in colony. All animals that were reintroduced into the colony were successfully reintegrated (Fig. 2). These animals showed normal in-colony behavior before and after infusion with saline. Once animals were reintroduced, 100% survived a single infusion of saline. However, survival dropped to approximately