KOPF CARRIER

May 2018

Kopf Carrier #94

PUBLISHED BY DAVID KOPF INSTRUMENTS ■ TUJUNGA, CALIFORNIA

Cannulation and microinjection stereotaxic surgeries in the prairie vole (*Microtus ochrogaster*)

Caitlyn J. Finton, Aubrey M. Kelly, Alexander G. Ophir
Department of Psychology, Cornell University
Correspondence: ophir@cornell.edu

1. Introduction

1.1. The Prairie Vole

Prairie voles (*Microtus ochrogaster*) are small Avicoline rodents native to central and mid-west United States and south-central provinces of Canada (Cassola, 2016). Prairie voles are a uniquely notable species because (1) a rich literature on their natural history and behavioral ecology exists (Fitch 1957; Getz and Hofmann 1986; Getz and Carter 1980), (2) prairie voles adapt extremely well to laboratory environments (Carter et al. 1986), (3) scientists can leverage many of the tools developed in other more classic rodent models due to the prairie vole's phylogenetic relationship to them (Donaldson and Young 2008; McGraw and Young 2010), and (4) they share some relatively rare but defining behaviors with humans (i.e., communal living, social monogamy, and biparental care)(Lukas and Clutton-Brock 2012; Kleiman 1977; Carter 1998; Dewsbury 1981). Indeed, prairie voles and several congener species (including meadow voles [M. pennsylvanicus], pine/woodland voles [M. pinetorium], montane voles [M. montanus], and Mandarin voles [M. mandarinus – sometimes called Lasipodomys mandarinus]) represent a tremendous opportunity to understand the evolution and expression of social behavior (McGraw and Young 2010; Insel and Shapiro 1992; Gewirtz and Yong-Kyu 2016), and are well suited to serve as models for several aspects of human social behavior and dysfunction (Carter 2007; Young 2001; Young et al. 2002; Carter 2005; Beery and Kaufer 2015).

Studies of natural history beginning in the mid-20th century have yielded a rich and well-documented behavioral ecology for voles (Fitch, 1957; Getz et al., 1981). At the same time, prairie voles are particularly exciting for translational research because, unlike many mammals, this species exhibits rare social characteristics similar to humans: social monogamy and biparental care (Getz et al., 1981; Kleiman, 1977). Knowledge of the natural history and behavior of this species has allowed researchers to design and conduct experiments specifically relevant to questions that address their evolved social behaviors that parallel those seen in humans. For example, prairie voles form monogamous pairbonds with the opposite sex in both the laboratory and the field (Williams et al. 1992; Getz et al., 1981). Moreover, effort to unravel the neurobiological mechanisms that govern this behavior has yielded major advances that can inform individualized treatment and prevention of a number of behavioral disorders characterized by social deficits, anxiety, and aggression (e.g., autism-spectrum and anxiety disorders) in both children and adults. Because prairie voles exhibit biparental care

and alloparental care, they are also an excellent species for understanding the mechanisms underlying parental care (Ahern et al., 2011; Kenkel et al., 2017). The relatively short developmental period of voles (21 days from birth to weaning; 45-60 days from birth to sexual maturity) and reliable laboratory breeding allow for the study of developmental manipulations on social behavior and the brain later in life (Prounis et al., 2015; Hammock, 2015). Prairie voles are also used for studying reproductive tactics and space use (Ophir et al., 2008; Okhovat et al., 2015), and for understanding addiction and reward-circuitry (Aragona et al., 2007; Ryabinin and Hostetler, 2016). Overall, prairie voles offer insight into the neural mechanisms underlying numerous behaviors ranging from affiliation to aggression, anxiety, and addiction. In short, studies using prairie voles are uncovering the complex neural circuitry underlying pairbond formation, monogamy, and other important elements of sociality (Young and Wang, 2004; Gobrogge and Wang, 2016; Amadei et al., 2017).

Although the prairie vole has been used for neuroscience experiments for decades, to our knowledge, there lacks a literary reference for details pertaining to stereotaxic surgeries. Here we hope to contribute to the transparency of surgical methods in order to facilitate manipulative studies of the brain in the research community.

