Linköping University | Department of Physics, Chemistry and Biology
Master thesis, 60 hp | Educational Program: Physics, Chemistry and Biology
Spring term 2019 | LITH-IFM-x-EX—19/3606--SE

Gustatory response to dietary ethanol in spider monkeys (*Ateles geoffroyi*)

Daniel Dausch-Ibañez

Examinator, Jennie Westander
Tutor, Matthias Laska

LINKÖPINGS UNIVERSITET

Avdelning, institution
Division, Department

Department of Physics, Chemistry and Biology
Linköping University

Datum

Date

2019-03-18

Språk
Language

☐ Svenska/Swedish
☒ Engelska/English

☐ ______________

Rapporttyp
Report category

☐ Licentiatavhandling
☒ Examensarbete
☐ C-uppsats
☐ D-uppsats
☐ Övrig rapport

☐ ____________

ISBN

ISRN: LITH-IFM-x-EX--19/3606--SE

Serietitel och serienummer **ISSN**

Title of series, numbering ____________________

URL för elektronisk version

Titel

Gustatory response to dietary ethanol in spider monkeys (*Ateles geoffroyi*)

Författare

Author: Daniel Dausch-Ibañez

Sammanfattning

Abstract

Recent studies suggest that frugivorous primates might display a preference for the ethanol produced by microbia in overripe, fermenting fruit as an additional source of calories. The present study therefore assessed taste responsiveness of eight spider monkeys (*Ateles geoffroyi*) to dietary ethanol and determined taste preference thresholds for ethanol and sugarcane alcohol, as well as relative taste preferences for ethanol presented in sweet-tasting solutions and in fruit matrices, respectively. A two-bottle preference test of short duration (1 min) was used, in which ethanol solutions between 0.05% and 3% were tested against either water, a sucrose solution, or pureed fruit. The determined taste preference thresholds for ethanol and sugarcane alcohol were both at 0.5% ethanol, and all tested concentrations above the threshold were significantly preferred over water. The spider monkeys significantly preferred sucrose solutions spiked with 0.1, 0.5 and 3% ethanol over non-spiked solutions of the same sucrose concentration (30mM and 60mM), but significantly rejected the ethanol-spiked 30mM solution when tested against a sweeter (60mM) sucrose solution without ethanol. The spider monkeys significantly preferred pureed fruits (mango, melon, and papaya) spiked with 3% ethanol when tested against the same fruits without ethanol, and the degree of preference for the ethanol-spiked puree increased with the sugar content of the fruit. These results show that spider monkeys display a preference for the taste of ethanol at concentrations found in fermenting fruit, but also determined that they are more attracted to sweet taste than to ethanol.

Keywords:

Alcohol, spider monkeys, taste responsiveness, taste preference thresholds

Contents

1 Abstract

Recent studies suggest that frugivorous primates might display a preference for the ethanol produced by microbia in overripe, fermenting fruit as an additional source of calories. The present study therefore assessed taste responsiveness of eight spider monkeys (*Ateles geoffroyi*) to dietary ethanol and determined taste preference thresholds for ethanol and sugarcane alcohol, as well as relative taste preferences for ethanol presented in sweet-tasting solutions and in fruit matrices, respectively. A two-bottle preference test of short duration (1 min) was used, in which ethanol solutions between 0.05% and 3% were tested against either water, a sucrose solution, or pureed fruit. The determined taste preference thresholds for ethanol and sugarcane alcohol were both at 0.5% ethanol, and all tested concentrations above the threshold were significantly preferred over water. The spider monkeys significantly preferred sucrose solutions spiked with 0.1, 0.5 and 3% ethanol over non-spiked solutions of the same sucrose concentration (30mM and 60mM), but significantly rejected the ethanol-spiked 30mM solution when tested against a sweeter (60mM) sucrose solution without ethanol. The spider monkeys significantly preferred pureed fruits (mango, melon, and papaya) spiked with 3% ethanol when tested against the same fruits without ethanol, and the degree of preference for the ethanol-spiked puree increased with the sugar content of the fruit. These results show that spider monkeys display a preference for the taste of ethanol at concentrations found in fermenting fruit, but also determined that they are more attracted to sweet taste than to ethanol.

Keywords:

Alcohol, spider monkeys, taste responsiveness, taste preference thresholds

2 Introduction

Ethanol is a natural by-product of the microbial fermentation of fruit sugars (Janzen 1977). Its production starts when overripe fruits lose their structural integrity so that microbia can invade the fruit pulp which contains the fruit sugars (Dominy 2004). Recent studies suggest that this so-called dietary ethanol might be used by frugivorous primates as a supplemental source of calories (Gochman et al. 2016). Some authors even claim that the predilection of nonhuman primates for alcohol-containing overripe fruits would reflect the evolutionary origin of human alcoholism (Dudley 2000; Dudley 2014). Other authors, however, emphasize that the ethanol content in overripe fruits is usually low and thus unlikely to substantially contribute calories and that frugivorous animals usually prefer ripe over overripe fruits (Levey 2004; Milton 2004; McGrew 2011).

Behavioral tests on the voluntary consumption of ethanol in a variety of nonhuman primate species yielded mixed results: Pigtail macaques *(Macaca nemestrina)* showed no preference for a 5% ethanol solution when tested against water, and clearly rejected higher ethanol concentrations of 10% and 20% (Anderson and Smith 1963). The same species, presented with 10% ethanol in orange juice, readily consumed this mixture when no alternative liquid source was offered (Elton et al. 1976). Squirrel monkeys *(Saimiri sciureus)* consumed 5% and 10% ethanol when no alternative liquid source was offered (Kaplan et al. 1982) and were found to accept ethanol concentrations of 1-4% when diluted in sucrose solutions, again, with no alternative liquid source offered (Mandillo et al. 1998). Rhesus macaques *(Macaca mulatta)* were found to prefer 2% and 4% solutions of ethanol over water, but not higher concentrations, in three-bottle choice tests (Kornet et al. 1990).

Chimpanzees *(Pan troglodytes)* have been observed to voluntarily ingest the fermented sap of the raffia palm from human-made receptacles which may contain ethanol concentrations up to 4% (Hockings et al. 2015). Aye-ayes *(Daubentonia madagascarensis)* and slow lorises *(Nycticebus coucang)* were found to prefer sucrose solutions spiked with 2% and 4% ethanol over sucrose solutions spiked with lower concentrations of ethanol in five-alternative choice tests (Gochman et al. 2016).

It should be emphasized that the majority of the studies mentioned above aimed at assessing the mechanisms underlying alcohol dependence and thus used concentrations of ethanol that are often higher than those found in fermenting fruit. According to previous publications, the usual range of ethanol concentrations found in naturally fermenting fruit ranges from 0.04% to 4.5% (Eriksson and Nummi 1982, Dudley 2002, 2004, Dominy 2004, Sanchez et al. 2004).

Additionally, these studies usually employed long-term access to ethanol so that post-ingestive factors (in the case of ethanol: intoxication effects) were sought for. Similarly, studies in mice *(Mus musculus)* (Belknap et al. 1993), Egyptian fruit bats *(Rousettus aegyptiacus)* (Sanchez et al. 2004; Korine et al. 2011), African elephants *(Loxodonta africana)* (Morris et al. 2006), tree shrews *(Ptilocercus lowii)* (Wiens et al. 2008), and frugivorous birds (Zungu and Downs 2017) usually employed unphysiologically high concentrations of ethanol and long-term exposure to the stimulus. Thus, there is a clear need for behavioral tests assessing the responsiveness of different species, in particular non-human primates, to ethanol concentrations that are naturally found in fermenting fruits and which clearly separate between consumption based on the taste of ethanol and on its post-ingestive effects, respectively.

Spider monkeys are highly frugivorous New World primates and specialize on consuming ripe fruit (Di Fiore et al. 2010; Gonzalez-Zamora et al. 2009). Thus, they are a particularly suitable species to further assess the taste responsiveness of nonhuman primates to dietary ethanol. Their responsiveness to sweet (Laska et al. 1996, 1998, 2001), sour (Laska et al. 2000, 2003), bitter (Laska et al. 2009), salty, and umami (Laska and Hernandez Salazar 2004; Laska et al. 2008) taste stimuli has been assessed in previous studies. The ethanol concentrations used in the present study ranged from 0.05% to 3%, as I tried to cover a broad part of the concentrations found in naturally fermenting fruit.

