The effect of the anesthetic tricaine (MS-222) on nerve activity in the anterior lateral line of the oyster toadfish, *Opsanus tau*

Lucy M. Palmer and Allen F. Mensinger

Biology Department, University of Minnesota Duluth, Duluth, MN 55812

Marine Biological Laboratory, Woods Hole, MA 02543

The effect of tricaine on toadfish

Allen F. Mensinger

Biology Department

10 University Dr

University of Minnesota Duluth

Duluth, MN 55812

218-726-7259

218-726-8142 (fax)

amensing@d.umn.edu
ABSTRACT

Inductive neural telemetry was used to record from microwire electrodes chronically implanted into the anterior lateral line nerve of the toadfish, *Opsanus tau*. Spontaneous neural activity and the response of lateral line fibers to water current were continually monitored from 17 primary afferent fibers before, during and after the administration of the anesthetic tricaine (MS-222). Significant decrease in spontaneous and evoked activity and increase in interspike interval was noted when anesthetic concentrations were 0.010%. Neural activity returned to control levels within approximately 90 min of anesthetic withdrawal. Decreasing the pH of the solution without the anesthetic caused transient heightened sensitivity indicating that tricaine and not the concurrent drop in pH was responsible for the decrease in sensitivity during anesthesia. During a secondary challenge with the anesthetic 24 hours after the first, fibers initially showed faster recovery however overall recovery kinetics were similar. Although high tricaine concentration was correlated with decreased neural sensitivity, the concentrations normally used to maintain anesthesia in the toadfish did not have significant effect on the evoked firing rate. Thus, given sufficient time to recover from the induction of surgical anesthesia, it may be possible to maintain the animal under light anesthesia while minimizing the physiological effects of tricaine.
INTRODUCTION

Anesthetics have been used for over a century to alleviate pain associated with surgical procedures (Corning 1885). Anesthetics act on nerve membrane excitability and transmitter release causing widespread depression of the central nervous system. Although it is unclear how cellular mechanisms act to induce anesthetic effects, it is hypothesized that the compounds bind specifically to neuronal membrane proteins and prevent the generation and conduction of nerve impulses (Winlow et al. 1992). Recent studies have illustrated that anesthetics target specific binding sites on Na⁺ channels (Bai et al. 2003) and other ligand gated ion channels.

Although a wide variety of anesthetics are available for use in warm blooded animals, tricaine (MS-222; M-Aminobenzoic Acid Ethyl Ester; tricaine methanesulfonate; metacaine methanesulfonate; or C₉H₁₁NO₂@CH₄O₃S) has been the drug preferentially prescribed for aquatic anesthesia. The anesthetic qualities of tricaine were recognized in the fisheries industry in the 1920s, and it is currently the only anesthetic approved for use in foodfish by the U.S. Food and Drug Administration. The compound is easily dissolved in aquatic mediums and can be rapidly absorbed across the gills providing quick, efficient and reversible anesthesia. Since tricaine is used as an anesthetic agent for a wide variety of aquatic physiology preparations, it is imperative to document the effect of the agent and its recovery kinetics. Tricaine has been shown to have suppressive effects on peripheral and central neurons in acute preparations of numerous sensory systems including electrosensors (Hensel et al. 1975; Spath and Schweickert 1977; Andrianov et al. 1991), supramedullary/dorsal neurons (Arnolds et al. 2002), and the lateral line (Hensel et al. 1975; Spath and Schweickert 1977). However, to accurately quantify the immediate and residual
effects of tricaine requires recording neural activity before, during and after its application. As most animal care protocols prohibit the removal of anesthesia in a restrained animal during invasive surgery, this creates an ethical dilemma. This has retarded efforts to determine both the immediate and residual effects of anesthetics using conventional recording techniques.