1.2. Stereotaxic Surgeries

In order to understand direct contributions of neural circuitry to various behaviors in the prairie vole, many researchers utilize stereotaxic surgeries such as cannulations or microinjections. These stereotaxic surgeries allow for controlled, direct manipulation of the brain in awake and behaving animals. Stereotaxic surgery via microinjection or cannulation allows researchers to study the direct effect of an injectable of choice (in liquid suspension) on behavior, or to determine the physiological or molecular effects of an injectable. In this case, cannulation involves inserting a hollow tube into the brain. Once inserted and secured, the injectable of choice can be infused through the cannula. Injections can be continual, periodically repeated, acutely repeated, or one time. Although cannulae are relatively stable after implantation, there is a chance that the cannula can be dislodged during either behavioral testing or the daily activities of the animal. As an alternative, microinjections are limited to one-time injections, but are not associated with head-gear and enable an animal to move naturally and freely after surgical recovery.

Whether with cannulation or microinjection, the injectable can be many things. Examples of injectables include a drug, purified hormone or other signaling molecule, a synthesized receptor agonist or antagonist, an oligonucleotide (oligo) construct designed to target a gene of interest, or a virally packaged construct capable of delivering genetic material that encodes a particular gene of interest or tools enabling chemogenetic manipulation. The cannulation and microinjection procedures make it possible to manipulate the whole brain via infusion of an injectable of choice into the ventricles (commonly referred to as intracerebroventricular [icv] injections) or with more precision in a site-specific way by targeting a particular region of the brain.

The power of cannulation and microinjection cannot be understated in terms of helping behavioral neuroscientists understand the mechanisms underlying animal behavior. For example, chemical lesions of brain areas can be achieved through these procedures (Alberts 1979) and have served as a cornerstone of behavioral neuroscience. Similarly, studies utilizing receptor antagonists delivered into the ventricles of prairie voles have elucidated neurochemical underpinnings of pairbond formation (Donaldson et al., 2010). Antisense oligonucleotides have been used to knockdown peptide production in specific brain regions.

For instance, this has been done in rats to inhibit nonapeptide receptor production in the septum, revealing a role for septal nonapeptide receptors in anxiety-like behavior (Landgraf et al., 1995). More recently, microinjections have been used to inject microparticle beads or nanospheres to effectively produce a slow-release implant effect in targeted brain regions. Choleris et al. (2007) infused microparticle beads containing antisense oligos into the medial amygdala of mice allowing for a timed release of antisense oligos over seven days, and identified the direct contribution of nonapeptide receptors in social recognition. Microparticle bead technology is of particular excitement to behavioral ecologists because it can theoretically allow neural manipulations of freely living animals under semi-natural or field conditions. Such studies can provide a complimentary line of research to laboratory studies, providing ecologically relevant information about natural behavior.

Various other techniques to study the brain can also be done with the use of stereotaxic surgeries. In prairie voles, implantation of microdialysis probes into specific brain regions have allowed for the measurement of hormone levels in the extracellular fluid in response to various stimuli (Curtis et al, 2003). More recently stereotaxic surgeries have been used in voles to implement optogenetic manipulations. For example, quite impressively, it is possible to bias a female prairie vole towards a male simply by activating a specific neural circuit via optogenetic stimulation (Amadei et al, 2017).

Below we discuss stereotaxic methods used in our lab to conduct cannulation and microinjection surgeries in prairie voles. Many of the procedures for these surgeries can be adapted for other stereotaxic surgical procedures. Finally, because of substantial species differences in responses to skull shape and composition and to anesthesia, translating surgical techniques across species or taxa can sometimes prove to be difficult. Thus, we discuss some differences between stereotaxic surgeries in prairie voles and surgeries in other species that are more commonly used for central manipulation.

2. Methods

2.1. Equipment

A complete list of equipment can be found in Table 1. For cannulation surgeries, all cannula components can be custom designed and ordered from PlasticsOne (Roanoke, Virginia, USA). This includes a guide cannula, which is implanted into the brain. Internal injectors are attached to polyethylene tubing to infuse substances through the cannula into the brain. Internal dummies remain inside the guide cannula at all times except during infusions. Finally, dust caps screw over the dummy and onto the cannula to cover the outside of the guide cannula, preventing unwanted particulates from entering the brain. Skull screws are used to secure the cannula and mount to the skull. Surgical screws and an accompanying screwdriver can also be purchased from PlasticsOne (screws: size 1/16, item number 39052; screwdriver: SD96). Cannulae are mounted to the skull using Loctite 454 gel cured with dental acrylic liquid. In the case of microinjections, we have used glass pipettes and Hamilton syringes (7000 series; Hamilton, Las Vegas Nevada). Custom needles from Hamilton (e.g., 26S, 1.5 in, 35 deg) have proven to be effective at accurately targeting our regions of interest. All equipment should be appropriately sterilized according to the Institutional Animal Care and Use Committee (IACUC) policy at the local institution.