It was therefore the aim of the present study to assess taste responses of spider monkeys to ethanol presented at concentrations found in fermenting, overripe fruits. To this end, a two-bottle preference test of short duration was employed (Richter and Campbell 1940a).

The first experiment aimed to determine the spider monkeys' absolute taste preference threshold and absolute taste preference for ethanol solutions between 0.05% and 3% tested against water. The second experiment assessed the animals' relative preference for ethanol by testing mixtures of ethanol and sucrose against sucrose solutions without ethanol. This experiment also determined the attractiveness of ethanol to spider monkeys relative to sucrose. The third experiment assessed the spider monkeys' taste preference for ethanol in fruit matrices by presenting the animals with ethanol-spiked pureed fruit against pureed fruit without ethanol.

3 Materials & Methods

3.1 Animals

Testing was carried out using five male and three female adult spider monkeys (*Ateles geoffroyi*) (Figure 1) aged between 4 and 25 years. Unfortunately, the age of the animals is onl approximative, as they come from rescue backgrounds, thus making any correlational analysis between their age and the results irrelevant. Not all animals participated in all three experiments, as some did not show any motivation to participate in the first experiment. They were kept at the field station UMA Doña Hilda Ávila de O'Farrill of the Universidad Veracruzana, near Catemaco, in the state of Veracruz, Mexico. Six of the animals were kept in enclosures under natural light conditions, while the other two were free roaming. All of them were fed fresh fruits and vegetables every day, in amounts that there were still leftovers present in the feeding areas the next morning. The animals kept in the enclosures had no access to water, as the water contained in the fruit was enough to quench their thirst. Some of the animals had participated in previous studies using the method described below (Larsson et al. 2014, Laska 1994, 1996, 1997, 1999, Laska et al. 2000, 2001, 2003, Laska and Hernandez Salazar 2004) and were already familiar with the procedure.

Figure 1: A spider monkey (Ateles geoffroyi)

3.2 Taste Stimuli

The stimuli used were ethanol (CAS# 64-17-5), sugarcane alcohol, and sucrose (CAS# 57-50-1). The pure ethanol was chemically synthesized whereas the sugarcane alcohol was distilled from *Saccharum officinarum*. The two substances clearly differed in both taste and smell as perceived by humans. Pure ethanol and 96% sugarcane alcohol were used for the first experiment while only pure ethanol was used for the experiments two and three. During the first and the second experiment, solutions were prepared using purified water, while in the third experiment, ethanol was diluted using pureed fresh fruit. The ethanol and the sucrose were obtained from Sigma-Aldrich (St. Louis, Missouri, USA), and the sugarcane alcohol was obtained from Alcoholera Orozco Hermanos (San Andrés Tuxtla, Veracruz, Mexico).

The stimuli were prepared using weight percentages, as equipment in the field allowed for more precision in that way.

3.3 Procedure

A two-bottle preference test of short duration (Richter and Campbell 1940a) was used to determine gustatory responsiveness of the spider monkeys to the ethanol and sugarcane alcohol solutions. The tests were performed between three and six times per day and per individual and took place in the morning before feeding and/or in the afternoon at least one hour after feeding, with 30-minute intervals between tests. Each pair of stimuli was presented ten times. Bottle position was pseudo-randomized between and within individuals to minimize the effect of possible position preferences. The spider monkeys were allowed to drink for 1 minute from a pair of 100mL graduated cylinders with metal drinking spouts (Figure 2). The volume consumed from each bottle was used to assess gustatory responsiveness to the presented stimuli. If an animal drank only from one bottle without trying the other one within the first 10 seconds of a given trial, the first bottle was removed so that the animal tried the second one, which was then also removed and then re-presented simultaneously with the first bottle.

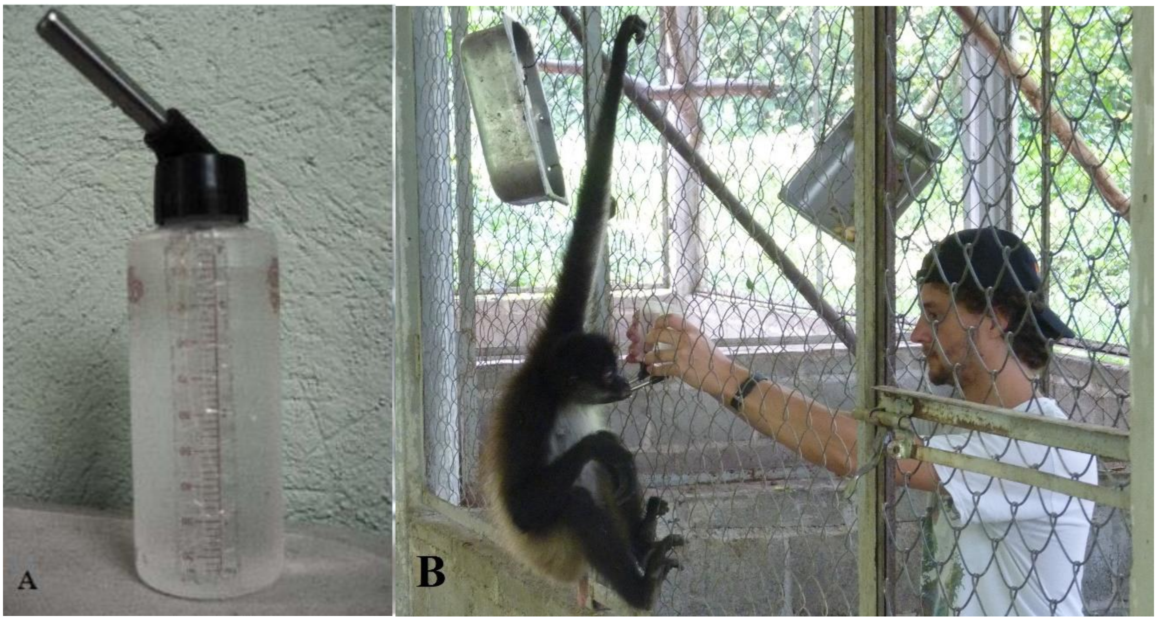

Figure 2: A) One of the cylinders used in the tests B) The two-bottle preference test

Determination of absolute preference and taste preference threshold for ethanol

To assess the taste preference threshold and the absolute preference for ethanol, six spider monkeys were given the choice between purified water and different concentrations of ethanol diluted in purified water.

Determination of absolute preference and taste preference threshold for sugarcane alcohol

To assess if the impurities in sugarcane alcohol, which were detectable for humans both as a taste and as a smell different from the pure ethanol, affected the absolute preference and taste preference threshold, the experiment was repeated using sugarcane alcohol instead of pure ethanol. With the sugarcane alcohol, dilutions were made so that the tested solutions contained 0.05% to 3% ethanol, and not 0.05% to 3% sugarcane alcohol, to make sure the results from both experiments were comparable.

In both aforementioned experiments aiming to assess taste preference thresholds, testing started at a concentration of 3% ethanol and decreased as follows: 2%, 1.5%, 1%, 0.5%, 0.1%, 0.05%, etc. until an animal failed to show a significant preference or aversion. To maintain the animals' motivation to cooperate when presented with less attractive concentrations, the testing did not follow the order of decreasing concentrations but was pseudo-randomized.

Determination of relative preference for ethanol

Relative preference for ethanol was assessed by presenting eight spider monkeys with a sucrose solution in one bottle and a sucrose solution spiked with ethanol (0.1%, 0.5%, and 3%) in the other bottle. The experiment was subdivided into three test series, with the sucrose concentration of the stimuli varying between the series.

In the first test series, both the spiked and the non-spiked stimuli had a sucrose concentration of 30mM.

In the second test series, the experiment was repeated with a sucrose concentration of 60mM for both stimuli. This was done in order to assess if the degree of sweetness had an effect on the spider monkeys' taste responsiveness to ethanol.

In the third test series, the experiment was repeated with a 60mM non-spiked sucrose solution and an ethanol-spiked (0.1%, 0.5%, and 3%) sucrose solution of 30 mM. This was done in order to assess if preference for the ethanol-spiked solution was due to it being hypercaloric compared to the non-spiked alternative in the previous test series.

