Department of Physics, Chemistry and Biology

Master Thesis

Facial expressions and other behavioral responses
to pleasant and unpleasant tastes in cats
(*Felis silvestris catus*)

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The behavior and facial expressions performed by cats have been reported to be visibly affected by the perceived taste quality of a food item. The goal of the present study was to assess how cats react to pleasant and unpleasant tastes. The facial and behavioral reactions of 13 cats to different concentrations of L-Proline and quinine monohydrochloride as well as mixtures with different concentrations of the two substances were assessed using a two-bottle preference test. The cats were videotaped during the tests and the frequency and duration of 50 different behaviors was analyzed in Noldus the Observer XT. The cats responded to tastes regarded as pleasant by having their eyes less than 50 % open for significantly longer periods of time compared to a water control. Tongue protrusions were also observed significantly more frequently when the cats sampled from a solution with a preferred taste compared to a water control. When encountering solutions of quinine monohydrochloride or mixtures containing quinine monohydrochloride the cats were observed to perform tongue protrusion gapes much more frequently compared to a water or L-Proline control. Even though the cats did not significantly differ in the number of times they licked at spouts containing the 50 mM L-Proline and 500 mM quinine monohydrochloride mixture compared to a 50 mM L-Proline, no masking effect could be confirmed as there was no increase in the acceptance of the mixture was observed. The present study suggests that the knowledge about behavioral responses to pleasant or unpleasant taste can be utilized in future studies on how cats perceive different tastes.

Nyckelord/Keyword:

Felis silvestris catus, cat, taste, behavior, L-Proline, quinine monohydrochloride

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1. Abstract

The behavior and facial expressions performed by cats have been reported to be visibly affected by the perceived taste quality of a food item. The goal of the present study was to assess how cats react to pleasant and unpleasant tastes. The facial and behavioral reactions of 13 cats to different concentrations of L-Proline and quinine monohydrochloride as well as mixtures with different concentrations of the two substances were assessed using a two-bottle preference test. The cats were videotaped during the tests and the frequency and duration of 50 different behaviors was analyzed in Noldus the Observer XT. The cats responded to tastes regarded as pleasant by having their eyes less than 50 % open for significantly longer periods of time compared to a water control. Tongue protrusions were also observed significantly more frequently when the cats sampled from a solution with a preferred taste compared to a water control. When encountering solutions of quinine monohydrochloride or mixtures containing quinine monohydrochloride the cats were observed to perform tongue protrusion gapes much more frequently compared to a water or L-Proline control. Even though the cats did not significantly differ in the number of times they licked at spouts containing the 50 mM L-Proline and 500 mM quinine monohydrochloride mixture compared to a 50 mM L-Proline, no masking effect could be confirmed as there was no increase in the acceptance of the mixture was observed. The present study suggests that the knowledge about behavioral responses to pleasant or unpleasant taste can be utilized in future studies on how cats perceive different tastes.

2. Introduction

Domestic cats (*Felis silvestris catus*) are one of our most popular and beloved companion animals and millions of cats are kept as pets in the United States alone. One of the most important aspects in the life of a pet cat is the feeding regime. Analyzing the behaviors associated with feeding helps us to better understand the needs of the cat and enhance the cats' quality of life. Providing palatable food for pet cats is an important part of creating a positive experience in the everyday life of the animal and in extension, a part of animal welfare (Boissy et al. 2009; Bradshaw, 1991). Cats are so sensitive to the palatability of their food that an unpalatable food item can be rejected to the extent that the cat develops medical

problems from starvation (Zaghini & Biagi, 2005). To understand how to best develop palatable food for cats one must raise the question; "What is palatable to a cat and how do we evaluate a cat's experience with a food item?". The most important aspect to consider is that the cats' behaviors and senses have evolved for a life as an obligate carnivore (Bradshaw et al., 1996; Bradshaw, 2006). The taste buds of the domestic cat are highly responsive to amino acids whilst the cats' reactivity for mono- and disaccharides is almost non-existent (Bradshaw et al., 1996). It has been reported that cats prefer the taste of the amino-acids L-proline, L-lysine and L-histidine (White & Boudreau, 1975; Bradshaw, 1996). Cats are, on the other hand, unable to detect the taste of sugar molecules and thus do not display behavioral responses to sugars (Li et al., 2006; Bradshaw et al., 1996). Tastes described as bitter by humans are usually avoided by cats. Bitter tastes can sometimes occur in commercial cat food because of the addition of important nutrients (Zaghini & Biagi, 2005). Quinine monohydrochloride, often described to have a distinct bitter taste, has been reported to be strongly disliked by cats (Carpenter, 1956). The behavior and facial expressions performed by cats have been reported to be visibly affected by the perceived taste quality of a food item (Bartlett et al., 1999; Berridge, 2000; Van den Bos et al., 2000). Behavioral responses to different taste qualities have been reported in a variety of mammalian species including humans, non-human primates, rats, rabbits, hamsters and horses (Ganchrow et al., 1979; Ganchrow et al., 1986; Brining et al., 1991; Steiner & Glaser, 1995; Steiner et al., 2001; Ueno et al., 2004; Jankunis & Whishaw, 2013). When experiencing tastes regarded as pleasant by the animal, facial expressions that involve smacking with the lips, tongue protrusion and relaxation of the facial muscles are commonly observed. Unpleasant tastes, in contrast, elicit facial expressions that commonly involve gaping mouth movements, head shakes and flailing with the limbs. The benefit of taking such behavioral factors related to the taste experience into consideration, as opposed to only looking at consumption data, is that we get a more precise idea on how the animal experience a taste and the underlying decisions in its food selection process (Grill & Norgren, 1978).

The goal of the present study was thus to assess how cats react to pleasant and unpleasant tastes. The study also aimed to find how cats reacted to mixtures of solutions with pleasant and unpleasant tastes and if it was possible to mask the unpleasant taste by using a pleasant one.

3. Methods

3.1 Animals

13 adult cats (*Felis silvestris catus*), 7 males and 6 females, of the breed Domestic Short Hair (DSH) were used in the study. All cats included in the study were bred for research purposes at Liberty Research, Inc. (NY) and were currently housed at the AFB International's Palatability Assessment Resource Center (PARC) where the study took place. The cats were all around 2.5 years old when the experiments were initiated in order to ensure that the cats had fully developed their senses related to the experience of taste as well as their preference for certain tastes (Bradshaw, 2006; Van den Bos et al., 2000). Both males and females used in the study were neutered in order to eliminate hormonal factors such as the oestrus cycle that could have an impact on the taste sensation (Van den Bos et al., 2000). Furthermore, all cats included in the study were healthy individuals without any history of allergies to the taste stimuli used in the tests. The cats participating had no history of serious injuries or behavioral problems. The cats were free from upper respiratory tract infections (URI) and were regularly monitored by animal technicians.

The 13 cats used in the study were selected from an original group of 42 palatability research cats based on their willingness to interact with the drinking spouts containing the taste solutions as well as on their calmness when placed in the testing arena. Cats with the desired qualities went on to have further acclimatization training and were observed to be able to drink from the spouts as well as regularly drinking both from the left and the right spout in a two-bottle preference test setting.

3.2 Taste Stimuli

Solutions and mixtures with different concentration levels of L-Proline (Figure 1) and quinine monohydrochloride (QHCl) (Figure 2) were used throughout the study to elicit behavioral responses based on the taste qualities of these solutions. L-proline was used in order to elicit behavioral responses indicating a pleasant taste experience. L-Proline is described by humans as having a complex sweet taste with components of both salty and sour (Schiffman & Sennewald, 1981). However, rats had difficulties to distinguish between the taste of L-Proline and sodium reduced Monosodium Glutamate which most commonly is identified as having an umami taste quality (Delay et al., 2007). Cats have been observed to prefer the taste of L-Proline when compared to other amino acids (White & Boudreau, 1975).

Solutions of QHCl (Product Information, Appendix 2) were used in order to elicit behavioral responses indicating an unpleasant taste experience. QHCl is described by humans as having a distinctly bitter taste

and is commonly used when investigating responses to bitter taste (Steiner & Glaser, 1984; Berridge, 2000; Jankunis & Whishaw, 2013). Similar to a variety of other mammals, cats are known to display an aversion towards bitter taste (White & Boudreau, 1975; Bradshaw et al., 1996).

Fig. 1. (left) Molecular structure of L-Proline. Fig. 2. (right) Molecular structure of quinine monohydrochloride.

3.3 Behaviors and Facial Expressions

Evaluations of the cats' facial and behavioral reactions to different concentrations of L-Proline and QHCl as well as mixtures with different concentrations of L-Proline and QHCl were performed during the study. The behaviors and facial expressions included in the study were based on the Universal Feline Behavior Coding Scheme from AFB International and on behaviors and facial expressions commonly reported to occur in mammals when experiencing pleasant or unpleasant taste (Van den Bos et al., 2000; Berridge, 2000, Steiner et al., 2001). Behaviors and facial expressions performed by the cats during the trials were analyzed from the obtained video material by performing continuous observations using Noldus The Observer XT (version 11.5) software. Behaviors and facial expressions used in the study were divided into seven different categories (Table 1). For a complete list of behaviors included in the study, see Appendix 1. The coding scheme was designed to accommodate usage of Noldus the Observer. State behaviors indicates behaviors that are both measured in duration and frequency whilst point behaviors only registers frequency of a behavior

In order to prevent observer bias in behavior coding a non-observer assigned code names to the different taste solutions used in the study. The person assigning the codes kept a register of which code represented a certain solution but the observer did not partake in this

information whilst still coding. The correct contents linked to the assigned code were revealed to the observer after behavior coding of a concentration series was finished. This procedure enabled the observer to be blindfolded for the properties of the taste solutions whilst at the same time ensuring that the solutions were always tested together with their control.

Fig. 3. The testing arena with the marked out area of interest. The area of interest is located within the half circle in the front part of the cage and below the upper line.

