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Caudal thoracic air sac cannulation in zebra finches for isoflurane anesthesia

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Abstract

Small songbirds such as the zebra finch are commonly used for studies on the neural mechanisms that underlie vocal learning. For these studies, survival surgeries are often performed that involve animal anesthesia and stereotaxic stabilization for localization of specific brain regions. Here we describe air sac cannulation as a novel method for delivering isoflurane gas to zebra finches for anesthesia during neurosurgery. Advantages of this method include that it leaves the bird's head free for stereotaxic targeting and does not interfere with the beak clamps that are often used to position and stabilize the head. It additionally allows for the use of the inhalant anesthetic, isoflurane, which is an appealing alternative to injectable anesthetics because it provides fast, minimally stressful induction, and low subject and personnel toxicity. The use of isoflurane also prevents overdosing and lengthy postoperative recovery times.

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1. Introduction

The zebra finch (*Taeneopygia guttata*) is a robust songbird. Adults typically weigh 12 g and are easily housed and bred year-round in a laboratory setting. Juvenile males require exposure to adult male courtship song during a defined developmental window in order to sing normal, attractive song when they, themselves, are adults (Immelmann, 1969). Distinct brain regions, referred to as 'song nuclei', in the males of this species and in other oscine songbirds are devoted to song learning and production (Nottebohm and Arnold, 1976; Nottebohm et al., 1982; Bottjer et al., 1984; McCasland, 1987; Sohrabji et al., 1990; Scharff and Nottebohm, 1991; Vates et al., 1996). All of these qualities combine to make the zebra finch an attractive experimental model for studies on the neurobiological basis of vocal learning (Konishi, 1989).

Many such neurobiological studies involve anesthetizing the birds to allow for surgical manipulation. Because these manipulations often require free access to the head of the bird, inhalant anesthetics delivered via a bell mask that fits over the head cannot be used. Additionally, many of the neural surgeries performed on zebra finches use stereotaxic instruments to target the song nuclei. Stereotaxic devices can restrict the use of tracheal intubation as a means to deliver inhalant anesthetics because the head is stabilized by clamping the beak, and the clamp can compress the tracheal tube. Further, successful tracheal intubation in young birds is difficult due to the small size of the target (unpublished observations). Consequently, intramuscular injection (i.m.) of an anesthetic agent such as equithesin, a mixture of pentobarbital and chloral hydrate, has been widely used.

There are several drawbacks to the use of chloral hydrate-based anesthetics. Because it is made from separate components, each batch of chloral hydrate-based anesthetic has a slightly different potency and, therefore, a dose–response curve must be determined every time a new batch is made. Once made, the potency of these batches can decline over

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time due to ingredient degradation. This can result in dosing uncertainties that further lead to the bird being inadequately anesthetized, which then requires additional doses that are often fatal (unpublished observations). Further, full recovery from chloral hydrate combinations is very slow (Harrison and Harrison, 1986), and it may take 5–7 h after the initial i.m. injection before the bird is alert and perching. This long duration of recovery puts the bird at risk of dangerous heat loss for an extended period of time and requires extensive post-anesthetic monitoring.

Many of these complications can be avoided by using an inhalant anesthetic technique. Isoflurane, an inhalant anesthetic, is an appealing alternative to injectable anesthetics because it allows for fast, minimally stressful induction, and current findings support earlier reports that isoflurane has the lowest probability for subject toxicity compared to other anesthetic agents (Harrison and Harrison, 1986; Raper et al., 1987; Duffy and Matta, 2000). Additionally, the low toxicity of isoflurane makes it safe to work with for those personnel who may be exposed to small amounts during anesthesia procedures (Harrison and Harrison, 1986). Consequently, we set out to develop a mean of delivering isoflurane when performing those cranial surgeries that do not allow for the use of a mask or tracheal intubation.

Air sac cannulation has been used in clinical veterinary medicine to anesthetize avian patients where the use of a mask or tracheal intubation was contraindicated (Whittiw and Ossorio, 1970; Korbel, 1998b; Good et al., 2001). Reports documenting cases of air sac cannulation involved birds ranging from 794 to 2254 g (Whittiw and Ossorio, 1970; Good et al., 2001; Jaensch et al., 2001). A recent publication states that air sac perfusion anesthesia can be used for birds with a body weight as low as 30 g (Korbel, 1998b). However, to our knowledge there are no reports documenting air sac cannulation in a bird as small as the zebra finch. Herein we describe a modified air sac cannulation technique to maintain anesthesia with isoflurane in zebra finches that weigh from 10 to 15 g. Once perfected, this technique is easy to use, enables fine control of anesthetic depth, and allows for fast, complication-free recovery.