In the third test series, the 0.1% and 0.5% ethanol-spiked sucrose solutions contained less calories than the non-spiked alternative, and the 3% ethanol-spiked sucrose solution contained more calories than the non-spiked alternative, based on caloric values of 4 kcal/g for sucrose and 7 kcal/g for ethanol (Cederbaum 2012).

Assessment of taste preference for alcohol in fruit matrices

To assess taste preference in conditions that aimed to mimic the overripe fruit found in the wild even better than a simple mixture of ethanol and sucrose, three different kinds of pureed fruit were presented to the spider monkeys which either were spiked with ethanol (3%) or not. Fruits used were mango (*Mangifera indica*), cantaloupe melon (*Cucumis melo*), and papaya (*Carica papaya*), as all three have different textures and sweetness and thus cover a certain range of the "flavour-palette" found in the spider monkeys' diet. The fruit was pureed thoroughly enough to be presented in the same bottles as used in the previous experiments.

3.4 Data analysis

For each test series or experiment, the volume of liquid consumed from each bottle was recorded for every trial and monkey. After ten trials, the volumes consumed from each bottle were converted into percentages relative to the total volume consumed from both bottles. The criteria for a preference at the individual level were as follows: an animal was only regarded as significantly preferring one of the two alternative stimuli if it (1) reached the criterion of 66.7% preference (relative to the total amount of liquid consumed) and (2) consumed more from the bottle containing the preferred stimulus in at least eight out of ten trials. According to the binomial test, the ratios 9/10 and 10/10 correspond to $p< 0.01$, and the ratio 8/10 corresponds to $p<0.05$.

4 Results

4.1 Determination of absolute taste preference and taste preference threshold for ethanol

Figure 3 summarizes the mean performance of the six spider monkeys in the two-bottle preference test when presented with ethanol concentrations ranging between 0.05% and 3% and purified water as the alternative stimulus. With concentrations between 0.5% and 3.0%, the animals clearly preferred the ethanol solutions over water and met both preference criteria. With the concentration of 0.1%, the animals still showed a preference for the ethanol but only met one of the two preference criteria, and with 0.05% ethanol they failed with both preference criteria. Appendix 1 shows the detailed results for each individual as well as the group average and standard deviation for each of the tested ethanol concentrations.

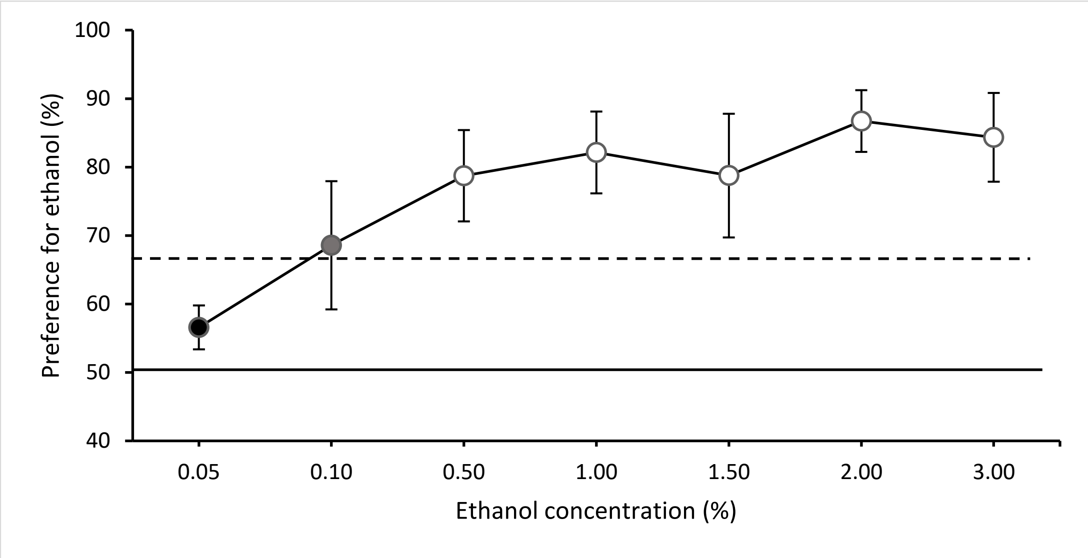

Figure 3: Taste responsiveness of six spider monkeys to various concentrations of ethanol tested against purified water as an alternative. Each symbol represents the mean value (±SD) of ten trials per animal and per concentration, for the six monkeys tested. White symbols represent concentrations for which both preference criteria (66.7% preference, and binomial test $p<0.05$) were met, grey symbols represent concentrations for which only of the two preference criteria was met, and black symbols represent concentrations for which none of the two preference criteria were met. The horizontal solid line represents chance level (50% preference) and the horizontal dotted line represents the first preference criterion (66.7% preference).

4.2 Determination of absolute taste preference and taste preference threshold for sugarcane alcohol

Figure 4 summarizes the mean performance of the six spider monkeys in the two-bottle preference test when presented sugarcane alcohol solutions containing ethanol concentrations ranging between 0.05% and 3% and purified water as the alternative stimulus. With concentrations between 0.5% and 3.0% ethanol, the animals clearly preferred the sugarcane alcohol solutions over water and met both preference criteria. With the ethanol concentration of 0.1%, the animals still showed a preference for the sugarcane alcohol but only met one of the two preference criteria, and with 0.05% ethanol they failed with both preference criteria.

Appendix 2 shows the detailed results for each individual as well as the group average and standard deviation for each of the tested ethanol concentrations.

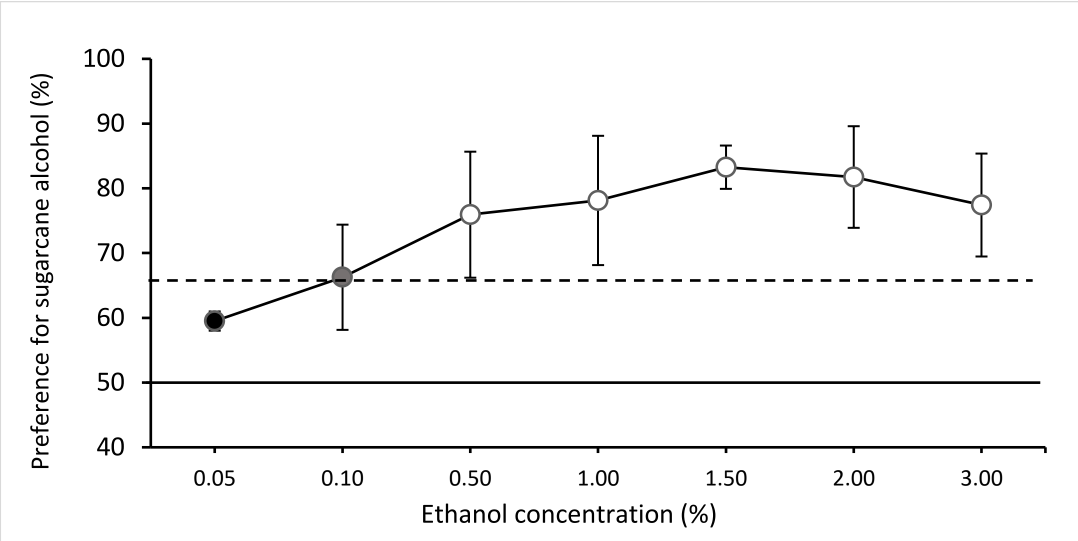

Figure 4: Taste responsiveness of six spider monkeys to various concentrations of sugarcane alcohol tested against purified water as an alternative. Each symbol represents the mean value (±SD) of ten trials per animal and per concentration, for the six monkeys tested. White symbols represent concentrations where both preference criteria were met (66.7% preference, and binomial test p<0.05), grey symbols represent concentrations where only of the two preference criteria was met, and black symbols represent concentrations where none of the two preference criteria were met. The horizontal solid line represents chance level (50% preference) and the horizontal dotted line represents the first preference criterion (66.7% preference).

4.3 Determination of relative taste preference for ethanol

Figure 5 summarizes the mean performance of the eight spider monkeys in the two-bottle preference test when presented with 30mM sucrose solutions spiked with 0.1%, 0.5%, and 3% ethanol, respectively, and a 30mM sucrose solution without ethanol as the alternative stimulus. With all three tested concentrations, the animals clearly preferred the ethanol-spiked solutions over the non-spiked alternative and met both preference criteria, not only as a group but also individually. Appendix 3 shows the detailed results for each individual as well as group means and standard deviation for the three tested ethanol concentrations.