Tab. 1. Behavior categories used in the study. The number of behaviors in a group shows how many different behaviors were included in a particular group of behaviors. A (1.) after the behavior group indicates that it is a state behavior group and a (2.) indicates that it is a point behavior group.

Behavior Group	Description	Number of behaviors in group
Location ^{1.}	Describes if the cat was located in the right or left part of the cage or if the cat was located in the area of interest (Figure 3).	3
Body Position ^{1.}	Describes if the cat was moving around, sitting etc.	9
Sniffing and Drinking ^{1.}	Describes if the cat was sniffing at a spout or the cage, drinking from spouts etc.	11
Oral Grooming ^{1.}	Different behavior including grooming with the tongue.	4

Eyes ¹ .	Describes to what degree the cat had its eyes open whilst sampling solution.	4
Ears ¹ .	Describes how the cats positioned its ears whilst sampling solution.	3
Point Behaviors ² .	A wide variety of behaviors including tongue movements, mouth movements etc.	25

For additional guidelines to the coding scheme, see Appendix 3.

3.4 Experimental Set-up

Two 17 ml metal spouts were mounted to the Plexiglas front door of a 75x61x72 cm metal cage as set-up for the two-bottle preference tests (Figure 4). An electric lick-o-meter counter (model 86063, Lafayette Instrument Company), located on the outside of the cage, was mounted to each of the spouts to count the number of times the cat came into direct contact with the spout. The cage had metal flooring to allow for the lick-o-meter circuit to be completed whenever the cat came into contact with one of the metal spouts. HD quality video (MP4) recording of each trial was collected by mounting a Sony (HDR-CX380) camera on a tripod placed 2.3 m in front of the cage.

Fig. 4. Metal cage used as observational arena seen from the front. In the center of the door, two metal spouts containing the solution were mounted. Lick-o-meters registering total number of interactions with its respective spout is mounted to each side.

Observations took place from 09:30 in the morning until 14:30 in the afternoon four days a week. The cats had access to food between 15:00 until 06:00, thus no food was accessible to the cats during hours when trials were run. The taste solutions were stored in glass jugs sealed with a lid and kept in a fridge at 6 °C. The solutions used during trials were removed from fridge storage 2 h before the onset of testing in order to present them at room temperature. The cats were divided into morning testers and afternoon testers. 6 of the 13 cat used were always tested in the morning and the rest of the cats were always tested in the afternoon. This was done in order to make the trials as similar as possible for the individual cat and exclude differences in performance due to variation in time of day. Each cat participated in only one trial per day.

The metal cage used as the experimental testing arena was rigged with spouts and solutions before the start of the observation. Each of the spouts was filled with 14 ml of liquid (Figure 6). Vinyl gloves were worn at all times when prepping the spouts and handling solutions. The lick-o-meters were checked twice before each trial so that the circuit was completed upon interaction (Figure 5). The circuit check was done by touching the upper part of the neck of the spout and the cage floor which allowed for full circuit check without contaminating the dispenser area of the spout. When the testing arena had been prepared the cat was transported in a cat carrier (Bargain Hound, 50 x 27 cm) from its housing area into the testing arena. The cat was kept in the cat carrier no longer than 5 min before the onset of testing. Cats that were not willing to use the cat carrier as means of transportation were instead transported to the testing arena via carrying them by hand. At all times when the cats were carried by hand, hand arm guards (BiteBuster) and protection gloves (ATG, MaxiCut) were used.

Fig. 5. (left) Performing circuit check to ensure that each interaction with the metal spout is registered on the lick-o-meter. Fig. 6. (right) Inserting the solution into the metal spout using a syringe. Vinyl gloves were worn in order to avoid contamination.

A single cat was placed in the testing arena for each trial (Figure 7; 8). Video recording was started just before placing the cat in the arena. The handler left the room as soon as the cat had been placed in the arena. No observers, other cats or personnel was allowed to be present during trials in order to avoid influencing the cat's behavior during testing. The trial then went on for 5 min before the handler returned. Upon returning the number of licks registered on the lick-o-meter was written down and the cat was then returned to its normal housing area.

Fig. 7. (left) Cat placed in the observational arena. Fig. 8. (right) Cat drinking of the solution from the metal spout.

After the cat had been returned, the remaining liquid in the spouts was emptied into a syringe in order to measure the residual volume. The spouts were then demounted from the testing arena and the cage cleaned. Cleaning was done by wiping the door, floor and walls of the cage first with a sterilizing wipe (Clorox Healthcare, Bleach Germicidal Wipes, 30.5 x 30.5 cm) and then with a paper towel soaked with distilled water in

order to dilute the sterilizer and remove any smell of bleach. The testing arena was then allowed to air dry for at least 20 min before being used again.

For a trial to be regarded as successful, the cat had to interact with at least one of the spouts in the cage by coming within a 2.5 cm radius from the spout and sniff at the spout. Trials in which the cat failed to do so were eliminated from the data set.

The study was subdivided into three different experiments in which the different solutions were tested. In *Experiment 1* the cats were presented with gradually increasing concentrations of L-proline solutions versus a control of distilled water.

In *Experiment 2* the cats were presented with gradually increasing concentrations of QHCl solutions versus a control of distilled water.

Experiment 1 & 2 were executed parallel to each other throughout the first part of the study. *Experiment 3* was performed in the second part of the study after the completion of *Experiment 1 & 2*.

In *Experiment 3*, the cats were presented with taste mixtures with a fixed concentration of QHCl and gradually increasing concentrations of L-proline versus L-Proline reference solutions.

During the first week of testing the cats went through a “water versus water” test in which both spouts in the testing arena were prepped only with distilled water. During the week of water versus water tests, each cat did partake in two testing sessions. The water versus water test was used as a baseline comparison to observe the behaviors and consumption patterns of the cats when no experimental solutions were presented. The water versus water test was then repeated after the completion of *Experiment 1 & 2* before *Experiment 3* was started and then repeated a third time after the completion of *Experiment 3*. As with the first water test, each cat participated in two sessions within each week that the water versus water test was performed. The “water versus water” test was performed to see if the behaviors and consumption patterns of the cats were altered during the process of data collection.

In order to ensure that the cats remained interested in the spouts and to make sure that the cats were able to operate the spouts throughout the study, a practice run was performed at the end of each week of testing. During the practice run, both spouts in the cage were filled with a chicken liver digest eliciting a high consumption response in cats. This procedure encouraged the cats to continue investigating the spouts, understand how to operate the spouts and not associating one spout as a positive and the other spout negative. After the chicken liver digest trial had been performed the

cats did not partake in any test for an additional two days to avoid the flavor of chicken liver digest influencing the upcoming trial.

3.5 Experiment 1: Taste reactivity to pleasant flavor (L-Proline); Experiment 2: Taste reactivity to unpleasant flavor (QHCl)

In *Experiment 1 & 2* of the study the cats were presented with one of the taste solutions, L-proline or QHCl respectively, in one of the spouts and a control of distilled water in the other spout. During *Experiment 1 & 2* five different concentrations of L-Proline and QHCl respectively were tested (Table 2). The cats were first presented with the solutions with the lowest concentration, 0.05 mM for L-Proline and 0.005 μ M for QHCl, and then the concentrations of the solutions were increased in increments of 10 times higher concentration with each week of testing, ending with the highest concentrations on the last week, 500 mM for L-Proline and 50 μ M for QHCl respectively. Going from lower to gradually higher concentrations was done in order to avoid affecting the cats' sensitivity to the different compounds.

The concentration levels for L-Proline included in the study were based on a previous study which found that the cats' preference for L-Proline peaks at a concentration of 50 mM (White and Boudreau, 1975). The concentrations for the L-Proline solutions used in the study were set to encircle this preference peak. The concentrations selected for QHCl were based on the findings from Carpenter (1956) who found that cats were able to distinguish and reacted to QHCl hydrochloride at concentrations as low as 5 μ M (0.000005 M). The concentrations selected for the solutions in this study were chosen to encircle that detection level for QHCl up to a concentration level strong enough so that possible aversive responses towards the solution were likely to occur.

Tab. 2. Concentrations used during Experiment 1 & 2.

Series	L-Proline	QHCl
1	0.05 mM	0.005 μ M
2	0.5 mM	0.05 μ M
3	5 mM	0.5 μ M
4	50 mM	5 μ M
5	500 mM	50 μ M

The cats were presented with each concentration of L-Proline and QHCl twice. Each week, the cats were presented with one concentration of L-proline and one concentration of QHCl. An equal number of observations with L-proline and QHCl were performed each day. In total, each cat

participated in 4 trials per week. The cats were alternated between solutions each day so that an individual cat was tested with L-proline every other day and QHCl every other day. This was done in order to avoid that the cats would perform the same test two days in a row but instead were confronted with a new stimulus each day and thus were more likely to investigate what was served in the spouts. Each week the cats were selected randomly to either start with L-Proline or QHCl. The time of day the cats were tested on, however, remained constant.

Each solution of the different concentration series used for testing L-Proline or QHCl solutions were derived from a 500 mM stock solution of L-Proline or a 5 mM stock solution of QHCl respectively. The stock solutions were prepared before the experimental onset of *Experiment 1 & 2*. The solutions used in the trials were diluted from the stock solutions to the desired concentration series a few days before the trials were carried out.

3.6 Experiment 3: Taste Reactivity to mixtures of L-Proline and QHCl

In *Experiment 3*, the cats were presented with a taste mixture of L-Proline and QHCl in one of the spouts and an L-Proline taste solution in the other spout in order to investigate the masking potential of L-Proline as well as the cats' behavioral response based on the taste qualities of these solutions when consuming a taste mixture of different compounds versus a single compound solution. The reported taste reactivity range in cats for L-Proline by White and Boudreau (1975) and QHCl by Carpenter (1956), as well as indications of taste reactivity range for the solutions in the current study, were used when deciding upon suitable concentrations for taste mixtures (Table 3). The concentration of L-Proline was increased with each mixture whilst the concentration level of QHCl remained constant in all of the mixtures. The cats were first presented with a mixture of 5 mM L-Proline and 50 μ M QHCl versus a solution of 50 mM L-Proline. The concentration of L-Proline in the mixtures was then increased with each week (Tab. 3). The control L-Proline solution was kept constant at a concentration of 50 mM throughout the experiment. The cats were tested with each mixture twice. In total, each cat underwent two trials per mixture. The mixture tests were performed under two consecutive days but as in previous tests each cat was only allowed to participate in one trial per day.

