PSYCHOPHYSICAL EVALUATION OF SWEET TASTE PERCEPTION IN MICE

By

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Despite differences in their molecular structure, many sugars, a subset of amino acids, and some synthetic compounds are "sweet-tasting" to humans and appear to possess a "sucrose-like" taste quality to non-human mammals. It has been proposed that in taste bud cells (TBCs), a family of receptors called the T1Rs mediates signal transduction of all of these "sweeteners." However, in a brief-access test with non-deprived mice, licking responses to sucrose were discernibly different from the responses to the amino acids tested.

Experiments detailed here were designed to test the ability of mice to distinguish between L-serine and various sugars in operant taste discrimination tasks. Mice were able to discriminate NaCl from sucrose (n=6) and L-serine (n=6) which served as training stimuli. Mice had difficulty discriminating sucrose from L-serine, maltose, fructose and glucose to varying degrees depending on the stimulus and the training history. For example, when concentration effects are taken into consideration, mice were unable to

discriminate sucrose from glucose or fructose suggesting that these sugars generate a unitary percept. However, these animals were able to discriminate sucrose from L-serine. Mice were also able to discriminate L-serine from glucose, fructose, and maltose, but only moderately so. Data gathered using a conditioned taste aversion assay suggest that L-serine generates a complex taste that includes a sucrose-like component and that this complexity may be at the source of the limited discriminability between L-serine and the sugars. To my knowledge, the qualitative complexity elicited by L-serine, which includes both a "sweet" and a "bitter" taste, has never been previously demonstrated before in rodents. L-serine's ability to evoke multiple taste percepts would explain much of the results observed in this dissertation including its lack of affective potency, its poor efficacy as a "standard" stimulus, and its discriminability from sugars.

In summary, these data suggest that all of these compounds share some qualitative similarities. Therefore, it is likely that some taste input arising from TBCs that express different T1R receptors converges somewhere along the gustatory neuraxis. However, the results of these experiments also imply that "sweet-tasting" L-amino acids, such as L-serine, also possess distinguishable taste characteristics.

CHAPTER 1
INTRODUCTION

Psychophysical Examination of Taste Quality in Non-Human Mammals

It is generally accepted that taste perception is comprised of only a few basic qualities (e.g., sweet, salty, sour, bitter; see Bartoshuk, 1988). However, the nature of these perceptions is difficult to study because these experiences cannot be directly measured and must be inferred from behavior. In animals, the systematic study of these perceptions is accomplished by the use of behavioral procedures that are designed to characterize the relationship between physical stimuli and sensation (Blough and Blough, 1977; Berkley and Stebbins, 1990; Spector, 2003). These psychophysical techniques allow for, among other things, the assessment of an animal's capacity to discriminate or generalize between two chemical compounds. If an animal treats a test stimulus similarly to a trained or conditioned stimulus (i.e., generalization), then the compounds are assumed to share some perceptual features. If an animal can be trained to discriminate between two compounds, then the stimuli involved **must** generate distinguishable neural signals in both the periphery and the brain. Identifying compounds that are behaviorally indiscriminable allows for them to be categorized into perceptual classes. Indeed, a group of stimuli that are mutually indiscriminable constitute the definition of a perceptual class. Below I propose the use of psychophysical procedures specifically designed to answer these questions. As argued above, the use of psychophysical methodology is the only way to rigorously and objectively examine the taste perception of animals.

Amino Acid Taste Perception

Explicit taste discrimination experiments using amino acids as stimuli are rare. Instead, researchers have tried to perceptually categorize amino acids in rodents by using the conditioned taste aversion (CTA) generalization paradigm to quantify the degree to which these compounds are similar to prototypical chemical stimuli thought to represent basic taste qualities (e.g., sucrose, NaCl, citric acid, quinine). In this procedure, an animal samples a novel taste stimulus followed by the injection of an agent that causes visceral malaise (usually lithium chloride; LiCl). After which, researchers measure whether the subsequent avoidance conditioned to the novel tastant generalizes to other test compounds. The results of such experiments suggest that a variety of amino acids appear to possess some degree of a qualitative similarity with the taste of sucrose.

Taken together, without regard to strain and species differences, results from CTA experiments demonstrate that a subset of D-amino acids and a subset of L-amino acids including L-alanine, L-proline, L-serine, and glycine (which does not have a chiral carbon) are all treated as possessing some degree of qualitative similarity with the taste of sucrose and are thus considered "sweet." In addition, when mixed with the epithelial sodium channel blocker, amiloride, aversions conditioned to monosodium glutamate (MSG) and L-aspartic acid have also been shown to generalize to sucrose in CTA tests (e.g., Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999; Heyer *et al.*, 2003). Other amino acids tested fail to fall into this category (including L-arginine, L-isoleucine, L-methionine, L-phenylalanine, L-tryptophan, D-alanine, and D-serine tested in various strain of mice; see Ninomiya *et al.*, 1984b; Kasahara *et al.*, 1987; Ninomiya *et al.*, 1992; and L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine,

and L-histidine tested in the golden hamster; see Nowlis *et al.*, 1980; Yamamoto *et al.*, 1988). A large body of psychophysical literature also suggests that these L-amino acids give rise, to some degree, to a sweet taste perception in humans (e.g., Solms *et al.*, 1965; Schiffman and Dackis, 1975; Schiffman and Clark, 1980; Schiffman *et al.*, 1981; Schiffman *et al.*, 1982; Haefeli and Glaser, 1990; Shallenberger, 1993). These data also suggest that other L-amino acids, such as L-threonine, may also share perceptual characteristics with sucrose. Indeed, the notion that rodents may also perceive L-threonine as "sucrose-like" (i.e., "sweet") is supported by the fact that L-threonine was shown to be preferred by Sprague-Dawley rats and by ddy mice, at certain concentrations, as assessed using a two-bottle preference test (Pritchard and Scott, 1982a; Iwasaki *et al.*, 1985). However, as detailed below, this fact, in and of itself, does not allow for a confident categorization of a taste compound into the perceptual class humans label sweet.

In general, rats unconditionally prefer those amino acids that have been shown to generalize with sucrose in conditioned taste aversion tests. Using the two bottle preference test, a variety of researchers have shown that these same L-amino acids seem to be favored, to varying degrees, by different strains of mice (Iwasaki *et al.*, 1985; Lush *et al.*, 1995; Bachmanov *et al.*, 2001b). Indeed, these measures are commonly used to determine whether a particular species or animal perceives a stimulus as "sweet" and/or as a measure of the relative intensity of that percept (e.g., Bachmanov *et al.*, 2001b). However, the use of preference measures to determine the relative qualitative similarity of a putative "sweet-tasting" stimulus to that of a prototypical sugar (i.e., sucrose) implies that if that compound shares a qualitative resemblance to a particular sugar, then it will be

unconditionally preferred. Additionally, it suggests that if a compound is not unconditionally preferred, it will not be perceived as "sweet."