2. Materials and methods

2.1. Subjects

All use of animals was approved by the Institutional Animal Care and Use Committee at the University of California, Los Angeles. Following pilot studies, we used 26 zebra finches from our breeding colony, of both sexes and ranging in age (59 days post-hatch to ~1 year) and weight (10–15.5 g).

2.2. Anesthetic equipment

A tabletop anesthesia device consisting of an oxygen flow-meter (range of flow from 0 to 4 l/min), a common gas out-

let, a Tec 3 vaporizer for isoflurane, and a F/air canister for scavenging waste anesthetic gas is used (Summit Medical Equipment Company, Bend, OR, USA). This anesthetic device is coupled to Y-tubing, both arms of which have a valve for opening or closing the tubing. One arm is connected to a customized mask used in the initial induction of anesthesia (see below). When the valve is open, gas flows to the mask. When the valve is closed, gas flows toward the second arm of the Y-tube. The second arm connects to a reservoir bag for positive pressure ventilation (0.25 or 0.5 l; Summit Medical), and to the catheter used for air sac cannulation. The connection to the catheter is made via a modified 1 cc syringe (Becton Dickinson & Co., Franklin Lakes, NJ, USA); the syringe is cut at the back and inserted directly into the tubing, thus serving as an adapter.

Positive pressure ventilation is achieved by gentle compression of the reservoir bag, known as 'bagging'. Bagging is critical for maintaining the bird under anesthesia via air sac cannulation because even the lowest setting of any oxygen flow-meter is too high for these small birds. The flow rate would not only exceed the bird's minute ventilation and result in apnea, but the flow of gas, combined with the resistance to gas flowing out of the bird's respiratory system via the trachea, would quickly exceed the bird's vital capacity (Schmidt-Nielson, 1991) and traumatic damage to tissues would result. Thus, it is necessary to direct the gas outflow first to the reservoir bag rather than sending it directly to the bird. Gentle compression of the bag sends a portion of the gas to the catheter. When the bag over-inflates, a sliding valve positioned at the bag inlet allows the excess gas to be vented to the scavenging device. A schematic of the connectivity between these components, from the oxygen tank to the catheter, is shown in Fig. 1.

A 20-gauge IV catheter (Surflo[®], Fisher Scientific, Pittsburgh, PA, USA) is modified for cannulation (Fig. 2). Three side vents are cut in the IV catheter with a scalpel blade to prevent accidental occlusion by any tissue of the flow of gas from the catheter into the air sac and to decrease the resistance and minimize a jet effect from gas flowing into the air sac. All side vents are made within 1 cm from the catheter tip. These side ports also serve as markers of the depth of catheter insertion into the air sac (see below). Suture (4-0 ethilon) with cutting needle (Ethicon Inc., Somerville, New Jersey) is used to stabilize the catheter once it is inserted. Oxygen (100%; AirLiquide Healthcare, Houston, TX, USA) is the carrier gas for the vaporized isoflurane (2%; IsoFlo[®], Abbott Laboratories, North Chicago, IL, USA). For anesthetic induction, a customized mask is made from a commercially available mouse mask (Summit Medical) and a modified 60 cc syringe (Becton Dickinson; Fig. 2). The syringe is modified by removing the plunger and then cutting the dispensing end of the barrel so that the tubing from the rodent face mask can be inserted into the syringe barrel. The tubing is pulled through such that the end of the rodent mask (intended to go over the snout) becomes parallel with the ~45 ml mark on the syringe. Parafilm[™] (American National Can, Chicago, IL)