Tab. 3. Mixture and control concentration per series during Experiment 3

Mixture	L-proline + QHCl	L-Proline
1	5 mM + 50 μ M	50 mM
2	25 mM + 50 μ M	50 mM
3	50 mM + 50 μ M	50 mM

4	250 mM + 50 μ M	50 mM
5	500 mM + 50 μ M	50 mM

A preparation of 500 mM L-Proline stock solution was done twice in *Experiment 3* of the study. Sufficiently enough solution remained of the original stock solution of QHCl from *Experiment 1 & 2* to cover the needs for *Experiment 3*, thus no new stock solution of QHCl was created.

3.7 Data Analysis

The number of licks registered during the sessions was compared using a Wilcoxon's test for pairwise observations. Behavioral data from the coded videos were obtained by running Behavioral Analysis and Lag Sequential Analysis in the Observer XT. The Behavioral Analysis indicated what behaviors occurred during certain state behavior intervals, such as "What other behaviors occurred whilst the cat performed the behavior drinking?", of the observation.

Three different forms of Behavioral Analysis were generated, each one evaluating a specific behavior interval; the first analysis form included intervals when the cats in some way sampled from the solutions by either sniffing the spout or direct ingestion (Sampling). The second form of analysis was more restrictive and focused only on behaviors occurring whilst sampling in the form of drinking directly from the spout (Drinking). The final form of Behavioral Analysis took into account the behaviors occurring during the individual cat's test session as a whole. Observed behaviors during this interval were ascribed to the solution located on the same side as the behavior occurred in (Overall).

Lag Sequential Analysis was generated after the end of a drinking interval with the parameters set to ignoring repeating criteria and a 20s delay. In other words, "what behaviors occurred in a 20 s interval after the cat had stopped drinking from a spout?". The generated data were then analyzed by using Wilcoxon's test for pairwise observations at behaviors of indicating a pleasant or an unpleasant taste experience between taste solution and control. The alpha level was set at 0.05.

Significant difference in interval duration or frequency, inevitably, skewed duration and frequency of behaviors occurring during these intervals. In order to account for behaviors being affected by significant differences in interval duration or frequency, behavioral data was normalized when needed. Normalization was done by dividing the behavioral data with the respective interval data. For example, the time the cat had its eyes between 50-100 % open when sampling L-Proline was

divided by the sampling time for L-Proline to achieve the normalized value.

4 Results

4.1 Experiment 1 – L-Proline

The cats licked significantly more often at spouts containing L-Proline at 50 (Wilcoxon, $p=0.017$) and 500 mM (Wilcoxon, $p=0.028$) compared to spouts containing water (Figure 9). This shows that the cats were sampling more extensively from spouts containing the L-Proline at these concentrations than they were sampling the water. However, the registered number of licks was slightly lower at 500 mM indicating a sampling peak at 50 mM. There was no significant difference in the number of licks between L-Proline at 5 mM, 50 mM and 500 mM (Wilcoxon, $p>0.05$). At 0.05, 0.5 and 5 mM L-Proline, the cats did not significantly differ in the number of times they licked at spouts containing L-Proline compared to spouts containing water (Wilcoxon, $p>0.05$). An increase in how often the cats licked the spout containing L-Proline was observed for L-Proline at 5 mM, however, the number of licks registered for the L-Proline at this level did not significantly differ from that of the water (Wilcoxon, $P>0.05$). The cats licked at the spouts containing water to a similar extent throughout the experiment.

Fig. 9. Registered number of licks in Experiment 1. Mean value ($\pm$ SE) of the total number of registered licks at spouts containing different concentrations of L-Proline (black squares) or water (white circles), respectively. An asterisk indicates a significant difference between L-Proline and water at the corresponding concentration.

In trials with L-Proline at 0.05 mM and 5 mM, no significant difference in the frequency or duration of behaviors was found between L-Proline and water during any of the evaluated intervals. Thus, neither differed from water with regard to the registered number of licks nor the facial expressions or other behavioral responses observed.

Significant differences in the frequency and duration of behaviors were observed with 50 and 500 mM of L-Proline versus water (Figure 10; 11). The cats licked significantly more often at the spouts containing L-Proline compared to water spouts containing water during tests at these concentrations.

During trials with L-Proline at 50 mM, the time spent sniffing at the spouts was significantly longer when the cats were sampling from spouts containing L-Proline compared to water (Wilcoxon, $p=0.033$). Whilst sampling from the L-Proline, the cats were, furthermore, observed to have their eyes 50-100 % open more frequently compared to when the cats were sampling from spouts containing water (Wilcoxon, $p=0.046$). The

duration of the cats having their eyes open between 50-100% (Wilcoxon, $p=0.05$), less than 50% (Wilcoxon, $p=0.017$) or closed (Wilcoxon, $p=0.028$) were all significantly longer when the cats were sampling from spouts containing L-Proline than when sampling water. Similarly, the duration of for how long the cat held its ears positioned upward was significantly longer when sampling from L-Proline compared to water (Wilcoxon, $p=0.028$). When normalizing the values, it was confirmed that the cats spent a significantly longer duration of their time with their eyes less than 50 % open when sampling L-Proline at 50 mM contra water (Wilcoxon, $p=0.028$). Furthermore, the frequency for which the cat started the behavior eyes 50-100% open also remained significantly higher even after normalizing the values. During sampling of 50 mM L-Proline the cats performed tongue protrusion behavior significantly more frequently than with water (Wilcoxon, $p=0.039$).

Fig. 10. Behaviors that were observed when the cats were located close to the spout containing L-Proline during trials with L-Proline at 50 mM versus water. Behaviors scoring above 0.5 were more frequent or occurred for longer periods of time when the cats were located at the same side as the spout containing L-Proline compared to the water side. White bars indicate significant difference between the L-proline and the water in a Wilcoxon's signed rank test whilst black bars indicate no significant difference.

Similarly, at 500 mM, the duration and frequency of sampling behaviors, such as drinking from spout or sniffing at the spout, were significantly higher when the cats were sampling from spouts containing L-Proline compared to water. The cats were observed to have their eyes 50-100% open (Wilcoxon, $p=0.013$; 0.019) and less than 50% open (Wilcoxon, $p=0.038$; 0.038) for a significantly longer period of time when both when sampling and when drinking L-Proline compared to when sampling or drinking water. When sampling from the L-Proline, the cats were observed to close their eyes significantly more often than when sampling water (Wilcoxon, $p=0.033$). Both when sampling and when drinking L-Proline the cats held their ears upward for significantly longer periods of time compared to when the cats were sampling from or drinking water (Wilcoxon, $p=0.019$; 0.034). Whilst drinking 500 mM L-Proline the cats also had their ears positioned upwards significantly more frequently

compared to when the cats were drinking water (Wilcoxon, $p=0.047$). Whilst sampling from 500 mM L-Proline the cats performed tongue protrusions (Wilcoxon, $p=0.012$), mouth smacks (Wilcoxon, $p=0.008$) and nose lick (Wilcoxon, $p=0.011$) significantly more often than when sampling water. When located close to spouts containing L-Proline the cats held a standing position for a significantly longer duration than they did when located close to the spout containing water (Wilcoxon, $p=0.007$).

Fig. 11. Behaviors that were observed when the cats were located close to the spout containing L-Proline during trials with L-Proline at 500 mM versus water. Behaviors scoring above 0.5 were more frequent or occurred for longer periods of time when the cats were located at the same side as the spout containing L-Proline compared to the water side. White bars indicate significant difference between the L-proline and the water in a Wilcoxon's signed rank test whilst black bars indicate no significant difference.

The cats did not show any significant difference in behavior between L-Proline and water in Lag Sequential Analysis in *Experiment 1*.

Tables 4 and 5 summarize all behaviors for which the cats displayed significant differences in either the duration (Table 4) or the frequency (Table 5) of behaviors between L-proline and water in *Experiment 1*.

Tab. 4. Behaviors observed to occur with significantly different duration in Experiment 1

Behavior	More Prominent in	Interval	p-Value
Sniffs Spout	50 mM L-Proline > Water	Sampling	0.033
Sniffs Spout	500 mM L-Proline > Water	Sampling	0.046
Drinking from Spout	500 mM L-Proline > Water	Sampling	0.012
Eyes open 50-100%	50 mM L-Proline > Water	Sampling	0.05
Eyes open 50-100%	500 mM L-Proline > Water	Sampling	0.013
Eyes open 50-100%	500 mM L-Proline > Water	Drinking	0.019
Eyes Open <50%	50 mM L-Proline > Water	Sampling	0.017
Eyes Open <50%	500 mM L-Proline > Water	Sampling	0.038
Eyes Open <50%	500 mM L-Proline > Water	Drinking	0.038
Eyes Closed	50 mM L-Proline > Water	Sampling	0.028
Ears Upward &/or Forward	50 mM L-Proline > Water	Sampling	0.028
Ears Upward &/or Forward	500 mM L-Proline > Water	Sampling	0.019
Ears Upward &/or Forward	500 mM L-Proline > Water	Drinking	0.034
Stand	500 mM L-Proline > Water	Overall	0.007

Table 5. Behaviors observed to occur with significantly different frequency in Experiment 1

Behavior	More Prominent in	Interval	p-Value
Eyes open 50-100%	50 mM L-Proline > Water	Sampling	0.046
Eyes Closed	50 mM L-Proline > Water	Sampling	0.027

Eyes Closed	500 mM L-Proline > Water	Sampling	0.033
Ears Upward &/or Forward	500 mM L-Proline > Water	Drinking	0.047
Mouth Smack	500 mM L-Proline > Water	Sampling	0.008
Tongue Protrusion	50 mM L-Proline > Water	Sampling	0.039
Tongue Protrusion	500 mM L-Proline > Water	Sampling	0.012
Nose Lick	500 mM L-Proline > Water	Sampling	0.011

4.2 Experiment 2 – QHCl

The cats did not lick at spouts containing QHCl significantly less often than they did at spouts containing water at any of the tested concentrations (Wilcoxon, $p > 0.05$) (Figure 12). Thus, no significant aversion nor preference for QHCl was found in this experiment. The cats both licked at spouts containing QHCl as well as spouts containing water to a similar extent throughout the experiment. A small increase in registered number of licks for both QHCl and water was observed at 0.5 μM QHCl but there was still no significant difference between the two.