The development of a chronically implantable electrode with telemetry tag (Mensinger and Deffenbaugh 2000) provides a mechanism to record spontaneous and evoked neural activity during anesthetic application and withdrawal. The lateralis system provides a good model to determine the effect of anesthesia on nerve excitability. The lateral line hair cells can be physiologically characterized and easily excited by external stimuli (Tricas and Highstein 1991; Montgomery and Coombs 1998; Engelman et al. 2002). We report for the first time the effect of tricaine application on the sensitivity of chronically recorded anterior lateral line afferent fibers.
MATERIALS AND METHODS

Animal husbandry

Adult toadfish [n = 15; 28 ± 1.4 standard error (SE) cm standard length; 675 ± 46 SE g wet weight] of either sex were obtained from the Marine Biological Laboratory (MBL), Woods Hole, MA. Experiments were conducted at the MBL and the University of Minnesota, Duluth. At the MBL, fish were maintained in large, flow-through seawater tanks and maintained at 20°C. In Duluth, fish were maintained at the same temperature in artificial seawater (Instant Ocean) in 800 l tanks equipped with a recirculating seawater system containing mechanical, biological, chemical and ultra violet filtration. All experimental procedures conformed to institutional animal care protocols.

Dose response curve

To assess the effect of tricaine on toadfish respiration and equilibrium, individual fish were transferred to 4 l aquaria containing a single concentration (range 0.0 % - 0.030 %) of tricaine in salt water. Respiration rate was quantified by counting the number of opercular movements per min. The toadfish were also manually inverted with a net approximately every three min to assess their ability to regain equilibrium. After 30 min of immersion in the anesthetic, fish were transferred to another 4 l aquaria containing anesthetic-free salt water and observed until pre-anesthetized respiration rates and equilibrium (if applicable) were restored.

Microwire electrode

Three channel electrodes consisting of microwires, an interconnect and a waterproof connector were custom fabricated for each implantation. The electrode tip consisted of three enamel insulated 20 µm diameter 10 % platinum/iridium microwires (Sigmund Cohn Corp).
Each microwire was affixed to a hard silver plated copper (enamel insulated) multistranded wire (25 µm diameter) (New England Wire Corp) with conductive silver paint (Silver Print Paint). The multistranded wires were soldered to individual silver wires (0.32 mm) that terminated into a multipin underwater connector. The anterior portion of the microwires were threaded through a 1 mm length of polymide tubing (0.18 mm O.D.; A-M Systems Inc) to maintain the recording sites in close proximity. All exposed wire/connections were encased in medical device adhesive (Loctite 3341) that was cured with ultraviolet light (Litex 660). The resistance of each electrode channel was determined with an impedance-test unit (FHC) and only electrodes with impedances between 0.5 and 1.0 MΩ were used.

**Electrode implant**

Fish were anesthetized by immersion in 0.005 % tricaine (3-aminobenzoic acid ethyl ester; Sigma) and paralyzed with an intramuscular injection of pancuronium bromide (Sigma) (600 µg / kg) dissolved in saline solution (10 %). A dorsal incision was made along the sagital crest of the cranium and the overlying muscle was retracted to expose the skull. A small craniotomy was performed lateral to the sagital crest and posterior to the transverse crest to expose the anterior branch of the anterior lateral line nerve. The electrode was inserted into the nerve just prior to its exit from the braincase. Potentials were differentially amplified (Dagan) and monitored on a portable computer using Chart5 for Windows software (AD instruments). The two channels that provide the highest fidelity signal were chosen for the experiments. Once a candidate fiber was located, the fish was left undisturbed for 30 min to ensure fiber stability.

Cyanoacrylate gel was used to affix the electrode to the skull and seal the craniotomy. The muscle was restored to its original position and the muscle, faschia and epidermis were
individually sutured to provide a watertight seal over the craniotomy and around the transdermal electrode lead. The differential amplifier was disconnected from the electrode and the cylindrical telemetry tag (15 mm dia x 38 mm; 8 g) was inserted into the waterproof electrode connector. The tag was sutured parallel to the dorsal fin on the dorsal surface of the fish.

**Neural recordings**

Extracellular recordings from lateral line primary afferent neurons were obtained using inductive telemetry. The inductive telemetry system consists of the attached transmitter tag and external receiver coils. The tag contains miniature 0.2 Farad capacitors, a circular inductive coil, high (4 kHz) and low (100 Hz) pass filters, and a differential amplifier (20 MΩ input impedance). The telemetry tag transmits the voltage signal as a magnetic field (50 µT, 200 kHz) which is detected by coils embedded in a cylindrical habitat (30 cm x 12 cm diameter) at a rate of 100 kHz. The habitat also serves as a recharging station and generates the inductive current (50 uT, 200 kHz) necessary to charge the tag’s capacitors. Thirty seconds of charging provides up to five min of telemetry. Neural activity was recorded and stored on a portable computer using Chart5 for Windows software (AD instruments) and analyzed offline with Spike2 software (Ver. 4.07; Cambridge Electronic Design).

