

# A Comparison Of The Analgesic Effects Of Fentanyl And Butorphanol In African Clawed Frogs (*Xenopus laevis*) Under Tricaine Methanesulfonate (MS222) Anaesthesia

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## Abstract

In this two-part study, the potency of two analgesics on nociception was assessed in African Clawed Frogs (ACFs). First, three different Pain Stimuli (PSs) were evaluated in the frogs during Tricaine Methanesulfonate (MS222) anaesthesia. Using the most effective PS from the preliminary study, the analgesic effects of three different doses of fentanyl and butorphanol were examined in frogs under MS222 anaesthesia.

Comparing the three different PSs (5% acetic acid onto skin, toe pinch by a clamp and pull on the ovaries), continuous Blood Pressure (BP) and Heart Rate (HR) recordings of the frogs indicated a sharp and reproducible increase in both parameters in response to acetic acid. That result clearly indicated an increased nociception during MS222 anaesthesia. Therefore, MS222 alone does not provide sufficient analgesia for painful interventions in ACFs. From all tested analgesic groups only 5 mg/kg butorphanol showed a short lasting decreased BP and HR response. In contrast, neither lower dosed butorphanol nor fentanyl in general reduced BP and HR response to a PS, only producing considerable side effects on the haemodynamic system.

These findings argue against using fentanyl as an analgesic in ACFs. Butorphanol significantly reduced the nociception in the high dose group. However, considering its limited duration of action and potential adverse effects, further analgesics (e.g., ketamine and metamizole) should be evaluated to improve intraoperative analgesia when using MS222 anaesthesia in ACFs.

**Key words:** Analgesic Effects; Fentanyl; Butorphanol; African Clawed Frogs; Tricaine Methanesulfonate (MS222);

## Introduction

*Xenopus laevis*, the African Clawed Frog (ACF), is a widely used laboratory animal and the most frequently used amphibian species in biomedical research [1-3]. *Xenopus* has retained its status as a widely used animal model with various uses in research. In addition to its ethological aspects, various qualities have made *Xenopus laevis* popular, e.g., its robustness, its simple

husbandry and the possibility of working with three different developmental stages (oocytes/larvae/adults) [4 & 5]. Of those three, oocytes are the most commonly used developmental stage. They are harvested via laparotomy and the excision of a small portion of the ovarian mass, which is the most common surgery for *Xenopus* and is repeatedly performed in female animals [6 & 7]. Surgeries are usually conducted under anaesthesia with MS222 with no additional analgesics. Currently, MS222 is the agent of choice for inducing general anaesthesia in fish and amphibians [8-11]. As an isomer of benzocaine, it is water-soluble and has a short induction time, a tolerance phase that can be variously prolonged and a short recovery phase [12]. With few exceptions and when given via immersion, MS222 is described as being a safe anaesthetic with a low impact on heart rate and oxygen saturation [13-15 & 19]. In pain research, amphibians have gradually established a small but adequate status as animal models, even though their ability for nociception and to pain perception, has been controversially discussed in the past [20-22]. In 1969, Kaplan declared that pain perception is not particularly developed in all poikilotherms due to their low phylogenetic position and underdeveloped brain [23]. However, according to Guénette et al., it is now commonly accepted that amphibians possess neuroanatomical pathways conducive of a complete nociceptive experience. In general, nociception is defined as the transmission of pain from a peripheral receptor, usually an unmyelinated nerve ending, to the central nervous system and brain processing is the final process where pain perception occurs in conscious animals [24]. According to several studies frogs have nociceptors in superficial and deep layers of the skin transducing mechanical and chemical noxious stimuli [25-28]. And furthermore frogs also have both myelinated and unmyelinated afferent fibres that compose the peripheral sensory nervous system [29]. These fibres, identified as large diameter A $\delta$  fibres and C fibres are similar to those found in mammals [24

& 30]. Pathways via the spinothalamic or trigeminal tract have been relatively underexplored, but studies have shown these are comparable with mammals [30 & 31]. These findings indicate that frogs perceive nociception during anaesthesia and therefore whenever painful surgical procedures are performed, a proper anaesthetic regime including sufficient analgesia must be chosen.

Commonly, general anaesthesia in animals including surgical tolerance is defined by inducing hypnosis (unconsciousness), muscle relaxation and analgesia. To cover all three aspects, combination of drugs is necessary in mammals. This allows lower doses of each drug, therefore less side effects and increased safety respectively. In the sense of a balanced anaesthetic regime it is therefore necessary based on the duration of the experiment and the painful stimuli to combine anaesthetics which induce hypnosis and muscle relaxation with analgesics to ensure a sufficient depth of anaesthesia and to ensure a highly controllable anaesthetic management [32].