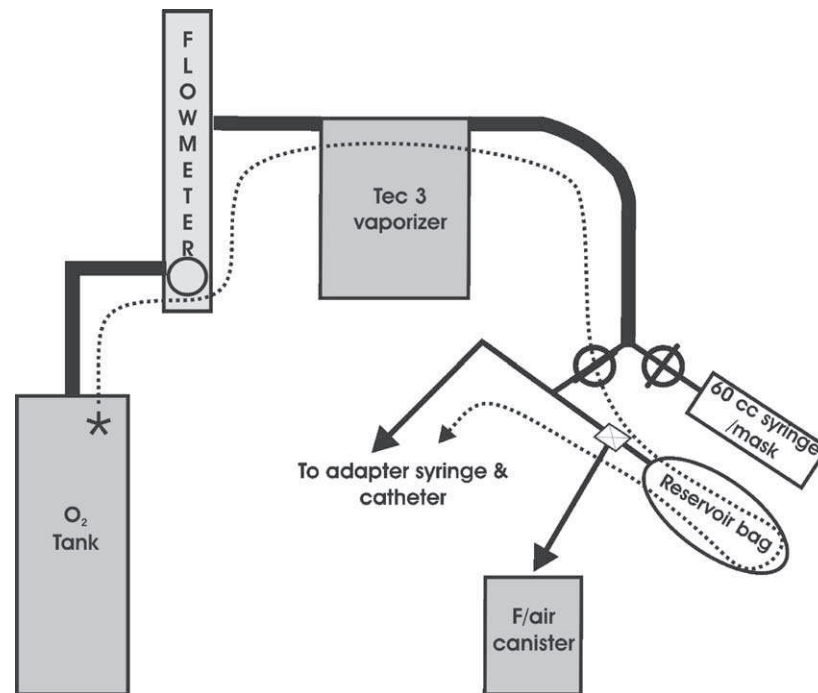


Fig. 1. Schematic shows connectivity between components of the anesthetic set-up. The dotted line indicates the path of fresh gas flow during delivery of anesthetic to the catheter (and bird). Flow begins with the oxygen carrier gas and is marked with an asterisk. Oxygen passes through the tubing to the flow meter, which indicates the rate of flow into the vaporizer. This rate is set by a dial on the meter (open circle). Vaporized isoflurane carried by oxygen exits the vaporizer and encounters the Y-tube. Each arm of the Y-tube is connected to a valve (circles with cross hatch). The valve on the right leads to the modified 60 cc syringe used as a mask for initial anesthetic induction. In this schematic, the right valve is closed and gas flows to the second arm of the Y-tube through the open left valve toward the small reservoir bag and catheter. Gas will flow principally to the bag because the deflated bag has low resistance. When the reservoir bag is partially inflated, gentle compression sends gas toward the catheter which will anesthetize the cannulated bird (not shown). Before the bag becomes fully inflated, excess gas should be vented to the F/air scavenger canister by rotating a sliding valve at the opening of the reservoir bag (box with cross-hatch). Full compression of the bag will now send this excess gas to the scavenger rather than to the bird.

is used to seal the juncture between the mouse mask tubing and the narrow end of the syringe and to secure their relative placement.

2.3. Anesthesia induction

In order to decrease the stress on the bird and to aid in catheter placement, the bird is first anesthetized with isoflurane (2%) in oxygen delivered at 0.2 l/min to the customized mask (Figs. 2 and 3). The Tec 3 vaporizer is fairly accurate at low flow rates, but the percentage of isoflurane in the gas mixture may be slightly higher than the dial reading at a flow rate of 0.2 l/min (Dorsch and Dorsch, 1994). The open end of the 60 cc syringe is gently placed over the awake bird's head. Usually the bird moves itself up the syringe such that its beak is near the opening of the mouse mask, whereby the bird inhales the anesthetic. Once the bird is anesthetized, the site of catheter insertion is prepared for cannulation. The bird's right leg is extended cranially and secured to the outside of the mask/syringe using first-aid adhesive (Fig. 3A). An ethanol swab (S|P® Alcohol Swab, Baxter, McGaw Park, IL, USA) is used to moisten the feathers of the abdomen on the right side. The feathers from both pterygiae (i.e. the tracks

in the skin in which the feathers grow) on the right side of the abdomen are plucked (Fig. 3B).

2.4. Catheter placement and stabilization

The caudal thoracic air sac is easily cannulated once the bird is prepared as described above. The catheter is inserted just caudal to the rib cage in the lateral abdominal apertium (i.e. the region where no feathers grow) between the tracks where the feathers were plucked previously. The caudal edge of the pectoral muscle is usually visible through the skin at the caudal edge of the rib cage and may be used as a visible landmark. The two lateral abdominal pterygiae have subcutaneous fat that visibly identifies their locations, and the catheter is inserted between them (Fig. 3C).