Immediately after surgery, the fish was placed in a recovery tank containing fresh saltwater and left undisturbed for a minimum of 90 min. The fish then were transferred into the recharging habitat and submerged (15 cm water depth) in a rectangular aquarium (56 cm x 36 cm x 20 cm) containing 20 L seawater. The location of the innervated neuromast was determined by gently maneuvering a small brush over the anterior lateral line while
concurrently recording from the fiber. The epidermal location that elicited the maximum increase in neural activity was determined as the innervated neuromast.

Both the spontaneous firing rate and evoked nerve firing rate were recorded during each trial. Spontaneous activity was reported as the non stimulated firing rate and was recorded for 1 min prior to lateral line stimulation. The evoked firing rate was reported as the absolute spike rate observed during the stimulus and was recorded in response to water current of 85 cm / s representing a turbulent free shear flow as described by Pope (2000). The water current was produced by a submersible water pump (Aquarium systems, MN 404) with a round outlet (1 cm dia). The water pump was positioned perpendicular to the right operculum, 6 cm from the epidermis and 8 cm off the substrate. The stimulus was applied for three consecutive 60 s periods with 60 s intervals between stimuli. All analyses (firing rate, spike amplitude) were conducted on the nerve activity in response to the first stimulus. To compare activity between fibers, firing rates were normalized according to the spontaneous and evoked rates recorded prior to the anesthesia (ie unanesthetized state).

Spontaneous and evoked neural activity were recorded from the unanesthetized toadfish 10 min after transfer to the experimental aquarium. At the completion of the 5 min stimulus cycle, tricaine was added to the aquarium and manually dispersed to produce an anesthetic concentration of 0.001 %. After 15 min exposure to the anesthetic, the stimulus cycle was repeated and spontaneous and evoked neural activity recorded. Additional tricaine was added to produce concentrations of 0.005 %, 0.010 %, 0.015 %, 0.020 %, 0.025 %, 0.030 % and evoked and spontaneous nerve activity were recorded 15 min after each addition. For each trial, fish were exposed to anesthetic for 110 min.
After the final stimulus at 0.030 %, the fish were removed from the anesthetic solution and placed in a holding tank containing anesthetic free saltwater for a maximum of 3 min while the experimental tank was drained and refilled with anesthetic free saltwater (20 L). The fish was transferred back to the experimental aquaria and the stimulus cycle repeated every 10 min during the first half hour and then at 15 minute intervals for a minimum recovery period of 90 min. Four fish were subjected to a second tricaine exposure approximately 24 hours after the initial post-surgical challenge using the same protocol.

**pH effects**

The effect of pH on the neural activity of the anterior lateral line was tested in three fish. The implanted fish were placed in anesthetic free saltwater and monitored for both spontaneous and evoked neural activity. The pH of the water was then sequentially lowered by the addition of HCl (1N, Fisher Scientific) in 0.1 to 0.2 increments that mimicked the pH decrease created by tricaine addition (e.g. 7.8, 7.7, 7.5, 7.2, 7.1, 7.0, 6.9). The pH of the solution was measured with a digital pH meter (Fisher Scientific, AB15 Meter).

The pH experiments were performed using the same protocol as the anesthetic experiments. After each HCl addition, the fish was left undisturbed and the stimulus cycle performed after 15 min. At the conclusion of the pH testing, the acidic experimental water was replaced with fresh saltwater (pH 7.8 – 8.2) and the stimulus cycle repeated at 10 min (first 30 min) and then 15 min intervals. After an additional recovery period of 1 hr, the fish underwent the anesthesia trial.