Fig. 12. Registered number of licks in Experiment 2. Mean value ($\pm$ SE) of the total number of registered licks at spouts containing different concentrations of QHCl (black squares) or water (white circles), respectively.

Behaviors that occurred significantly more often or for a longer duration were observed with all of the tested concentrations of QHCl. At the lowest tested concentration of QHCl, 0.005 μ M, the frequency of the cats closing their eyes was significantly higher when sampling QHCl than when sampling water (Wilcoxon, $p=0.038$).

When sampling from the spouts with water the cats folded their ears outward for significantly longer periods of time than they were when sampling from spouts containing QHCl at 5 μ M (Wilcoxon, $p=0.043$). Furthermore, the cats folded their ears outward more frequently whilst sampling from spouts containing water compared to QHCl (Wilcoxon, $p=0.038$).

At the highest tested concentration of QHCl, 50 μ M, the cats were observed to perform tongue protrusion gapes (Figure 13) significantly more often when located close to a spout containing QHCl compared to water (Wilcoxon, $p=0.039$). While drinking from 50 μ M QHCl, a significantly lower frequency of tongue protrusions was observed compared to when the cats were drinking from spouts with water (Wilcoxon, $p=0.031$). Whilst drinking from the water, tongue protrusions were more common compared to when the cats were drinking from the QHCL (Wilcoxon, $p=0.031$). The cats held their ears positioned upwards for significantly longer periods of time whilst drinking from the water compared to when drinking from 50 μ M QHCl (Wilcoxon, $p=0.022$). Both during sampling or drinking 50 μ M QHCl, the cats held their ears in different positions for significantly longer periods of time than during sampling or drinking water (Wilcoxon, $p=0.041$; 0.046).

Fig. 13. Behaviors that were observed when the cats were located close to the spout containing QHCl during trials with QHCl at 50 μ M versus water. Behaviors scoring above 0.5 were more frequent when the cats were located at the same side as the spout containing QHCl compared to the water side. White bars indicate significant difference between the QHCl and the water in a Wilcoxon's signed rank test whilst black bars indicate no significant difference.

The cats did not show any significant difference in behavior between QHCl and water in Lag Sequential Analysis in *Experiment 2*.

Tables 6 and 7 summarize all behaviors for which the cats displayed significant differences in either the duration (Table 6) or the frequency (Table 7) of behaviors between QHCl and water in *Experiment 2*.

Tab. 6. Behaviors observed to occur with significantly different duration in *Experiment 2*

Behavior	More Prominent in	Interval	p-Value
Sniffs Other	5 μ M QHCl > Water	Overall	0.023
Ears Upward &/or Forward	50 μ M QHCl < Water	Drinking	0.022
Ears Lowered &/or Outward	5 μ M QHCl < Water	Sampling	0.043

Ears in Different Position	50 μ M QHCl > Water	Sampling	0.041
Ears in Different Position	50 μ M QHCl > Water	Drinking	0.046
Tuck/Crouch	0.5 μ M QHCl > Water	Overall	0.028

Tab. 7. Behaviors observed to occur with significantly different frequency in Experiment 2

Behavior	More Prominent in	Interval	p-Value
In Area of Interest	0.5 μ M QHCl > Water	Overall	0.020
Sniffs Other	5 μ M QHCl > Water	Overall	0.014
Eyes Closed	0.005 μ M QHCl > Water	Sampling	0.038
Ears Lowered &/or Outward	5 μ M QHCl < Water	Sampling	0.038
Tongue Protrusion	50 μ M QHCl < Water	Sampling	0.031
Tongue Protrusion Gape	50 μ M QHCl > Water	Overall	0.039
Stand	0.5 μ M QHCl < Water	Overall	0.047
Movement	0.005 μ M QHCl < Water	Overall	0.008
Movement	5 μ M QHCl < Water	Overall	0.023
Sit	0.005 μ M QHCl < Water	Overall	0.009
Sit	0.5 μ M QHCl > Water	Overall	0.033

3.3 Experiment 3 – Mixtures

The cats performed significantly more licks at spouts containing the L-Proline at 50 mM without the addition of QHCl compared to spouts containing mixtures with either 5 (Wilcoxon, $p=0.018$), 25 (Wilcoxon, $p=0.013$), 50 (Wilcoxon, $p=0.033$) or 250 mM L-Proline (Wilcoxon, $p=0.016$), and 50 μ M QHCl (Figure 14). In trials when mixtures with 500 mM L-Proline and 50 μ M QHCl were tested, the cats did not significantly differ in the number of times they licked spouts containing the mixture compared to spouts containing the 50 mM L-Proline control (Wilcoxon, $p>0.05$).

Fig. 14. Registered number of licks in Experiment 3. Mean value ($\pm$ SE) of the total number of registered licks at spouts containing different concentrations of the L-Proline and QHCl mixture (black squares) or 50 mM L-Proline control (white circles), respectively. An asterisk indicates a significant difference between the L-Proline and QHCl mixture and the L-Proline control at the corresponding concentration.

With mixtures containing 5 mM L-Proline and 50 μ M QHCl the time the cats spent drinking from the spout was significantly shorter for spouts containing the mixture compared to the 50 mM L-Proline control (Wilcoxon, $p=0.021$) (Figure 15). Whilst sampling or drinking from a 5 mM L-Proline and 50 μ M QHCl mixture the cats were observed to hold their eyes 50-100% (Wilcoxon, $p=0.041$; 0.015) or less than 50 % open (Wilcoxon, $p=0.013$; 0.017) for significantly shorter periods of time compared to the L-Proline control. When normalizing the values, it was confirmed that the cats spent a significantly shorter duration of their time with their eyes either 50-100 % (Wilcoxon, $p=0.028$) or, less than 50 % open (Wilcoxon, $p=0.017$) when sampling 5 mM L-Proline and 50 μ M QHCl mixture compared to the 50 mM L-Proline control. Furthermore, the cats were observed to close their eyes significantly less frequently whilst drinking from a 5 mM L-Proline and 50 μ M QHCl mixture, compared to the L-Proline control (Wilcoxon, $p=0.046$).

Fig. 15. Behaviors that were observed when the cats were located close to the spout containing the mixture during trials with 5 mM L-Proline and 50 μ M QHCl mixture versus an L-Proline control. Behaviors scoring above 0.5 occurred for longer periods of time when the cats were located at the same side as the spout containing the mixture compared to the L-Proline control side. White bars indicate significant difference between the 5 mM L-Proline and 50 μ M QHCl and the 50 mM L-Proline control in a Wilcoxon's signed rank test whilst black bars indicate no significant difference.

When increasing the concentration of L-Proline to 250 mM in the mixture, the time the cats spent drinking from spouts containing the 250 mM L-Proline and 50 μ M QHCl mixture remained significantly shorter than the time spent drinking from spouts containing the L-Proline control (Wilcoxon, $p=0.028$) (Figure 16). The behavior tongue protrusion gape was seen significantly more often when the cats were located close to the spout containing the 250 mM L-Proline and 50 μ M QHCl mixture compared to when the cats were located close to the spout containing the L-Proline control (Wilcoxon, $p=0.034$). Just as in trials with the 5 mM L-Proline and 50 μ M QHCl mixture, the duration of the cats having their eyes

less than half open was significantly longer during sampling from the L-Proline control compared to when the cats were sampling from the 250 mM L-Proline and 50 μ M QHCl mixture (Wilcoxon, $p=0.033$). In trials with the 250 mM L-Proline and 50 μ M QHCl mixture, however, the cats also had their eyes closed for significantly longer periods of time when sampling from the L-Proline control compared to when the cats were sampling from the 250 mM L-Proline and 50 μ M QHCl mixture (Wilcoxon, $p=0.047$).

Fig. 16. Behaviors that were observed when the cats were located close to the spout containing the mixture during trials with 250 mM L-Proline and 50 μ M QHCl mixture versus an L-Proline control. Behaviors scoring above 0.5 were more frequent or occurred for longer periods of time when the cats were located at the same side as the spout containing the mixture compared to the L-Proline control side. White bars indicate significant difference between the 250 mM L-Proline and 50 μ M QHCl and the 50 mM L-Proline control in a Wilcoxon's signed rank test whilst black bars indicate no significant difference.

With mixtures of 500 mM L-Proline and 50 μ M QHCl, the cats did not significantly differ in regards to how many times they licked the spouts containing the mixture and the L-Proline control. The cats did not show any significant difference in behavior between L-proline and QHCl mixtures and the L-Proline control in Lag Sequential Analysis in *Experiment 3*.

Tables 8 and 9 summarize all behaviors for which the cats displayed significant differences in either the duration (Table 8) or the frequency (Table 9) of behaviors between the L-proline and QHCl mixtures and the L-Proline control in *Experiment 3*.