Currently, very little literature can be found that discusses intraoperative pain management in the belief that MS222, as an ideal anaesthetic, guarantees hypnosis, muscle relaxation and analgesia [14, 18 & 33]. Therefore, the aim of this study was to clarify whether different Pain Stimuli (PSs) lead to nociception during anaesthesia with MS222. According to Dincklage, the Nociceptive Flexion Reflex Threshold best predicts movement and heart rate responses to noxious stimuli under general anaesthesia [34]. However, as this measurement system is not validated for poikilothermic animals, we used in accordance with Richter and Arras et al., the changes of Blood Pressure (BP) and Heart Rate (HR) response as an indicator for nociception [35 & 36]. In the first part of the study, three different PSs were evaluated to identify the most sensitive and reproducible stimulus for nociception under MS222 anaesthesia. In the second part of the study, two opioids, fentanyl and butorphanol were evaluated at three different doses using the most reproducible nociceptive stimulus to investigate their analgesic potency under MS222.

## Animals, Materials and Methods

### Animals and Husbandry

In this explorative, not blinded study, 42 (n=6 in 7 groups; 1 preliminary and 6 analgesic testing groups) non breeding female ACFs with a mean body weight of  $122.9 \pm 17.4$ g (expressed as mean  $\pm$  SD) were used.

The frogs were obtained from an experimental breeding colony and were group-housed in 300 L aquariums, with a maximum number of 25 animals per group [a & b]. The aquarium water was carbon-filtrated, dechlorinated tap water at a temperature of  $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and a room temperature of  $23^{\circ}\text{C}$ . The maintained pH value of the water was  $8.2 \pm 2$ . The water quality of the aquariums was monitored monthly by bacteriological analysis and every second month by measurement of nitrate (5 - 10 mg/L) and nitrite (0.025 - 0.1 mg/L). Every week, approximately one-third of the water was replaced.

A cycle of 14 h of light followed by 10 h of dark was maintained.

The animals were fed an alternating commercial pelleted diet and fresh beef heart twice a week [c]. Each aquarium offered two half pipes as enrichment items. Prior to anaesthesia, the animals were starved for at least 24 h.

The study was approved by the governmental institution of Baden-Württemberg, Regierungspräsidium Tübingen (case number: 35/9185.81-3) and performed in accordance with the German Animal Welfare Act.

### Anaesthesia

On the day of the experiment, the frogs were caught from the aquarium and placed in a non-transparent 10L bucket filled with 2L dechlorinated, carbon-filtrated water. For transportation, the bucket was covered and sudden movements avoided.

The experiments were conducted from 8 am to 4 pm under standardised room temperature conditions with an average room temperature of  $23^{\circ}\text{C}$ . Prior to the surgery, anaesthesia was induced by immersion of the frog in 1 g/L MS222 (tricaine methanesulfonate) in a covered dark container for 30 minutes [d]. As the pure solution of MS222 has a pH of 1-2, it was buffered to a pH of 7.0 by adding sodium bicarbonate [e]. Once the corneal, swallowing, wiping and righting reflexes were no longer evident, the frogs were weighed and then placed at a  $10^{\circ}$  incline. Anaesthesia was maintained via continuous flushing of 0.5g/L MS222 at an infusion rate of 1 mL/30sec [f]. The tube of the syringe pump was placed on top of the incline operation field and rinsed the backside of the animal [f]. To keep their skin moist, the animals were covered with a soft wet gauze pad and rinsed with frog ringer (0.65% saline solution) at least 3 times per minute [g& h].

### Experimental protocol

As experiments were final, operations were not conducted under sterile surgical conditions. Each frog was placed on its back, and a paramedian incision of 3cm was made in the cranial third of the body to open the coelom. A small silicon tube was inserted into the superficial abdominal vein to enable the intravenous application of the placebo or the two different opioids [i]. For HR and systolic BP measurement, a micro-tip catheter was placed in the heart ventricle. To gain access to the heart, it was necessary to remove the main part of the chest cartilage. The ventricle was punctured by a 20G cannula and the micro-tip catheter was quickly inserted and fixed by a prepared purse-string suture [j, k & l]. The catheter was connected to a recording device that monitored the intraventricular BP and HR every two seconds [m]. After the surgical procedures, a 10-minute period of rest was taken, during which the skin was constantly kept moist by rinses with the frog ringer.

In the preliminary study, three different PSs according to a randomized study plan were applied to six animals:

- 1) An Acetic acid Test (AAT): 5% acetic acid was dropped onto the skin of the thigh and washed off with 20mL of frog ringer after 5 sec [n].
- 2) A pinch by a bulldog clamp in the dactyls of the forelimbs for

5 sec.

3) A pull on the ovaries by forceps for 5 sec. After the first PS, the placebo (500µL frog ringer) was given intravenously. Then, each PS was given twice at intervals of 10 minutes in a randomized sequence.