I recently conducted a set of experiments assessing the affective potency of sucrose, L-serine, and glycine using a brief-access taste test in a gustometer, the details of which are covered in Chapter 2 of this dissertation. Suffice it to say, these data do not support the supposition that L-serine (and to a lesser extent, glycine) is unconditionally preferred by non-deprived mice on the basis of taste. Thus, if the aforementioned contention regarding the correspondence between "sweet" taste quality and preference is correct, then, despite suggestions from the CTA literature, L-serine could not be considered "sweet."

That said, it should be noted here that the two-bottle preference test and the brief-access taste test only assess the motivational properties of a taste stimulus, not its qualitative characteristics per se. For example, rats prefer low concentrations of NaCl in two-bottle tests and avoid high concentrations, but this does not mean that the former are "sweet" and the latter are "bitter." Qualitative perception is best inferred from tasks in which taste serves as a cue for some other event (e.g., reinforcement) that will generate a trained directed response regardless of the hedonic characteristics of the taste stimulus.

"Sweetener" Transduction

An understanding of the neural basis of sweetener and amino acid taste perception has been propelled by remarkable discoveries regarding the molecular biology of transduction processes in the mammalian peripheral gustatory system. Specifically, a gene family has been identified which encodes for three 7-transmembrane spanning G-protein coupled receptors (T1R1, T1R2, and T1R3) that bind with sugars, synthetic sweeteners, amino acids, and in some species "sweet" proteins (e.g., Hoon *et al.*, 1999;

Bachmanov *et al.*, 2001a; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001; Sainz *et al.*, 2001; Li *et al.*, 2002). Initial calcium imaging studies of "receptor-ligand" interactions in a heterologous expression system, suggested that individual T1Rs are not functional, but that they, similar to other class C G-protein coupled receptors, combine into heterodimeric receptor complexes. Dimerization, however, has yet to be explicitly demonstrated in this sub-family of receptors. The T1R3 receptor has been shown to combine with T1R1 or T1R2 to form functional "heteromers." The T1R2+3 complex was shown, *in vitro*, to be activated by a variety of both natural and synthetic sweeteners as well as "sweet-tasting" D-amino acids (Nelson *et al.*, 2001; Zhao *et al.*, 2003). A similar study revealed that the combination of mouse T1R1 and T1R3 gives rise to a "heteromeric" receptor complex that interacts with most of the twenty common L-amino acids (Nelson *et al.*, 2002). A more recent *in vitro* study suggested that T1R3 may also function independently as a low affinity receptor, binding with high concentrations of natural but not synthetic sweeteners (Zhao *et al.*, 2003). Results from experiments on the neural and behavioral consequences of the deletion (i.e., knock-out) of one or more of the genes encoding for the T1R receptors in mice are summarized in Table 1-1. Collectively, these data confirm, *in vivo*, that sugars and L-amino acids bind, selectively, with different T1R receptor complexes.

These heteromeric receptors were first purported to be expressed, principally, in non-overlapping sets of taste bud cells. The receptor, T1R1, was reported to be mainly expressed in fungiform papillae (anterior tongue) and in the palate (in ~20-30% of the taste bud cells (TBCs) in 100% of the buds in these receptor fields; Hoon *et al.*, 1999). In the posterior tongue, it was purported to be rarely found in the taste buds of the

circumvallate papillae (in less than 5% of TBCs in the less than 10% of circumvallate taste buds that express T1R1; Hoon *et al.*, 1999) and only modestly in foliate papillae (in ~10% of TBCs in the ~30% of foliate taste buds that express T1R1; Hoon *et al.*, 1999). In contrast, T1R2 was reported to be expressed mainly in the circumvallate and foliate papillae (in ~20-30% of the TBCs in 100% of the buds in these papillae; Hoon *et al.*, 1999). It is almost non existent in the taste buds of the fungiform papillae (in less than 1% of fungiform taste buds; Hoon *et al.*, 1999; but see below) and only modestly expressed in the palate (in less than ~5% of TBCs in the ~20% of palatal taste buds that express T1R2; Hoon *et al.*, 1999). The receptor T1R3, however, was purportedly expressed in ~30% of TBCs in all three taste bud containing papillae (Nelson *et al.*, 2001; see Table 1-2 for a summary of the results from all of the heretofore mentioned expression pattern studies). Double-label *in situ* hybridization studies showed that virtually all T1R3-expressing cells in circumvallate and foliate papillae express T1R2 (Max *et al.*, 2001; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001). Conversely, all T1R2 expressing cells in circumvallate and foliate papillae, as well as in the palate, were also reported to express T1R3 (Montmayeur *et al.*, 2001; Nelson *et al.*, 2001). Moreover, most of the taste cells in fungiform papillae that express T1R3 also express T1R1 (Nelson *et al.*, 2001). These experiments, however, also implied that there exists a population of cells in fungiform papillae and in the palate that express T1R3 without T1R1 or T1R2 (Nelson *et al.*, 2001).

These expression data imply that the nerves innervating the circumvallate and fungiform receptor fields (i.e., the fields of the chorda tympani and glossopharyngeal nerves, respectively) would be differentially sensitive to sugars and amino acids. For

example, the chorda tympani nerve, which innervates fungiform papillae, which preferentially expresses the T1R1+3 receptor complex, should be relatively more responsive to L-amino acids than to sugars or D-amino acids. On the other hand, the glossopharyngeal nerve, which innervates the circumvallate and foliate papillae, which preferentially expresses the T1R2+3 receptor complex, should be relatively more responsive to sugars and D-amino acids than to L-amino acids. However, the response properties of the mouse whole chorda tympani and glossopharyngeal nerves do not correspond to these predicted patterns (e.g., Ninomiya and Funakoshi, 1989; Ninomiya *et al.*, 1993; Ninomiya *et al.*, 2000; Danilova and Hellekant, 2003). This may suggest the existence of other receptors that are responsive to these ligands or it may suggest that the aforementioned expression data are inaccurate or incomplete. Indeed, more recent data do conflict with the previous studies on the expression pattern of these receptors. Contrary to these reports, the expression of the receptor, T1R1, was shown to be greater (i.e., in a larger number of cells) than that of T1R2 and T1R3 in circumvallate papillae, albeit with a lower signal intensity than the other two receptors (Kim *et al.*, 2003). Using double-label *in situ* hybridization, these researchers found that, in the circumvallate, almost all cells that express T1R2 and the majority of cells that express T1R3 also express T1R1 (Kim *et al.*, 2003). They also reported that, in fungiform papillae, half of the T1R3 expressing cells also express T1R2. As mentioned above, prior work suggested that the expression of the T1R2 receptor in fungiform papillae is rare. Moreover, expression of T1R1 and T1R2 overlapped in fungiform papillae. Since every T1R2 positive cell ubiquitously expressed T1R3, then T1R1 + T1R2 double positive cells must also express T1R3. Therefore, in both receptor fields, TBCs that co-express all

three T1R receptors can be found (i.e., T1R1, T1R2, and T1R3; Kim *et al.*, 2003). Interestingly, in the circumvallate, 66% of the T2R receptor expressing cells[1] also expressed the receptor T1R1. The co-expression of the other two receptors with the various T2R receptors was not assessed.