**Statistical Analysis**

All statistical analysis was performed using GraphPad Software, San Diego CA. Samples were tested for normality using the method of Kolmogorov and Smirnov. Bartlett’s
test was used to assure that the standard deviations (SD) of the groups were equal in order to use parametric statistical tests. When the SDs were unequal when computed by Bartlett’s test, non-parametric statistical analysis was used.
RESULTS

Behavioral effects of tricaine anesthesia

The behavioral effects of tricaine anesthesia on toadfish are summarized in Table 1. Both control and fish exposed to 0.001 % MS-222 respired at approximately 20 cycles min\(^{-1}\) and remained upright and relatively motionless on the aquarium bottom. If manually inverted with a net, the fish would immediately right themselves within 5 s. At 0.005 %, fish were slightly less responsive to contact with the net and were slower to return to an upright position after inversion. Immersion in 0.010 % tricaine decreased respiration rate by 10 % and fish spontaneously lost equilibrium in approximately 20 min. The respiration rate continued to decrease with increasing concentration of anesthetic until gilling ceased at 0.030 %. At concentrations greater or equal to 0.015 %, fish lost equilibrium within 5 min. After transfer to anesthetic free salt water, respiration recovery preceded balance restoration. Recovery time was correlated with anesthetic dosage with 14 min necessary for gilling to resume and 23 min needed for equilibrium recovery after exposure to 0.030 % tricaine.

Effects of Tricaine on firing frequency

The firing rate of both stimulated and spontaneously active lateral line fibers decreased in response to increasing anesthetic concentrations (Fig. 1). Response functions were obtained from 17 anterior lateral line afferent fibers in 15 chronically implanted fish. Six fibers were silent (exhibited no spontaneous activity) while 11 fibers were spontaneously active (average spontaneous activity of 20.8 ± 5 SE Hz). Spontaneous activity decreased with increasing tricaine concentration. At tricaine concentrations 0.010 %, spontaneous activity was significantly lower than control rates (P < 0.01; Tukey-Kramer). Evoked activity was only slightly depressed at 0.001 % and 0.005 % before declining significantly (P < 0.01;
Tukey-Kramer) below control values at concentrations of 0.010%

![Figure 1](image)

**Figure 1**
All fish regained equilibrium and normal gilling within 23 min of anesthetic withdrawal. Seven fibers (4 spontaneous and 3 silent) were monitored for up to 105 min after anesthetic removal (Fig. 2). Spontaneous and evoked activity returned to 90% of the pre-anesthetized firing rates within 90 min of tricaine removal.
The effect of tricaine application and withdrawal is illustrated for a single spontaneously active fiber (Fig. 3). This fiber had a pre-anesthetized evoked firing rate of 46 Hz which decreased to 4 Hz in 0.030 % tricaine. The fiber returned to 43 Hz within 90 min of anesthetic removal.
Figure 3

Exposure to increasing tricaine concentration resulted in an increase of the interspike interval in both silent and spontaneous fibers. Figure 4 plots the interspike interval during anesthetic exposure of two representative fibers. When stimulated, the silent fiber showed a gradual lengthening of the interspike interval from pre-anesthesia (mean = 14.6 ± 0.6 ms) to 0.010 % tricaine (mean 34.8 ± 1.3 ms). However, at tricaine concentrations greater than or equal to 0.015% there was a considerable lengthening of the interspike interval (range 78.3 ± 3.1 to 131.9 ± 6.0 ms). The interspike interval of the spontaneous fiber at rest and during stimulation also increased in response to greater tricaine concentrations. Action potential generation remained relatively constant during stimulation until tricaine concentrations reached 0.015% when the interval increased significantly (Kruskal-Wallis, \( P < 0.001 \)) from...
47.7 ± 2.9 to 65.2 ± 2.7 ms. The maximum interspike interval reached 105.6 ± 4.5 ms at 0.030% during stimulation and the fiber exhibited a change in firing pattern.

Figure 4
Repeated anesthesia effects

Figure 5 illustrates the effect of a secondary anesthesia exposure to four spontaneously active fibers. The time course of depression was similar during both trials. During the recovery period following the secondary challenge, the fibers initially showed faster recovery however by 60 min all fibers had regained approximately the same level of sensitivity. The fibers in the secondary challenge displayed heightened sensitivity during the final 15 min of the observation period.
Figure 5

Solution pH

As the incremental addition of tricaine decreased the pH of the saltwater solution, the affect of lowering the pH on firing activity without anesthetic was tested. Three spontaneously active fibers were exposed to sequentially decreasing pH and spontaneous and...
evoked rates were recorded. The increasing acidity initially caused a slight depression in both evoked and spontaneous firing rates, however as the pH decreased below 7.5, the spontaneous fibers showed a transient increased sensitivity to stimulation. After the fish were returned to the original pH, spontaneous activity returned to normal however evoked sensitivity remained slightly depressed. The fish then underwent the anesthesia trial and the fibers showed the typical depression in neural activity related to increased tricaine concentration (Fig. 6).
DISCUSSION

The neural activity of the anterior lateral line afferent fibers was affected by exposure to the anesthetic tricaine. When stimulated, both silent and spontaneously active fibers experienced a decrease in neural activity in response to increasing tricaine concentrations. These effects were transitory as both spontaneous activity and neural sensitivity recovered in the majority of fibers within 90 min of anesthetic withdrawal.

