Different Reactions of Zebra Finches and Bengalese Finches to a Three-Component Mixture of Anesthetics

Authors: Ikebuchi, Maki, Okanoya, Kazuo, and Bischof, Hans-Joachim

Source: Zoological Science, 37(2) : 159-167

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zs190055

ZOOLOGICAL SCIENCE **37**: 159–167 (2020)

Different Reactions of Zebra Finches and Bengalese Finches to a Three-Component Mixture of Anesthetics

Maki Ikebuchi[1]*, Kazuo Okanoya[1,2], and Hans-Joachim Bischof[3]

[1]*Cognition and Behavior Joint Research Laboratory, RIKEN Center for Brain Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*
[2]*Department of Life Sciences, Graduate School of Art & Science, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan*
[3]*Behavioral Biology, University of Bielefeld, Morgenbreede 45, 33501 Bielefeld, Germany*

Kawai et al. (2011) recently introduced a mixture of three anesthetic agents (here called MMB) that has an effect similar to ketamine/xylazine in mice, which might allow more effective reaction to changes in the animal condition, as an antagonist is available, and which can be used without license for handling narcotic drugs. Using Kawai's study as a baseline, we tested whether this anesthesia and its antagonist can also be used in avian studies. In the present study, we used two species, the zebra finch and the Bengalese finch, of the avian family Estrildidae. In zebra finches, anesthesia effects similar to the use of ketamine/xylazine and to those obtained in mice can be reached by the use of MMB if a higher dose is applied. MMB leads to more variable anesthesia, but has the advantage of a longer time window of deep anesthesia. An antagonist to one component of MMB reduced the awaking time, but was not as effective as in mice. For Bengalese finches, MMB cannot be generally recommended because of difficult handling and high mortality rate when used without antagonist, but could be used for perfusions instead of pentobarbital.

Key words: medetomidine, midazolam, butorphanol, M/M/B, ketamine/xylazine, *Taeniopygia guttata*, *Lonchura striata*

INTRODUCTION

To use appropriate kinds of anesthesia in animal experimentation is important for several reasons, e.g., to reduce pain for the experimental animals, to guarantee a steady narcosis over the experimental time course, and to prevent interaction with other drugs used during the experiment. Pentobarbital sodium has been one of the broadly used anesthetics for animal experimentations including perfusions. However, this anesthetic is not the best choice for surgery and perfusion because it does not have an analgesic effect. It is also not easy to keep the appropriate anesthetic depth because the necessary dose for reliable anesthesia is very close to the lethal one (Flecknell, 2010). Recently, a ketamine/xylazine mixture anesthetic is often used for animal experimentation because it has both sedative and analgesic effects and it produces a relatively safe and reliable anesthesia (Coles, 2007; Doneley, 2011; Fish et al., 2008; Grimm et al., 2015). Ketamine, however, was denoted as a narcotic drug in Japan in 2007, so that a narcotic handling license is necessary for its use. Kawai et al. (2011) suggested a mixture of three anesthetic agents (here called MMB) that have an effect similar to ketamine/xylazine in mice and other mammals (Konno et al., 2012; Nakatsukasa et al., 2015). This mixture may also facilitate a faster reaction to worsened animal conditions, as an antagonist is available.

Medetomidine is an α2-adrenergic receptor agonist, Midazolam a $GABA_A$ receptor agonist, and Butorphanol a k-opioid receptor agonist. A mixture of these components, which have different modes of action, is able to keep the animal under anesthesia for a limited period of time (Coles, 2007; Flecknell, 2010; Kawai et al., 2011). One of the components, medetomidine, can be antagonized by atipamezole. Injection of this antagonist is said to shorten the awaking time in mice to about 5–10 minutes, which makes the three-component mixture attractive for experiments in which the animals have to survive after the treatment under anesthesia (Kawai et al., 2011; Konno et al., 2012).

The present publication examines whether MMB can be used for avian studies. We examined the effect of the drug mixture in two species of finches (Estrildidae), zebra finches (*Taeniopygia guttata castanotis*) and Bengalese finches (*Lonchura striata* var. *domestica*), the first one established as a model species for early learning (Griffith and Buchanan, 2010), the second one known as a well-examined example for song learning in birds (Okanoya, 2004a, b). To make the effects as well as the dose comparable to those obtained in the mouse studies, we used the composition of the drugs recommended by Kawai et al. (2011) for mice as the basis of our experiments. We developed a classification scale, based on behavioral cues, which characterizes the different depth levels of the anesthesia, similar to a scale developed by

* Corresponding author. E-mail: maki.ikebuchi@riken.jp
doi:10.2108/zs190055

Guedel (1937) for ether anesthesia. We also compared the efficiency of MMB with that of ketamine/xylazine. Finally, we tested the effect of the antagonist atipazemole, which to our knowledge has not been used as yet in avian research.

MATERIALS AND METHODS

Animals

The original research reported herein was performed under guidelines established by the animal experimentation committee of RIKEN. (Permission H28-2-212(2) from the RIKEN Safety Management Division, Bio Safety and Ethics Section, Sep. 5, 2016).

Thirty-nine adult zebra finches (♂17, ♀22, weight: 14.6 ± 1.86 g (AV ± SD)) and 52 Bengalese finches (♂25, ♀27, weight: 15.3 ± 1.64 g (AV ± SD)) were used for this study. The birds were kept in bird cages (37 × 42 × 44 cm (l/h/d)) housing on average 8 birds at the RIKEN research center under a 15/9 hour light/dark cycle, a temperature 25°C, and around 50% humidity. Birds had ad libitum access to food (grass seeds, greens, minerals) and water.

Preparation of anesthetics and antagonist

The mixture of the three anesthetics (here called MMB) was composed of Medetomidine (Domitol, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), Midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan), and Butorphanol (Vetorphale, Meiji Seika Pharma Co., Tokyo, Japan). The Ketamine and Xylazine (K/X) mixture was composed of Ketalar® (Daiichi Sankyo Co., Ltd., Tokyo, Japan) and Celactal® (Bayer Yakuhin Co., Ltd., Osaka, Japan). Atipamezole (Antisedan, Nippon Zenyaku Kogyo Co., Ltd.) was used as a medetomidine antagonist. All original drugs were stored at room temperature.