The fact that sugars and L-amino acids bind, selectively, with different T1R receptor complexes suggest that theses compounds may be perceptually distinct and as a result, behaviorally discriminable. Indeed, the early reports on the pattern of T1R receptor expression provide a degree of receptor complex segregation that could support this discriminability. However, depending on the degree of receptor expression overlap on single TBCs, the data reported by Kim *et al.* predict that mice would have at least some difficulty discriminating between sugars and L-amino acids. Indeed, these data, reported by Kim *et al.*, portray a more balanced receptor expression pattern, at least across the circumvallate and fungiform papillae, and correspond more closely to the response properties of gustatory nerves than do data from the previous studies.

As an important caveat, it should be noted that peripheral nerve responses only index the nature of the signal arising from the initial processing of taste input. The central gustatory system can amplify, attenuate, or alter features of the peripheral signal (e.g., convergence). Moreover, knowledge regarding a neural response, whether of peripheral or central origin, does not in and of itself, necessarily reveal how those signals are translated into behavior (and the associated inferred perceptions).

The complexities of these interpretive issues are exemplified by the opposing postulations regarding "bitter" taste perception made by researchers who study receptor

[1] Mix probes of T2R5, T2R8, T2R18, and T2R19 were used for analysis of T2Rs expression

expression and those who study the response properties of TBCs. A family of G-protein-coupled taste receptors (T2Rs) were shown to bind with a structurally diverse class of "bitter-tasting" compounds (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Matsunami *et al.*, 2000). It was suggested that these receptors evolved to help animals avoid ingesting toxic or otherwise harmful substances. Although each one of the receptors in this family is thought to be relatively specific for its ligand, many appear to be co-expressed in subsets of TBCs. This latter finding led researchers to hypothesize that mammals could not discriminate between "bitter-tasting" stimuli, because a given TBC could potentially be stimulated by a wide variety of compounds (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000). Caicedo and Roper (2001) suggested, however, that TBCs are more narrowly tuned than predicted from the receptor co-expression. This conclusion was based on their assessment of the intracellular calcium responses in TBCs, *in situ*. Each "bitter-responsive" TBC assayed responded to only one, or at most only a few of the five compounds tested (Caicedo and Roper, 2001). These researchers suggested that rats could likely discriminate between the "bitter" compounds tested, based on the apparent specificity of TBCs.

Spector and Kopka (2002) tested these predictions and demonstrated that rats could not behaviorally discriminate between denatonium benzoate and quinine hydrochloride. Data from this study strongly suggested that these two ligands produce a unitary taste sensation. These results appear to support the molecular findings, which indicted that numerous T2Rs are co-expressed in subsets of TBCs. Nevertheless, the specificity of TBCs observed in the periphery (based on Ca^{++} imaging measurements) might indeed exist. However, any segregation may be negated by a convergence of the signals

generated by the TBCs into a single neural channel somewhere along the gustatory neuraxis.

Summary

Conclusions regarding the taste quality of amino acids based on data from CTA experiments suggest that a subset of amino acids are perceptually similar to sucrose and some other sugars and results from two-bottle intake experiments show that these amino acids are preferred by mice. However, although L-amino acids are thought to bind exclusively with the T1R1+3 receptor complex, strain preference behavior measured in the two-bottle intake test seems to depend on an anomaly in the T1R2+3 complex, leaving open the possibility that other factors were influencing the relative preference for L-amino acids in this test. Moreover, data gathered in our laboratory question whether or not these stimuli are actually preferred by mice at all on the basis of taste and as a result question the very nature of the taste quality evoked by these compounds. Indeed, the molecular biology of "sweetener" transduction appears to provide a neurobiological basis for behavioral discriminability (i.e., perceptual distinction). Thus, my objective was to determine the degree to which receptor specificity predicts the relative discriminability of various "sweeteners," by testing whether C57BL/6J (B6) mice can discriminate between sucrose and L-serine, as well as a variety of other sugars and putative sweeteners using an operate discrimination paradigm. To my knowledge, explicit discrimination experiments in rodents with these ligands have never been conducted. These tasks were designed to assess whether pairs of putative sweeteners would be treated by animals as perceptually identical. If compounds are distinguishable, however, these discrimination tasks provide little information on the basis of the discriminability. Moreover, even if animals can discriminate between two compounds, they may also find them to be similar, relative to

other taste qualities (Spector, 2003). Therefore, to determine the relative similarity of these "sweeteners," as well as to provide information to aide in the interpretation of the discrimination data, CTA generalization tests where also conducted with a theoretically relevant subset of the stimuli.

The identification of compounds that share perceptual features ***and*** that are behaviorally indiscriminable allows for a confident categorization of a taste compound into a perceptual class (e.g., sweetness). Moreover, such data can be related back to the molecular biology of "sweet" taste in search of clues regarding the functional organization of the "normal" murine gustatory system.

Table1-1: Consequences of the "knockout" of various T1R receptors on neural and taste-related behavioral responses

STIMULUS	Chorda Tympani Nerve Recording				Brief-Access Taste Test[#]			
	T1R1 KO	T1R2 KO	T1R3 KO	T1R2+3 KO	T1R1 KO	T1R2 KO	T1R3 KO	T1R2+3 KO
L-serine	?	?	?	?	**I**	Normal	**0**	?
L-serine + 30 mM IMP*	**0**	Normal	**0**	?	?	?	?	?
L-alanine	?	?	?	?	**I**	Normal	**0**	?
L-alanine + 30 mM IMP*	**0**	Normal	**0**	?	?	?	?	?
D-tryptophan	Normal	**0**	**0**	**0**	Normal	**0**	**0**	?
Sucrose	Normal	**I**	**I**	**0**	Normal	**I**	**I**[#]	**0**
Glucose	Normal	**I**	**I** ** (Normal)	**0**	Normal	**I**	**I**	**0**

I = Impaired responsiveness; 0 = No responsiveness; ? = Not tested; * IMP alone produced no nerve response; ** All results are from Zhao *et al.*, 2003 except for those in parentheses, which are from Damak *et al.*, 2003.

Behavioral data, not gathered in a brief-access test, from two separate studies (Damak *et al.*, 2003 and Delay *et al.*, 2006) suggest that mice, lacking the receptor T1R3 can detect the presence of sucrose.

Table 1-2: % of taste buds and % TBCs in papillae

	Circumvallate			Foliate			Fungiform			Palate		
Receptor	TB	TBCs	refs	TB	TBCs	refs	TB	TBCs	refs	TB	TBCs	refs
T1R1	< 10%	5%	1	~30%*	~10%*	1	**100%**	~20-30%	1	**100%**	~20-30%	1
T1R2	**100%**	~20-30%	1	**100%**	~20-30%	1	< 1%	N/A	1	~20%	< 5%	1
T1R3	**100%**	~30%	2	**100%**	~30%	2	**100%**	~30%	2	**100%**	~30%	2

Data derived from published reports (see reference below and in text) of the percentages of taste buds expressing a given taste receptor and the percentages of TBCs within those taste buds (see text for more details).

[1]All data from Hoon *et al.*, 1999 are derived mouse and rat tongues.

*****These cells were reported to have a much weaker signal relative to T1R2 positive cells in the foliate, or T1R1 cells in fungiform papillae or in the palate.