Tab. 8. Behaviors observed to occur with significantly different duration in Experiment 3

Behavior	More Prominent in	Interval	p-value
Drinking from Spout	5 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Drinking	0.021
Drinking from Spout	250 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Drinking	0.028
Eyes open 50-100%	5 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Sampling	0.041
Eyes open 50-100%	5 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Drinking	0.015
Eyes open <50%	5 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Sampling	0.013
Eyes open <50%	5 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Drinking	0.017
Eyes open <50%	250 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Sampling	0.033
Eyes Closed	250 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Sampling	0.047
Ears Up- and/or Forward	5 mM L-Proline and 50 μ M QHCl Mixture < 50 mM L-Proline Control	Sampling	0.005

Ears Up- and/or Forward	5 mM L-Proline and 50 µM QHCl Mixture < 50 mM L-Proline Control	Drinking	0.021
Stand	250 mM L-Proline and 50 µM QHCl Mixture < 50 mM L-Proline Control	Overall	0.013

Tab. 9. Behaviors observed to occur with significantly different frequency in Experiment 3

Behavior	More Prominent in	Interval	p-value
Drinking Dripped Solution from Floor or Door	250 mM L-Proline and 50 µM QHCl Mixture < 50 mM L-Proline Control	Sampling	0.034
Sniffs Other	250 mM L-Proline and 50 µM QHCl Mixture < 50 mM L-Proline Control	Overall	0.024
Eyes Closed	5 mM L-Proline and 50 µM QHCl Mixture < 50 mM L-Proline Control	Drinking	0.046
Tongue Protrusion Gape	250 mM L-Proline and 50 µM QHCl Mixture > 50 mM L-Proline Control	Overall	0.034
Lip Lick	500 mM L-Proline and 50 µM QHCl Mixture < 50 mM L-Proline Control	Overall	0.048
Movement	250 mM L-Proline and 50 µM QHCl Mixture < 50 mM L-Proline Control	Overall	0.032
Sit	250 mM L-Proline and 50 µM QHCl Mixture < 50 mM L-Proline Control	Overall	0.035

5. Discussion

The results of the present study show that cats performed behaviors indicative of a pleasant taste experience whilst sampling L-Proline at 50 and 500 mM. During *Experiment 1*, the cats were observed to differ significantly between L-Proline and water in behaviors such as: sniffing at the spouts, eyes less than 50% open, tongue protrusions, nose licks and mouth smacks.

In *Experiment 2*, significant differences in the behavior of the cats between QHCl and water included; the occurrence of tongue protrusions and tongue protrusions gapes.

A preference for solutions of L-Proline without the addition of QHCl was observed during *Experiment 3*. Only with the strongest concentration of L-Proline at 500 mM in the mixture with 50 μ M QHCl was there no significant difference in how often the cats licked at the spouts between the mixture and the 50 mM L-Proline control. However, no masking effect for L-Proline could be confirmed as rather than the cats starting to accept the mixture, they instead decreased in the number of times they licked at the spout containing the control. The cats were observed to differ significantly between the L-Proline and QHCl mixtures and the L-Proline control in behaviors such as eyes less than 50% open and tongue protrusion gapes.

In 1975, White & Boudreau examined the preference of cats for the taste of L-Proline. Their results regarding the cats' consumption of L-Proline is in line with the registered number of licks for L-Proline from *Experiment 1* in the present study. Notably, in both studies the sampling of L-Proline peaked at 50 mM and then slightly decreased with increased concentration.

In both *Experiment 1* and *Experiment 3* the cats responded to preferred concentrations of L-Proline by having their eyes less than 50 % open for significantly longer periods of time (Figure 17). Non-human primates have been reported to show a similar response to pleasant tastes by having their eyes slightly, but not fully, closed in a relaxed manner (Steiner & Glaser, 1984). A commonly reported response to pleasant taste in both newborn humans, non-human primates and rabbits is that the animal tends to have a relaxed facial expression (Steiner, 1973; Ganchrow et al., 1979; Ganchrow et al., 1983; Steiner & Glaser, 1984). It stands to believe that the cats having their eyes less than 50 % open is a form of relaxed facial expression in this species. This indicates that cats, just as the previously mentioned mammals, often respond to pleasant taste with a relaxed facial expression. Another possibility is that holding the eyes less than half open is a behavioral response to pleasant taste specific to cats.

The fact that eyes less than 50 % open whilst sampling L-Proline occurred in both *Experiment 1* and *Experiment 3* further underlines the importance of this behavior as a part of the cats' behavioral response repertoire to pleasant taste. It is important to mention that the behavior "eyes less than 50 % open" should not be confused with the "eye squinch" often seen as a reaction to unpleasant taste in human infants (Steiner, 1973 via Steiner et al., 2001). The eye squinch refers to the eyes being shut and a visible compression of the periorbital muscles.

Tongue protrusions (Figure 18) are one of the most commonly described reactions to pleasant taste and have been reported to occur in both humans, non-human primates, rats, rabbits and horses (Grill & Norgren, 1978; Ganchrow et al., 1979; Steiner & Glaser, 1995; Steiner et al., 2001; Ueno et al., 2004; Jankunis & Whishaw, 2013). The results from this study show that cats are no exception to this. Tongue protrusions were significantly more frequent when sampling from spouts containing preferred concentrations of L-Proline compared to water or mixtures of L-Proline and QHCl. The prevalence of tongue protrusions conveys important information about the taste experience of cats since it is such a widely identified behavioral response to pleasant taste.

With a high concentration of L-Proline, mouth smacks (Figure 20) and nose licks (Figure 21) became significantly more frequent compared to lower concentrations of this tastant. In hominoid apes and newborn humans the behavior lip smack, similar to the behavior mouth smack, is indicative of a pleasant taste experience (Steiner & Glaser, 1995). Rats have also been reported to perform mouth smacks as a response to pleasant taste (Grill & Norgren, 1978). Nose licks, on the other hand, have a more debatable disposition. Van den Bos et al. (2000) reported that nose licks in cats were more prevalent when the cats were consuming low flavor foods and defined the nose lick in cats as an indicator of an unpleasant or at least less pleasant taste experience. Nose licks in the present study, however, occurred during sampling of something that the cats preferred to consume. Furthermore, the nose licks occurred together with other behaviors usually considered as indicative of a pleasant taste experience. One explanation that coincides with the findings of Van den Bos et al. is that the high concentration of L-Proline might elicit side tastes, possibly triggering both pleasant and unpleasant sensations. If this is the case, however, why did nose licking behavior not occur significantly more often during any of the tests with QHCl? Steiner et al., 2001 reported that a behavior defined as "an upwards tongue protrusion" in New World monkeys, similar to the nose lick behavior in cats, was indicative of a pleasant taste experience. The results from the present study coincides

more with the results from Steiner et al. (2001) that the nose lick behavior is indicative of a pleasant taste experience.

The cats were sniffing (Figure 19) at high concentrations of L-Proline for significantly longer durations than they were sniffing at water or L-Proline and QHCl mixtures. This suggests that the cats were able to detect the odor of the amino acid. L-Proline is known to have a detectable odor for humans, spider monkeys, and mice, when presented at mM concentrations (Laska, 2010; Wallén et al. 2012). Sniffing at a food item is an important part in the food selection process of cats (Hullar et al., 2001). In a study by Becques et al. (2014) sniffing behavior directed towards a food item was described as an indicator of hesitation in the food selection process of cats. If two different food items are available to the cat and the cat finds the smell for at least one of the food items not enticing enough, the cat will sniff at both choices and possibly continue sampling by tasting both food items (Hullar et al., 2001). If the cat is more certain of its choice, however, it will more likely only sample from the first approached choice if the cat finds it palatable enough. In the present study, the cats sniffed approximately equally often at spouts containing L-proline at lower concentrations as they did at spouts containing water. This indicates possible indecision in their choice and that the odor components were not enticing enough, or perhaps not detectable. The observation that the cats sniffed longer at spouts containing high concentrations of L-Proline than at spouts containing water, indicates that they found the odor appealing enough to evaluate longer whether or not to consume the solution.

Fig. 17. (left) The cat has its eyes less than 50% open whilst sampling the solution. Fig. 18. (middle) The Cat performs a tongue protrusion where it sticks its tongue straight out. Fig. 19. (right) The cat samples from the spout by sniffing at it.

Fig. 20. (left) The cat performs a mouth smack where it just slightly opens its mouth and then closes it again. Fig. 21. (right) The cat performs a nose lick where it sweeps its tongue over the nose.

Behaviors associated with a negative taste experience whilst sampling QHCl were not found. The negative impact of QHCl on the taste experience as a whole for the cats was more clearly observed during tests with mixtures of L-Proline and QHCl. The changes that occurred both in regards to behavior as well as the registered number of licks shows that QHCl had negative effects on how the cats experienced the mixture.

In a study by Carpenter (1956), cats showed an aversion towards QHCl at 5 μ M, which was not observed in the present study. One possible explanation for the difference between the results is that in the study by Carpenter the cats were exposed to QHCl for 48 h whilst in the present study the cats only had access to QHCl for 5 min. It is possible that with a 48 h exposure the cats' behavior was affected by postingestive factors so that variables other than taste alone may have influenced the aversion towards QHCl in cats reported by Carpenter.

Hullar et al. (2001) reported that cats that find the taste of a food item unpalatable tended to sample both of the two available options. This might have been the case in the present study, too, with the cat trying to establish a choice between the two solutions and therefore investigating both solutions equally often. It is possible that there is a floor effect in regards to the cats' consumption of QHCl in this study. That is, the cats could not reject QHCl more than they already did within the parameters of the current study.

Even though no aversion was found in *Experiment 2*, it is interesting to note the significant differences in behavior occurring during these trials. The tongue protrusions that are often connected to a pleasant taste experience were observed to occur more often whilst the cats were sampling water compared to QHCl. As stated by Ganchrow et al. (1983), however, in newborn humans water evokes the least intense facial

expressions in terms of behavioral response to taste. Rather than an increase in tongue protrusions whilst sampling water it might instead be so that there is a decrease in tongue protrusions whilst sampling QHCl.