The catheter with its stylet is held at a shallow angle, and the direction of insertion is cranio-lateral in order to avoid damage to major organs (e.g. liver, heart, lungs; Fig. 3C). The stylet of the catheter is metal with a sharp beveled tip, which allows for insertion through the body wall but can cause potentially fatal tissue damage and bleeding if inserted too deeply. The total depth of insertion of the stylet is roughly 1 mm. The stylet should not be advanced any further once its



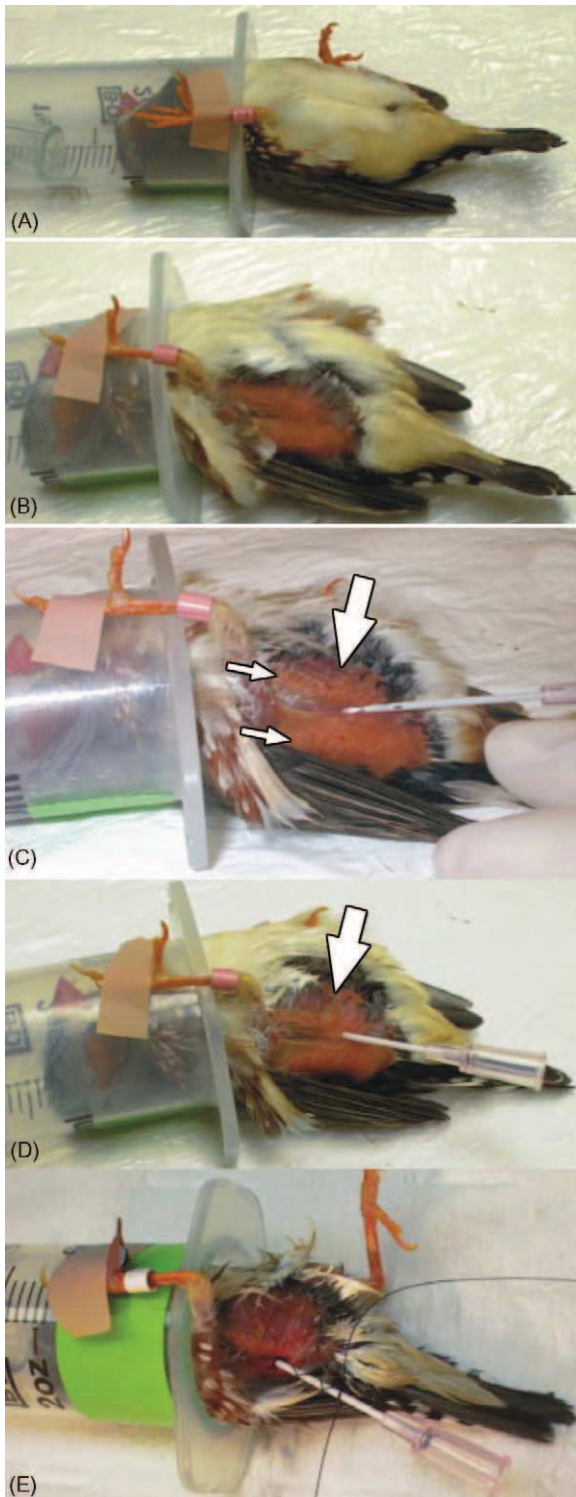
Fig. 2. Photographs show customized mask (A, B) and customized catheter (B, C) used to administer gas anesthetic. (A) A mask used for the initial anesthetic induction is made from a pre-fabricated mouse mask (Summit Medical) and an altered 60 cc syringe (Becton Dickinson; see Section 2). (B) Closer view of the customized mask shows the prefabricated mouse mask inside the modified syringe. Arrow points to the end of the mouse mask. A 20-gauge IV catheter (Surflo[®], Fisher Scientific, Pittsburgh, PA) with side vents is used for air sac cannulation. (C) While the catheter is still partially in its case three side vents are cut with a scalpel blade. All vents are placed within the first centimeter from the tip of the catheter. The arrow points to two vents on one side of the catheter. A third vent is cut on the opposite side. These vents prevent accidental occlusion of the catheter from tissue, and serve as a partial gauge of depth of catheter insertion. Ruler is shown for reference.