Other equipment includes heating pads that are used to provide warmth to voles under anesthesia and also during post-op recovery. An electric razor is used to shave fur from the top of the head. In some instances, hair removal products (such as Nair, Church & Dwight, Ewing, New Jersey) can be used instead of a razor. Sterile drapes should be

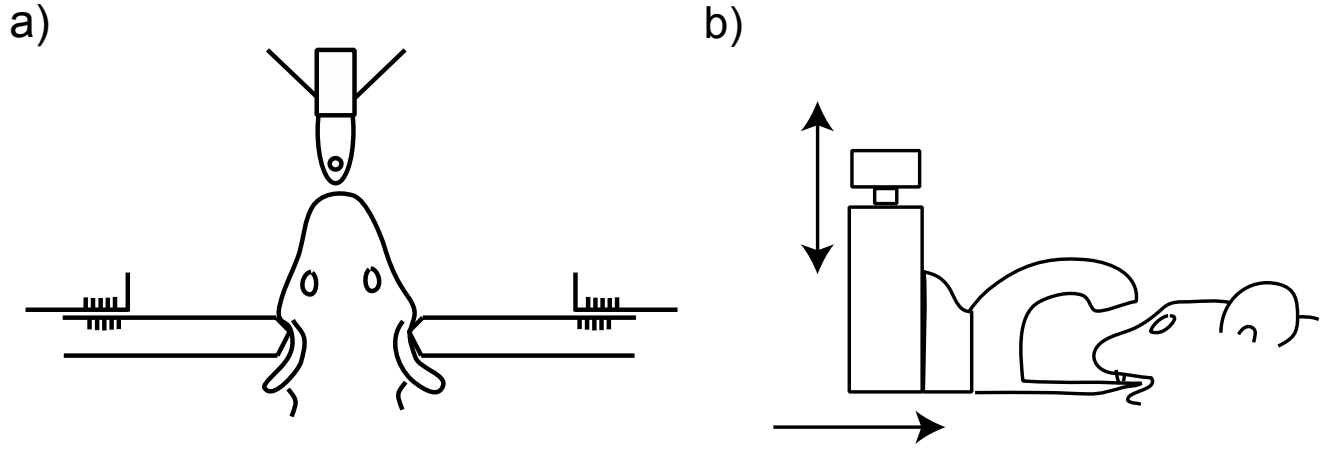
used accordingly for aseptic technique. Betadine is used as an antiseptic for disinfecting the skin prior to incisions of the scalp. It is important to use an ocular lubricant during anesthesia while blink reflex and tear production is decreased to protect the eyes from drying out. Sterile cotton tipped applicators are used for applying Betadine and also for drying the skull prior to cannula implantation. A cauterizer is helpful for marking implantation locations. We use a dremel for drilling holes into the skull for placement of screws and the guide cannula, or for creating holes to allow for microinjection; a dental drill is also appropriate for this purpose.

2.2. Anesthesia and fitting the vole to the stereotax

We use isoflurane to anesthetize our prairie voles during surgery. Although injectable ketamine is often used to anesthetize mice and rats, we have had far more success using isoflurane (see 'Troubleshooting' below). Importantly, there appears to be individual differences in isoflurane metabolism in the voles, so breathing should be constantly monitored throughout the procedure and isoflurane levels adjusted accordingly. In our setup, medical grade oxygen (O_2) flows through an isoflurane vaporizer into a custom built Plexiglas box, which is where our animals are initially placed for anesthesia delivery. A multi-valve manifold system allows for controlled direction of the anesthetic flow such that isoflurane + O_2 can be delivered to the box, directly to the stereotax, or to both simultaneously. Voles are anesthetized with isoflurane vapor delivered at 2-4% of O_2 in the box for 3 minutes, and are then transferred to the stereotax.