The proportion of the single components of the MMB mixture (0.3/4/5) was chosen in accordance with information from previous studies (Kawai et al., 2011). To prepare the injection, we combined 0.3 ml of medetomidine (original concentration: 1 mg/ml), 0.8 ml of midozolam (original concentration: 5 mg/ml) and 1 ml of butopharmol (original concentration: 5 mg/ml), then added saline (0.9% NaCl in distilled water) to a final volume of 5 ml. To reach the proposed MMB dose (0.3/4/5 mg/kg) as used in the mouse study, the total injection volume was 0.05 ml/10 g. We used this dose (named here "1.0* MMB") as a basis for the doses used in our experiments, to make them comparable to the mouse studies. The 1.2* MMB, for example, stands for the 1.2 fold of the mouse dose, or 1.5* MMB for the 1.5 fold of the mouse dose.

The composition of the ketamine/xylazine mixture in zebra finches (K/X, 1.67/33.3 mg/kg) and in Bengalese finches (K/X, 1.25/25 mg/kg) followed a protocol developed in our laboratory. We combined 0.1 ml of ketamine (original concentration 50 mg/ml) and 0.5 ml of xylazine (original concentration 20 mg/ml) and filled up with saline to 1.0 ml injection solution. For anesthesia, we used 0.033 ml/10 g for zebra finches, and 0.025 ml/10 g for Bengalese finches.

The antagonist (0.06 ml, original concentration 5 mg/ml) was filled up to 5 ml with saline. From this stock, we used 0.05 ml/10 g, a dose comparable to that of the mouse study (Kawai et al., 2011), as basis for the amounts which were injected into the zebra finches and the Bengalese finches, therefore tagged as "1* ", for the mouse dose equivalent, "5* " for the 5 fold of the mouse dose, etc.

Injection procedure and observations

The experimental birds were chosen at random from the colony, weighed and transferred from the home cage to smaller experimental cages (16 × 30 × 22 cm (l/h/d)). The birds were then assigned to one of the experimental groups and the anesthetic to be tested was injected into the pectoral muscle, which is most commonly used for intramuscular injections in birds (Doneley, 2011) because it is the biggest muscle.

When we injected an antagonist, it was applied to the pectoral muscle of the opposite body side. Terumo™ Tuberculin syringes and 27G needles (R·B (regular bevel, 12°)), length: 25 or 38 mm) were used for injection. The finest scaling of the syringe was 0.01 ml, and because it was possible to adjust the injection volume also quite precisely to the middle between the two bars, the calculated injection volumes were corrected to the nearest 0.005 ml step.

After injection, the birds were transferred to the experimental cage and an infrared lamp was attached for warming. We checked the birds' condition after 2, 5, 10, 15, 30, 45, 60, 75, 90 min and every 30 min after 90 min until the birds woke up. The same schedule, truncated after 60 min, was used in shorter experiments. The exact number and gender of the birds assigned to the different subgroups of the experiment are depicted in Table 1.

At each check, we evaluated the depth and duration of the anesthesia by observing the behavior of the birds and tested the foot withdrawal reflex by pinching the toes with help of thumb and forefinger.

We scored the depth of anesthesia by these behavioral observations, as shown in Table 2, which is inspired by a classification of the effects of ether anesthesia suggested by Guedel (1937) and extended by criteria used by Kawai (2011). We also controlled the birds' breathing and other bodily conditions while and after we injected anesthetics or the antagonist. Birds are not able to sit on a perch when they are anaesthetized, but can do it in normal sleep (e.g., Galton and Shepherd, 2012). Thus, when we noticed that the birds started to wake up, we tried whether they were able to sit on the perch and if so, this was taken as the time for being awake (Score 1 at Table 2).

Experimental schedules

We did three experiments using zebra finches and three similar ones with Bengalese finches. First, we tested the efficiency of the anesthetic. Second, we sought to find the appropriate antagonist doses. Third, we examined the interaction of the anesthetic dose and the timing of the antagonist injection. Although this schedule was principally the same for both species, we describe it separately for both species because there were some differences according to details (Table 1).

Efficiency of the anesthetics (Exp 1.1, Exp. 2.1, Table 1)

Fifteen zebra finches were used to test the efficiency of the anesthetics. The MMB doses tested were the 1.2* and the 1.5* of the mice dose. The dose used was 0.033 ml/10 g (K/X: 1.67/33.3). These doses were selected on the basis of our previous experience concerning the different effects of anesthetics, for example nembutal and ketamine/xylazine, in mice, zebra finches and Bengalese finches.

Thirteen Bengalese finches were tested using the identical time schedule time schedule. The dose used for the Bengalese finches was 0.025 ml/10 g (K/X: 1.25/25). We injected 3 birds each with 1.0* and 0.7*, 2 birds with 0.8* the MMB mouse dose, and 5 birds with K/X.

Anesthetic–antagonist interaction (Exp 1.2, 2.2, Table 1)

To test how the antagonist atipamezole altered the anesthesia pattern induced by the anesthetic mixture, 9 zebra finches received a dose of MMB 1.5* of that used in mice. From these, 3 birds each got an injection of the antagonist after 60 minutes into the opposite pectoralis muscle of a 1-, 5-, and 10* dose of that used for mouse.

Nine Bengalese finches were injected with 1.0* MMB mouse dose. Three birds each got an injection of the antagonist after 60 minutes into the opposite pectoralis muscle of a 1-, 5-, and 10* dose of that used for mouse.

Table 1. Experiments performed with zebra finches (1.1–1.3) and Bengalese finches (2.1–2.3). For each experiment, the anesthetic used and the number of birds is depicted. Further explanation see text. The results of experiments 1.2 and 2.2. are compared also with those of the corresponding groups of experiments 1.3 and 2.3, respectively (*1 and *2). "a": The results of these groups are used for a comparison between awaking times with and without antagonists.