beveled tip is within the body. Once the stylet has just passed through the abdominal muscles, the catheter is threaded down the stylet and advanced into the air sac deep to the ribcage. The catheter itself does not readily induce traumatic damage if manipulated carefully and can therefore be advanced into the air sac to a depth of approximately 1 cm without incident. When the catheter is in the air sac, there is no resistance to advancing the catheter. Once the catheter is advanced into the air sac and none of the side vents are outside the bird's body, the stylet is completely removed from the catheter and discarded (Fig. 3D). Correct placement of the catheter is checked by connecting the tubing from the Y-arm to the catheter via

the syringe adapter, increasing the flow rate to 0.6 l/min, and using the valves on the Y-tubing to direct the anesthetic gas to the reservoir bag and catheter. Once the reservoir bag is partially inflated, it is gently squeezed; when the catheter placement is appropriate, the thorax expands. When the catheter is improperly placed, subcutaneous pockets of air or expansion of the abdomen are observed (Korbel, 1998b).

Once the catheter is correctly inserted and checked, the tubing is disconnected from the catheter and the anesthetic gas is again directed to the mask while the catheter is stabilized with suture (4-0 ethilon) using a Finger-Trap style of suture pattern (Fig. 3E). The Finger-Trap friction suture is

initiated by taking a single bite, approximately 1 mm wide, through the skin just cranial to the catheter insertion site. This suffices as the anchor for the suture pattern. The catheter is prevented from being pulled out of place by virtue of friction from the lengths of suture material that are wrapped twice and then tied with a square knot around the portion of the catheter that remains outside the body.



2.5. Anesthesia maintenance

Once the bird has been cannulated and the catheter stabilized, the tubing from the anesthetic machine is reconnected to the catheter, the anesthetic gas is directed to the reservoir bag and catheter, and the flow rate is adjusted to 0.6 l/min. Positive pressure ventilation is achieved by gentle compression of the reservoir bag, known as ‘bagging’. Bagging is used to deliver the gas to the air sac whence it passes through the lungs and out the bird’s mouth. The amount of thoracic expansion during each bagging should be carefully monitored to ensure that the air sacs do not over-inflate. When using the smaller 0.25 l reservoir bag, the sliding valve on the bag must be frequently opened and the bag emptied by expelling the gas directly into the scavenger. For the duration that the bird is anesthetized, the bird’s viability and depth of anesthesia are gauged by close observation of breathing rate and regularity and by the bird’s muscle tone and feather erectness. Maintaining the bird under anesthesia requires that one person give his or her attention solely to monitoring the bird and adjusting the gas mixture and rate of bagging accordingly. We have found an appropriate plane of anesthesia to be one in which the bird is unresponsive to toe-pinch, the feathers are relaxed and down, and the bird is breathing regularly at a rate of approximately 0.5 Hz. Following initial induction at 2%, we have maintained such a plane of anesthesia with a gas mixture of 1.5 ± 0.3 vol% isoflurane, a concentration similar to those recommended for isoflurane delivered to other species of birds via a mask (Korbel, 1998a; Ludders, 2001), for the duration of our surgeries which have ranged from 50 min to 3 h.

2.6. Recovery

Once the surgery is complete, the bird is returned to mask anesthesia and the tubing is removed from the catheter. This is done so that the bird can be maintained under anesthesia

Fig. 3. Photographs show initial induction of anesthesia via the customized mask through stabilization of the catheter following air sac cannulation. (A) A male zebra finch is anesthetized with isoflurane delivered to its face with the customized mask. The right leg is raised and temporarily secured (here, via adhesive bandage) to the outside of the 60 cc syringe to reveal the area of catheter insertion. (B) Feathers are plucked and skin swabbed with ethanol prior to stylet and catheter insertion. (C) The insertion site is approximately 2–3 mm caudal to the visible edge of the pectoral muscle (large arrow) in juvenile and adult birds (59 days to one year of age). Two lateral abdominal pterygiae (tracks from which the feathers were plucked previously) have subcutaneous fat that visibly identifies their locations (small arrows), and the catheter is inserted between them. The stylet is held at a very shallow angle, and the direction of insertion is cranio-lateral. (D) Once the stylet has passed through the abdominal muscles, just deep to the ribs, such that none of the side-vents in the catheter remain outside the bird’s body, the catheter is pushed off the stylet and advanced into the air sac. Large arrow indicates the caudal edge of the pectoral muscle, used as a cranio-caudal landmark, as in C, above. (E) After checking catheter placement (see Section 2), 4-0 ethilon suture with a cutting needle (Ethicon) is used to stabilize the catheter with a Finger-Trap style suture pattern.