The anesthetized animal should be fit to the stereotax quickly before the transient effects of isoflurane wear off. To begin, place the animal on a platform to raise the body to an appropriate level. Platforms can be purchased; however, a microcentrifuge tube rack or a calipers box can also be used. We use non-rupture ear bars with a 45° tip with a 0.8 mm radius. The ear bars should be positioned on the stereotax in the approximate location, with one bar secured and the other left loose. Fit the animal in the apparatus by securing one ear bar first, then adjusting the other ear bar. The animal should be placed against the secured ear bar and the loose bar should be slid into place until the animal is secure. Once the animal is secured, the head position can be moved to align with the bite plate, by placing firm pressure on the ear bars toward the animal and simultaneously sliding the ear bars until the head is in the ideal position. Care should be taken to avoid placing too much pressure on the animal, while also ensuring the animal is sufficiently secured. Ultimately, ear bars should be symmetrical and should fit snugly in the ears of the animal (Figure 1a).

Figure 1. Diagram of fitting a prairie vole in the ear bars (A) and bite plate (B) of a stereotax.



The bite plate (prairie vole adaptor, Kopf Model 928) is then slid toward the animal so that the maxillary incisors fit into the hole of the bite plate (Figure 1b). It can be helpful to use fine tipped forceps to prop open the animal's mouth when positioning the upper teeth into the hole of the bite plate and to ensure the animals tongue is kept clear of the apparatus. Lower or raise the bite plate to level the head with the rest of the body; do this with great care to avoid causing the animal pain. Once in position, the nose bar is gently screwed down to secure the vole's head. The nose piece should hold the animal's head in place with the least pressure possible.

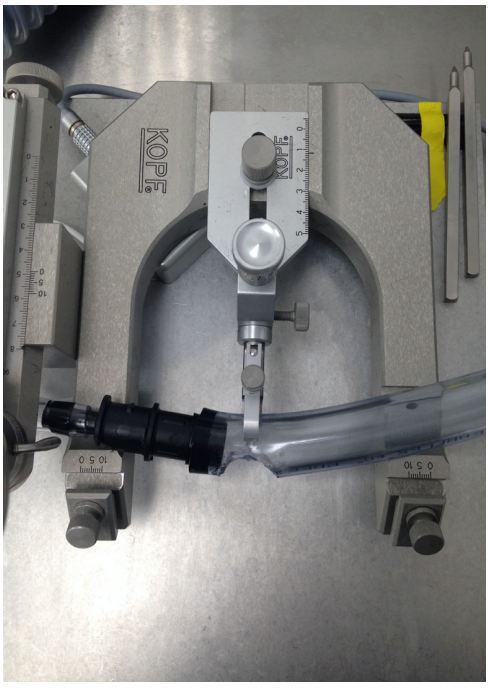
Unfortunately, anesthesia masks are currently available only for mice and rats and these do not adapt well for the use of prairie voles. However, an anesthesia mask can be constructed using PVC tubing and stepped tubing connectors (or reduction couplers), (see section 2.5 for details). The tubing is connected to the isoflurane/valve rig and attached over the prairie vole adaptor (bite plate; see Figure 2). This set up is very effective at keeping the animal anesthetized once the animal is in place, but it can also contribute to the challenge of quickly securing the anesthetized animal into the stereotax before it wakes.

2.3. Cannulation

Once the vole is securely in the stereotax, it is covered with a sterile drape, and the fur is removed from the top of its head. Using a cotton-tipped applicator, rub the scalp with Betadine following good surgical practices. With a scalpel, make a ¾ inch incision to expose the skull and then scrape connective tissue off of the skull (Figure 3a). A cotton-tipped applicator should be used to dry the skull. For cannulation surgeries, it is important for the skull to be clear of all connective tissue and completely dry so that the Loctite mount can successfully adhere. A polishing buffing pad or scalpel can be used to roughen the surface of the skull; this will improve the stability of the cannula mount. To ensure a clear work-

Figure 2. Example of anesthesia mask constructed from PVC tubing and stepped tubing connectors (or reduction couplers). The tubing is connected to the isoflurane/valve rig and attached over the prairie vole adaptor (Kopf adaptor model 928). There is a small slit in the back of the PVC tube, allowing the PVC tube to fit over the bite plate while still fitting under the nose bar.

a)



b)