One of the more commonly reported reactions to bitter taste is different varieties of the tongue protrusion gape (Figure 22) during which the animal opens its mouth and then sticks its tongue out. This behavior has been observed in both non-human primates, rats, rabbits and horses (Grill & Norgren, 1978; Ganchrow et al., 1979; Steiner & Glaser, 1984; Ueno et al., 2004; Jankunis & Whishaw, 2013). The cats in the present study were observed to perform the behavior tongue protrusion gape when both located close to a spout containing QHCl as well as when located close to a spout containing an L-Proline and QHCl mixture. A possible explanation to why this behavior did not occur significantly more often during sampling of QHCl might be that the cat immediately retracted from the spout and thus tongue protrusion gapes were not registered whilst the cat was sampling QHCl.

Fig. 22. The cat performs a tongue protrusion gapes where it sticks its tongue out and at the same time gapes with its mouth.

Individual differences between the cats both in terms of preference for different solutions as well as behavioral differences were observed. Such variation is to be expected, however, as individual preferences are common in the food selection process. Earlier experience of different food items is one of the most important aspect in terms of individual food preferences in cats (Bradshaw, 1991; Bradshaw et al., 2000). The cats used in the present study, however, have all been raised with the same type of diet and variation due to earlier experience was reduced to a minimum. Individual differences in perceived palatability due to genetic differences have, however, been reported in cats (Bradshaw et al., 1996).

Behavioral responses to different tastes have been described to have an important communicative value in human infants (Steiner, 1974; Ganchrow et al., 1983; Steiner & Glaser, 1984) When resources allows,

cats are known to live in social groups (Liberg & Sandell, 1988). It stands to believe that behavioral reactions to taste would play a part in the social communication of cats. The cats in the present study were always tested with the different solutions when alone. The implication that behavioral reactions to taste have a social function could possibly affect the occurrence of behavioral responses to different tastes. For future studies, it would be interesting to repeat the experiment but with another cat or a human present in the room as the cat samples from the solutions.

The cats were not subjected to stressful or in any way harmful procedures. The results of this study are part of the process to further develop the quality of the everyday food for our companion animals. As mentioned earlier, such improvements in the everyday life of our companion animals in extension, are a part of improving animal welfare.

One of the aims for the present study was to expand upon the current knowledge on cats behavioral reactions to taste as a complementary parameter for future studies when evaluating palatability. The cats responded to tastes regarded as pleasant by having their eyes less than half open for significantly longer periods of time compared to the water control. Furthermore, the cats were also found to perform tongue protrusions, mouth smacks and nose licks significantly more frequently as a reaction to tastes regarded as pleasant compared to the water control. Both during tests with QHCl and tests with L-Proline and QHCl mixtures, the behavior tongue protrusion gape was visibly affected and occurred significantly more often when the cat was located close to a spout containing QHCl or a mixture containing QHCl compared to water or L-Proline controls. The present study suggests that the knowledge about behavioral responses to pleasant or unpleasant taste can be utilized in future studies on how cats experience different tastes.

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Appendix.

Appendix 1 – Product Information Sheet L-Proline

SIGMA-ALDRICH			
Material Safety Data Sheet			
Version 3.0 Revision Date: 07/15/2007 Print Date: 03/06/2012			
1. PRODUCT AND COMPANY IDENTIFICATION			
Product name	: L-Proline		
Product Number	: 81709		
Brand	: Sigma		
Company	: Sigma-Aldrich 3050 Spruce Street SAINT LOUIS MO 63103 USA		
Telephone	: +1 800-325-5832		
Fax	: +1 800-325-5052		
Emergency Phone #	: (314) 776-6555		
2. COMPOSITION/INFORMATION ON INGREDIENTS			
Synonyms	: (S)-Pyrrolidine-2-carboxylic acid		
Formula	: C ₅ H ₉ NO ₂		
Molecular Weight	: 115.13 g/mol		
CAS-No.	EC-No.	Index-No.	Concentration [%]
L-Proline			
147-85-3	205-702-2	-	-
3. HAZARDS IDENTIFICATION			
Emergency Overview OSHA Hazards No OSHA Hazards			
HMIS Classification Health Hazard: 0 Flammability: 0 Physical hazards: 0			
NFPA Rating Health Hazard: 0 Fire: 0 Reactivity Hazard: 0			
Potential Health Effects			
Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.		
Skin	May be harmful if absorbed through skin. May cause skin irritation.		
Eyes	May cause eye irritation.		
Sigma - 81709		Sigma-Aldrich Corporation www.sigma-aldrich.com	
		Page 1 of 5	

Ingestion	May be harmful if swallowed.
4. FIRST AID MEASURES If inhaled If breathed in, move person into fresh air. If not breathing give artificial respiration. In case of skin contact Wash off with soap and plenty of water. In case of eye contact Flush eyes with water as a precaution. If swallowed Never give anything by mouth to an unconscious person. Rinse mouth with water.	
5. FIRE-FIGHTING MEASURES Flammable properties Flash point no data available Ignition temperature no data available Suitable extinguishing media Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide. Special protective equipment for fire-fighters Wear self contained breathing apparatus for fire fighting if necessary.	
6. ACCIDENTAL RELEASE MEASURES Personal precautions Avoid dust formation. Environmental precautions Do not let product enter drains. Methods for cleaning up Sweep up and shovel. Keep in suitable, closed containers for disposal.	
7. HANDLING AND STORAGE Handling Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection. Storage Keep container tightly closed in a dry and well-ventilated place.	
8. EXPOSURE CONTROLS / PERSONAL PROTECTION Contains no substances with occupational exposure limit values. Personal protective equipment Respiratory protection Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Hand protection For prolonged or repeated contact use protective gloves. Eye protection Safety glasses	
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<p>Hygiene measures General industrial hygiene practice.</p>																					
<p>9. PHYSICAL AND CHEMICAL PROPERTIES</p> <p>Appearance</p> <table> <tr> <td>Form</td><td>powder</td></tr> <tr> <td>Colour</td><td>white</td></tr> </table> <p>Safety data</p> <table> <tr> <td>pH</td><td>6.0 - 7 at 115 g/l at 25 °C (77 °F)</td></tr> <tr> <td>Melting point</td><td>228 °C (442 °F)</td></tr> <tr> <td>Boiling point</td><td>no data available</td></tr> <tr> <td>Flash point</td><td>no data available</td></tr> <tr> <td>Ignition temperature</td><td>no data available</td></tr> <tr> <td>Lower explosion limit</td><td>no data available</td></tr> <tr> <td>Upper explosion limit</td><td>no data available</td></tr> <tr> <td>Water solubility</td><td>115 g/l at 20 °C (68 °F) - completely soluble</td></tr> </table>		Form	powder	Colour	white	pH	6.0 - 7 at 115 g/l at 25 °C (77 °F)	Melting point	228 °C (442 °F)	Boiling point	no data available	Flash point	no data available	Ignition temperature	no data available	Lower explosion limit	no data available	Upper explosion limit	no data available	Water solubility	115 g/l at 20 °C (68 °F) - completely soluble
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Upper explosion limit	no data available																				
Water solubility	115 g/l at 20 °C (68 °F) - completely soluble																				
<p>10. STABILITY AND REACTIVITY</p> <p>Storage stability Stable under recommended storage conditions.</p> <p>Materials to avoid Strong oxidizing agents</p> <p>Hazardous decomposition products Hazardous decomposition products formed under fire conditions. Carbon oxides, nitrogen oxides (NOx)</p>																					
<p>11. TOXICOLOGICAL INFORMATION</p> <p>Acute toxicity no data available</p> <p>Irritation and corrosion no data available</p> <p>Sensitisation no data available</p> <p>Chronic exposure no data available</p> <p>Signs and Symptoms of Exposure To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.</p> <p>Potential Health Effects</p> <table> <tr> <td>Inhalation</td><td>May be harmful if inhaled. May cause respiratory tract irritation.</td></tr> <tr> <td>Skin</td><td>May be harmful if absorbed through skin. May cause skin irritation.</td></tr> </table>		Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.	Skin	May be harmful if absorbed through skin. May cause skin irritation.																
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<p>Sigma - 81709</p> <p>Sigma-Aldrich Corporation www.sigma-aldrich.com</p> <p>Page 3 of 5</p>																					

Eyes	May cause eye irritation.	
Ingestion	May be harmful if swallowed.	
12. ECOLOGICAL INFORMATION		
Elimination Information (persistence and degradability)		
no data available		
Ecotoxicity effects		
no data available		
Further information on ecology		
no data available		
13. DISPOSAL CONSIDERATIONS		
Product		
Observe all federal, state, and local environmental regulations.		
Contaminated packaging		
Dispose of as unused product.		
14. TRANSPORT INFORMATION		
DOT (US)		
Not dangerous goods		
IMDG		
Not dangerous goods		
IATA		
Not dangerous goods		
15. REGULATORY INFORMATION		
OSHA Hazards		
No OSHA Hazards		
TSCA Status		
On TSCA Inventory		
DSL Status		
All components of this product are on the Canadian DSL list.		
SARA 302 Components		
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.		
SARA 313 Components		
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.		
SARA 311/312 Hazards		
No SARA Hazards		
Massachusetts Right To Know Components		
No Components Listed		
Pennsylvania Right To Know Components		
L-Proline	CAS-No. 147-85-3	Revision Date

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New Jersey Right To Know Components		
L-Proline	CAS-No. 147-85-3	Revision Date
California Prop. 65 Components		
This product does not contain any chemicals known to State of California to cause cancer, birth, or any other reproductive defects.		
16. OTHER INFORMATION		
Further Information		
Copyright 2007 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only.. The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.		
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Appendix 2 – Product Information Sheet Quinine Monohydrochloride

SIGMA-ALDRICH

sigma-aldrich.com

Material Safety Data Sheet

Version 4.1
Revision Date 07/23/2010
Print Date 03/05/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Quinine monohydrochloride dihydrate
Product Number : W297607
Brand : Aldrich
Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA
Telephone : +1 800-325-5832
Fax : +1 800-325-5052
Emergency Phone # : (314) 776-6555

2. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards

Target Organ Effect, Skin sensitizer

Target Organs

Eyes, ears, Nerves., Gastrointestinal tract, Pancreas., Skeletal muscle., Cardiovascular system.