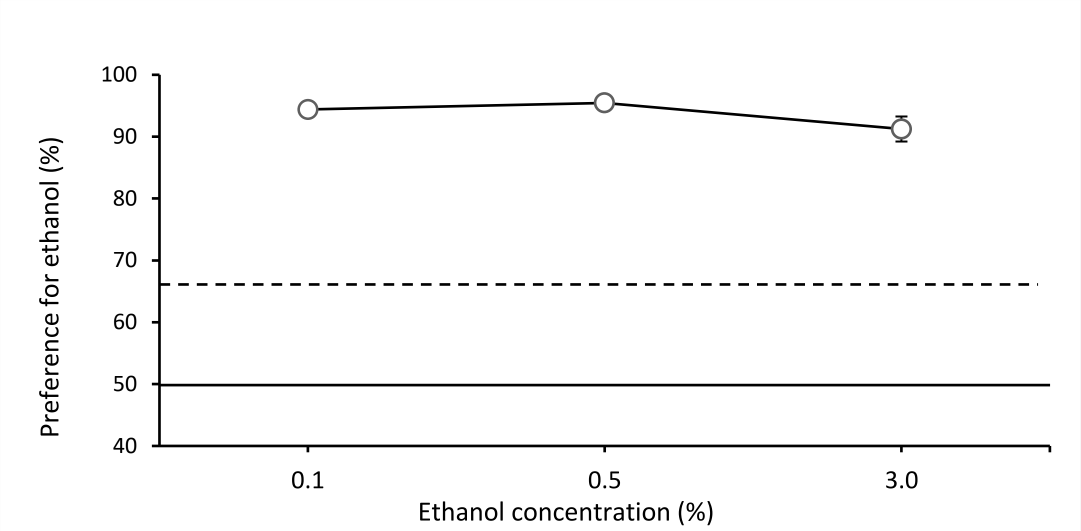

Figure 5: Taste responsiveness of eight spider monkeys to various concentrations of ethanol diluted in a 30mM sucrose solution tested against a 30mM sucrose solution without ethanol as the alternative. Each symbol represents the mean value (±SD) of ten trials per animal and per concentration, for the eight monkeys tested. White symbols represent concentrations where both preference criteria were met (66.7% preference, and binomial test $p<0.05$). The horizontal solid line represents chance level (50% preference) and the horizontal dotted line represents the first preference criterion (66.7% preference).

Figure 6 summarizes the mean performance of the eight spider monkeys in the two-bottle preference test when presented with 60mM sucrose solutions spiked with 0.1%, 0.5%, and 3% ethanol, respectively, and a 60mM sucrose solution without ethanol as the alternative stimulus. With all three tested concentrations, the animals clearly preferred the ethanol-spiked solutions over the non-spiked alternative and met both preference criteria, not only as a group but also individually. Appendix 4 shows the detailed results for each individual as well as group means and standard deviation for the three tested ethanol concentrations.

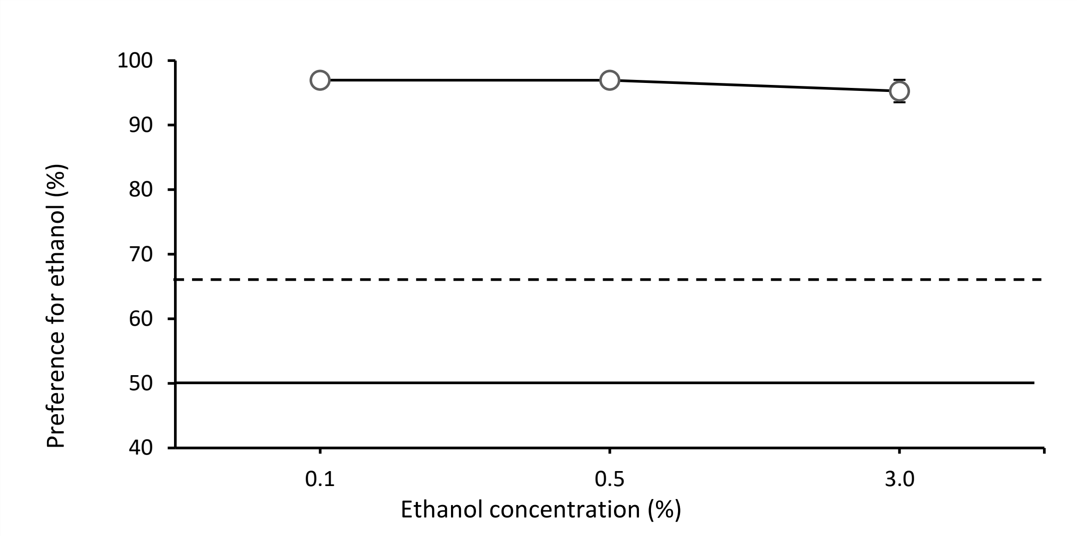

Figure 6: Taste responsiveness of eight spider monkeys to various concentrations of ethanol diluted in a 60mM sucrose solution tested against a 60mM sucrose solution without ethanol as the alternative. Each symbol represents the mean value (±SD) of ten trials per animal and per concentration, for the eight monkeys tested. White symbols represent concentrations where both preference criteria were met (66.7% preference, plus binomial test $p<0.05$). The horizontal solid line represents chance level (50% preference) and the horizontal dotted line represents the first preference criterion (66.7% preference).

Figure 7 summarizes the mean performance of the eight spider monkeys in the two-bottle preference test when presented with 30mM sucrose solutions spiked with 0.1%, 0.5%, and 3% ethanol, respectively, and a 60mM sucrose solution as the alternative stimulus. With all three tested concentrations, the animals clearly rejected the ethanol-spiked solutions over the alternative and met both rejection criteria, not only as a group but also individually. Appendix 5 shows the detailed results for each individual as well as group means and standard deviation for the three tested ethanol concentrations.

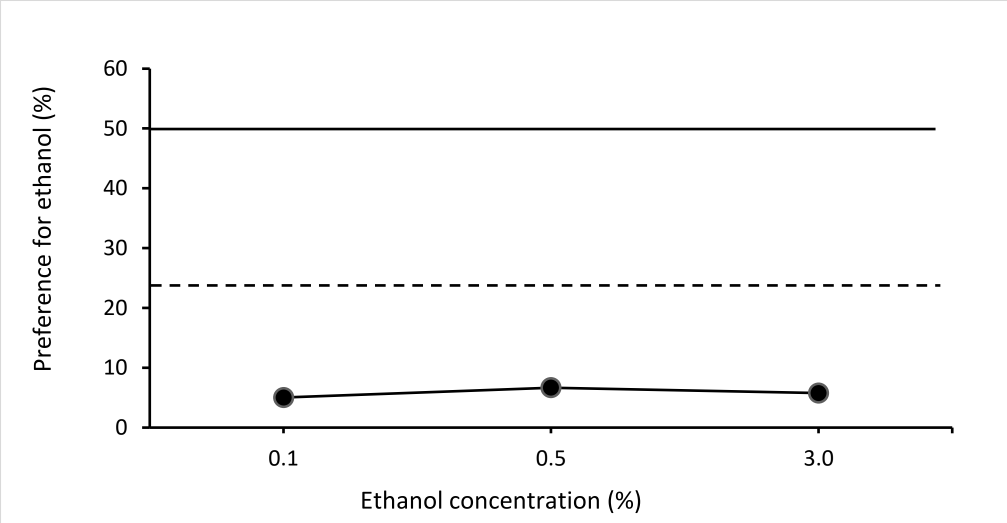

Figure 7: Taste responsiveness of eight spider monkeys to various concentrations of ethanol diluted in a 30mM sucrose solution tested against a 60mM sucrose solution without ethanol as the alternative. Each symbol represents the mean value (±SD) of ten trials per animal and per concentration, for the eight monkeys tested. Black symbols represent concentrations where none of the two preference criteria were met. The horizontal solid line represents chance level (50% preference) and the horizontal dotted line represents the first rejection criterion (66.7% rejection).