In the second part of the study, after identification of the most sensitive and reproducible PS, additional fentanyl and butorphanol were examined in three different doses each in accordance with literature [o & p] [37-42]. The chosen doses for fentanyl were 0.05, 0.25 and 0.5 mg/kg, and for butorphanol 0.05, 1.0 and 5.0mg/kg, respectively. Six randomly allocated animals were investigated per dosage group.

Painful test stimuli using the formerly identified PS were executed before application of the analgesics (PS 1) and thereafter in 10 minutes intervals (until 50 minutes after application, PS 2-5). BP and HR were continuously observed before and after applying the PS. The data one minute prior to a PS were taken as baseline reference value. During the minute directly after the PS, the peak value of both BP and HR were highlighted. The difference between maximum and baseline, delta ( $\Delta$ ), showed the increase in BP and HR.

At the end of the experimental protocol, 1 mL of heart blood was taken directly before euthanasation of the frog by an intracardiac overdose of thiopental (100mg/kg) [q] [9, 15 & 43]. Drug serum level was measured from the blood sample afterwards.

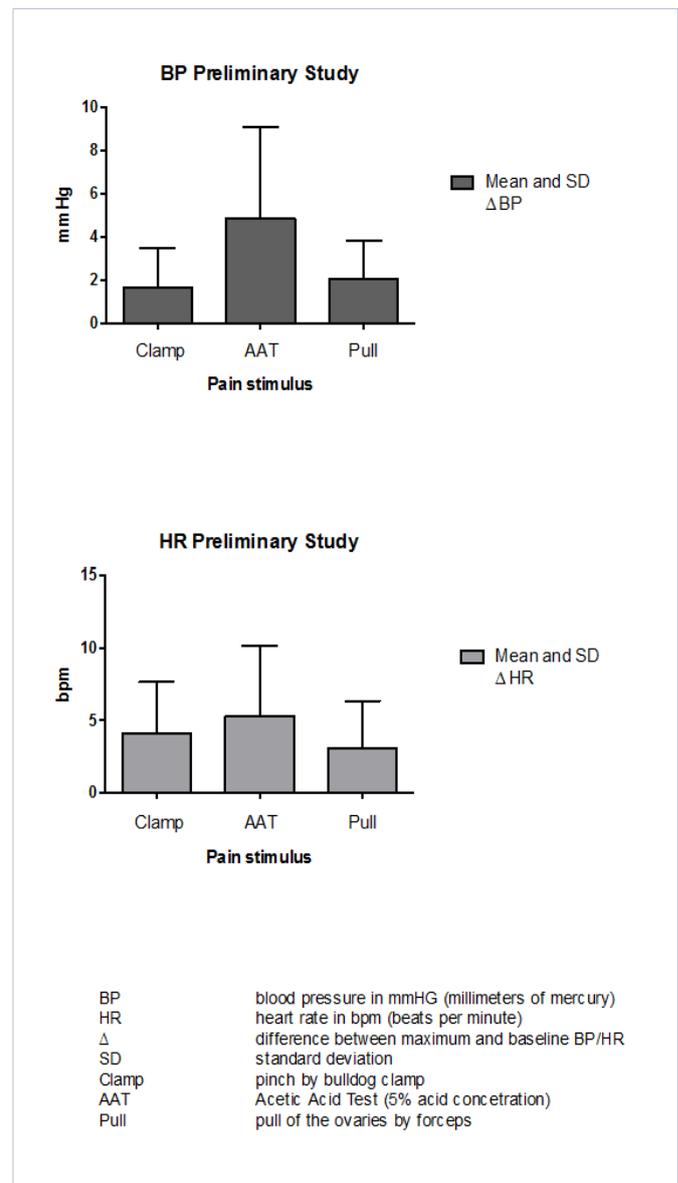
### Statistical evaluation

The statistical analysis was performed using the software package SPSS [r]. Descriptive statistics regarding the distribution of the group differences in systolic BP and HR are given by the mean and standard deviation as well as by the percentage change. As the study was explorative, no adjustment for multiple comparisons was necessary. For pair wise group comparisons, either paired sample t-tests (comparison of the five different PS) or independent paired t-tests were conducted in an explorative manner and at a significance level of 0.05 (two-sided). To compare the three different serum levels of fentanyl and butorphanol, a one-way analysis of variance (ANOVA) and (in case of statistical significance) post hoc tests (Tuckey-HSD) were performed (significance level of 0.05; two-sided). Illustrations are given by dynamic plots (with mean and error bars) or box plots.