while the suture is removed. Once the suture is removed, the catheter is removed, and no closure of the insertion site is necessary. At this point, the anesthetic gas is turned off and the bird is allowed to breathe 100% O₂ through the mask at the 0.2 l/min flow rate for several minutes (even after the bird has awakened) while the isoflurane is flushed from its respiratory system. Within minutes, the bird should be alert and perching.

2.7. Pulse oximetry

Pulse oximetry is a non-invasive method used to monitor the percentage of haemoglobin saturated with oxygen. Although pulse oximetry was not regularly used to monitor birds during surgeries involving air sac perfusion, it was used initially here to determine the appropriateness of the procedure and to compare O₂ saturation levels during mask-administered anesthesia with those during air sac cannulation anesthesia. For this monitoring, a one-sided infrared probe was held in position over the right brachial artery until a stable reading was obtained while the bird was under anesthesia delivered via a mask or via air sac cannulation and the bird was breathing spontaneously in between bagging. Both readings were taken while the bird was delivered a mixture of 2.0 ± 0.5 vol% isoflurane in 100% O₂ as a carrying gas.

2.8. Heart rate

We recorded heart rate during mask-administered isoflurane anesthesia and during anesthesia via air sac cannulation in the same bird. Meditrace[®] self-adhesive heart-monitoring electrodes were cut down to size and affixed to the bird. One electrode was applied over the cranial portion of the right pectoral muscle and the other over the left portion of the abdomen. Two electrical leads were connected to the electrodes via alligator clips. The electrical signal from the heart muscle activity was differentiated and amplified using a model 410 amplifier (Brownlee Precision Co., San Jose, CA, USA). Signals were collected and analyzed using a data acquisition board (AT-MIO-16E2, National Instruments, Austin, TX, USA) and acquisition and analysis programs custom written by Felix E. Schweizer using LabView software (National Instruments). Heart rate was monitored for an individual bird for >30 min while under mask anesthesia and then during catheter placement and anesthesia via air sac cannulation.

3. Results

We have successfully used the air sac cannulation method in 23 birds. Our first challenge was to identify and cannulate the air sac with accuracy. This skill developed rapidly by checking catheter placement, as described above. Following the initial development of this technique, we have experi-

enced two unsuccessful cases, both involving blood loss. In one, blood was observed in the catheter as it was advanced. The catheter was immediately withdrawn, the surgery was discontinued, and the bird recovered. In the other case, the stylet was advanced too far resulting in fatal blood loss. These problems occurred only once for an individual experimenter and were not reencountered in subsequent surgeries. In a few other cases, the catheter slipped out of the air sac when the bird was being situated in the stereotaxic device (Herb Adams Engineering, Glendora, CA, USA), but these birds were successfully re-cannulated. Catheter slippage can be avoided by increasing the tightness of the Finger-Trap friction suture on the catheter (Fig. 3E).

3.1. Respiration

Because the fresh gas flow rate delivered to the air sac exceeds the bird's minute ventilation, over-ventilation of the bird occasionally resulted in apnea due to low partial pressure of CO₂ in arterial blood (PaCO₂) and consequent loss of respiratory center stimulation (Korbel, 1998b; Ludders, 2001). When such apnea occurred, bagging (see Section 2) was interrupted and the PaCO₂ was allowed to rise until spontaneous breathing returned, at which time the ventilation was resumed. With practice, we found that this reversible apnea could be avoided by allowing the bird to take approximately three breaths in between bagging.

3.2. Pulse oximetry

The pulse oximetry reading during anesthesia via mask on the two birds that were tested was consistent over a period of 5-min and at a level of >90%. After this stable reading was obtained, one of the two birds was cannulated so as to compare oxygen saturation levels obtained during maintenance of anesthesia with mask versus cannulation. Cannulated anesthesia was maintained for over 10-min after which a pulse oximetry reading indicated O₂ saturation of >90%, identical to that obtained during delivery of anesthesia by mask in the same bird.