GHS Label elements, including precautionary statements

Pictogram

Signal word : Warning

Hazard statement(s)

H302 : Harmful if swallowed.
H317 : May cause an allergic skin reaction.

Precautionary statement(s)

P261 : Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264 : Wash skin thoroughly after handling.
P270 : Do not eat, drink or smoke when using this product.
P272 : Contaminated work clothing should not be allowed out of the workplace.
P280 : Wear protective gloves.
P301 + P312 : IF SWALLOWED: Call a POISON CENTER or doctor/ physician if you feel unwell.
P302 + P352 : IF ON SKIN: Wash with plenty of soap and water.
P321 : Specific treatment (see supplemental first aid instructions on this label).
P330 : Rinse mouth.
P333 + P313 : If skin irritation or rash occurs: Get medical advice/ attention.
P363 : Wash contaminated clothing before reuse.
P501 : Dispose of contents/ container to an approved waste disposal plant.

HMIS Classification

Health hazard: 2
Chronic Health Hazard: -
Flammability: 0
Physical hazards: 0

NFPA Rating

Health hazard: 2

Fire: 0
Reactivity Hazard: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.
Skin May be harmful if absorbed through skin. May cause skin irritation.
Eyes May cause eye irritation.
Ingestion May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Molecular Weight : 396.92 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
(8α,9R)-6'-Methoxycinchonan-9-ol monohydrochloride			
6119-47-7	205-001-1	-	-

4. FIRST AID MEASURES

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters

Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions

Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

Environmental precautions

Do not let product enter drains.

Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling

Avoid contact with skin and eyes. Avoid formation of dust and aerosols.

Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage

Keep container tightly closed in a dry and well-ventilated place.

Light sensitive.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection

For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator. For higher level protection use type OVI/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection

Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection

Complete suit protecting against chemicals. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	white

Safety data

pH	no data available
Melting point	115 - 116 °C (239 - 241 °F) - dec.
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Chemical stability

Stable under recommended storage conditions.

Conditions to avoid

no data available

Materials to avoid

Strong oxidizing agents

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas

11. TOXICOLOGICAL INFORMATION

Acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
May cause allergic skin reaction.

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
prolonged or repeated exposure can cause: Blood disorders, Vomiting, Diarrhoea, Asthma

Additional information

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available		
13. DISPOSAL CONSIDERATIONS		
Product Offer surplus and non-recyclable solutions to a licensed disposal company.		
Contaminated packaging Dispose of as unused product.		
14. TRANSPORT INFORMATION		
DOT (US) Not dangerous goods		
IMDG Not dangerous goods		
IATA Not dangerous goods		
15. REGULATORY INFORMATION		
OSHA Hazards Target Organ Effect, Skin sensitizer		
DSL Status All components of this product are on the Canadian DSL list.		
SARA 302 Components SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.		
SARA 313 Components SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.		
SARA 311/312 Hazards Acute Health Hazard, Chronic Health Hazard		
Massachusetts Right To Know Components No components are subject to the Massachusetts Right to Know Act.		
Pennsylvania Right To Know Components		
(8a,9R)-6'-Methoxycinchonan-9-ol monohydrochloride	CAS-No. 6119-47-7	Revision Date
New Jersey Right To Know Components		
(8a,9R)-6'-Methoxycinchonan-9-ol monohydrochloride	CAS-No. 6119-47-7	Revision Date
California Prop. 65 Components This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.		
16. OTHER INFORMATION		
Further Information Copyright 2010 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only. The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.		

Appendix 3 – Coding Scheme Guidelines

Table 1. Behaviors and facial expressions - Universal Feline Behavior Coding Scheme

Guidelines

Start Recording: Start to record at the beginning of each video. If the cat is not visible at the start of the video, press the suspend button in the Observer and let the program be in suspend mode until **both eyes of the cat** can be observed. As soon as both eyes can be seen the coder will click the suspend button to stop the suspend mode and then start coding as usual.

The 2 second rule: The 2 second rule states that the cat must have been in the behavioral state for at least two seconds for the behavior to be scored. If the animal is in the behavioral state for less than 2 seconds the behavior is not to be coded in cases where the 2 second rule applies. **The 2 second rule is used as a behavior validation**, that is, the 2 second rule is a rule of thumb for when a behavior should be registered. The 2 second rule does, however, **not** indicate **when** to start coding a behavior. **The behavior should always be coded at the onset of the behavior**. This rule is used in order to confirm that the cat has entered a different behavioral state and not just merely paused in its ongoing behavioral state. The 2 second rule applies to the following state behavior groups, including modifiers;
Location and Body Position

The 2.5 cm rule: The 2.5 cm rule states that a behavior is to be coded if the cat is within the range of 2.5 cm from an object that the cat is interacting with. This applies to the spouts, walls, door and floor. If the cat is further away than 2.5 cm from the object that the cat has been interacting with, the state behavior is replaced with a stop state behavior. Example; The cat extends its head for a sniff. When the cat is 2.5 cm from the floor the behavior is coded. When the cat moves its head away to the extent that the head is now further away from the floor than 2.5 cm “No sniff/drink” is to be coded. State events where the 2.5 cm rule applies does not follow the 2 second rule. The 2.5 cm rule applies to the following state behavior group;
Sniffing & Drinking

The eye priority rule: The eye priority rule applies when coding eyes. It states that if the eyes of the cat **differ** in how much of the eye is visible to the observer, it's the eye with the **smallest area visible** to the observer that sets which eye behavior is to be coded. If only one of the eyes is visible to the observer, it's the visible area of **the eye that can be observed** that is to be coded.

Area of interest: All behaviors included in the coders guide are **only to be coded when the cat is within the area of interest** (Pic) defined by the guide. The only exceptions to this rule are so called “off-behaviors” that should be coded when the cat is out of the area of interest. The “off behaviors” are used to stop other event behaviors in their category to make sure that previous event behaviors are stopped and that other event behaviors are represented correctly when analyzing the data. The cat is said to be in the area of interest when the cat's nose is within the vertical plane of the curved area indicated on the floor extending between the front

corners and upward to 4/5 the height of the cage. The floor arch extends from the corners of the cage and peaks at the center of the cage floor.

Figure 3. The testing arena with the marked out area of interest. The area of interest is located within the half circle in the front part of the cage and below the upper line.

Feline Coded Behaviors for Drinking Tests

State Behavior = duration (indicate start and stop)	Point Behavior = occurs at a single point in time
---	---

Location		
	The area of interest is a half circle arching in the center of the cage and going out towards the intersecting corners of the cage and the Plexiglas door. The cat's nose is located in the area of interest for at least 2 seconds. Behaviors are only to be coded when the cat is located within the area of interest. (Fig 3.)	
1	In area of interest	Code: When the cats' nose is in the area of interest.
	<i>Modifiers for In area of interest</i>	
	Nose Left	Nose entirely in left half of cage Code: When the nose crosses the middle line of the cage.
	Nose Right	Nose entirely in right half of cage Code: When the nose crosses the middle line of the cage.

2	Out of Area of Interest	<p>The nose of the cat is not located in the area of interest. When the cat is out of the area of interest, no behaviors are to be coded.</p> <p>Code: When the cat's nose is outside of the area of interest.</p>
Body Position		
3	No coding (Out of Area of Interest)	<p>Cat is not located in the area of interest.</p> <p>Code: When the cat's nose is outside of the area of interest.</p>
4	Stand	<p>The cat is stationary and has its legs in an upright position and at least 3 of the paws are in direct contact with the floor.</p> <p>Code: At onset of behavior after verifying that the cat will be stationary for at least 2 seconds.</p>
5	Stand up	<p>The cat stands in an upright position and supports itself on its back paws or haunches. None of the front paws are in direct contact with the floor. If the cat stands up against the Plexiglas door with its nose outside the area of interest the location state "Out of area of interest" is coded together with "Stand up".</p> <p>Code: When the last front paw leaves the floor so that both front paws are off the floor simultaneously.</p>
6	Sit	<p>The hindquarters of the cat are in direct contact with the floor whilst its front legs and the upper body remain in an upright position.</p> <p>Code: When the hindquarters touches the floor.</p>
7	Tuck/Crouch	<p>The shoulders and hips are in upright position but the legs are not fully erected as in a stand. The cat may still support itself a bit with the paws, have its paws tucked in or have the legs extended along the floor. The body of the cat is lowered to the floor and the cat supports its weight ventrally.</p> <p>Code: When the hindquarters are in contact with the floor and the cat has its upper body in a lowered position.</p>
8	Lie down	<p>Weight bearing is either ventral or lateral and none of the legs are fully erected. Either the hip, the shoulder or both are not in upright position but slightly rotated to the side.</p> <p>Code: When the hindquarters and the elbows of the cat simultaneously are in contact with the floor.</p>
9	Movement (Step/Walk)	<p>Movement is defined as a directed step or walk performed by the cat. For simplicity, each individual step longer than 2.5 cm is regarded as movement. Movement is not to be coded if the cat is only shifting its weight, pawing at the floor or if the hindquarters are still in direct contact with the floor. The cat must take a step.</p> <p>Code: The first paw in the step is no longer in direct contact with the floor. The movement is said to have come to a stop when the cat has been stationary for more than 2 seconds. If the cat has been stationary for less than 2 seconds the behavior is coded as continuous movement.</p>