Despite the wide use of the anesthetic, most studies have only examined the kinetics of induction and recovery and few studies have correlated the application of tricaine with neural activity. Hensel et al (1975) indicated that deep anesthesia (induced by 0.005 % – 0.100 % tricaine) in the dogfish (Scyliorhinus canicula) caused a complete inhibition of anterior lateral line neural activity. Experiments conducted by Spath and Schweickert (1977) showed a 70 % reduction in the neural activity of the anterior lateral line in the freshwater fish Tilapia sp after 3 minute exposure to 0.007 % tricaine.

Depending on the protocol, investigators may elect high concentrations of anesthesia for rapid immobilization during short procedures (e.g. fish tagging, blood collection) or lower doses for longer, acute experiments (e.g. electrophysiological studies). Often a significantly higher dose is needed to induce the surgical plane of anesthesia however this state can be maintained by lower doses. Thus the dosage, kinetics and recovery time of tricaine administration varies widely among aquatic species. For example, atlantic killifish (Fundulus hteroclitus) are immobilized within 1.5 min in 0.0008 % tricaine (McFarland 1960), salmonids can be effectively anesthetized after immersion in 0.005 % tricaine for 3 min (Laird and Oswald 1975), marine snapper (Pagrus auratus) are anesthetized after exposure to 0.010 % (Ryan 1992), and channel catfish (Ictalurus punctatus) are anesthetized after immersion in
0.0004 % tricaine for 60 min (Small 2002). Additional variability may be dependent on temperature, size and season.

Toadfish required greater concentrations of anesthetic that other species. Loss of equilibrium was not achieved in toadfish until exposed to tricaine concentrations 0.010 %. Following the 110 minute anesthetic application, all fish had ceased breathing and were allowed to spontaneously recover by transfer to anesthetic free water. Fish began to spontaneously gill within 10 to 15 min of anesthetic removal and recovered equilibrium within 30 min. The recovery of lateral line sensitivity followed with fish exhibiting 90 % of pre-anesthetized neural activity within 90 min of anesthetic withdrawal.

Variations in anesthetic concentration and time course can be attributed to behavioral and physiological factors. The gill membranes are the site of tricaine absorption (Oswald 1978), therefore greater respiration rates may cause a more rapid induction of anesthesia. Other gill membranes have a higher affinity for dissolved particulates (Hunn and Allen 1974; Maren et al. 1968) and thus may facilitate tricaine absorption. Anesthesia potency is directly related to lipid solubility (Ross and Ross 1999), therefore fish with greater lipid content of nerves may experience faster nerve penetration and blockade of sodium channels. Furthermore, diffusion through body tissue may also influence the speed of onset. To reduce complications due to handling and restraint, anesthesia was induced in unrestrained, “free swimming” toadfish. Therefore, tricaine was “self-induced” and dependent on the respiration rate of the fish in contrast to gill irrigation employed by most physiological studies. Therefore, the kinetics of induction and recovery should be considered conservative with faster times likely achieved with direct gill irrigation.
The dampening effect of tricaine on the firing activity of the lateral line nerve was illustrated by the increase in interspike interval in response to increasing anesthesia. Increased tricaine concentrations caused a significant delay in interspike interval at concentrations > 0.010%. The silent fiber changed from 15 to 35 ms interspike intervals to broadly spaced action potentials with intervals greater than 100 ms. Spontaneous fibers also displayed elongated interspike interval at high tricaine concentrations. As spike timing is paramount for stimulus discrimination, it is apparent that high anesthesia concentrations can interfere with sensory discrimination (Zakon, 2003). For example, at 0.030% tricaine, the spontaneous fiber responded to constant water current with an elongated rhythmic bursting which contrasted sharply with the previous firing patterns. It is apparent that anesthesia may influence the sensory information relayed to the central nervous by altering the temporal and spatial pattern of action potentials.