## Results

### Preliminary study

Results from five animals were evaluated. One frog was excluded due to cardiovascular depression while baseline data monitoring. Overall, each PS provoked an increase in the HR and systolic BP of the anaesthetised *Xenopus* (Figure 1). The delta of the BP was  $1.65 \pm 1.82$  mmHg (mean  $\pm$  SD) after pinches by the bulldog clamp, which corresponds to an increase of 6.0%. The delta of the HR was  $4.10 \pm 3.54$ bpm, which is an increase of 10.5%.



**Figure 1:** Preliminary Study: Comparison of three different pain stimuli under MS 222 anaesthesia

The AAT resulted in a delta of  $4.86 \pm 4.22$  mmHg for the BP and a delta of  $5.30 \pm 4.81$  bpm for the HR. The increases in percent were 17.4% and 14.4%, respectively.

The pull of the ovaries by forceps provoked a delta of  $2.05 \pm 1.76$  mmHg for the BP and a delta of  $3.10 \pm 3.24$  bpm for the HR, which represents increases of 7.4% and 8.2%, respectively.

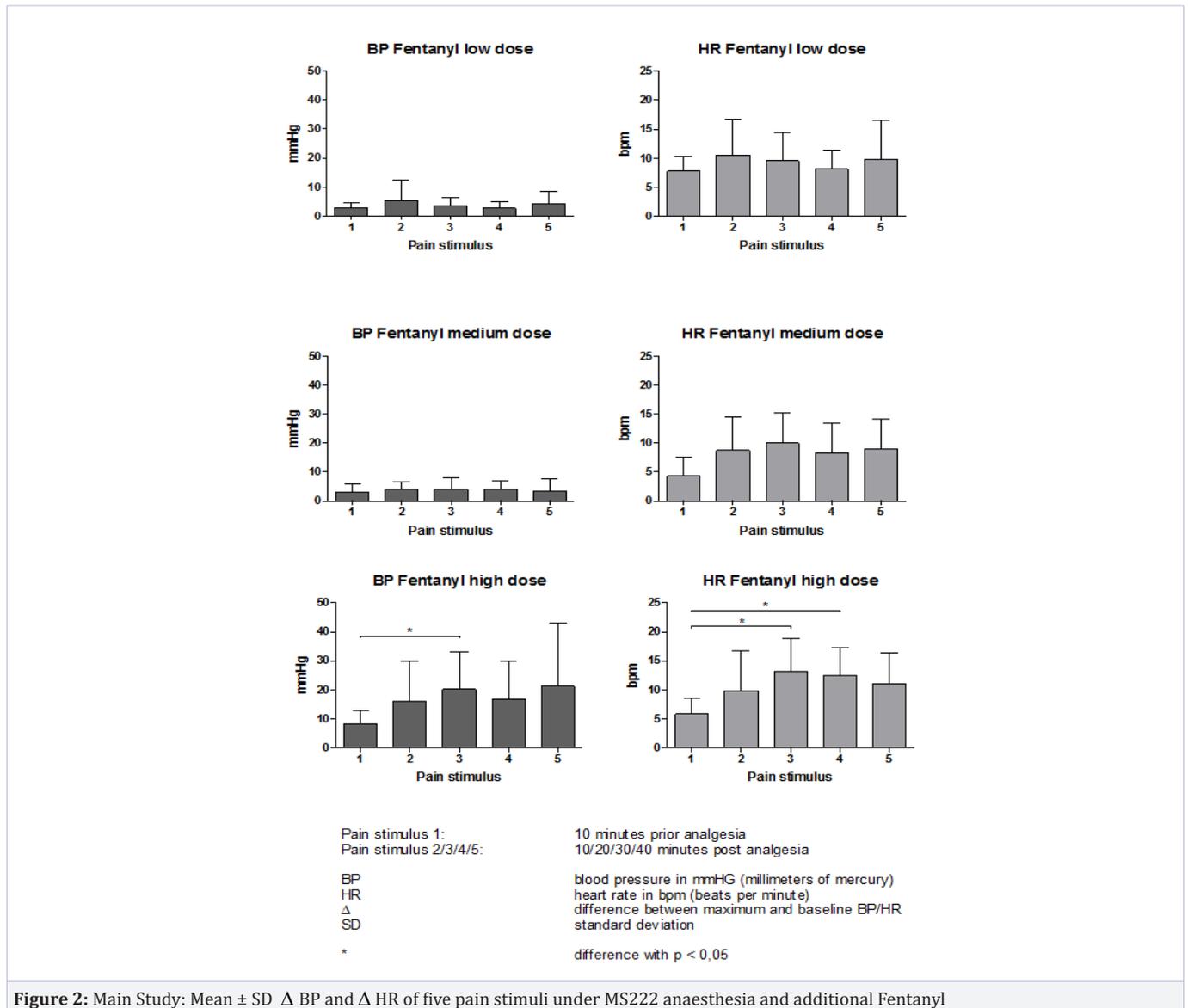
There with, the results of the preliminary study identified the AAT being the most reproducible nociceptive stimulus during MS222 anaesthesia. It was taken to test the analgesic potency of additional butorphanol and fentanyl in the further study design.