Zebra Finches

Exp.1.1		Anaesthetic Dosage	Antagonist Dosage	Antagonist Inj.-Time (min)	♂+♀	♂	♀
	a	*1.5 MMB			5	3	2
	a	*1.2 MMB			5	2	3
	a	K-X			5	2	3
Total 1.1					15	7	8

Exp 1.2		Anaesthetic Dosage	Antagonist Dosage	Antagonist Inj.-Time (min)	♂+♀	♂	♀
		*1.5 MMB	*1	60	3	1	2
			*5	60	3	1	2
	⁎1		*10	60	3	1	2
Total 1.2					9	3	6

Exp 1.3		Anaesthetic Dosage	Antagonist Dosage	Antagonist Inj.-Time (min)	♂+♀	♂	♀
	a	*1.5 MMB	*10	60	3	1	2
	a			90	3	2	1
	a			120	3	1	2
	⁎1 a	*1.2 MMB	*10	60	3	2	1
	a			90	3	1	2
	a			120	3	1	2
Total 1.3					15	7	8

	♂+♀	♂	♀
Totals 1, 1 - 3	39	17	22

Bengalese Finches

Exp. 2.1	Anaesthetic Dosage	Antagonist Dosage	Antagonist Inj.-Time	♂+♀	♂	♀
	*1 MMB			3	1	2
	*0.8 MMB			2	0	2
	*0.7 MMB			3	2	1
	K-X			5	3	2
Total 2.1				13	6	7

Exp.2. 2		Anaesthetic Dosage	Antagonist Dosage	Antagonist Inj.-Time (min)	♂+♀	♂	♀
		*1 MMB	*1	60	3	1	2
			*5	60	3	2	1
	⁎2		*10	60	3	2	1
Total 2.2					9	5	4

Exp. 2.3		Anaesthetic Dosage	Antagonist Dosage	Antagonist Inj.-Time (min)	♂+♀	♂	♀
		*1.2 MMB	*10	60	3	1	2
				90	3	1	2
				120	3	3	0
	⁎2	*1.0 MMB	*10	60	3	2	1
				90	3	1	2
				120	3	1	2
				180	3	1	2
				240	3	2	1
		*0.8 MMB	*10	60	3	1	2
				90	3	2	1
				120	3	1	2
Total 2.3					30	14	16

	♂+♀	♂	♀
Totals 2, 1 - 3	52	25	27

Table 2. Classification of anesthesia depth based on a scale developed by Guedel (1937). Surgery is possible at stages 5 and 6, at stage 7, the survival of the bird is endangered. After injection, birds quickly go from score 1 to the final score (5–7). When waking up, they slowly go from the highest score back to score 1.

Score	Description	Status
1	Bird is able to stay on perch	fully awake
2	open eyes, but can't stay on perch	drowsy
3	eyes open on touching but then close immediately	light anaesthesia
4	pinching toes: opens eyes and leg retraction	leaving full anaesthesia
5	pinching toes: leg retraction, eyes not opening	anaesthesia gets lower
6	pinching toes: feathers moving, no leg retraction	deep anaesthesia
7	pinching toes: no reaction	anaesthesia too deep

Interaction of anesthetic dose and delay time between anesthetic and antagonist injection (Exp 1.3, 2.3, Table 1)

Nine zebra finches received an injection of 1.2*MMB, another 9 birds were injected with a 1.5*MMB. After these injections, 3 birds of each anesthetic dose got an injection of the antagonist (10* concentration) after 60 min, 3 birds after 90 min, and 3 birds after 120 min.

Nine Bengalese finches were injected with 0.8* MMB, 15 others were injected the 1.0* MMB and 9 others were injected the 1.2* MMB dose. Subsequently, 3 birds of each MMB dose group were injected the antagonist 10* mouse dose after 60, 90, and 120 min, respectively, after anesthesia injection. The 1.0* group was extended by two antagonist injection times, 180 and 240 min, again 3 birds per group. These additional groups were introduced later because the experiments with the lower delay times indicated that a longer delay would be more appropriate for Bengalese finches.

Data analysis

Data were analyzed using the Excel statistics for Windows (BellCurve for Excel, Social Survey Research Information Co. Ltd.,

Japan).

For zebra finches, we compared the scores from each anesthesia condition (factor 1) and time from injection (factor 2) using 2-way ANOVA, followed by determination of the simple main effect of condition and with Bonferroni correction for multiple comparison tests. We also tested the difference of the waking up time between birds injected with anesthetic only, and birds that received both, anesthetic and antagonist, using ANOVA followed by Bonferroni correction for multiple comparison tests.

For Bengalese finches, we did not make statistical tests for experiment 1 because most of the birds died in the course of the experiment irrespective of the doses they received. We used the same statistics as described above for experiment 3 and parts of experiment 2.

RESULTS

Zebra finches

General reactions to anesthetic and/or antagonist injection

In seven of 34 zebra finches, MMB injections caused breath changes indicating health problems. These changes included breathing with beak clapping, production of sound with breathing, and also a change of the beak color. Some of the birds showed this at an early stage of the experiment, around 2–5 min from injection, others later, between 45 and 90 min from injection. We also observed vomiting in very rare cases, but this did not worsen the condition of the bird if the vomit was immediately cleared from the beak. In many cases, the body temperature of the birds decreased after some time, although heat was provided by the heating lamp, so that it was advisable to at least check the bird's temperature manually and to increase the heating. Such a decrease of the body temperature was also observed with K/X injections after some time, but in contrast to the MMB cases, we did not observe breath and beak color changes.

In addition to the immediate reduction of the anesthesia depth and shortening of the awaking time, the antagonist injections elicited in all birds some strong behavioral reactions. Two to 5 minutes after injection, the birds started to breathe much more deeply, so that the breathing movements included the whole body. The birds returned to normal breath 10 to 90 minutes later. Eleven of 24 the birds exhibited strong wing vibrations and/or foot movements similar to running from about 2–5 min up to 30 minutes after antagonist injection. Fourteen of 24 birds even appeared as trying to hop around the floor with open or closed eyes starting 45 min and ending up to 120 min after the injection. Such behaviors were not observed in birds after MMB and K/X anesthesia.

Efficiency of the anesthetics (Exp 1.1, Table 1)

Figure 1 shows that with all anesthetics and concentrations tested, the anesthesia level increased very fast, the peak of the anesthesia score was reached latest after 15 minutes. The highest score of 7 (deepest anesthesia) was observed in two birds of the 1.5* MMB dose group and one of the 1.2* group. The mean initial scores of both groups were comparable with those of the K/X group. Thereafter, there was a short drop from the peak values in both MMB groups at about 30–45 minutes, not in the K/X group. After reaching the peak values again, the downward slope of the curves, that means the duration of the anesthesia and the awaking time, was very different between the three groups.