[2] All data Nelson *et al*., 2001[1] are derived from adult mouse tongues

CHAPTER 2
EXPERIMENT 1: THE RELATIVE AFFECTIVE POTENCY OF GLYCINE, L-SERINE AND SUCROSE AS ASSESSED BY A BRIEF-ACCESS TASTE TEST IN INBRED STRAINS OF MICE[2]

Background

The molecular biology pertaining to the transduction of both sugars and synthetic sweeteners as well as "sweet-tasting" D-amino acids, reviewed in detail in Chapter 1, is consistent with the electrophysiological and behavioral phenotypes expressed by different inbred strains of mice, but such a correspondence regarding "sweet-tasting" L-amino acids (and glycine) is less straightforward. It has been known for many years that mouse strains can be differentiated according to their intake of and nerve responsiveness to natural and synthetic sweeteners. In general, "taster" mice have lower preference thresholds for sweeteners in two-bottle tests and their chorda tympani nerves (CT) are more responsive to sucrose, saccharin, and various "sweet-tasting" D-amino acids (especially D-phenylalanine) when compared with "non-taster"[3] mice (Capretta, 1970; Pelz *et al.*, 1973; Fuller, 1974; Ninomiya *et al.*, 1984; Lush, 1989; Capeless and Whitney, 1995; Bachmanov *et al.*, 1996; Frank and Blizard, 1999; Inoue *et al.*, 2001; Nelson *et al.*, 2001). These taster/non-taster phenotypes in mice were genetically linked to a single chromosomal locus referred to as *Sac* that was later discovered to encode for the T1R3

[2] A version of this Chapter has been published previously in Chemical Senses, Vol. 29 No. 6 © Published by Oxford University Press. All rights reserved.

[3] The phenotypic descriptors "taster" and "non-taster" may at first glance seem to denote ageusic vs. non-ageusic strains, however this nomenclature is commonly used in the literature to categorize mouse strains with varying degrees of sensitivity (i.e., low behavioral threshold vs. high behavioral threshold) to compounds such as sucrose and/or sodium saccharin.

receptor (e.g., Fuller, 1974; Ramirez and Fuller, 1976; Lush, 1989; e.g., Capeless and Whitney, 1995; Lush *et al.*, 1995). Taster and non-taster mouse strains have different alleles of the *Tas1r3* gene that give rise to receptors with slightly different amino acid sequences (e.g., Bachmanov *et al.*, 2001a; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001). Interestingly, the taster and non-taster allele of *Tas1r3* generates receptors that are functionally similar when combined with T1R1, but the non-taster form of the T1R3 receptor displays impaired binding when combined with T1R2 (Nelson *et al.*, 2002; Damak *et al.*, 2003). Thus non-taster mouse strains possess a dysfunctional T1R2+3, but an apparently normal T1R1+3 receptor complex. Indeed, there is evidence that L-amino acids, which bind with the T1R1+3 receptor, stimulate the CT comparably in both taster and non-taster mice, with the possible exception of L-proline (Ninomiya *et al.*, 1984; Inoue *et al.*, 2001). Yet, two-bottle preference for some "sweet-tasting" L-amino acids and glycine appears to depend on the "taster" status of the mouse strain based on testing with sugars (Lush, 1989; Capeless and Whitney, 1995; Lush *et al.*, 1995; Bachmanov *et al.*, 2001b). These behavioral findings are curious considering that L-amino acids are believed to bind primarily with the T1R1+3 receptor which, as noted above, is thought to display similar binding properties in both taster and non-taster mice (Nelson *et al.*, 2002).

In light of the apparent tension between the predicted behavior of mouse strains based on the molecular biology of amino acid taste transduction and the observed behavior seen in the two-bottle preference test, we examined the relative effectiveness of sucrose, glycine and L-serine to stimulate licking in C57BL/6J (B6), SWR/J (SWR), DBA/2J (D2) and 129P3/J (129) mice in a brief-access taste test. As noted above, inbred

mice vary in their preference for all three of these compounds as assessed in two-bottle intake tests, and there is evidence that these compounds possess some common perceptual properties with respect to taste quality (i.e., "sweet") in at least some rodents. If glycine and L-serine generate concentration–response functions that emulate sucrose, then it would suggest that these compounds are similar in their affective potency.

In addition, we sought to examine the generality of the response profiles generated by these compounds by including taster (B6 and SWR) and non-taster (129 and D2) mouse strains in the experimental design allowing us to make inferences regarding the effect of the non-taster form of the *Tas1r3* allele on taste-guided behavior (Capretta, 1970; Pelz *et al.*, 1973; Fuller, 1974; Lush, 1989; Capeless and Whitney, 1995; Bachmanov *et al.*, 1996; Max *et al.*, 2001; Nelson *et al.*, 2001). With some notable exceptions (Glendinning *et al.*, 2002; Zhang *et al.*, 2003; Zhao *et al.*, 2003), most of the work conducted to date involving strain comparisons of unconditioned behavioral responsiveness to these compounds has been based on two-bottle intake tests (water versus taste compound). Although taste certainly influences the behavior in that test paradigm, postingestive events can also influence intake. The brief-access taste test involves the measurement of licking during very short trials with a sapid solution increasing the confidence that the responses are based on the oral sensory features of the stimulus. Many trials of various concentrations of the taste stimulus are presented during a session and concentration–response functions are derived. The taste solutions are delivered in randomized blocks to minimize systematic carry-over effects and to mitigate the influence of postingestive factors on the response to a given stimulus in the set.

Methodological Details

Subjects

A total of 120 male naive mice (Jackson Laboratories, Bar Harbor, Maine) from four different strains, C57BL/6J (B6), SWR/J (SWR), 129P3/J (129), and DBA/2J (D2), served as subjects (n=30/strain). Within each strain, animals were randomly assigned to one of 3 stimulus groups (n=10/group). The mice were housed individually in polycarbonate shoebox cages in a colony room where the lighting was controlled automatically (12h: 12h). Testing and training took place during the lights-on phase. Mice were habituated to the laboratory environment for seven days before testing and were ~ 8 weeks of age at the start of testing. During this time, food and purified water (Elix 10; Millipore, Billerica, MA) were available *ad libitum*. During periods when the animals were placed on a water-restriction schedule, mice that dropped below 80% of their free-feeding weight received 1 ml supplemental water 2 hours after the end of the testing session. All procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

Taste Stimuli

All solutions were prepared daily with purified water and reagent grade chemicals, and were presented at room temperature. Test stimuli consisted of 5 concentrations of sucrose (0.0625, 0.125, 0.25, 0.5, and 1.0 M; Fisher Scientific, Atlanta, GA), L-serine (0.25, 0.5, 0.75, 1.0, and 1.5 M; Sigma-Aldrich, St Louis, MO), glycine (0.25, 0.5, 0.75, 1.0, and 1.5 M; Sigma-Aldrich, St Louis, MO) and purified water. Sucrose was chosen because 1) it is a prototypical natural sweetener that is commonly used in taste experiments, 2) it has been used to differentiate taster (B6 and SWR) from non-taster (D2 and 129) mice in two-bottle preference tests, and 3) binds with the T1R2+3, but not the

T1R1+3, receptor complex. L-serine and glycine were chosen because 1) there is evidence that at least in some rodents these compounds share a perceptual quality with sucrose, 2) they are preferred by some rodents at mid-range concentrations in two-bottle preference tests, and 3) appear to bind primarily with the T1R1+3, but only poorly, if at all, with the T1R2+3 receptor complex.