There was also a concurrent decrease in spike amplitude at the higher anesthetic concentrations. During exposure to 0.030% tricaine, spike amplitude was reduced to approximately 25% compared to pre-anesthetic amplitudes. It is probable that the anesthetic blocks ion channels (Bai et al. 2003), decreasing the amount of ion transfer and causing a decreased spike amplitude. Pre-anesthetic spike amplitude was restored in most fibers within 90 min, indicating the potential removal of the blocking agent from the ion channels.

There are a number of conflicting reports on the effects of repeated anesthesia. For example, McFarland and Klontz (1969) reported that repeated exposure caused an increase in tolerance to tricaine, whereas Spath and Schweickert (1977) concluded fish experience a decrease in neural response to repeated anesthesia. Re-application of the anesthetic 24 hours after the initial experiment do not influence the induction time, however lateral line sensitivity
appeared to recover faster. However, due to the small sample size (n=3) and high intrafish variability during the recovery period, further work will be needed to verify these results.

Tricaine has a sulphonated side-chain which enhances its solubility in water, but also increases the acidity of the medium. The natural buffering capacity of saltwater partially offset this effect, however the pH dropped approximately one log unit during the experiment. To verify the decrease in neural activity was the result of the anesthetic and not an artifact of increasing acidity, neural activity was monitored during decreased pH levels. In contrast to decreased sensitivity during anesthetic addition, decreasing pH led to transient heightened sensitivity of the lateral line nerve which is consistent with the observations of Wedemeyer (1970) that low pH solutions produced by tricaine can act as an irritant. Therefore, the decrease in nerve activity after exposure to tricaine was the result of the anesthetic and not the decreasing pH of the solution.

The protocol to gradually increase anesthetic dosage rather than challenge each fish with a single concentration and allow subsequent recovery was done to minimize the complications of multiple challenges with anesthetic. It also provided a shorter window (< 3 hr) to compare pre- and post-anesthetic activity then if the same protocol was repeated for seven concentrations (> 14 hr). It is possible that individual challenges to anesthetic concentrations would result in different recovery kinetics. Recovery of nerve activity is of paramount importance following anesthetic application. Since sensitivity, interspike interval and amplitude returned within 90 min after prolonged and relatively high anesthetic levels, recovery following exposure to lower concentrations would probably proceed faster.

All experimental fish were exposed to tricaine and pancuronium bromide during the electrode implant. Therefore, the experimental exposure to anesthesia was the second
application of tricaine experienced by the toadfish. The concurrent use of a paralytic allowed lower doses of tricaine (0.005 %) to be applied during the surgery than during the experiment. The results of this study indicate low anesthesia levels do not significantly alter lateral line sensitivity and all experiments were conducted at least 2 hours after surgery to provide sufficient recovery time from both the paralytic and the tricaine. As this time was subsequently shown by the experimental results to be sufficient for the recovery of lateral line sensitivity, the neural response during the experimental application of the anesthesia should be comparable to a naive toadfish. In both control fish exposed to tricaine for the first time and in post-surgery fish, the time course of the observable effects of the anesthetic was similar. Toadfish were relatively resistant to concentrations 0.005 % however at 0.010 % tricaine, fish had difficulty regaining equilibrium and at 0.015 % fish lost equilibrium and became unresponsive to stimulus. Although spontaneous neural activity from dropped sharply within 15 min of exposure to 0.005 %, evoked activity remained equal to pre-anesthetic levels. Both spontaneous and evoked activity were significantly reduced at 0.010 %.

It should be noted that efferent nerve activity could have a compounding effect on the reported afferent nerve activity. It is possible that the observed decrease in afferent firing rate is due to activation of the efferent system in response to anesthesia. However, in accordance with current knowledge of anesthesia action, it is more probable that the decrease in afferent firing rate is due to the anesthetic acting directly on the peripheral receptor or nerve. More research is required to determine the exact mode of anesthesia action.