### Main Study: Analgesic addition to MS222

Using the AAT as test pain stimulus under MS222 anaesthesia, the systolic BP and HR increased by 10.3% and

18.9%, respectively, after the first PS prior to application of low dose fentanyl (0.05mg/kg). The second stimulation 10 minutes after drug application (PS 2) even showed a further increase of both parameters: 21.5% for the systolic BP and 37.6% for the HR compared to baseline. However, data variance was quite high, no statistically significant differences were observed between any of the recorded time points within the low dose fentanyl group (Figure 2). Administration of the medium dose of fentanyl

(0.25mg/kg) did not obviously influence BP or HR increase after a PS. The paired t-test showed no significant differences between the effects of the low and medium fentanyl dose (Figure 2). Application of the high fentanyl dose (0.5mg/kg) even showed a statistically significant increase in BP and HR 20 minutes after drug application (PS 3) compared to stimulation prior to the analgesia injection.



**Figure 2:** Main Study: Mean ± SD Δ BP and Δ HR of five pain stimuli under MS222 anaesthesia and additional Fentanyl

As shown in figure 3, the drug serum level difference between the low, medium and high doses both of fentanyl ( $p < 0.006$ )

and butorphanol ( $p < 0.000$ ) could be statistically significantly confirmed.

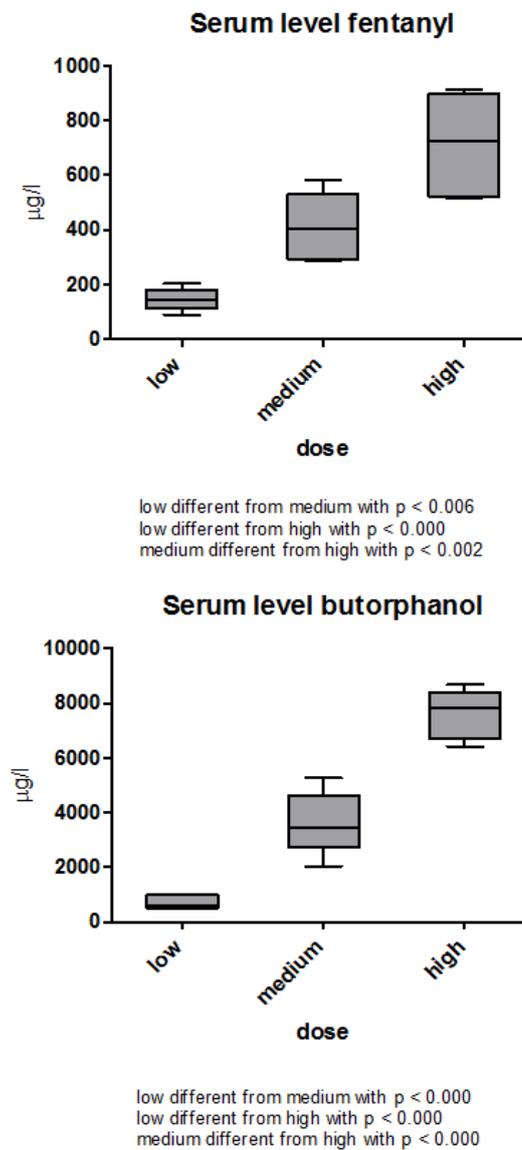


Figure 3: Main study: Comparison of the different serum levels of Fentanyl and Butorphanol

The BP and HR results for butorphanol are shown in figure 4. The low dose of butorphanol (0.05mg/kg) had no statistically significant effect on BP or HR. Interestingly, the application of the medium dose of butorphanol (1.0mg/kg) affected the blood pressure significantly, it even increased from PS 3 on compared to PS 1. However, HR was not significantly changed.

Only the high dose of butorphanol (5mg/kg) significantly decreased BP (HR not significantly) at PS 2 after drug administration compared to PS 1 before. This effect could not be reproduced though at the later time points PS 3-5 when an increase of cardiac data was observed again.