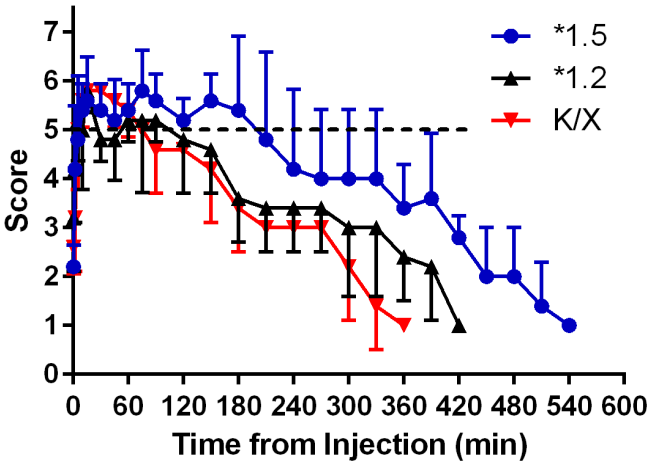

Fig. 1. Time course of the anesthesia of zebra finches induced by two dosages of MMB (1.2* and 1.5* of the mouse dose) and K/X, respectively (Exp. 1.1., means and Std. deviations.). After a fast increase of anesthesia depth to about score 6, the time course of the reduction of anesthetic depth is slowest in the 1.5* MMB group, followed by the 1.2* group and the K/X group.

On average, the birds of the 1.5* group retained an anesthesia level over 5 for about three hours, those of the 1.2* group and of the K/X group for about 60–90 and 60–75 minutes, respectively. Score 1, fully awake, was reached in the 1.5* group as late as 492 ± 40 min (mean ± SD) after initiation of the anesthesia.

In the 1.2* group, birds were on average awake around 390 ± 52 min from injection. The average awaking time of the K/X group was 324 ± 25 min from injection. Statistical treatment of the data: see Supplementary Text S1.

K/X anesthesia also reached an anesthetic depth between 5 and 6. Four of 5 birds kept a score 5 until 150 to 210 min after induction of the anesthesia. One of the 5 birds showed a score of 3 already at 90 minutes.

Anesthetic–antagonist interaction (Exp 1.2, Table 1)

Figure 2 shows the changes of the anesthesia level (1.5* MMB) over time for zebra finches with a 10*, 5*, and 1* mouse dose of the antagonist, injected 60 minutes after the anesthetic. The 10* group showed a quick (2–10 min) reduction of the anesthesia to the score of 3 after injection. Thereafter, this level was kept for about 3 hours and the birds needed a long time, on average 220 ± 35 min after antagonist injection (average 280 min after anesthetic injection) until waking up. This was, however, earlier than without antagonist (492 ± 40 min).

The anesthesia curves of the 5* and the 1* antagonist group also looked quite smooth but there were some individual differences in the 5* and even more in the 1* group. The birds that received the 5* antagonist dose exhibited a score of 3 at 5 min from injection. However, one of them showed a score of 4 five minutes later and then scored between 3 and 4 until 150 min after injection. The average waking up time of the 5* group was 400 ± 92 min, that of the 1* group could not be determined because one bird was saved from death by an additional dose of antagonist. Statistical treatment of the data: see Supplementary Text S1.

Interaction of anesthetic dose and delay time from anesthetic to antagonist injection (Exp. 1.3, Table 1)

The interaction between anesthetic dose and delay time was tested in two groups, one with 1.2*, the other with 1.5* MMB doses (Fig. 3). The antagonist was the 10* mouse dose in both cases. The birds of both groups scored around 5 before antagonist injection, and showed a reduction to score 3, 5 min after antagonist injection, regardless of anesthesia doses and antagonist injection timing. Thereafter, they kept scores of 3 or 2 until around 220 min, but there was a large individual variation in the course of anesthesia depth.

Concerning the waking up times, there were no significant differences between any of the groups (2way-ANOVA, factor1: anesthetic doses, factor2: delay of antagonist injection, $F1_{1,12} = 0.078$, $P = 0.784$, $F2_{2,12} = 0.490$, $P = 0.0624$, $F1 \times F2_{2,12} = 0.726$, $P = 0.504$).

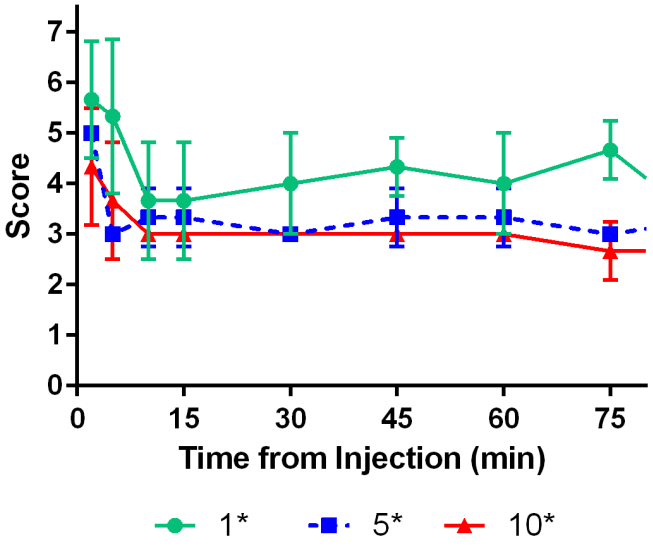

Fig. 2. Changes of the anesthesia level (1.5* MMB) over time for zebra finches with a 10*, 5*, and 1* mouse dose of the antagonist, injected 60 minutes after the anesthetic. The scores drop within 10 minutes from scores 5–6 to around 3 in the 5* and the 10* group and remains there. In the 1* group, the anesthesia level goes below 4 after 10 minutes, but then increases to 4–5.