Invasive procedures present a dilemma for the sensory physiologist as ethical consideration demands anesthesia which can negatively impact the sensitivity of the targeted
system. Our studies suggest that low doses (0.001 %) do not significantly impact lateral line sensitivity. If higher doses are necessary to invoke the surgical plane of anesthesia, then switching to lower maintenance doses and allowing sufficient recovery time could minimize the effect of the anesthetic.
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REFERENCES


FIGURE LEGENDS

Figure 1: The normalized firing rate of lateral line fibers (n=17, 11 spontaneous and 6 silent) is plotted versus increasing tricaine concentrations (see text for details). Firing rate is normalized as a percent of the pre-anesthetized firing activity. Spontaneous activity (solid) represents the resting discharge rate from spontaneously active lateral line fibers. Evoked activity (open) is the firing rate from both spontaneous and silent fibers in response to water current. Error bars = 1 SE.

Figure 2: Induction and recovery of lateral line afferent nerve fibers during application and withdrawal of anesthesia. (A) The tricaine concentration of the toadfish bathing solution is plotted versus time (min). (B) Evoked neural activity from both spontaneously active (solid; n=3) and silent fibers (open; n=4) in response to water current is plotted versus time (min). Firing rate is normalized as a percent of the pre-anesthetized firing activity. The arrow indicates the time at which fish were transferred to anesthetic-free solution. The long dashed line represents the pre-anesthetic firing rate. Error bars = 1 SE.

Figure 3: Evoked neural activity from an anterior lateral line afferent fiber during anesthesia application. The left column shows the fiber activity 15 min after exposure to the tricaine concentration on the left of each trace. The right column shows fiber activity following transfer to anesthetic-free saltwater with the time after the transfer on the left of each trace.

Figure 4. Interpike histograms were constructed for approximately 300 consecutive impulses and were compiled in 2 ms bins for a silent lateral line fiber during stimulation and a spontaneous fiber at rest and during stimulation. The tricaine concentration during each test is shown in upper corner of the right panel.
Figure 5. (A) The tricaine concentration of the toadfish bathing solution is plotted versus time (min). (B) The normalized firing rate of spontaneous anterior lateral line fibers (N=3) at rest (triangle) and during water current stimulation (circle) is plotted versus time (min) following addition and withdrawal (arrow) of tricaine. Neural activity was monitored during the initial challenge at 0 hrs (solid) and again during a second tricaine exposure 24 hours later (open). The long dashed line represents the pre-anesthetic firing rate.

Figure 6. (A) The pH of the toadfish bathing solution is plotted versus time (min). The pH of the solution was adjusted every 15 min to mimic effect of adding tricaine. It was restored to 8.1 at 110 min. (B) Spontaneous (open) and evoked (solid) neural activity from anterior lateral line fibers is plotted versus time (min) during pH decrease and subsequent restoration to normal (8.1) at 110 min (circle). The experiment was then repeated (triangles) during tricaine administration and concurrent pH decline. The anesthetic was removed and the pH restored to 8.1 at 110 min. Each data point represents the mean of 3 fish.
Table 1. Behavioral observations during 30 min exposure to tricaine. Animals were then transferred to anesthetic free salt water to monitor recovery. Experiments were performed on three to five fish per concentration.

<table>
<thead>
<tr>
<th>Tricaine Concentration (%)</th>
<th>Behavioral Observation</th>
<th>Respiration Rate (% of resting)</th>
<th>Respiration Recovery (min)</th>
<th>Equilibrium Recovery (min)</th>
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<td>0.000</td>
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<td>100</td>
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<td>No effect</td>
<td>100</td>
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<td>NA</td>
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<td>0.005</td>
<td>Delayed righting response (&lt; 5 s)</td>
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<td>0.5 ± 0.3</td>
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<td></td>
<td>Decreased reaction to stimuli</td>
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<tr>
<td>0.010</td>
<td>Prolonged delay in righting (&gt; 5 s)</td>
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<td>3.0 ± 1.7</td>
<td>4.3 ± 1.7</td>
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<td></td>
<td>Partial loss of equilibrium</td>
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<td>0.015</td>
<td>Loss of equilibrium (LOE)</td>
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<td>2.6 ± 1.2</td>
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<td>9.7 ± 2.2</td>
<td>10.0 ± 3.5</td>
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<td>LOE, irregular breathing hyperextension of operculum</td>
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<td>23.0 ± 0.7</td>
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NA = not applicable

Values represents means ± SE.
Respiration rate was quantified before (resting) and at the conclusion of tricaine exposure