Sniffing and Drinking		
		The cat is not sniffing or licking at or drinking from the interior of the cage or the spouts. No ingestion can be observed. Not drinking or sniffing is also to be used when the cat is outside the area of interest.
10	Not drinking or sniffing	Code: When the cat is more than 2.5 cm from something that it can sniff or drink.
		The cat is interacting orally with the interior of the cage without ingesting.
11	Non-ingestive lick	Code: When the cat starts licking in an area that is not located close to the spouts or the floor below the spouts.
	<i>Modifiers for Non-ingestive lick</i>	
	Non-ingestive lick Left	<i>Lick whilst the nose is entirely in the left half of the cage.</i>
	Non-ingestive lick Right	<i>Lick whilst the nose is entirely in the right half of the cage.</i>
		The cat is ingesting liquid from the spout either via extending its tongue and then retracting it in a lapping motion so that the tongue comes in direct contact with the end of the spout or by putting the end of the spout in its mouth and suckle upon it.
12	Drinking from Spout (ingestive)	Code: At the first lick or when the mouth comes in contact with the spout.
	<i>Modifiers for Drinking from Spout</i>	
	Drinking from spout Left	<i>Ingesting from the spout located to the left from the observers point of view.</i>
	Drinking from spout Right	<i>Ingesting from the spout located to the right from the observers point of view.</i>
		The cat is drinking from either the door or the floor directly below the spout. The tongue is extended and then retracted back into the cats' mouth. Whilst extended, the tongue comes in contact with dripped solution on the surface of the cage and results in ingestion.
	Drinking dripped solution from floor or door (ingestive)	Code: At the first lick or when the mouth comes in contact with solution on the door or floor just below the spouts.
	<i>Modifiers for Drinking dripped solution from floor or door</i>	

	Drink dripped solution Left	ingesting whilst the nose is entirely in the left half of the cage.
	Drink dripped solution Right	ingesting whilst the nose is entirely in the right half of the cage.
13	Sniffs Spout	The cat is sniffing the spout. The head and neck is visibly extended and directed towards the spout or the dripped solution. No ingestion is observed. Code: When the cat is sniffing and within 2.5 cm of the spout.
<i>Modifiers for Sniffs Spout</i>		
	Sniffs spout Left	head, neck and nose indicate sniffing within 2.5 cm of the left spout
	Sniffs spout Right	head, neck and nose indicate sniffing within 2.5 cm of the right spout
14	Sniffs Other	The cat is sniffing the interior of the cage. The head and neck is visibly extended and directed towards a point in the cage. No ingestion is observed. Code: When the nose of the cat is within 1 inch of the object of interaction.
<i>Modifiers for Sniffs Other</i>		
	Sniffs Other Left	When the nose is entirely in the left half of the cage.
	Sniffs Other Right	When the nose is entirely in the right half of the cage.
Oral Grooming		
15	Not grooming	Not licking any part of body Code: When the cat has not groomed itself for at least 2 seconds.

16	Grooming	<p>The tongue of the cat is in direct contact with the body of the cat. Grooming does not include scratching or ingestive behaviors as nose lick, lip lick or whisker lick.</p> <p>Code: When the tongue or paw first comes in direct contact with the rest of the cats body in a grooming motion.</p>
	<i>Modifiers for Grooming</i>	
	<i>Licks body other than face or paw</i>	<p><i>Licks a body part other than face or paw. The cat performs a stroking motion with its head allowing it to lick the fur on the body.</i></p> <p>Code: When the tongue first comes in contact with the body.</p>
	<i>Lick paws only</i>	<p><i>The cats' tongue is in direct contact with any of its paws without the paw coming in direct contact with the facial area or the head of the cat.</i></p> <p>Code: When the tongue touches the paw.</p>
	<i>Facial Grooming</i>	<p><i>The cat moves its paw so it comes in direct contact with the facial area or the head of the cat. If paw licking is also performed, code as facial grooming.</i></p> <p>Code: When the paw first touches the head.</p>
	<i>Miscellaneous</i>	<i>Miscellaneous grooming behavior, described</i>
Eyes (during sniffing spout and ingestive behaviors only)		
17	No Eyes (not recording)	The cat is neither ingesting liquid nor interacting with the spouts.
18	Eyes open 50-100%	<p>The eyes of the cat have a rounded shape and only slight muscular contraction in the area around the eyes can be observed. More than half of the full area of the eyes is visible to the observer.</p> <p>Code: At onset of behavior.</p>
19	Eyes open <50%	<p>The eyes of the cat are not fully open, rather the cat is squinting. A contraction can be observed that narrows the eye down to a slit rather than a rounded shape. The area of the eye visible to the observer is then less than 50 % of the area that can be observed when the cat has its eyes fully open.</p> <p>Code: At onset of behavior.</p>
20	Eyes closed	<p>The eyes are closed and not visible to the observer forming a solid line in the cats face as opposed a slit or a rounded shape. The eye itself should in no way be observable. Only when the eyes are completely shut the behavior for closed eyes is to be coded.</p> <p>Code: At onset of behavior.</p>
21	Eyes Undetermined	The eyes of the cat are not visible to the observer.
Ears (during sniffing spout and ingestive behaviors only)		

22	No Ears (not recording)	The cat is neither ingesting liquid nor interacting with the spouts.
23	Ears upward &/or forward	Both ears of the cat are perked upward, attentive, or forward. The ears form a 50 to 90° angle with the scalp . Code: At onset of behavior.
24	Ears lowered &/or outward	The bases of the ears are further apart than when forward; possibly turned outward. The ears form an angle below 50° with the scalp . Code: At onset of behavior.
25	Ears different positions	The ears are held in different positions compared to the scalp. Example; one ear is in the upright position whilst the other one is folded outward. Code: At onset of behavior.
Point Behaviors		
1	Licks lip (formerly "microlick")	The tongue is extended upwards and out of the mouth towards the nose but does not reach the nose or the whiskers and instead only covers the bottom of the upper lip ("microlick"). Code: At peak of behavior.
2	Licks nose	Flicks tongue quickly over the nose only. The tongue is extended upwards and covers any part of the cats' nose . The tongue goes up to the nose and then back into the mouth without continuing into a whisker lick. The mouth is generally less open in a nose lick than in a whisker lick. Code: At peak contact with the nose.
3	Licks whiskers	The cat sweeps its tongue to the side of its face (may start as nose lick). The mouth is in most cases more open than it would be in a nose lick. If the tongue is extended upward to whiskers without the sweeping motion and without any nose contact it is also to be coded as a whisker lick. Code: At peak of behavior when the tongue is extended over the whiskers or when the tongue comes into contact with the whiskers without first sweeping over the nose.
4	Paws cage	The cat lifts one or both of its front paws depending on its body position. The motion is then continued so that one or both of the front paws touches any part of the cage or its interior except the floor. Code: When the paw touches the cage.
5	Rubs with body	The body of the cat is performing a stroking motion resulting in direct contact with the interior of the cage or the spout. Code: At onset of behavior.
6	Hunch	The cat arches its back upwards but does not rub its body against the interior of the cage. Cat can be seen as using legs to push their back up. Slight extension of legs might be observed.

		Code: Just before the cat lowers it back again out of the hunch.
7	Rubs with face	The cat performs a stroking motion with its head resulting in the facial area coming in direct contact with the interior of the cage or a spout. Code: At onset of behavior.
8	Scratch	The cat lifts one of its paws to result in a quick stroking motion in direct contact to the rest of the body of the cat. The movement might be repeated several times. No oral grooming can be observed. Code: At the start of the first scratch when the paw touches the body. Code once at onset of behavior.
10	Shake body	The cat performs a rapid side to side motion with its body resulting in a shaking movement that involves the head and moves beyond the shoulder area of the cat involving its back into the movement. Code: At the start of the shake.
11	Shake head	The cat performs a rapid side to side motion with its head resulting in a shaking movement that involves the head and can reach down to the shoulder area of the cat. The movement does, however, never reach beyond the shoulder area. Code: At the start of the shake.
12	Head Twist	The cat performs a single rapid side to side motion with its head. The motion is less extensive than that of a head shake. Head Twist is a slower, more controlled, movement than "Shake Head". Code: At the start of the twist.
13	Tongue protrusion	The tongue is visible and directed forward out of the mouth and not reaching upwards towards the lip or nose of the cat. The tongue is not in contact with any object. Code: When tongue is visible.
14	Tongue Protrusion Gape	The tongue is visible and directed forward out of the mouth whilst the cat has its mouth wide open in a gape. The tongue is not reaching upwards towards the lip or nose of the cat. The tongue is not in contact with any object. Code: At peak of behavior.
15	Air Lick/ Pre –Drink Lick	The cat performs a lapping motion with its tongue out in the air before the onset of drinking. The tongue does not touch the spout or any part of the cage. Code: At peak of behavior.
16	Flehmen	The cat opens its mouth without closing it immediately. The open mouth is held open and there is no closing in between as you

		would see with mouthing. Code: Once at the starting point of the behavior when the mouth starts to open.
17	Mouthing	The cat performs a repeated chewing motion with its lower jaw and might slightly open its mouth in between the movements of the jaw. Mouthing is performed without any form of ingestion. Mouthing may, however, follow directly after ingestion has been performed. During mouthing, the tongue is not visible. Code: Once at the starting point of the behavior when the movement is first visible and not for each individual jaw movement.
18	Mouth Smack	The cat performs a single chewing motion with its lower jaw and might slightly open its mouth. Mouth smack is performed without any form of ingestion. Mouth smack may, however, follow directly after ingestion has been performed. During mouth smack the cat may push its lip slightly outward. Code: At peak of behavior. Each instance of mouth smack is to be coded.
19	Wretch	Cat heaves but does not vomit
20	Vomit	Cat vomits
21	Flinch/Startle	Cat reacts suddenly to environmental stimulus. The cat performs a snapping motion with its body that interrupts the currently ongoing behavior. After the snap, the ears are in upright position and eyes fully open.
22	Miscellaneous	Miscellaneous Point Behavior
23	Bite	The cat bites on the spout. The cat has put a part of the spout into its mouth and closes its jaws around it.
24	Paw Shake	The cat lifts a single paw and performs a rapid shaking motion with its leg. Code: When the shaking motion is first observed.
25	Paw Curl	The cat lifts a single paw and curls the paw and/or its leg towards its body. The leg of the cat is positioned in a distinct C-shape . The behavior includes the whole leg. If the cat only bends its paw it is not to be coded as a paw curl. Code: When the curling motion is first observed.