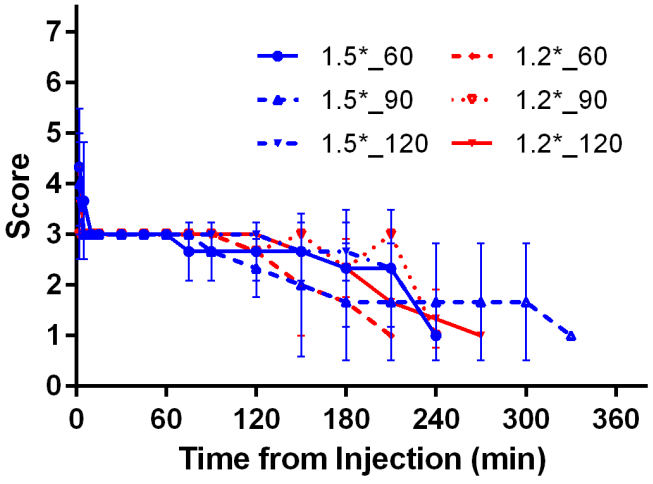

Fig. 3. Interaction of anesthetic dose and delay time from anesthetic to antagonist injection in zebra finches. For 60 minutes, there was no difference between treatments concerning anesthesia scores. Thereafter, variation between groups increased strongly with time.

Comparison of awaking times with and without antagonists (Exp 1.1 and 1.3, Table 1)

Figure 4 shows a comparison of the time for waking up of birds from experiment1 that received only the anesthetic (MMB 1.5* and 1.2*, and K/X, each group $n = 5$), with those from experiment 3, injected with anesthetic (each group $n = 9$, MMB 1.5* and 1.2*) and antagonist (10*). We tested in each case the time span between injection of the anesthetic and waking up (score 1).

The 1.5* MMB group needed significantly longest for waking up (ANOVA, $F_{4,28} = 13.395$, $P < 0.001$, multiple comparison test 1.5* vs. 1.5*a (plus antagonist), 1.5* vs. 1.2*a and 1.5* vs. K/X; $P < 0.001$, 1.5* vs. 1.2*: $P = 0.045$, others; n. s., Fig. 4). Injection of the antagonist did not, as observed in mammals, lead to immediate waking up. However, the birds woke up significantly earlier than without antagonist, about 2–3 hours earlier in the 1.5* MMB group and 1 hour earlier in the 1.2* MMB group.

Summary of zebra finch results

Side effects like beak clapping, breathing with sound, or movements occurred more frequently with MMB injections and application of antagonist compared with K/X. However, the 1.2* MMB mouse dose was generally appropriate for a quite long surgery period of up to 90 minutes. The risk to overdose was lower than with the 1.5* dose, but there was a higher risk for stronger fluctuation of the anesthetic depth, which rarely even lead to short episodes of awakening. The 1.5* MMB anesthesia has a longer duration compared with K/X, this may be advantageous in cases of complicated surgery and is in any case appropriate for perfusions. The time window for surgery with K/X was quite variable, but there were no obvious differences to MMB injections in the behavior of the birds during anesthesia and after waking up. Both, the 5* and the 10* antagonist dosages led to a fast reduction

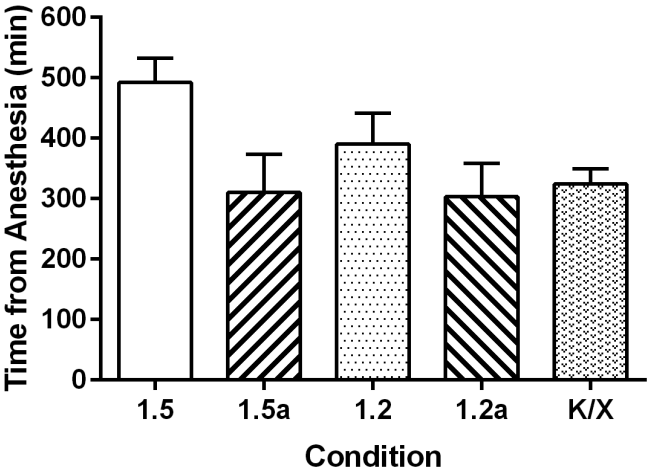

Fig. 4. Comparison of awaking time of zebra finches after anesthesia with and without antagonists. The antagonist reduced the awaking time to levels also observed with K/X anesthesia.

of the anesthesia to around score three. However, only the 10* dose of antagonist shortens the awaking time of MMB reduced anesthesia substantially. It can thus be recommended for use with MMB anesthesia in zebra finches. But, the shortening is not comparable with that observed in mice (10–15 minutes). The antagonist can be applied at any time of the experiment. Its efficiency is not dependent on the time delay between anesthetic and antagonist injection. Time of awaking exhibits large interindividual differences.

Bengalese finches

General reactions to anesthetic and/or antagonist injection

The Bengalese finch behavioral reactions to MMB injection were very similar to those observed in zebra finches. Breathing changes were observed in five of 52 Bengalese finches after MMB injection, including breathing with beak clapping (three birds), production of sound with breathing (three birds), and also a change of the beak color (one bird). Some birds showed this at an early stage of the experiment, starting around 2 min from injection, others as late as 45 min and 510 min from injection. In many cases, the body temperature of the birds was decreasing over time, so that the heating had to be increased, and it was advisable to check the bird´s temperature manually.

Birds with K/X anesthesia also showed decreasing body temperature, but we did not observe breathing and beak color changes.

In addition to an immediate reduction of the anesthesia depth and shortening of the awaking time, antagonist injections elicited some strong behavioral reactions in all birds. From 2–5 minutes after injection, all birds but four started to breathe much deeper, so that the breathing movements appeared to affect the whole body. In four birds, these changes began 10–15 min after antagonist injection. The birds returned to normal breath 10–120 min after antagonist application, mostly after 30–45 min. A few birds showed breathing with beak clapping, production of sound, and one exhibited also a change of the beak color. We also observed vomiting in two birds, but this did not worsen the condition of the bird if the vomit was cleared from the beak. Twenty-nine of 39 birds with the antagonist exhibited strong wing vibrations and/or foot movements similar to running. These behaviors started 2–5 min after antagonist injection and stopped 30 to 90 min later. Five of 39 birds even looked as if they were attempting to hop around the floor with open or closed eyes for short periods of time.

Efficiency of the anesthetics (Exp 2.1, Table 1)

The progression of the MMB anesthesia in Bengalese finches was very different from that in zebra finches, and also from K/X anesthesia (Fig. 5).