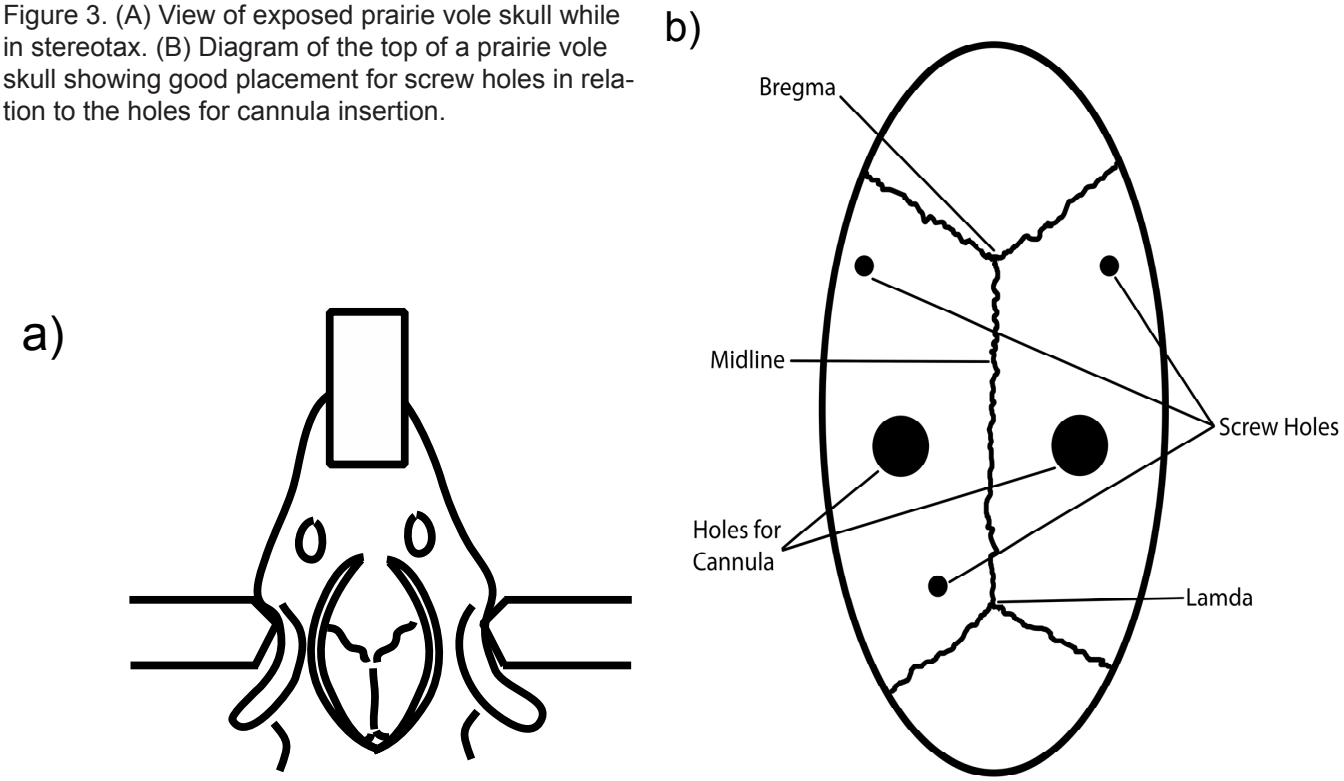
space, we use bulldog clamps to hold the skin away from the area of the skull where screws and the cannula will be implanted.

The surface of the vole's head is then leveled by referencing lambda and bregma. For ease of visualization, we locate bregma through the use of a stereotaxic microscope. The stereotax is next advanced to the desired coordinates with respect to bregma. We mark the location of where the cannula will be inserted using a cauterizer. In addition to the coordinates where the cannula will be inserted, we cauterize marks for the desired location for the skull screws (Figure 3b). We typically use 2 – 3 skull screws, depending on the target region and the size of the cannula. These will help stabilize the cannula on the skull. A small dremel bit (0.8 mm diameter, engraving bit 105) is used to drill the holes for the skull screws, whereas a larger dremel bit (1.6 mm diameter, engraving bit 106) should be used for drilling the holes for the cannula. The screws should be put in place using a small screwdriver before lowering the cannula in order to avoid hitting the cannula after it has been advanced into the brain. There should be about an eighth of an inch between the head of the screw and the skull in order to avoid damaging the underlying cortex and to give the dental acrylic and Loctite gel something on which to bind.

After the cannula and the screws are in place, the cannula is affixed by building layers of Loctite cured with dental acrylic liquid. Start by applying the Loctite gel around the cannula and over the skull screws. Then using a cotton tipped applicator, dab the dental acrylic liquid over the Loctite. The Loctite will be cured and hardened within seconds. Repeat this process and continue adding layers of Loctite until the screws are completely encapsulated and the base of the guide cannula is mostly covered and firmly in place (2-4 layers depending on the size of the cannula).