Procedure

We used a brief-access procedure similar to that described by Glendinning *et al*. (2002). Testing took place in a lickometer referred to as the Davis rig (Davis MS-160, DiLog Instruments, Tallahassee, FL; see Smith, 2001). This device allowed the mouse access to a single tube containing a taste stimulus for a brief period of time (5 s) and then after a 7.5-s inter-presentation interval, a different tube was offered. The stimulus array for each compound tested included the five different concentrations detailed above and purified water contained in separate bottles. A given trial started after the first lick. Presentation order was randomized without replacement in blocks so that every concentration of a stimulus and water was presented exactly once before the initiation of the subsequent block. Unconditioned licking responses were recorded for later analysis. Sessions were 30 min in duration during which mice could initiate as many trials as possible. The animals were first trained to lick a stationary tube of water for 30 min in the Davis rig after being placed on ~23.5-h restricted water access schedule. Animals then received 2 days of testing with five stimulus concentrations and purified water while maintained on the water-restriction schedule. This was done to familiarize the animals with the stimulus array. The water bottles were then replaced on the home cages and the mice were tested for three days non-deprived.

Data Analysis

For data gathered when animals were water-deprived, a Tastant/Water Lick Ratio was calculated. This ratio was derived by taking the average number of licks per trial for each concentration and dividing it by the average licks per trial when water was delivered. This ratio controls for individual differences in lick rates and for differences in motivational state. The Tastant/Water Lick Ratio is useful for analyzing responses of animals highly motivated to lick due to the restricted water access schedule. When animals were non-deprived, the average number of licks per trial for each concentration was collapsed across test sessions and divided by that animal's maximum potential lick rate per trial based on the mean of the inter-lick interval (ILI) distribution measured during training (only inter-lick intervals greater than 50 and less than 200 ms were used), yielding a Standardized Lick Ratio (see Glendinning *et al.* 2002). Standardizing the licking response in this fashion controls for individual differences in maximal lick rates.

The ratio scores were analyzed with two-way strain x concentration analyses of variance (ANOVAs). When a strain main effect or a strain x concentration interaction was significant, 1-way ANOVAs were conducted to test for simple effects. Differences between strains at each concentration were evaluated using Tukey's honestly significant difference test. Differences between Standardized Lick Ratio scores in response to a given concentration and those measured for water were tested with matched-t-tests. The conventional $P \leq .05$ was applied as the statistical rejection criterion. Only mice that had at least 1 trial at every concentration were included in the analysis of a given stimulus.

Results

Standardization Data

Because there can be within-strain and between-strain differences in the local lick rate as well as in the motivational response to the water restriction schedule, it is important to account for these factors in any licking measure of taste responsiveness. As recommended by Glendinning *et al.* (2002), the Tastant/Water Lick Ratio was calculated for animals tested when under the water-restriction schedule and the Standardized Lick Ratio was calculated for animals tested when non-deprived to statistically control for non-taste influences in licking. Table 2-1 contains the means values representing licks during water trials used in the calculation of the Tastant/Water Lick Ratio for the various strains and compounds. A two-way ANOVA on water licks revealed a significant main effect of strain [$F(3,107) = 40.7$, $P < .001$] and test solution [$F(2,107) = 9.15$, $P < .001$] as well as a significant interaction [$F(6,107) = 5.31$, $P < .001$]. One-way ANOVAs were conducted within each taste compound to test for strain differences in water licks. There was a significant main effect of strain on the mean number of licks to water when mice were tested with sucrose [$F(3,36) = 15.6$, $P < .001$], L-serine [$F(3,36) = 15.4$, $P < .001$] and glycine [$F(3,36) = 19.6$, $P < .001$] in the water restriction condition. Interestingly, when mice were water deprived, one-way ANOVAs conducted within each strain to test for differences in water licks to the stimuli revealed that the non-taster strains increased licks to water when tested with L-serine relative to licks taken in the other stimulus conditions (Ps $\leq .001$). The taster strains did not significantly differ in their responses to water across stimulus conditions.

Table 2-2 contains the means of the individual values representing the ILI observed when water-restricted animals were licking water from a stationary spout. These means exclude the mice that were not included in the analysis of responses under non-deprived conditions (n = 93). The reciprocal of these values were multiplied by 5000 to derive the estimated maximum possible licks during a 5-s trial and used in the calculation of the Standardized Lick Ratio for various strains and compounds. As expected, a two-way ANOVA revealed a significant effect of strain [$F(3,81) = 52.1$, $P < .001$] but no significant stimulus effect [$F(2,81) = 0.1$, $P = .909$] or interaction [$F(6,81) = 0.7$, $P = .657$]. Collectively, the results from these analyses confirm the necessity for standardizing the licking data across animals and strains.

Sucrose

When animals were water deprived, a two-way ANOVA of the Tastant/Water Lick Ratios revealed a significant main effect of strain [$F(3,36) = 18.1$, $P < .001$), a significant main effect of concentration [$F(4,144) = 5.9$, $P < .001$] and a significant interaction [$F(12,144) = 10.4$, $P < .001$]. Strain differences at each concentration are delineated in Table 2-3. Confirming what is apparent in Figure 2-1, separate one-way ANOVAs for each strain revealed that only the 129 mice showed a significant monotonically increasing concentration-response function [$F(4,36) = 11.9$, $P < .001$]. Although we did not expect to find meaningful results in the water-restriction condition considering that mice will usually lick water at a maximal rate making it difficult to ascertain a response to appetitive stimuli and we did not expect to see an aversive response profile elicited by these “sweet-tasting” compounds, it appears that the 129 mice did suppress licking to water relative to the other strains (see table 2-1) and, as a result, increased their Tastant/Water Lick Ratio to the stimulus [$F(3,36) = 15.6$, $P < .001$]. There were some

significant concentration-dependent effects on the Tastant/Water Lick Ratio for the other 3 strains (All *F*s >3.0, all *P*s < .05), but it is obvious that these functions were relatively flat and generally equal to or below a value of 1.0. The 129 mice had significantly higher ratios at all five concentrations compared with the B6 and D2 mice and at the four highest concentrations compared with the SWR mice (all *P* values < .05); the latter three strains did not differ.