The 0.7* MMB group reached a score of 5 after 10 minutes. Thereafter, two of them showed the score below 5 and they kept this score for a moment, then went even higher, to score 5. Eventually, they showed the score below 5, again. One of them remained at a level of 5 after injection and died without awaking from the anesthesia. The birds of the 0.8* and 1.0* groups scored 5 after ten minutes. Then, the score was gradually reduced to 4 within 60 minutes. Thereafter, with some short fluctuations reaching scores of 3 (starting to wake up), the birds remained at a level of 5 after injection and died without awaking from the anesthesia.

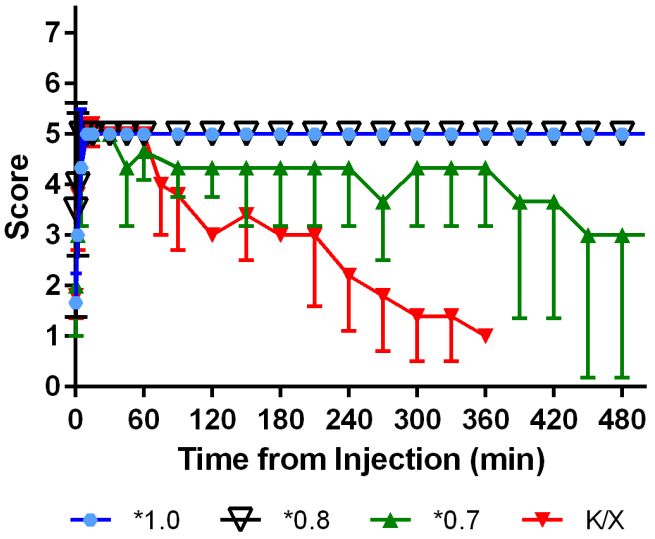

Fig. 5. Bengalese finch anesthesia scores over time after anesthetic injections of different concentrations. Anesthesia levels of 5–6 were reached after less than 5 minutes. Birds with 1.0* and 0.8* MMB did not recover, 0.7* MMB did not induce reliable anesthesia. K/X initiated a reliable anesthesia for 60 minutes.

Finally, only two of eight birds woke up completely, one with a 1.0* dose, one with a 0.7* dose. Another one with a 0.7* dose also woke up completely around 10 hours after injection, but died another seven hours later.

When we recognized that there was no sign of waking up in most of the birds even after 10 to 15 hours, we injected a 10* dose of the antagonist to rescue their lifes. This in most cases reduced the anesthesia level to 3, but did not prevent the birds from dying. There was no change of breathing or other behavioral reaction before the birds died.

Birds with K/X anesthesia expressed scores over 5 after 10 min from anesthetic injection. Seventy-five min after injection, peak scores were 5–6, and after 120 min, three of the five birds showed scores from 3 to 4, the other two birds scored 3. All birds woke up between 210 and 360 min (mean ± SD: 274 ± 58 min) after injection.

Anesthetic-antagonist interaction (Exp 2.2, Table 1)

Figure 6 shows the changes of the anesthesia level (1.0* MMB) over time for Bengalese finches with a 10*, 5*, and 1* mouse dose of the antagonist, injected 60 minutes after the anesthetic. Birds with an injection of a 1* dose of the antagonist 60 minutes after application of 1.0* MMB showed scores of 3–4, 15 minutes later. One of these birds returned to score 5 after 45 min, and then remained at this score. The two other birds of this group kept a score of 3, but the eye opening that characterizes this score was incomplete and the birds appeared to return to higher anesthesia levels over time. To prevent these birds from dying, we made a second antagonist injection between 90 to 210 min after the first one. All three birds then woke up completely after 360 min.

Birds with a 5* dose of antagonist showed scores of 3 to 4, 15 minutes after injection. One bird of this group woke up completely 330 minutes later. The two others returned to a higher score of 4, 30 minutes after injection and then went

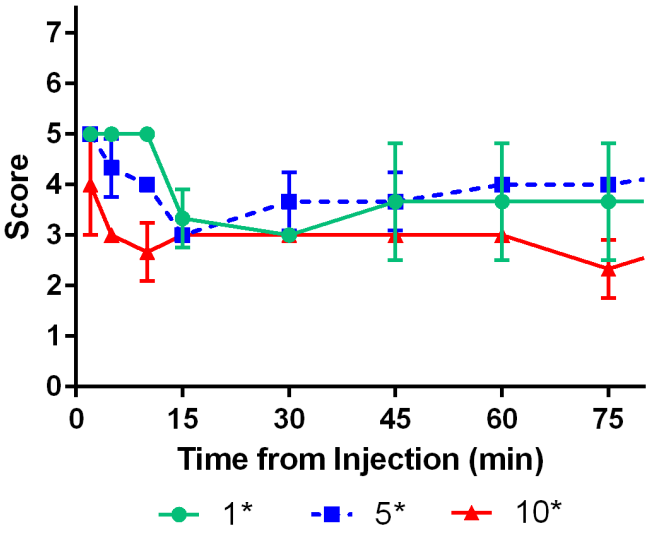

Fig. 6. Injections of antagonist 60 minutes after anesthesia induction (1.0* MMB) in Bengalese finches. The birds of all groups return to a level between 2 and 4 after about 15 minutes, but do not awake or awake much later (see text).

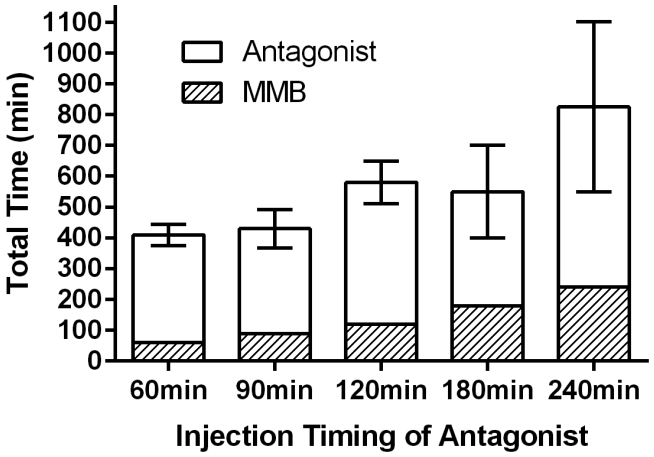

Fig. 7. Awaking times (score 1) of Bengalese finches after injection of the 10* antagonist at different time delays from application of 1.0* MMB. Striped part of columns, time from anesthetic injection to antagonist application. Blank part, time from antagonist injection until awaking. Note that the awaking times from antagonist injection also increased with increasing time span between anesthetic and antagonist injection, and also note the increase of the standard deviation.

even higher, to score 5 after 90–120 min. As in the previous group, we injected more antagonist 120 min after the 1st antagonist injection to rescue the life of these birds. They woke up completely, one 540 min, the other 780 min after the 1st antagonist injection.