4.4 Assessment of taste preference for ethanol in fruit matrices

Figure 8 summarizes the mean performance of the eight spider monkeys in the two-bottle preference tests when presented with pureed fruit spiked with 3% ethanol, and a non-spiked pureed fruit as the alternative. With all three fruit types tested (mango, melon, and papaya), the animals as a group significantly preferred the ethanol-spiked pureed fruits over the non-spiked alternative and met both preference criteria. Appendix 6 shows the detailed results for each individual as well as group means and standard deviation for the three tested ethanol concentrations.

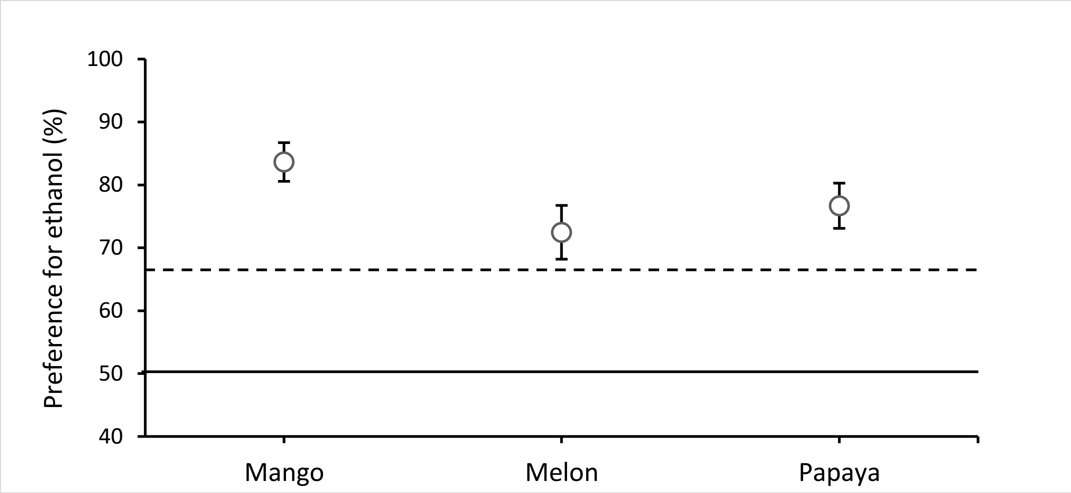

Figure 8: Taste responsiveness of eight spider monkeys to pureed fruit spiked with 3% ethanol tested against plain pureed fruit as the alternative. Each symbol represents the mean value (±SD) of ten trials per animal and per concentration, for the eight monkeys tested. White symbols represent concentrations where both preference criteria were met (66.7% preference, plus binomial test p<0.05). The horizontal solid line represents chance level (50% preference) and the horizontal dotted line represents the first preference criterion (66.7% preference)

5 Discussion

5.1 Taste preference threshold and absolute taste preference for ethanol

The results of the present study show that spider monkeys significantly preferred ethanol solutions between concentrations of 0.5% and 3% when tested against water. The range of concentrations tested here corresponds to the range of ethanol concentration found in naturally fermenting fruit (Eriksson and Nummi 1982, Dudley 2002, 2004, Domini 2004, Sanchez et al. 2004). This result is remarkable as to my knowledge, no primate species tested so far has been found to prefer ethanol solutions when tested against water, except for Rhesus macaques (*Macaca mulatta*) (Kornet et al. 1990, Vivian et al. 1999).

Ethanol has been found to interact with sweet taste receptors (Hellekant et al. 1997, Danilova & Hellekant 2000) and activating a sucrose-responsive gustatory neural pathway (Lemon et al. 2004). Nevertheless, this interaction also happens in primates that do not prefer ethanol over water and therefore, it is unclear whether this contributes to the taste responsiveness to ethanol displayed by different primate species. Also, spider monkeys might recognize ethanol as an additional source of calories besides fruit-sugars, as Gochman et al. (2016) suggested.

5.2 Taste preference threshold and absolute taste preference for sugarcane alcohol

Impurities in sugarcane alcohol did not affect the taste preference threshold for ethanol nor the taste preference for ethanol at all tested concentrations above the taste preference threshold. An increase in preference could have been expected as sugarcane alcohol contains a small amount of sugars and ethanol has been shown to increase gustatory nerve response to sucrose (Danilova & Hellekant 2000). Furthermore, small impurities in drinks have repeatedly been shown to affect human taste perception, for example minerals in drinking water (O'Mahony 1972, Whelton et al. 2007), and such a difference in taste perception, and thus taste preference, could have been expected in the present study.

5.3 Relative taste preference for ethanol

The results of the present study show that spider monkeys significantly preferred ethanol-spiked sucrose solutions over non-spiked sucrose solutions of the same sucrose concentration. These findings are in line with Danilova and Hellekant's (2000) electrophysiological findings, as they found that ethanol increased sweetness sensation when mixed with sweet solutions. Nonetheless, when the non-spiked stimulus was sweeter than the ethanol-spiked alternative, the animals in the present study significantly rejected the ethanol-spiked stimulus with all tested ethanol concentrations. This indicates that spider monkeys have a stronger preference for sucrose than for ethanol and that they choose the sweeter solution regardless of total caloric value. Previous research has shown that in food preference tests, spider monkeys preferred the more caloric food available (Laska 2000b). In the present study, spider monkeys chose sweet taste over calories when presented with sucrose-ethanol solutions. Thus, the findings of the present study do not support claims that a presumed predilection of non-human primates for alcohol-containing overripe fruits would reflect the evolutionary origins of human alcoholism (Dudley 2000, Dudley 2014), as in the present study, the spider monkeys clearly preferred the sweeter, non-alcohol-containing stimuli over the less sweet, alcohol-containing ones.

5.4 Taste preference for ethanol in fruit matrices

The results of the present study show that the spider monkeys preferred the pureed fruit spiked with ethanol over the non-spiked alternative. However, the preference was not as strong as in the first two test series of the second experiment. The ethanol-spiked fruit puree was preferred to a higher degree in fruits with a higher sugar content and a lower water content (See *Table 1*). These results might simply reflect the monkey's taste preference regarding the different fruits, which are in line with Laska's (2000b) findings. Otherswise, these findings could indicate that a higher sugar and /or calorie content increased the preference for ethanol in spider monkeys, which is in line with Danilova and Hellekant's (2000) findings, as they found that ethanol increased sweetness sensation when mixed with sweet solutions. Both options could explain the preference for ethanol increasing with the sugar content of the fruits.

Table 1: Water content, sugar content, caloric value, and measured preference for the fruit puree spiked with ethanol for the 3 fruit types tested in experiment 4. Source: McCance and Widdowson's The Composition of Foods: Edition 6 (2002)

	Water content (%)	Sugar content (%)	Kcal/100g	Preference for ethanol (%)
Mango	82.4	13.8	57	83.6
Papaya	88.5	8.8	36	76.7
Melon	92.1	4.2	19	72.5

It is important to point out that this result does not indicate that spider monkeys would prefer overripe, ethanol-containing fruit, rather than ripe fruit. Indeed, in this experiment, the ethanol-spiked and the non-spiked stimuli had the same sugar content, as it was not possible to reduce the sugar content of the ethanol-spiked stimulus in a way to match the sugar transformation by bacteria in in overripe fruit, without altering the puree's texture. As a result, one of the major differences in taste between ripe and overripe fruit, i.e. the sugar content difference, was not covered by this experiment. This experiment only allows to conclude that spider monkeys preferred the ethanol-spiked stimulus in fruit matrices, and thus still preferred the ethanol-spiked stimulus even when diluted in a complex-tasting matrix.

5.5 Comparison between spider monkeys and other primates

Only few studies so far assessed taste responses to ethanol in primates. Most of these studies focused on long-term intake of unphysiologically high concentrations of ethanol and investigated long-term effects of ethanol intake and the development of alcohol dependence in primates. These studies have nonetheless shown that ethanol can be attractive at least when mixed with an attractive (usually sweet) taste substance, to several primate species such as squirrel monkeys (*Saimiri sciureus*) (Mandillo et al. 1998) and rhesus monkeys (*Macaca mulatta*) (Meisch & Henningfield 1977).