The dummy can then be inserted into the cannula, and the dust cap can be screwed onto the cannula. If necessary, apply sutures to close the incised scalp around the cannula mount. Apply Betadine over the sutured skin

Figure 3. (A) View of exposed prairie vole skull while in stereotax. (B) Diagram of the top of a prairie vole skull showing good placement for screw holes in relation to the holes for cannula insertion.



before preparing to remove the vole from the stereotax.

2.4. Microinjection

The protocol for microinjection is very similar to that just described for cannulation. In this case, the microinjection device (e.g., a glass pipette, Hamilton syringe, etc) will be connected to the injection arm above, and the arm should be rotated out of position before securing the animal. The animal should be secured into the stereotax as just described, and then an incision can be made sufficiently exposing the skull and the reference point (e.g., bregma). The skull should be cleared of connective tissue and dried. The injection arm should be rotated into place and the tip of the microinjection device should be aligned with bregma along the anterior-posterior and medal-lateral axes. The coordinates should be recorded and used to calculate the desired coordinates for the injection, at which point the injection arm should be positioned appropriately. The tip of the microinjection device should be lowered just above the skull and the positions should be marked (e.g., with a cauterizer or other effective method). The injection device should be raised and cleared allowing access to drill holes into the skull at the desired location. We use the dremel 106 engraving cutter (or 107 if a larger hole is needed) for this purpose. The injection arm should be returned to the original position with accuracy (possibly realigned with bregma) and then inserted to the desired coordinates. Once the injection is complete, the injection arm should be retracted slowly until it is clear of the animal. Sutures (e.g., absorbable monofilament, reverse cutting 3/8 size 4-0) should be used to close the incision under anesthetic and the animal should be placed for recovery.

2.4. Recovery

Prior to removing the vole from the stereotax, the isoflurane vapor should be slowly decreased to 0%. Remove any drapes, and then unscrew the nose bar on the bite plate. Carefully lift the vole's jaw up to slide the maxillary incisors out of the bite plate. Lastly, remove the ear bars. The vole can then be returned to its cage, which should be placed on a heating pad for the first 30 minutes of recovery. Post-op pain management protocols should be followed according to institutional policy.

2.5. Troubleshooting

As is the case with many species, prairie voles offer unique challenges for conducting stereotaxic surgeries. Ketamine is the most frequently used anesthetic for rodents. However, in addition to the challenges associated with using a controlled substance, we have found that the efficacy of ketamine to anesthetize a prairie vole is highly variable, despite controlling for weight. The real-time control of the isoflurane dose throughout surgery has resulted in zero animals prematurely waking up during surgery, and has resulted in very minimal mortality. From our experience, prairie voles are better able to metabolize low amounts of isoflurane delivered with O_2, resulting in a quicker recovery. For these reasons, we recommend an isoflurane delivery system for anesthesia. However, if the use of ketamine is a preferred method of anesthetic, we have used a ketamine / xylazine mixture with success in the past. The specific doses should be developed in consultation with the appropriate veterinary staff, however, we have used a vigorously mixed 10:1 mixture of ketamine (20 mg/ml) and xylazine (2 mg/ml). The cocktail should be administered at 0.0025 - 0.0045 ml/g body weight, which will deliver 50-90 mg of ketamine and 5-9 mg of xylazine per kg. 0.3 ml of anesthetic will usually be sufficient for one animal.

Unfortunately, an anesthesia mask customized to fit the Kopf 928 prairie vole adaptor does not currently exist. We tried several permutations of combining the prairie vole, mouse, and rat adaptors with rat and mouse

anesthesia masks. Unfortunately, no combination resulted in the vole's head being completely secure. Thus, we manufactured our own anesthesia mask using PVC tubing. This is a sufficient and an affordable alternative to custom designing and building an appropriately sized anesthesia mask for the prairie vole adaptor. We use ¾ inch diameter PVC tubing as the pass-through mask. A large hole to place over the snout of the vole was approximately 25 mm wide and 19 mm high and cut as an oval. This should be large enough to secure the animal, while being small enough to trap the anesthetic gas in the apparatus. Opposite of the larger hole, a small 4 x 2 mm hole was cut so that the bite place could be inserted directly into the tube. The bite plate should fit snugly within this smaller hole. The relatively large PVC tube is connected to stepping tube connectors (¾ inch to ½ inch) to connect to smaller and more flexible PVC tubing (½ inch diameter) that either delivers anesthetic to the animal or channels the anesthetic away to a vacuum system. The flow of anesthetic many need to be adjusted based on the different volumes of tubing used.