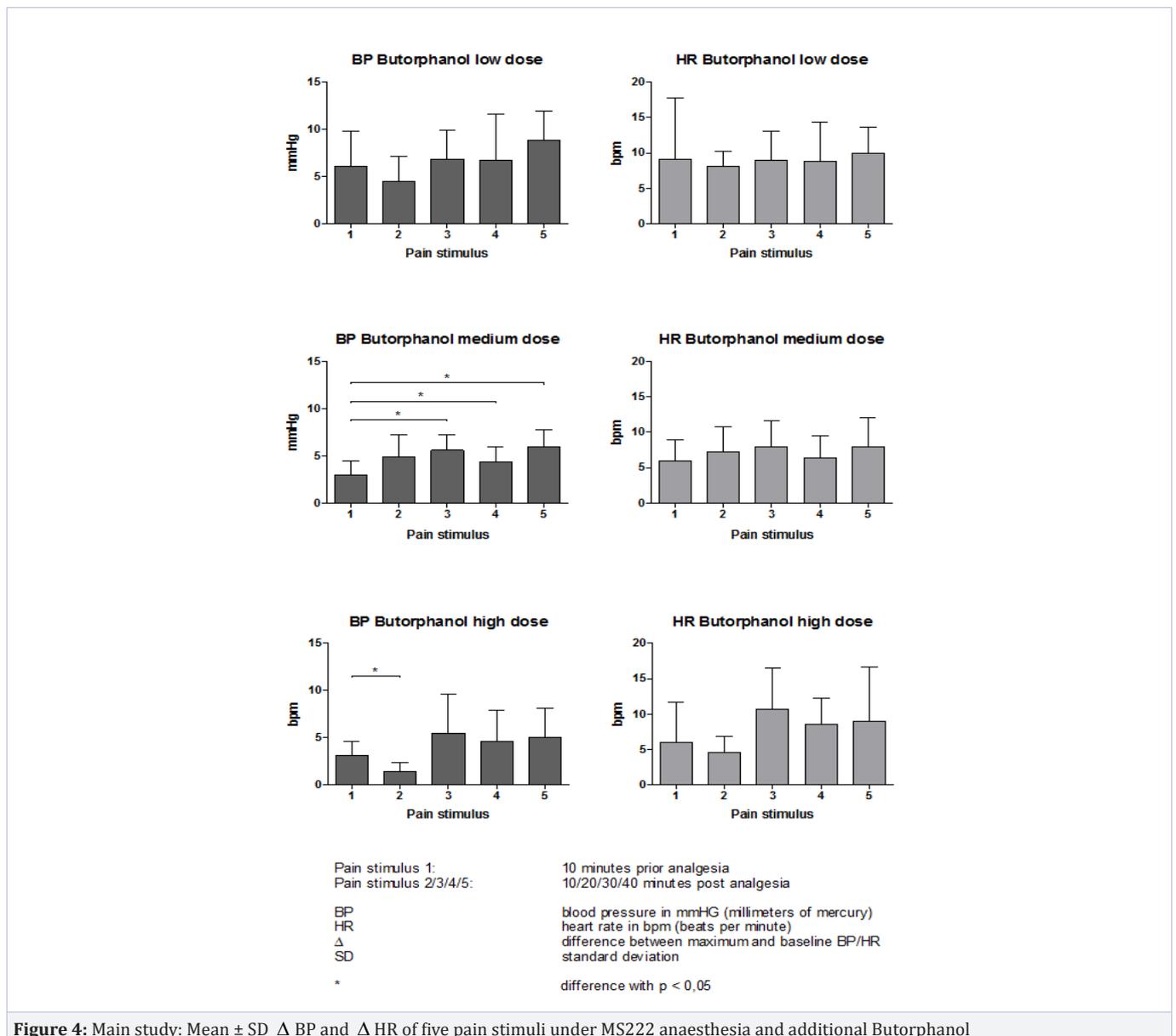


Figure 4: Main study: Mean ± SD Δ BP and Δ HR of five pain stimuli under MS222 anaesthesia and additional Butorphanol

## Discussion

As there is only poor current knowledge about intraoperative pain management in the ACF, the aim of this study was to clarify whether different Pain Stimuli (PSs) lead to nociception during anaesthesia with MS222. We used invasive systolic blood pressure and heart rate monitoring as indicators for intraoperative nociception. First, we evaluated the most sensitive and reproducible stimulus for nociception under MS222 anaesthesia before the analgesic potency of two additional opioids, fentanyl and butorphanol, was investigated at three different doses. Due to animal welfare aspects we abdicated an extra placebo control group. The preliminary study, performed with no additional analgesia, provided important information regarding that point instead. However, this was limited by the lack of potential sensitisation assessment.

In the past, several anaesthetic agents with different routes of administration have been examined in amphibians. Eugenol, benzocaine and MS222 induce general anaesthesia via immersion [18, 33 & 44-46]. In this field, MS222 is currently the agent of choice [12, 17, 24, 47 & 48]. It is an isomer of benzocaine, is characterized by better water solubility and has an average half-life of 3.2h. [8, 18 & 49]. Different doses of MS222 can be found in the literature: [12] 0.5g/L [50], 1-3g/L [51] or 1.5g/L [17]. the exact mechanism of action has not been determined, but a block of sodium currents is certainly provoked [12 & 52]. After 30 minutes of immersion, a tolerance phase is reached in which the withdrawal, corneal and righting reflexes are lost [12]. To achieve a surgically tolerant anaesthesia though, including muscle relaxation, hypnosis and analgesia, most anaesthetic agents must be used in combination. However, some studies have asserted that this is not necessary with MS222 [18 & 33].