When non-deprived, all strains clearly showed a concentration dependent increase in licking to sucrose (see Figure 2-2; $F(5,170)=531.9$, $P < .001$), but their concentration-response functions significantly differed (strain x concentration interaction: $F(15,170) = 10.9$, $P < .001$). Strain differences at each concentration are delineated in Table 2-4. The SWR mice were significantly more responsive to lower sucrose concentrations compared with D2 and 129 mice. At the lowest concentration tested (0.0625 M), the Standardized Lick Ratio was significantly greater than that for water in the SWR and B6 (both *t*s > 2.2, *P*s < .05), but not the D2 and 129, strains (both *t*s < -0.2, *P*s > .7). As the sucrose concentration was raised, however, D2 and 129 mice steeply increased their responsiveness to sucrose and eventually equaled or surpassed the licking in SWR mice. B6 mice had a concentration response profile somewhat in between the SWR and the 129 and D2 mice. At the lower concentration, B6 mice were statistically indistinguishable from all of the mice including the SWR, but at the two highest concentrations they were significantly less responsive compared with the other three strains.

L-serine

When mice were water deprived, there was a significant strain effect ($F(3,36) = 9.8$, $P < .001$) on the Tastant/Water Lick Ratio, and a significant strain x concentration interaction (see Figure 2-1; $F(12,144) = 6.6$, $P < .001$). Surprisingly, the D2 mice

actually decreased their lick rate as the L-serine concentration was raised (Figure 2-1; $F(4,36) = 15.3$, $P < .001$), whereas the other strains displayed relatively flat functions. Strain differences at each concentration are shown in Table 2-3.

When animals were non-deprived, there was no significant difference in the Standardized Lick Ratio between the strains ($F(3,21) = 0.1$, $P = .9$), but there was a significant effect of concentration ($F(5,105) = 4.2$, $P = .002$), though the increase was relatively minor; there was no significant strain x concentration interaction (see Figure 2-2).

Glycine

When animals were water deprived, there was a significant strain effect ($F(3, 35) = 10.6$, $P < .001$) on the Tastant/Water Lick Ratio, and a significant strain x concentration interaction ($F(12,140) = 5.7$, $P < .001$). Strain differences at each concentration are delineated in Table 2-3. As was the case with sucrose, separate one-way ANOVAs indicated that only the 129 mice increased their Taste/Water Lick Ratio monotonically as a function of concentration ($F(4,36) = 7.1$, $P < .001$); see Figure 2-1). This increase in licking was first significantly greater than 1.0 at the 0.75 M concentration ($P = .022$). There were some significant concentration-dependent effects on the Tastant/Water Lick Ratio for B6 and SWR mice (All Fs > 4.6, all Ps < .01), but it is obvious that the functions for these strains as well as for the D2 mice were relatively flat and generally below a value of 1.0.

When mice were non-deprived, there was a significant effect of strain ($F(3, 26) = 5.8$, $P = .004$) on the Standardized Lick Ratio, and a significant strain x concentration interaction ($F(15,130) = 2.9$, $P = .001$). Strain differences at each concentration are delineated in Table 2-4. Separate one-way ANOVAs of the Standardized Lick Ratios for

each strain revealed that 129 ($F(5,20) = 8.1$, $P < .001$), B6 ($F(5,40) = 3.3$, $P < .05$), and D2 ($F(5,40) = 2.5$, $P < .05$) mice changed their lick rate as a function of concentration, but the modest increases were apparently limited to higher concentrations (see Figure 2-2). For example, matched t-tests indicated that the 129 strain did not display significantly elevated licking relative to water until the glycine concentration reached 1.5 M ($P < .05$). For B6 and D2 mice, no concentration significantly differed from water. The SWR mice did not significantly change their licking as a function of concentration ($F(5,30) = 0.6$, $P = .678$).

Discussion

Overall, as assessed by the brief-access taste test, the amino acids, L-serine and glycine, paled in comparison to sucrose in their ability to generate licking in the mouse strains examined. Collapsed across strain, non-deprived animals licked L-serine and glycine at a mean rate of only 15.4% and 21.4%, respectively, of the maximum possible in the 5-s trial at the highest concentration tested (1.5 M). In striking contrast, 1.0 M sucrose (the highest concentration tested) elicited an average licking rate, collapsed across strain, that was more than 5 times higher than that seen for L-serine and nearly 4 times higher than that seen for glycine. The relatively broad concentration range used in this study weakens the possibility that the design failed to capture the dynamic range of responsiveness. Thus, the results presented here suggest that the taste-related affective potency of sucrose is far superior to that of glycine or L-serine.

Although neither amino acid was remarkably effective at stimulating licking in non-deprived mice relative to sucrose, glycine generated concentration-dependent increases in licking in water-restricted 129 mice, whereas L-serine did not. For the D2 non-taster mice, we actually observed a concentration-dependent decrease in the

Tastant/Water Lick Ratio in response to L-serine when these mice were water-deprived. Given that L-serine is thought to possess a sucrose-like taste quality, this finding was unexpected and suggests that L-serine may also bind with other receptors that lead to aversive responses (e.g. T2Rs), at least in the D2 strain. Other researchers have reported higher levels of L-serine licking relative to water by B6, 129X1/SvJ and CB6 (BALB/c x B6 hybrids) mice in a brief-access test (Zhang *et al.*, 2003; Zhao *et al.*, 2003). These discrepancies are likely the result of methodological differences between the studies. More specifically, in the prior work, both food and water intake was limited in a controlled fashion, based on procedures described by Glendinning *et al.* 2002, to achieve a motivational state that would promote stimulus sampling but would not lead to the asymptotic lick rates generally observed under 24-h water deprivation regimens. Based on the present results, it appears that without the additional effects of nutrient restriction, the gustatory properties of L-serine and glycine alone stimulate only slight, if any, licking behavior in the mouse strains tested here.

The profile of strain differences in responsiveness to the compounds tested here was more complex than previously reported. When non-deprive mice were tested with sucrose, in general the "taster" strains (B6 and SWR) were modestly more responsive at lower concentrations compared with the "non-taster" mice (129 and D2), but even this difference failed to reach significance for the B6 strain. As the concentration was progressively raised, the responsiveness of SWR and B6 taster mice converged with that seen in the D2 non-taster mice. Notably, the 129 non-taster mice licked the two highest concentrations of sucrose significantly more than did all three of the other strains. In

general, these results are consistent with findings obtained by other researchers (i.e., Glendinning *et al.*, 2005b).

When mice were water deprived, sucrose, as expected, produced licking rates comparable to water in all strains except for the 129 mice. The 129 mice, in fact, nearly doubled their rate of stimulus responsiveness relative to water at 1.0 M. This same pattern was seen with glycine, with the 129 mice responding to the compound at nearly 1.5 times the rate of water at 1.5 M. Interestingly, the D2 non-taster mice displayed concentration-dependent decreases in their L-serine tastant/water lick ratio when water deprived, whereas the other strains had relatively flat curves. It appears when mice were water-deprived the non-taster strains were less motivated to lick L-serine relative to sucrose and glycine, whereas all three stimuli were treated similarly by the taster strains. Collectively, these findings suggest that the phenotypic descriptors "taster" and "non-taster" do not necessarily apply to the responsiveness seen at higher concentrations of putative sweeteners, at least in the brief-access test.