Finally, cannulation in prairie voles can be challenging due to their lack of a calcified skull (Donaldson et al., 2010). The skull of a prairie vole is thinner than the skulls of rats and mice, and there is a greater risk that the cannula mount may crack the skull, resulting in minor movements and instability in the cannula and mount. We found that dental cement and dental acrylic to affix the cannula to the skull was not strong enough to keep it stable. However, we have had great success with stability by using Loctite cured with dental acrylic liquid.

3. Concluding Remarks

Prairie voles are an increasingly popular species for understanding the neural basis of affiliation, stress-coping, and reward-motivated behaviors. Using stereotaxic techniques, such as cannulation or microinjections, scientists can directly manipulate areas of the prairie vole brain to reveal the underlying mechanisms of behavior. It is important to utilize a comparative approach and to study the neural underpinnings of behavior in numerous species to gain insight into how the brain has evolved (Kelly and Ophir, 2015). The refinement of stereotaxic techniques for use in nontraditional organisms, such as the prairie vole, has resulted in elegant experiments that contribute to a growing body of work that aims to understand convergent and divergent mechanisms of behavior and the unique ways in which the brain has changed over evolutionary time.

4. References

Ahern TH, Hammock EA, Young LJ (2011). Parental division of labor, coordination, and the effects of family structure on parenting in monogamous prairie voles (*Microtus ochrogaster*). *Dev Psychobiol* 53, 118-131.

Alberts DJ (1979). Induction of mouse-killing in non-killer rats by temporary chemical lesions in the medial hypothalamus. *Phys Psych* 7(1), 64-66.

Amadei EA, Johnson ZV, Kwon YJ, Shpiner AC, Saravanan V, Mays WD, Ryan SJ, Walum H, Rainnie DG, Young LJ, Liu RC (2017). Dynamic corticostriatal activity biases social bonding in monogamous female prairie voles. *Nat Lett* 546, 297-301.

Aragona BJ, Detwiler JM, Wang Z (2007). Amphetamine reward in the monogamous prairie vole. *Neurosci Lett* 418, 190-194.

Beery AK, Kaufer D (2015). Stress, social behavior, and resilience: insights from rodents. *Neurobiology of stress* 1, 116-127.

Carter CS (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 23, 779-818.

Carter CS (2005). The chemistry of child neglect: Do oxytocin and vasopressin mediate the effects of early experience?. *Proc Nat Acad Sci* USA 102, 18247-18248.

Carter CS (2007). Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders?. *Behavioural Brain Research* 176, 170-186.

Carter CS, Getz LL, Cohen-Parsons M (1986). Relationships between social organization and behavioral endocrinology in a monogamous mammal. *Advances in the Study of Behavior* 16, 109-145.

Cassola F (2016). *Microtus ochrogaster*. (errata version published in 2017) The IUCN Red List of Threatened Species 2016: e.T42631A115196932. dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T42631A22347375.en

Choleris E, Little SR, Mong JA, Puram SV, Langer R, Pfaff DW (2007). Microparticle-based delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice. *PNAS* 104, 4670-4675.

Curtis JT, Stowe JR, Wang Z (2003). Differential effects of intraspecific interactions on the striatal dopamine system in social and non-social voles. *Neurosci* 118, 1165-1173.

Dewsbury DA (1981). An exercise in the prediction of monogamy in the field from laboratory data on 42 species of muroid rodents. *Biologist* 63, 138-162.

Donaldson ZR, Spiegel L, Young LJ (2010). Central vasopressin V1a receptor activation is independently necessary for both partner preference formation and expression in socially monogamous prairie voles. *Behav Neurosci* 124, 159-163.

Fitch HS (1957). Aspects of reproduction and development in the prairie vole. *University of Kansas Publications, Museum of Natural History* 10, 129-161.

Gewirtz JC, Kim YK (Eds.). (2016). *Animal Models of Behavior Genetics*. Springer.

Getz LL, Carter CS (1980). Social organization in *Microtus ochrogaster* populations. *Biologist* 62, 56-69.

Getz LL, Carter CS, Gavish L (1981). The mating system of the prairie vole, *Microtus ochrogaster*: Field and laboratory evidence for pair-bonding. *Behav Ecol & Sociobio* 8, 189-194.