In contrast, the results of our preliminary study showed an increase in HR and systolic BP after a painful stimulus.

Why is it important to control pain during surgery? Briefly, besides its ethical aspect, proper analgesia provides a deeper anaesthesia using lower doses. Regarding the cardiovascular system, acute pain sensation leads to a release of catecholamines, which results in an increase in HR and constriction of the peripheral vessels. These effects cause an increase in BP. In 2008, Arras demonstrated mild-to-moderate post-laparotomy pain states in laboratory mice by recording their HR and HR variability [36].

In the preliminary study, three different pain stimuli were examined in frogs under MS222. A standardised pinch by a bulldog clamp in the cloves of the forelimb is a somatic PS. The average percentage increase in BP was 6.0%, and that of the HR was 10.5%. Although the increase did not exceed 15%, a twitch of the forelimb could be recognised at the end of the experiment, when anaesthetic level got lighter. The pulling of the ovaries by forceps is a visceral PS, and it provoked a 7.4% increase in BP and an 8.2% increase in HR. The AAT is a somatic PS again. Currently, it is the method of choice for assessing nociception in amphibians [7, 18, 29 & 53-56]. Briefly, the AAT is regularly performed on awake animals with an increasing series of acid concentrations to examine the nociceptive threshold of the animal. In our study, we applied the lowest evocative acid concentration (5%) which was dropped onto the lateral area of the lower leg for 5sec where 61% of afferent fibres have their receptive field [29, 45, 54 & 55]. The percentage increase in BP in the preliminary study was 17.4%, and that of the HR was 14.4%. Of the three different PS, the AAT was the most effective and the best to standardise. This conclusion supports the results of earlier clinical trials in the literature [7, 18, 21, 22, 29, 54 & 55].

The increases in sympathetic tone after a PS demonstrate that amphibians probably have intact and active nociceptive pathways under MS222 anaesthesia resulting in intraoperative stress. This is certainly not a proof for pain perception in conscious animals. However, in 2004 Sneddon named seven indicating criteria whether an animal is capable of perceiving pain: 1) Nociceptors, 2) Brain structure, 3) Pathways to a higher brain structure, 4) Opioid receptors and substances, 5) Reduction of the nociceptive response with analgesics, 6) Avoidance learning and 7) Suspension of normal behaviour. All of these criteria refer to the class of amphibians [57]. Amphibian nociception has been recently reviewed in detail elsewhere [58]. Three different groups of primary afferent fibres have been identified in amphibians [27, 57 & 59]. Stevens assumes that there are no major differences between the amphibian and mammalian nociceptive afferent fibres [22]. Thirty-nine percent of all primary afferent fibres become excited by the AAT, which are mainly the A $\delta$  and C fibres [29]. Nociceptive information is transmitted via ascending pathways from the spinal cord to the brain. However, the brain structures differ significantly. In regard to amphibian pain perception, the biggest difference from mammalian pain perception is the lack of cortical tissue in both the cerebrum and limbic regions. Consequently, the appreciation of pain is

presumably diminished in amphibians rather than in mammals [58].

In Sneddon's numeration, point 5 is a reduced nociceptive response in conscious animals after analgesia [57]. In our study, two different opioids, fentanyl and butorphanol, were tested in three different doses each in anaesthetised *Xenopus* to enable the use of BP and HR to assess intraoperative stress and antinociception and therewith help to refine animal welfare due to post-operative pain and faster healing.

To ensure that the analgesic agent was totally absorbed, it was given intravenously, although the practicality of this approach might be questioned and therefore is a limitation of this study and may especially affect the time course of action of the drugs.

Fentanyl is a strongly lipophilic derivate of phenylpiperidin and has a high selectivity to the  $\mu$ -opioid-receptor in mammals. Its potency is 80-120 times higher than that of morphine, and its duration of effect is relatively short, ranging from 20-30 min. The side effects are strong sedation, hypothermia, bradycardia, respiratory depression and vomitus. Dosage data for amphibians in the literature range from 0.5mg/kg s.c. to 1mg/kg s.c. [37-39 & 60]. In rodents, Fentanyl is commonly used as an analgesic in combination with midazolam and medetomidine to induce general anaesthesia [61].