The birds of the 10* antagonist group showed a score of 3 after 2 to 5 min from antagonist injection. These birds kept this condition for several hours and woke up 350 ± 34.6 min later without additional antagonist injection.

Interaction of anesthetic dose and delay time from anesthetic to antagonist injection (Exp 2.3, Table 1)

The relation between MMB dosage and timing of the antagonist injection was tested with three MMB doses 0.8*, 1.0* and 1.2*. The antagonist (10* mouse dose) was injected 60, 90 and 120 min after application of the anesthetic. Due to the results of these tests, we added two delay times of 180 and 240 minutes to this experiment.

An ANOVA did not reveal significant differences of awaking times due to concentration of anesthetic and of time span between anesthetic and antagonist injection. There were, however, some tendencies: birds of the 0.8* anesthetic group woke up earlier (320 ± 132 min) than those of the 1.0* and the 1.2* group (383 ± 76 min and 457 ± 104 min). Within the 1.0* group (Fig. 7), the awaking time after antagonist injection increased from 60 min to 240 min delay between anesthetic and antagonist injection. Likewise, the standard deviation increased (delay time 60 min: 350 ± 34 min, 90 min: 340 ± 63 min, 120 min: 460 ± 69 min, 180 min: 370 ± 151 min, 240 min: 585 ± 276 min).

One of the birds with antagonist injection group of 240 min died after 120 min from injection without waking up. Another one was not in a good health condition for a while after this experiment and needed care for one week.

Summary of Bengalese finch results

While the K/X anesthesia lead to a reliable and stable anesthesia with a score of 5 for at least 60 minutes, Anesthesia with 0.7* MMB had a very irregular time course, and the birds with higher dosages did not recover properly and died some time after the experiment, if no antagonist was used. Both, the 1* and the 5* antagonist doses were too low to give reliable effects. A 10* dose of the antagonist was necessary in Bengalese finches for surviving the MMB anesthesia. It was possible to inject additional antagonist if the condition of the bird appeared to become critical. As seen in the zebra finches, the efficiency of the antagonist was not much affected by differences of the anesthetic dosage and the delay between anesthetic and antagonist injection. Unexpectedly, the awaking times did not become shorter with longer delays of antagonist injection. There was even a tendency for a prolongation of awaking time with longer delays, and the effect of the antagonist was more variable.

DISCUSSION

Compared with the results of the use of MMB in mice, zebra finches needed generally a higher dose of the anesthetic, and the concentration of the antagonist had to be 5–10-fold higher than that used in mice to be effective. The best results were obtained with the 1.5* mouse dose, which gave an anesthesia deep enough for surgery for up to 3.5 hours, much longer than the average deep anesthesia of one hour obtained with K/X. The very long time for waking up (up to nine hours) could be shortened substantially by injection of the 10* antagonist. The birds did not wake up in a few minutes as is observed in mice, but the anesthesia depth dropped from 5 to the less dangerous level of three within about 10 minutes, and the awaking time also was substantially shortened. Thus, a 1.5* dose of MMB in combination with the antagonist has advantages over the K/X anesthesia if a surgery window of more than one hour is needed.

For Bengalese finches, the dosage leading to anesthesia was even lower than that used in mice, but the anesthesia was not reliable independent of the dosage. In contrast to zebra finches, there was a significant drop of anesthesia depth, after reaching a peak score within 2–10 minutes, and a more steady higher level was attained not earlier than about 30–45 minutes. Finally, almost all birds did not survive the anesthesia unless the antagonist was given in a dose 10 times higher than that used in mice. Even with the antagonist, it was sometimes necessary to inject the birds a second time with antagonist to prevent them from death.

Given the fact that MMB as anesthetic caused very unpredictable effects in the Bengalese finches and the ten-fold dose of the antagonist (sometimes given more than once) was mandatory to keep the birds alive, this drug composition cannot be recommended as anesthetic for this species. The 1.0* mouse dose may be applied for sacrificing the Bengalese finches or to perform perfusions because the initial anesthesia is deep enough for such treatment, but not for longer lasting surgery because of the high risk of unwanted death.

Why the metabolism of zebra finches and Bengalese finches reacts so different to the application of MMB and also of the antagonist Atipamezole is not known. We also do not know why the effects of K/X are very similar in the two species, in contrast to those of MMB. To our knowledge, there are no studies on the effect of narcotic drugs in small birds like finches (Murphy and Fialkowski, 2001). A study with medetomidine and midozolam anesthesia in the much bigger pigeon (*Columba livia*) (300–400 g) reports results similar to those we obtained in the zebra finches and also stresses that the drug effects are somewhat variable and unpredictable (Pollock et al., 2001).

Given the results in pigeons, weight is thus probably not the reason for the unforeseen reactions in the Bengalese finches. These are slightly bigger than zebra finches, but slightly lower doses of MMB and K/X are necessary for anesthesia. Also, both species are closely related, belonging to the same family (Estrildidae) of the avian order Passeriformes. Thus, there might be subtle differences in the metabolism of the two species which cause the differential effects in the anesthetic action of the drugs used.

We propose that the differences between K/X and MMB anesthesia may be related to the retention time of the drugs within the body and the mechanisms of drug clearance. Xylazine has a half life of 1–6 min, ketamine of 45 minutes. The half life of medetomidin is also short, about 30 minutes, but midazolam has a half life of 1.5–2.5 h, and butorphanol of 4–6 hours. This may already explain why K/X gives much shorter periods of deep anaesthesia, and the short half life may also suggest that K/X is not as stressful as MMB for the organism.