Getz LL, Hofmann JE (1986). Social organization in free-living prairie voles, *Microtus ochrogaster*. *Behav Ecol & Sociobio* 18, 275-282.

Gobrogge K, Wang Z (2016). The ties that bond: neurochemistry of attachment in voles. *Curr Opin Neurobiol* 38, 80-88.

Hammock EA (2015). Developmental perspectives on oxytocin and vasopressin. *Neuropsychopharmacology* 40, 24-42.

Insel TR, Shapiro LE (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc Nat Acad Sci* 89, 5981-5985.

Kelly AM, Kingsbury MA, Hoffbuhr K, Schrock SE, Waxman B, Kabelik D, Thompson RR, Goodson JL (2011). Vasotocin neurons and septal V1a-like receptors potentially modulate songbird flocking and responses to novelty. *Horm Behav* 60, 12-21.

Kelly AM, Ophir AG (2015). Compared to what: what can we say about nonapeptide function and social behavior without a frame of reference. *Current Opinion in Behavioral Sciences* 6, 97-103.

Kenkel WM, Perkeybile AM, Carter CS (2017). The neurobiological causes and effects of alloparenting. *Dev Neurobiol* 77, 214-232.

Kleiman D (1977). Monogamy in mammals. Q. *Rev. Biol.* 52, 39-69.

Lukas D, Clutton-Brock T (2012). Cooperative breeding and monogamy in mammalian societies. *Proc Royal Soc B*

Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F, Engelmann M (1995). V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities, and anxiety-related behavior in rats. *J Neurosci* 15, 4250-4258.

McGraw LA, Young LJ (2010). The prairie vole: an emerging model organism for understanding the social brain. *Trends Neurosci* 33, 103-109.

Okhovat M, Berrio A, Wallace G, Ophir AG, Phelps SM (2015). Sexual fidelity trade-offs promote regulatory variation in the prairie vole brain. *Science* 350, 1371-1374.

Ophir AG, Wolff JO, Phelps SM (2008). Variation in neural V1aR predicts sexual fidelity and space use among male prairie voles in semi-natural settings. *Proc Natl Acad Sci USA* 105, 1249-1254.

Prounis GS, Foley L, Rehman A, Ophir AG (2015). Perinatal and juvenile social environments interact to shape cognitive behaviour and neural phenotype in prairie voles. *Proc B* 282, 1-9.

Ryabinin AE, Hostetler CM (2016). Prairie voles as a model to screen medications for the treatment of alcoholism and addictions. *Int Rev Neurobiol* 126, 403-421.

Williams JR, Catania KC, Carter CS (1992). Development of partner preferences in female prairie voles (*Microtus ochrogaster*): the role of social and sexual experience. *Horm Behav* 26, 339-349.

Young LJ (2001). Oxytocin and vasopressin as candidate genes for psychiatric disorders: lessons from animal models. *American Journal of Medical Genetics Part A* 105, 53-54.

Young LJ, Pitkow LJ, Ferguson JN (2002). Neuropeptides and social behavior: animal models relevant to autism. *Molecular Psychiatry* 7, S38-S39.

Young LJ, Wang Z (2004). The neurobiology of pair bonding. *Nat Neuro* 7, 1048-1054.

Table 1. List of equipment required for stereotaxic surgery.

General Surgery Equipment	
Scalpel	
Needle driver	
Foreceps, needle nose	
Foreceps, blunt nose	
Sterile drapes	
Heating pad	
Sterile cotton-tip applicators	
Ocular lubricant	
Glass bead sterilizer	
Sutures	
Bulldog clamps	
Electric razor	
Betadine	
Stereotaxic Surgery Equipment	
Dissecting microscope	Meiji Techno
Illuminator	Dolan-Jenner
Isoflurane vaporizor	Surgivet
Isoflurane	
Medical grade oxygen	Airgas
Dremel	Dremel
Dremel 105 engraving cutter, 0.8 mm diameter	Dremel
Dremel 106 engraving cutter, 1.6 mm diameter	Dremel
Cauterizer	
Non-rupture ear bar, 45° tip, 0.8 mm radius	Kopf
Bite plate, prairie vole adaptor, Kopf model 928	Kopf
Cannulation Equipment	
Guide cannula	PlasticsOne
Dummy cannula	PlasticsOne
Internal injector	PlasticsOne
Dust cap	PlasticsOne
Skull screws, size 1/16	PlasticsOne
Screwdriver	PlasticsOne
Loctite 494 gel	Loctite
Dental acrylic	