3.3. Heart rate

Fig. 4 shows example traces of the differentiated heart-beat signals from two birds, each anesthetized with isoflurane delivered via the customized mask and then switched to anesthesia delivered via an air sac cannula. As suggested by Fig. 4A, there was no overall difference in the stability of heart rate between the two situations for the same individual. We generated a plot of the inter-beat intervals over time for each delivery method (Fig. 4B). The slope of a line fitted to each plot did not differ significantly from zero. Heart rate was somewhat slower during air sac delivery of anesthesia; in the illustrated example, the inter-beat interval was 111 ± 3 ms (heart rate = 9.1 ± 0.2 Hz) during mask delivery versus 140 ± 7 ms (heart rate = 7.2 ± 0.3 Hz) during air sac

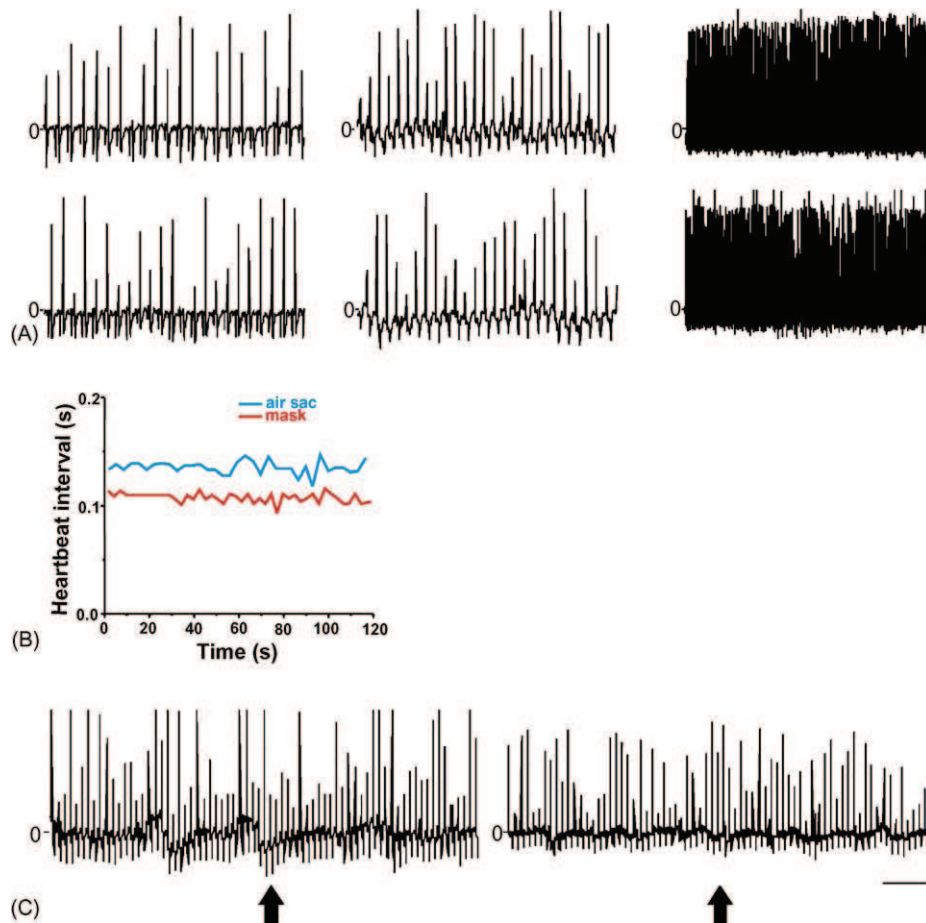


Fig. 4. Comparison of differentiated heartbeat signals obtained from two birds, each during delivery of anesthetic via mask or via air sac cannulation. (A) Heartbeat frequency during mask delivery (top traces) vs. air sac delivery (bottom traces) of anesthetic gas. Left traces are from one bird; middle and right traces are from another bird. (B) Graph shows interval between heartbeats plotted against time for one bird when masked (red) and when cannulated (blue). Rate is stable over the course of 2-min for each mode but slightly slower during mask delivery of isoflurane than during air sac delivery (see Section 3). (C) Heartbeat signal obtained from the second bird during two instances in which apnea was observed during air sac delivery of anesthetic. In each, arrows point to the moment when the bird stopped breathing; apnea was of ~ 1 s duration. Despite the temporary cessation in breathing, heartbeat frequency was not visibly disrupted. Scale bar = 0.5 s for left and middle traces in A; 1 s for C, and 20 s for right traces in A.

delivery. Heartbeat frequency during anesthesia via cannulation was stable despite the reversible apnea that was sometimes observed (Fig. 4C), as described above.