Voluntary alcohol intake has also been reported in different primate species, both in the wild and in experimental setups. Free-ranging chimpanzees (*Pan troglodytes verus*) have been observed ingesting the fermented sap of the raffia palm (*Raphia hookeri*) which contains up to 6.9% alcohol (Hockings et al. 2015). Captive vervet monkeys (*Chlorocebus aethiops*) also voluntarily ingested 6% ethanol solutions even when *ad-libitum* water was available (Juarez et al. 1993). Rhesus monkeys (*Macaca mulatta*) have also been observed to voluntarily consume ethanol solutions at concentrations between 0.5% and 6% (Kornet et al. 1990, Vivian et al. 1999). Finally, Gochman et al. (2016) reported that slow lorises (*Nycticebus coucang*) and aye-ayes (*Daubentonia madagascariensis)* voluntarily consumed fermented nectar (containing up to 3.8% ethanol) and preferred the highest available ethanol concentration (5%) in a multiple-test choice paradigm.

In most of these studies, the highest concentrations of ethanol accepted by the monkeys ranged from 4% to 6.9%. These ethanol concentrations are markedly higher than the ones found in most naturally fermenting overripe fruit (Eriksson and Nummi 1982, Dudley 2002, 2004, Domini 2004, Sanchez et al. 2004). Also, the fermented raffia palm sap acquires this unusually high ethanol concentration because the fermentation happens in man-made receptacles. My results are in line with the results from the aforementioned studies as the spider monkeys tested here displayed a significant preference for the 3% ethanol solution when tested against water, and an even stronger preference for an ethanol-sucrose mixture when tested against a non-spiked sucrose solution of the same concentration.

In humans, the average taste detection threshold obtained from L.J. Van Gemert's "Flavour thresholds" (2011), is 0.43% ethanol, but the results from the different studies vary tremendously. For example, Rothe et al. (1972) determined a taste detection threshold for ethanol in humans of 0.001%, while Richter (1941) reported 3% for the same substance.

The taste preference threshold determined for spider monkeys in the present study is 0.5%. This indicates that the spider monkeys' taste sensitivity to ethanol is close to that of humans, given that the monkeys' taste preference threshold is very similar to the average human taste detection threshold. Nonetheless, taste preference thresholds are only a conservative approximation of an animal's ability to perceive a given taste and are usually higher than the taste detection thresholds which are commonly determined using sophisticated signal detection methods.

5.6 Comparison between spider monkeys and non-primate mammals

Among non-primate mammals, the most tested animal for alcohol consumption is the rat (*Rattus norvegicus domesticus*). Richter and Campbell (1940b) showed that in a two-bottle preference test, rats preferred ethanol at concentrations of 1.8% to 4.4% over water. In the present study, the spider monkeys significantly preferred ethanol concentrations between 0.5% and 3%, which indicates a lower taste preference threshold in spider monkeys than in rats.

Egyptian fruit bats (*Rousettus aegyptiacus*) have been shown to increase consumption of fruit when these contained 0.1% ethanol, but only during the summer (Korine et al. 2011). Furthermore, fruit consumption did not change with ethanol concentrations of 0.01%, 0.3%, and 0.5%, and fruit consumption significantly decreased with ethanol concentrations of 1% and 2% (Sanchez et al. 2004; Korine et al. 2011). In the present study, the spider monkeys significantly preferred ethanol-spiked pureed fruit at all tested ethanol-concentrations (0.1%, 0.5%, and 3%), which suggests a higher tolerance to ethanol in the spider monkeys compared to the Egyptian fruit bats. The ethanol tolerance in Egyptian fruit bats seems to be lower than the ones reported in several primate species, indicating that tolerance and preference for ethanol might depend more on phylogenetic relatedness than on dietary specialization.

Taste preference for ethanol has also been tested in sheep (*Ovis aries*). Goatcher and Church (1970) determined that sheep showed no preference for ethanol even though they exhibit a "remarkably high" tolerance to it, which might indicate an absence of taste receptors sensitive to ethanol. Goatcher and Church (1970) estimated the sheep's lower detection threshold at an ethanol concentration of 1.56%. As the determined taste preference threshold for ethanol in spider monkeys in this study was 0.5%, this suggests that spider monkeys have a higher taste sensitivity to ethanol than sheep, and possibly other ruminants.

5.7 Comparison between spider monkeys and non-mammals

Only very few non-mammal species have been tested so far regarding their taste preference for ethanol. Zungu and Downs (2017) tested three bird species (cape white-eye, *Zosterops virens;* speckled mousebird, *Colius striatus;* and the red winged starling, *Onychognathus morio*) in a two-choice test paradigm, and none of them showed a preference for the ethanol-enriched diet.

Bees (*Apis mellifera*) have been reported to show a preference for ethanol-spiked sucrose solutions up to 2.5% ethanol but find the taste of ethanol aversive when diluted in water (Mustard et al. 2019).

The results from the present study differ from the aforementioned two studies as the tested spider monkeys showed a significant preference for ethanol, both when diluted in water and in a sucrose solution.

5.8 Conclusion

The spider monkeys in the present study have shown a preference for ethanol concentrations between 0.1% and 3% diluted either in water, sucrose solutions, or pureed fruit, when tested against alcohol-free alternatives of the same diluent. The spider monkeys had a stronger preference for sucrose than for ethanol, regardless of the total caloric content of the stimuli. Spider monkeys are one of the few tested primates so far that show a preference for ethanol solutions over water. Their sensitivity appears to be in line with previous findings in other species, including humans. Spider monkeys in the present study had a lower preference threshold than previously tested rats, and a higher sensitivity than previously tested sheep. The results do not support the notion that spider monkeys might use ethanol as an additional source of calories.

6 Societal & ethical considerations

The experiments reported here comply with the *American Society of Primatologists' Principles for the Ethical Treatment of Primates* and also with current Swedish and Mexican laws. The experiments were performed according to a protocol approved by the ethical board of the Federal Government of Mexico's Secretariat of Environment and Natural Resources (SEMARNAT; Official permits no. 09/GS-2132/05/10).

All the experiments performed in the present study relied on the voluntary cooperation of the animals. All spider monkeys participated in the experiments on their own will and were not forced in any way to consume any substance if they did not want to. The caretakers and a veterinarian were present every day at the field station and monitored the health and behaviour of every animal. None of the stimuli used (ethanol, sugarcane alcohol, and sucrose) were presented in quantities high enough to induce inebriation, or for a long enough duration to induce long-term side effects, such as cirrhosis, in the animals.

Animal studies help us to better understand alcohol dependence in humans. This study addressed the taste of alcohol, rather than its intoxication or dependence effects, hoping to approach the subject from a different and, so far, understudied angle. Furthermore, increasing knowledge regarding taste perception in non-human primates might also contribute to better welfare, for example in appropriate diet or enrichment, in captive animals.

7 Acknowledgements

I would like to thank my supervisors, Professor Matthias Laska and Dr. Laura Teresa Hernandez Salazar, for their assistance and support during this study. I would also like to thank my dear classmate Emmi Aineslahti, PhD-student José Eduardo Reynoso Cruz, and all the caretakers at the UMA Doña Hilda Ávila de O'Farrill of the Universidad Veracruzana field station for their help during the data collection.

8 References

Anderson WD, Smith OA (1963) Taste and volume preferences for alcohol in *Macaca nemestrina*. Journal of Comparative and Physiological Psychology 56, 144–149. doi:10.1037/h0048296

Belknap JK, Crabbe JC, Young ER (1993) Voluntary consumption of ethanol in 15 inbred mouse strains. Psychopharmacology 112, 503–510. doi:10.1007/BF02244901

Cederbaum, A.I. (2012) Alcohol Metabolism. Clinics in Liver Disease. doi:10.1016/j.cld.2012.08.002

Danilova V, Hellekant G (2000) The taste of ethanol in a primate model: II. Glossopharyngeal nerve response in *Macaca mulatta*. Alcohol 21, 259–269. doi:10.1016/S0741-8329(00)00094-X

Di Fiore A, Link A, Dew JL (2008) Diets of wild spider monkeys. In: Spider monkeys: The biology, behavior and ecology of the genus *Ateles*. Campbell CA (ed). Cambridge University Press, Cambridge, UK, pp 81-137. doi:10.1017/cbo9780511721915.004

Dominy NJ (2004) Fruits, fingers, and fermentation: The sensory cues available to foraging primates, in: Integrative and Comparative Biology. Society for Integrative and Comparative Biology, pp. 295–303. doi:10.1093/icb/44.4.295

Dudley R (2000). Evolutionary origins of human alcoholism in primate frugivory. The Quarterly Review of Biology 75, 3–15. doi:10.1086/393255

Dudley R (2002) Fermenting fruit and the historical ecology of ethanol ingestion: Is alcoholism in modern humans an evolutionary hangover? Addiction 97, 381–388. doi:10.1046/j.1360-0443.2002.00002.x

Dudley R (2004) Ethanol, fruit ripening, and the historical origins of human alcoholism in primate frugivory, in: Integrative and Comparative Biology. Society for Integrative and Comparative Biology, pp. 315–323. doi:10.1093/icb/44.4.315

Dudley R (2014) The drunken monkey: why we drink and abuse alcohol. University of California Press.