Longer retention times also may indicate that the clearance of the drugs may be different in the two species. The main organ for drug metabolization is the liver (Salonen, 1989), and at least in mammals, Medetomidine is contraindicated in animals with cardiovascular, respiratory, liver, or renal dysfunction (Sinclair, 2003).

We therefore believe that a reduced liver function could be the reason for the problems arising in Bengalese finches. Due to own observations, this species and its wild ancestor, the white-rumped munia (*Lonchura striata*), very often develop a fatty liver, and this may lead to a reduction of the decomposition of drugs. The longer retention time of the drug within the body may explain why the Bengalese finches were not fully recovering from the anesthesia. One has to take into account that the lack of nutrition and water supply may become dangerous for the small birds. The stress caused by the lack of these supplies may have added to the damaging effect of the anesthetic drugs. This may explain why with too long survival times even the injection of the antagonist did not show an effect.

Another, not mutually exclusive explanation for the observed effects may be due to an ecological adaptation of the zebra finches. Dehydration as described above, leads to a reduction of efflux of urine from the kidney and may thus also contribute to a reduction of drug degradation. Zebra finches, in comparison to Bengalese finches, suffer less from dehydration because of their adaptation to arid climates (Sossinka, 1972), and may thus be more resistant against the effects of dehydration by long lasting anesthesia.

We also do not have an explanation at present for the difference in effectiveness of the antagonist atipamezole between mice and birds. Although we applied up to the 10-fold dose of the antagonist, the awaking time was not shortened to the same extent as it was observed in mice. In Bengalese finches, we found that it prevents the MMB injected anesthetic from being sacrificed by the drug, in zebra finches, the awaking time was significantly reduced, but never to a few minutes as observed in mice (Kawai et al., 2011). As stated above, medetomidine has a quite short half life, and therefore the antagonist may also be more effective if the anesthesia periods are short. At the times when we applied the antagonist, medetomidine might not contribute much to the overall anesthetic effect in birds, and thus a reduction of this component does not promote awaking as much as in mice. To confirm these ideas, however, the effects of timing of the injections have to be examined more closely.

To summarize, compared with the ketamine/xylazine mixture that we have been using for years, the use of MMB in combination with the antagonist has advantages in the zebra finches because it allows longer surgeries. However, the use of MMB demands more careful control of every parameter, because more often than with K/X anesthesia because unforeseen, sometimes critical, incidents may occur. With longer anesthesia times, one has to keep in mind that nutrition and water loss has to be taken into account. For Bengalese finches, our results clearly show that anesthesia with MMB is not the method of choice. In this species, the negative effects by far outnumber the positive ones. MMB may be used for perfusions, but even in this case the fluctuations of anesthesia with MMB suggest to use K/X instead.

ACKNOWLEDGMENTS

The paper was partly prepared and written during a stay by H.-J. B. at the RIKEN research institute (Wako) on the invitation of K.O.

Supported by a grant from MEXT, grant # 17H01015 to K.O.

COMPETING INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

M.I.: study design, experiments, writing, K.O.: fund raising, supervision, H.-J. B.: publication design, writing.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available online. (URL: https://bioone.org/journals/supplementalcontent/10.2108/zs190055/10.2108.zsj.37.159.s1.pdf)

Supplementary Text S1. Statistical calculations.

REFERENCES

Coles BH (2007) Essentials of Avian Medicine and Surgery. Wiley-Blackwell, Oxford

Doneley B (2011) Avian Medicine and Surgery in Practice: Companion and Aviary Birds. Manson Publishing Ltd, London

Fish RE, Brown MJ, Danneman PJ, Karas AZ (2008) Anesthesia and Analgesia in Laboratory Animals. 2nd ed, Academic Press, New York

Flecknell P (2010) Laboratory Animal Anaesthesia. 3rd ed, Academic Press, London

Galton PM, Shepherd JD (2012) Experimental analysis of perching in the European starling (Sturnus vulgaris: Passeriformes; Passeres), and the automatic perching mechanism of birds. J Exp Zool 317: 205–215

Griffith SC, Buchanan L (2010) The zebra finch: the ultimate Australian supermodel. Emu 110: v–xii

Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA (2015) Veterinary Anesthesia and Analgesia: The Fifth Edition of Lumb and Jones. 5th ed, Wiley-Blackwell, Boston

Guedel AE (1937) Inhalational Anaesthesia - A Fundamental Guide. Macmillan, New York

Kawai S, Takagi Y, Kaneko S, Kurosawa T (2011) Effect of three types of mixed anesthetic agents alternate to ketamine in mice. Exp Anim 60: 481–487

Konno K, Horiuchi S, Isoe K, Matsudai H, Fujiwara H, Furuya M, Takashima H (2012) Evaluation of the combination of three anesthetics in rats and rabbits. Annual Report of Hatano Research Institute 35: 53–59

Murphy JP, Fialkowski J (2001) Injectable Anesthesia and Analgesia of Birds. In "Recent Advances in Veterinary Anesthesia and Analgesia: Companion Animals" Ed by RD Gleed, JW Ludders, International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA

Nakatsukasa E, Natsume R, Nakamoto C, Takada H, Fei P, Suzuki Y, Sakimura K (2015) The usefulness of the combination of three anesthetics in rat embryo transplantation. Niigata Med J 129: 751–759

Okanoya K (2004a) Song syntax in Bengalese finches: proximate and ultimate analyses. Adv Stud Behav 34: 297–346

Okanoya K (2004b) The Bengalese finch: a window on the behavioral neurobiology of birdsong syntax. Ann NY Acad Sci 1016: 724–735

Pollock CG, Schumacher J, Orosz SE, Ramsey EC (2001) Sedative effects of Medetomidine in pigeons (*Columba livia*). J Avian Med Surg 15: 95–100

Salonen JS (1989) Pharmacokinetics of Medetomidine. Acta Vet Scand Suppl 85: 49–54

Sinclair MD (2003) A review of the physiological effects of α2-agonists related to the clinical use of Medetomidine in small animal practice. Can Vet J 44: 885–897

Sossinka R (1972) Langfristiges Durstvermögen domestizierter Zebrafinken. J Ornithol 113: 418–426

(Received April 15, 2019 / Accepted November 5, 2019 / Published online April 6, 2020)