The taster and non-taster classification is based on the preference behavior of various mouse strains to low concentrations of sweeteners in long-term two-bottle intake tests. The brief-access taste test differs from the two-bottle intake test in interpretively important ways. In the brief-access test, immediate responses to small volumes of stimuli are measured raising the confidence that the behavior is driven by taste (see Spector, 2003). Indeed, Spector *et al.* (1996) demonstrated that when rats are deprived of gustatory input from the 7th and 9th cranial nerves innervating the oral cavity, they show essentially flat concentration–response curves for sucrose when tested using a brief-access paradigm providing further evidence that behavior measured using a brief-access

procedure is taste-guided. In contrast, in the two-bottle test, intake is usually measured 24 h after stimulus presentation allowing for postingestive factors to influence the outcome. Moreover, differences in stimulus preference at high concentrations are difficult to detect with the two-bottle preference test because of ceiling effects. Typically, preference ratios approach an asymptotic value of 1.0 at very low concentrations for normally preferred stimuli, after which differences are difficult to discern. Other researches have used a shorter-term one-bottle intake test (e.g., 6 hr.) where ceiling effects and position preferences are avoided or at least minimized (e.g., Blizard *et al.*, 1999). But while the results obtained using the one-bottle test are consistent with those seen when using the two-bottle intake procedure, neither test avoids the confounding effects of viscerosensory input. On the other hand, the brief-access taste test does not appear to be as sensitive to changes in behavior at low concentrations, at least when several higher concentrations are available during the session. Thus, these various procedures have different dynamic ranges of sensitivity. Accordingly, it would appear that, behaviorally speaking, the taster/non-taster distinction is limited to low concentrations of sweeteners. This is consistent with sucrose and glucose detection thresholds measured with an operant procedure in which the hedonic value of the taste stimulus is rendered irrelevant (Eylam and Spector, 2004). Interestingly, in the Eylam and Spector study, the threshold values for glycine measured with the same procedure in the same mice did not distinguish taster and non-taster strains in as straightforward a manner. That is, non-taster 129 mice had significantly higher glycine thresholds relative to B6 mice. However, the glycine thresholds for non-taster D2 mice did not differ from those for the taster B6 and SWR mice. In stark contrast, in our study, at the higher

concentrations, the 129 mice were the most responsive strain tested in this report. These findings further highlight the difference between suprathreshold responsiveness and threshold sensitivity (cf., Bachmanov *et al.*, 1997).

If the T1R family of receptors mediates "behavioral attraction," as postulated by some (Zhao *et al.*, 2003), then activation of either receptor complex should elicit appetitive behavior. However, compounds that bind with the T1R2+3 complex are apparently much more effective, at least as measured by the assay used in our study. Sucrose, which was shown to stimulate the T1R2+3 complex in a heterologous system (HEK 293), generated licking at rates at least four times higher than any other compound tested. Partial support for this dissociation comes from the fact that glycine, which was also shown to stimulate the T1R2+3 complex, but to a lesser extent, in general elicited slight increases in licking at high concentrations resembling its modest ability to bind with the receptor (see Nelson *et al.*, 2002), at least in those mice that sampled all of the concentrations. We found no evidence that L-serine, a compound that binds with the T1R1+3 heteromer, but not with the T1R2+3, is an effective behavioral stimulus in the brief-access test in non-deprived mice. As noted above, there is evidence that L-amino acids can stimulate significant degrees of licking in mice that have restricted food and water access. Thus, it would appear that the affective value of stimuli that bind with the T1R1+3 receptor depends upon the nutritional/physiological status of the animal, whereas stimuli that bind with the T1R2+3 receptor do act like general "attractants."

The behavioral results presented here do not relate to the electrophysiological response properties of the CT nerve in an obvious way (Frank and Blizard, 1999; Inoue *et al.*, 2001). While all three stimuli used in our study reportedly evoke very clear

concentration-related increases in CT responsiveness in B6 and 129 mice, the concentration-response functions for glycine and L-serine in non-deprived mice from these strains in the brief access test had very shallow slopes. Moreover, while the magnitude of CT responses to sucrose is greater in B6 compared with 129 mice even at high concentrations, the 129 mice displayed more vigorous sucrose licking than the B6 mice at the 0.5 and 1.0 M concentrations in the brief-access test. It is conceivable that a subclass of CT fibers might display a better correspondence with the hedonic value of these stimuli and this relationship might be obscured in whole-nerve analyses (cf., Frank and Pfaffmann, 1969). However, it is likely that the affective potency of these stimuli is based on more than just input from the CT. Input from other peripheral nerves and the central neural circuits that translate those signals into behavior must be considered. Thus, while non-taster strains might have an impaired peripheral signal for certain sweeteners that stimulate the T1R2+3 receptor complex, the way that input is processed by the brain can also differ from taster strains in a manner that could augment behavior. Likewise, a robust peripheral signal for glycine or L-serine or any taste stimulus does not guarantee that a given behavioral response will be generated.

In summary, we found that sucrose was the most effective compound tested, followed by glycine, and lastly L-serine in generating licking in the brief-access taste test. The order of affective potency seems to be related to the ability of the stimulus to activate the T1R2+3 receptor complex. Furthermore, strain differences in responsiveness to these compounds suggest that the current understanding of "sweet-tasting" ligand transduction is insufficient in entirely explaining the observed response profiles. For example, the fact that the 129 mice licked at rates greater than the D2, B6 and SWR mice to the higher

concentrations of sucrose would not have been predicted by the current molecular biological findings or CT nerve recordings. Apparently, the taster/non-taster distinction which has been shown to be dependent on the polymorphism of the *Tas1r3* gene encoding for the T1R3 receptor is limited to low concentrations of sucrose, whereas responsiveness to higher concentrations of the sugar is related, at least in part, to other genes that might affect stimulus processing anywhere along the gustatory neuraxis. It would be instructive to repeat the behavioral tests conducted here in congenic, transgenic and/or knock-out mice in which the *Tas1r3* gene has been manipulated keeping the genetic background constant to examine the explicit role of the T1R3 variants in behavioral responsiveness to mid-range and high concentrations of sugars, synthetic compounds and amino acids. The results of our study also call into question the very nature of the perceptual quality elicited by these amino acids. As noted above, there is evidence from conditioned taste aversion generalization experiments that rodents treat glycine and L-serine as possessing a sucrose-like taste quality (Nowlis *et al.*, 1980; Pritchard and Scott, 1982b; Kasahara *et al.*, 1987). Yet, in the brief-access test with the non-deprived mice tested here, the responses to sucrose were discernibly different than those to the amino acids. Thus, it would appear that while the perception evoked by glycine and L-serine might share some qualitative characteristic with sucrose, these amino acids might also generate additional qualities that impact upon their affective value at least in certain species and strains. For example, saccharin is both sweet and bitter tasting to humans depending on concentration (Bartoshuk, 1979; Schiffman *et al.*, 1979). Experiments designed explicitly to test the ability of these mice to distinguish between sucrose, glycine, L-serine, and other L-amino acids and sugars in operant taste

discrimination tasks, in addition to a more comprehensive examination of conditioned taste aversion generalization profiles should help refine the characterization of the qualitative similarities and differences of these taste stimuli. Such behavioral experiments can provide a functional context to guide the interpretation of findings from more molecular levels of analysis.

Table 2-1: Mean number of licks to water ± SEM taken by the four strains when tested with sucrose, L-serine, or glycine when mice were water deprived.