Elton RH (1976) Drinking patterns of pigtailed macaques. Journal of Studies on Alcohol 37, 1548–1555. doi:10.15288/jsa.1976.37.1548

Eriksson K, Nummi H (1982) Alcohol accumulation from ingested berries and alcohol metabolism in passerine birds. Ornis Fennica 60, 2–9.

Food Standards Agency (2002) McCance and Widdowson's The Composition of Foods, Sixth summary edition. Cambridge: Royal Society of Chemistry.

Goatcher WD, Church DC (1970) Taste responses in ruminants. I. Reactions of sheep to sugars, saccharin, ethanol and salts. Journal of Animal Science 30, 777–783. doi:10.2527/jas1970.305777x

Gochman SR, Brown MB, Dominy NJ (2016) Alcohol discrimination and preferences in two species of nectar-feeding primate. Royal Society Open Science 3. doi:10.1098/rsos.160217

González-Zamora A, Arroyo-Rodríguez V, Chaves OM, Sanchez-Lopez S, Stoner KE, Riba-Hernández P (2009) Diet of spider monkeys (*Ateles geoffroyi*) in mesoamerica: Current knowledge and future directions. American Journal of Primatology. doi:10.1002/ajp.20625

Hellekant G, Danilova V, Ninomiya Y (1997) Primate sense of taste: behavioral and single chorda tympani and glossopharyngeal nerve fiber recordings in the rhesus monkey, *Macaca mulatta*. Journal of Neurophysiology 77, 978–93. doi:10.1152/jn.1997.77.2.978

Hockings K, Bryson-Morrison N, Carvalho S, Fujisawa M, Humle T, McGrew W, Nakamura M, Ohashi G, Yamanashi Y, Yamakoshi G, Matsuzawa T (2015) Tools to tipple: Ethanol ingestion by wild chimpanzees using leaf-sponges. Royal Society Open Science 2. doi:10.1098/rsos.150150

Janzen DH (1977). Why fruits rot, seeds mold, and meat spoils. The American Naturalist, 111(980), 691–713. https://doi.org/10.1086/283200

Juarez J, Guzman-Flores C, Ervin F, Palmour RM (1993) Voluntary alcohol consumption in vervet monkeys: Individual, sex, and age differences. Pharmacology, Biochemistry and Behavior 46, 985–988. doi:10.1016/0091-3057(93)90232-I

Kaplan JN, Hennessy MB, Howd RA (1982) Oral ethanol intake and levels of blood alcohol in the squirrel monkey. Pharmacology, Biochemistry and Behavior 17, 111–117. doi:10.1016/0091-3057(82)90271-4

Korine C, Sánchez F, Pinshow B (2011) Effects of ethanol on food consumption and skin temperature in the egyptian fruit bat (*Rousettus aegyptiacus*). Integrative and Comparative Biology (Vol. 51, pp. 432–440). https://doi.org/10.1093/icb/icr012

Kornet M, Goosen C, Ribbens LG, Van Ree JM (1990) Analysis of spontaneous alcohol drinking in rhesus monkeys. Physiology and Behavior, 47, 679–684. https://doi.org/10.1016/0031-9384(90)90077-H

Kwiecinski GG, Griffiths TA (1999). Rousettus egyptiacus. Mammalian Species, (611), 1-9. https://doi.org/10.2307/3504411

Laska M, Carrera Sanchez E, Rodriguez Rivera JA, Rodriguez Luna E (1996) Gustatory thresholds for food-associated sugars in the spider monkey (*Ateles geoffroyi*). American Journal of Primatology 39, 189–193. doi:10.1002/(sici)1098-2345(1996)39:3<189::aid-ajp4>3.0.co;2-v

Laska M, Carrera Sanchez E, Rodriguez Luna E (1998) Relative taste preferences for food-associated sugars in the spider monkey (*Ateles geoffroyi*). Primates 39, 91–96. doi:10.1007/BF02557747

Laska M, Hernandez Salazar LT, Rodriguez Luna E, Hudson R (2000) Gustatory responsiveness to food-associated acids in the spider monkey (*Ateles geoffroyi*). Primates 41, 213-221. doi:10.1007/BF02557803

Laska M, Hernandez Salazar LT, Rodriguez Luna E (2000b) Food preferences and nutrient composition in captive spider monkeys, *Ateles geoffroyi*. International Journal of Primatology 21, 671–683. doi:10.1023/A:1005517421510

Laska M, Hernandez Salazar LT (2004) Gustatory responsiveness to monosodium glutamate and sodium chloride in four species of nonhuman primates Journal of Experimental Zoology Part A: Comparative Experimental Biology 301, 898–905. doi:10.1002/jez.a.118

Laska M, Kohlmann S, Scheuber HP, Hernandez Salazar LT, Rodriguez Luna E (2001) Gustatory responsiveness to polycose in four species of nonhuman primates. Journal of Chemical Ecology 27, 1997–2011. doi:10.1023/A:1012286719241

Laska M, Persson Suorra J, Rivas Bautista RM, Hernandez Salazar LT (2008) Taste difference thresholds for monosodium glutamate and sodium chloride in pigtail macaques (*Macaca nemestrina*) and spider monkeys (*Ateles geoffroyi*). American Journal of Primatology 70, 839–847. doi:10.1002/ajp.20558

Laska M, Rivas Bautista RM, Hernandez Salazar LT (2009) Gustatory responsiveness to six bitter tastants in three species of nonhuman primates. Journal of Chemical Ecology 35, 560–571. doi:10.1007/s10886-009-9630-8

Laska M, Scheuber HP, Hernandez Salazar LT, Rodriguez Luna E (2003) Sour-taste tolerance in four species of nonhuman primates. Journal of Chemical Ecology 29, 2637–2649. doi:10.1023/B:JOEC.0000008009.85523.7a

Levey DJ (2004) The evolutionary ecology of ethanol production and alcoholism, in: Integrative and Comparative Biology. Society for Integrative and Comparative Biology, pp. 284–289. doi:10.1093/icb/44.4.284

Mandillo S, Titchen K, Miczek KA (1998) Ethanol drinking in socially housed squirrel monkeys. Behavioural Pharmacology 9, 363-368. doi:10.1097/00008877-199807000-00008

McGrew WC (2011) Natural ingestion of ethanol by animals: why? In Schiefenhövel W, Macbeth H (eds) Liquid bread: beer and brewing in cross-cultural perspective. Berghahn, New York, pp 13-19. https://doi.org/10.5860/choice.49-1550

Meisch RA, Henningfield JE (1977) Drinking of ethanol by rhesus monkeys: Experimental strategies for establishing ethanol as a reinforcer. pp. 443–463. doi:10.1007/978-1-4615-9038-5_29

Milton K (2004) Ferment in the family tree: does a frugivorous dietary heritage influence contemporary patterns of human ethanol use? in: Integrative and Comparative Biology. Society for Integrative and Comparative Biology, pp. 304–314. doi:10.1093/icb/44.4.304

Morris S, Humphreys D, Reynolds D (2006) Myth, Marula, and elephant: an assessment of voluntary ethanol intoxication of the African elephant (*Loxodonta africana*) following feeding on the fruit of the Marula tree (Sclerocarya birrea). Physiological and Biochemical Zoology 79, 363–369. doi:10.1086/499983

Mustard JA, Oquita R, Garza P, Stoker A (2019) Honey bees (*Apis mellifera*) show a preference for the consumption of ethanol. Alcoholism Clinical & Experimental Research, 43, 26-35. doi:10.1111/acer.13908

O'Mahony M (1972) Purity effects and distilled water taste. Nature 240, p.489. doi:10.1038/240489a0

Richter CP (1941). Alcohol as a food. Quarterly Journal of Studies on Alcohol, 1(4), 650-662.