3.4. Discussion

Here we report a novel means of inducing isoflurane anesthesia using air sac cannulation. This technique works well on birds that are one-third the body mass of other species for which air sac cannulation has been described previously (10 g versus 30 g; Korbel, 1998b). The technique is easy to use and allows fine control of anesthetic depth, and fast, complication-free recovery. Indeed, time-to-perch after discontinuous gas inhalation has ceased is within minutes, as compared to the hours it takes for zebra finches to completely recover from chloral hydrate anesthesia (unpublished observations). Among the many advantages, this method allows

access to the head of the bird, prevents mortality due to overdose, and dramatically decreases post-operative recovery and monitoring time. We found no need to monitor heart and pulse oximetry each time, relying instead on observations of respiratory depth and rate. While we have only used this method with isoflurane, similar methods could be used for other inhalant anesthetics.

As mentioned above, zebra finches and other small songbirds are often subjects of neuroanatomical study because of their capacity to learn song. An interconnected network of discrete brain regions that subserves song development and production has been identified, but the neural mechanisms for song learning within and between these regions are incompletely understood. The present anesthetic technique is suitable for introducing neuroanatomical tracers to these brain regions or to otherwise experimentally manipulate neural tissue for survival studies.

One research area in which isoflurane use may not be suitable is for in vivo studies of auditory or visual physiology. In mammals, auditory responsiveness is blunted or lost in cortical neurons when isoflurane, but not pentobarbital, is the anesthetic (Cheung et al., 2001). Similarly, visual response properties are compromised in the primary visual cortex of cats when isoflurane, but not halothane, is the anesthetic (Villeneuve and Casanova, 2003). Because of species-specific differences, however, further work should be done to specifically evaluate these functions in isoflurane-anesthetized songbirds. For example, Budgerigars are birds of a different order than songbirds, yet they share the rare behavioral trait of vocal learning. Not all auditory responsiveness is lost in isoflurane anesthetized Budgerigars as pallial/cortical neurons fire preferentially to acoustic playback of the bird's own contact call over other auditory stimuli (Plummer and Striedter, 2000). In Budgerigars, these preferential neural responses appeared more robust under isoflurane anesthesia than under urethane or ketamine anesthesia. However, these neural responses, recorded with extracellular electrodes from pallial/cortical neurons of isoflurane-anesthetized Budgerigars, appear weaker than those recorded in equithesin-anesthetized zebra finches (Theunissen and Doupe, 1998). Thus, it remains to be demonstrated whether isoflurane or another inhalant anesthetic is suitable for performing survival studies of auditory physiology in oscine songbirds.

Endotracheal intubation has been used successfully in chicks to deliver isoflurane anesthetic (Roberson et al., 2000) and we have been able to similarly anesthetize zebra finches. However, our impression is that the air sac is easier to target than is the trachea. During air sac cannulation, but not during endotracheal intubation, the bird can be anesthetized via mask delivery, thereby alleviating procedural stress. Further, air sac cannulation leaves the bird's head free for neurosurgery and the catheter free from the potential compression that can be caused by the beak stabilization bars used for stereotaxic targeting of brain regions. In no case did we lose a cannulated bird because of overdose or obstruction of the airway due to emesis or aspiration. Moreover, the quick recovery period associated with inhalant anesthetic spares the bird from heat loss, decreases the need for warming devices during recovery, and spares personnel from long-term monitoring of the bird during recovery.

Unlike Roberson et al. (2000), we do not use cautery during our surgeries. Those authors note that cautery is not advised while the 100% oxygen carrier gas is in use because of the potential for oxygen-enriched fires. Like Roberson, we find that the drawback of needing two individuals to perform surgeries, one for the anesthesia and the other for the procedure itself, is overcome by the reliability of this technique, the increase in animal survival, and the decreased time for post-operative monitoring. The trade-off between these factors will vary for each research and clinical setting, and thus should be explored on a case-by-case basis.

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