Butorphanol is a partial  $\mu$ -agonist/antagonist and a partial  $\kappa$ -agonist in mammals. The efficacy is dose-dependent, and high doses can have an antagonist effect. Its plasma half-life depends on species specific liver enzyme metabolism [60]. Data concerning the dosage vastly vary in the literature. For some authors it is the agent of choice for analgesia at a dose of 0.05-1 mg/kg i.v., p.o., i.m. or s.c. for a duration of 12h, while others recommend 0.2-0.4 mg/kg i.m. or even 25 mg/kg i.p [40-42]. In comparison to rodents, butorphanol is recommended for use in mice by Cagle et al. in combination with dexmedetomidine, tiletamine, zolazepam and by Kawai et al., in combination with medetomidine and midazolam [62 & 63]. It was shown that additional butorphanol improves the intraoperative analgesia and the efficacy of the anaesthesia.

For the refinement of animal experiments and the aim of giving proper dosage guidelines, we assayed three different dosages in accordance with the references. The results of our study have to be judged as explorative data using n=6, not reference-based and statistically pre-calculated. Therefore, some data show quite a wide variance. In our study, all three doses of fentanyl showed no analgesic effect. In contrast, BP and HR even increased to higher levels after painful stimulation than without analgesia, irrespective of the fentanyl dose, signifying a possible induction of hyperalgesia. In sum, the drug showed no analgesic effect but considerable side effects on the haemodynamic system of the animals. The use of fentanyl is therefore not advisable in frogs.

Butorphanol did not show any significant effect on BP and HR in the low dose group, whereas in the medium dosed group a significant BP increase could be observed compared to PS 1. In

conclusion, neither a low dose nor a medium dose of butorphanol provided efficient analgesia in ACF. The only significant decreases in BP and HR after a noxious input, meaning the only demonstrable analgesic effect of this study, were temporarily observed with the high butorphanol dose 10 min after its application at PS 2. However, in consideration of the short duration of the analgesic effect by simultaneous cardiovascular depressive side effects, its practical benefit was not particularly evident.

In summary, we conclude that ACFs have intact and active nociceptive pathways under anaesthesia with MS222. The current anaesthetic agent of choice in amphibians induced a good unconsciousness and muscle relaxation but insufficient analgesia during the painful stimulations in our study [12, 17, 24, 47 & 48]. The two opioids fentanyl and butorphanol did not effectively relieve this pain. Therefore, further non-opioid analgesics (e.g., ketamine, metamizole) should be evaluated to improve intraoperative analgesia in ACFs under MS222 anaesthesia. To stay with these examples, dosages in the literature range from 20-210 mg/kg i.m., s.c., i.v. for ketamine [9]. Dosage recommendations for metamizole in amphibians are extremely rare and follow the ones for mammals: 20-100 mg/kg i.m., slowly i.v [64]. In our first preliminary trial we observed a markedly lower increase of BP and HR after a PS at a relatively low dose of 20 mg/kg ketamine given i.v. In conclusion, this might be a reference point for further studies, particularly using ketamine in the ACF for improving intraoperative analgesia with fewer side effects on the cardiovascular system.

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## Declaration of conflicting interests

The authors declare no conflicts of interest to disclose.

## Notes

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- b. Tierforschungszentrum, Universität Ulm, Ulm, Germany.
- c. Krallenfrosch Extrudat 3mm, Provimi Kliba AG, Kaiseraugst, Switzerland.
- d. Ethyl-3-aminobenzoatmethanesulfonate 98%, Sigma-Aldrich Chemistry, St. Louis, Missouri, USA.
- e. Natriumhydrogencarbonat, Merck KGaA, Darmstadt, Germany.
- f. Syringe Pump, Harvard Apparatus, Holliston, Massachusetts,

USA.

- g. Gazin®, Lohmann & Rauscher GmbH&Co.KG, Rengsdorf, Germany.
- h. Isotone Kochsalzlösung 0.9%, B. Braun-Melsungen-AG, Melsungen, Germany.
- i. Silicon tube 7mm, FMI Föhr-Medical-Instruments-GmbH, Seeheim, Germany.
- j. Sterican® Gr.1 G20x1½"/0.90x40mm, B. Braun-Melsungen-AG, Melsungen, Germany.
- k. Mikro-Tip® catheter transducers, Millar Instruments, Inc., Houston, Texas, USA.
- l. PDS II 6-0 BV-1, violet monofilament absorbable suture, Johnson & Johnson Medical GmbH, Ethicon Deutschland, Norderstedt, Germany.
- m. Type "Aria", FMI Föhr-Medical-Instruments-GmbH, Seeheim Germany.
- n. Essigessenz, Surig, Speyer & Grund GmbH&Co.KG, Mainz, Germany.
- o. Fentanyl-hameln 50mikrogramm/mL Injektionslösung, Hameln pharmaceuticals GmbH, Hameln, Germany.
- p. Morphasol®-4 ad. us. vet. Injektionslösung, Albrecht GmbH, Aulendorf, Germany.
- q. Thiopental-Rotexmedica, 0.5g, Trockensubstanz, Rotexmedica GmbH, Trittau, Germany.
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