Richter CP, Campbell KH (1940) Taste thresholds and taste preference of rats for five common sugars. The Journal of Nutrition 20, 31–46. doi:10.1093/jn/20.1.31

Richter CP, Campbell KH (1940b) Alcohol taste thresholds and concentrations of solution preferred by rats. Science 91, 507–508. doi:10.1126/science.91.2369.507

Rothe M, Wölm G, Tunger L, SIEBERT HJ (1972) Schwellenkonzentrationon von Aromastoffen und ihre Nutzung zur Auswertung von Aromaanalysen. Nahrung 16, 483-495.

Sanchez F, Korine C, Pinshow B, Dudley R (2004) The possible roles of ethanol in the relationship between plants and frugivores: first experiments with Egyptian fruit bats in: Integrative and Comparative Biology. Society for Integrative and Comparative Biology, pp. 290–294. doi:10.1093/icb/44.4.290

Van Gemert L.J. (2011) Flavour Thresholds; Compilations of flavour threshold values in water and other media. 2nd Edition. Oliemans Punter & Partners BV, The Netherlands.

Vivian JA, Higley JD, Linnoila M, Woods JH (2006) Oral ethanol self-administration in rhesus monkeys: behavioral and neurochemical correlates. Alcoholism: Clinical and Experimental Research, 23(8), 1352–1361. https://doi.org/10.1111/j.1530-0277.1999.tb04357.x

Whelton PA, Dietrich AM, Burlingame GA, Schechs M, Duncan SE (2007) Minerals in drinking water: Impacts on taste and importance to consumer health. In Water Science and Technology (Vol. 55, pp. 283–291). https://doi.org/10.2166/wst.2007.190

Wiens F, Zitzmann A, Lachance MA, Yegles M, Pragst F, Wirst FM, von Holst D, Guan SL, Spanagel R (2008) Chronic intake of fermented floral nectar by wild treeshrews. PNAS 105: 10426-10431. https://doi.org/10.1073/pnas.0801628105

Zungu, M.M., Downs, C.T., 2017. Effects of Ethanol on Fruit Selection by Frugivorous Birds. African Zoology 52, 69–72. doi:10.1080/15627020.2016.1276856

Appendix

Monkeys	Ethanol concentration in spiked solution						
	0.05%	0.1%	0.5%	1.0%	1.5%	2.0%	3.0%
Gruñon	65.0%	87.4%	89.0%	92.1%	93.7%	94.5%	94.7%
Lucas	46.3%	87.4%	89.0%	92.1%	93.7%	94.5%	94.7%
Cejitas	58.5%	73.2%	87.9%	95.4%	94.3%	96.1%	94.9%
Mary	64.9%	82.2%	88.0%	84.2%	89.2%	88.9%	93.7%
Patas	48.8%	32.0%	49.3%	58.4%	44.2%	76.8%	60.6%
Kika	55.9%	49.1%	69.2%	70.7%	57.4%	69.5%	67.5%
Group mean	56.6%	68.6%	78.7%	82.1%	78.8%	86.7%	84.3%
SD	7.9%	22.9%	16.3%	14.6%	22.1%	11.0%	15.9%
SE	3.2%	9.4%	6.7%	6.0%	9.0%	4.5%	6.5%

Appendix 1: Taste responsiveness of six spider monkeys to various concentrations of ethanol tested against purified water as an alternative. Individual ten-trial average, group mean, group standard deviation (SD), and group standard error (SE).

Monkeys	Ethanol concentration in spiked solution						
	0.05%	0.1%	0.5%	1.0%	1.5%	2.0%	3.0%
Gruñon	64.0%	73.1%	89.1%	92.7%	84.3%	95.7%	85.7%
Lucas	58.3%	75.8%	93.6%	94.0%	90.2%	94.7%	87.1%
Cejitas	63.8%	79.4%	94.7%	94.7%	91.5%	94.1%	92.9%
Mary	57.9%	86.5%	87.0%	93.4%	86.3%	91.3%	89.9%
Patas	57.9%	38.0%	41.2%	40.7%	70.1%	52.2%	66.3%
Kika	55.0%	44.8%	50.0%	53.2%	77.2%	62.5%	42.6%
Group mean	59.5%	66.3%	75.9%	78.1%	83.3%	81.7%	77.4%
SD	3.6%	19.9%	23.8%	24.5%	8.2%	19.2%	19.5%
SE	1.5%	8.1%	9.7%	10.0%	3.3%	7.8%	7.9%

Appendix 2: Taste responsiveness of six spider monkeys to various concentrations of sugarcane alcohol tested against purified water as an alternative. Individual ten-trial average, group mean, group standard deviation (SD), and group standard error (SE).

Monkeys	Tested ethanol concentration		
	0.1%	0.5%	3.0%
Gruñon	96.5%	97.2%	94.1%
Lucas	92.5%	97.0%	98.2%
Cejitas	94.9%	95.8%	96.9%
Brutus	95.5%	96.0%	94.0%
Mary	93.7%	93.8%	89.5%
Frida	97.8%	96.0%	87.8%
Kika	93.9%	93.4%	88.9%
Patas	90.3%	94.4%	80.6%
Group mean	94.4%	95.5%	91.2%
SD	2.4%	1.4%	5.7%
SE	0.8%	0.5%	2.0%

Appendix 3: Taste responsiveness of eight spider monkeys to various concentrations of ethanol diluted in a 30mM sucrose solution tested against a 30mM sucrose solution without ethanol as the alternative. Individual ten-trial average, group mean, group standard deviation (SD), and group standard error (SE).

Monkeys	Tested ethanol concentration		
	0.1%	0.5%	3.0%
Gruñon	97.2%	96.9%	97.6%
Lucas	97.2%	95.7%	95.5%
Cejitas	98.1%	98.7%	98.3%
Brutus	96.0%	94.4%	98.3%
Mary	95.8%	97.7%	96.6%
Frida	98.9%	98.4%	83.4%
Kika	94.5%	96.5%	95.6%
Patas	98.0%	97.4%	96.9%
Group mean	97.0%	97.0%	95.3%
SD	1.4%	1.4%	4.9%
SE	0.5%	0.5%	1.7%

Appendix 4: Taste responsiveness of eight spider monkeys to various concentrations of ethanol diluted in a 60mM sucrose solution tested against a 60mM sucrose solution without ethanol as the alternative. Individual ten-trial average, group mean, group standard deviation (SD), and group standard error (SE).

Monkeys	Tested ethanol concentration		
	0.1%	0.5%	3.0%
Gruñon	10.1%	10.7%	12.5%
Lucas	3.9%	5.7%	4.0%
Cejitas	1.3%	2.8%	2.4%
Brutus	3.7%	6.5%	3.2%
Mary	3.1%	7.4%	5.0%
Frida	1.4%	1.2%	1.4%
Kika	3.8%	3.1%	3.6%
Patas	2.9%	2.5%	2.7%
Group mean	5.0%	6.7%	5.8%
SD	2.7%	3.2%	3.5%
SE	1.0%	1.1%	1.2%

Appendix 5: Taste responsiveness of eight spider monkeys to various concentrations of ethanol diluted in a 30mM sucrose solution tested against a 60mM sucrose solution without ethanol as the alternative. Individual ten-trial average, group mean, group standard deviation (SD), and group standard error (SE).

Monkeys	Tested fruit type		
	Mango	Melon	Papaya
Gruñon	91.2%	78.8%	81.5%
Brutus	87.0%	77.0%	66.7%
Lucas	85.0%	84.6%	82.4%
Cejitas	93.0%	85.9%	83.0%
Mary	81.9%	75.7%	88.1%
Frida	66.8%	51.8%	56.9%
Kika	75.7%	59.9%	78.4%
Patas	88.5%	66.1%	76.5%
Group mean	83.6%	72.5%	76.7%
SD	8.7%	12.1%	10.2%
SE	3.1%	4.3%	3.6%

Appendix 6: Taste responsiveness of eight spider monkeys to pureed fruit spiked with 3% ethanol tested against plain pureed fruit as the alternative. Individual ten-trial average, group mean, group standard deviation (SD), and group standard error (SE).