	Sucrose	L-serine	Glycine
B6	32.2 ± 1.4	30.6 ± 2.3	32.3 ± 2.2
SWR	41.8 ± 2.2	44.1 ± 1.9	48.3 ± 1.5
129	22.9 ± 1.7	34.0 ± 1.9*	24.8 ± 2.3
D2	34.8 ± 2.5	47.1 ± 1.9*	32.8 ± 2.9

Asterisk indicates that the 129 and D2 "non-taster" mice took significantly higher numbers of licks to water when tested with L-serine relative to sucrose or glycine.

Table 2-2: Mean of the inter-lick interval (ILI) distribution (ms) ± SEM observed in the four strains of mice trained to lick either sucrose, L-serine, or glycine.

	Sucrose	L-serine	Glycine
B6	121.3 ± 1.1	122.0 ± 1.5	121.3 ± 1.9
SWR	98.0 ± 1.2	98.6 ± 1.4	94.4 ± 1.0
129	108.3 ± 1.1	109.8 ± 0.8	108.6 ± 1.7
D2	104.4 ± 2.3	105.9 ± 1.8	104.7 ± 2.8

For a given strain, the observed ILI value did not significantly differ whether tested with sucrose, L-serine, or glycine.

Table 2-3: Strains listed in order of mean Tastant/Water Lick ratio, for sucrose, L-serine, and glycine when mice were water deprived.

Sucrose[1]							L-serine[1]							Glycine[1]						
0.0625 M:	129	≥	SWR	=	D2	=	B6	**0.25 M:**	129	=	B6	=	SWR	=	D2	**0.25 M:**	129	=	B6	=	SWR	=	D2
0.125 M:	129	>	SWR	=	D2	=	B6	**0.5 M:**	SWR	≥	129	=	B6	=	D2	**0.5 M:**	129	≥	SWR	=	B6	=	D2
0.25 M:	129	>	SWR	=	D2	=	B6	**0.75 M:**	129	≥	SWR	=	B6	=	D2	**0.75 M:**	129	>	B6	=	SWR	=	D2
0.5 M:	129	>	SWR	=	D2	=	B6	**1.0 M:**	129	=	B6	=	SWR	>	D2	**1.0 M:**	129	>	D2	=	B6	=	SWR
1.0 M:	129	>	SWR	=	D2	=	B6	**1.5 M:**	SWR	=	129	=	B6	>	D2	**1.5 M:**	129	>	D2	=	SWR	=	B6

[1]At concentrations at which the ANOVA detected a significant strain effect, strains falling under the same line did not significantly differ in Tukey's HSD posthoc comparisons ($P<0.05$).

Table 2-4: Strain listed in order of mean Standardized Lick ratio, for sucrose, L-serine, and glycine when mice were non-deprived.

	Sucrose[1]											
Water:	SWR	=	B6	=	D2	=	129					
0.0625 M:	SWR	≥	B6	=	D2	=	129					
0.125 M:	SWR	≥	B6	=	129	=	D2					
0.25 M:	SWR	≥	129	≥	B6	>	D2					
0.5 M:	129	>	SWR	=	D2	=	B6					
1.0 M:	129	>	D2	=	SWR	=	B6					

	L-serine[2]											
Water:	B6	=	SWR	=	D2	=	129					
0.25 M:	129	=	B6	=	D2	=	SWR					
0.5 M:	D2	=	SWR	=	B6	=	129					
0.75 M:	B6	=	129	=	D2	=	SWR					
1.0 M:	B6	=	129	=	SWR	=	D2					
1.5 M:	B6	=	129	=	SWR	=	D2					

	Glycine[1]											
Water:	SWR	=	B6	=	D2	=	129					
0.25 M:	B6	=	D2	=	129	=	SWR					
0.5 M:	B6	=	129	=	D2	=	SWR					
0.75 M:	B6	=	129	=	SWR	=	D2					
1.0 M:	129	≥	B6	=	D2	=	SWR					
1.5 M:	129	≥	B6	=	D2	=	SWR					

[1]For sucrose and glycine, at concentrations at which the ANOVA detected a significant strain effect, strains falling under the same line did not significantly differ in Tukey's HSD posthoc comparisons ($P<0.05$).

[2]There were no significant strain effects for L-serine.

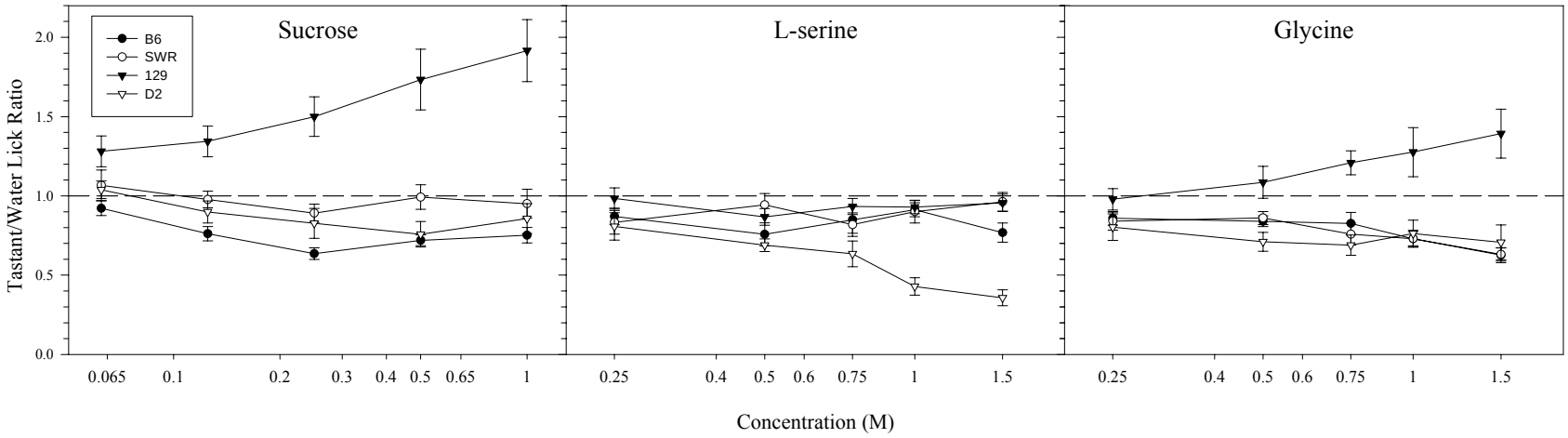

Figure 2-1: Mean (± SE) Tastant/Water Lick Ratio as a function of sucrose, L-serine, and glycine concentration for four different inbred strains of mice (n=10/stimulus/strain). The Tastant/Water Lick Ratio was calculated by dividing an animal's average licks to a given taste stimulus across trials by the average licks to water. The dashed line on the graph represents a Tastant/Water Lick Ratio of 1.0, which indicates licking to the taste stimulus was equivalent to licking to water. This ratio controls for differences in oral motor competence and physiological state. These animals were tested in 3 consecutive sessions while on a 23.5 h water restriction schedule.

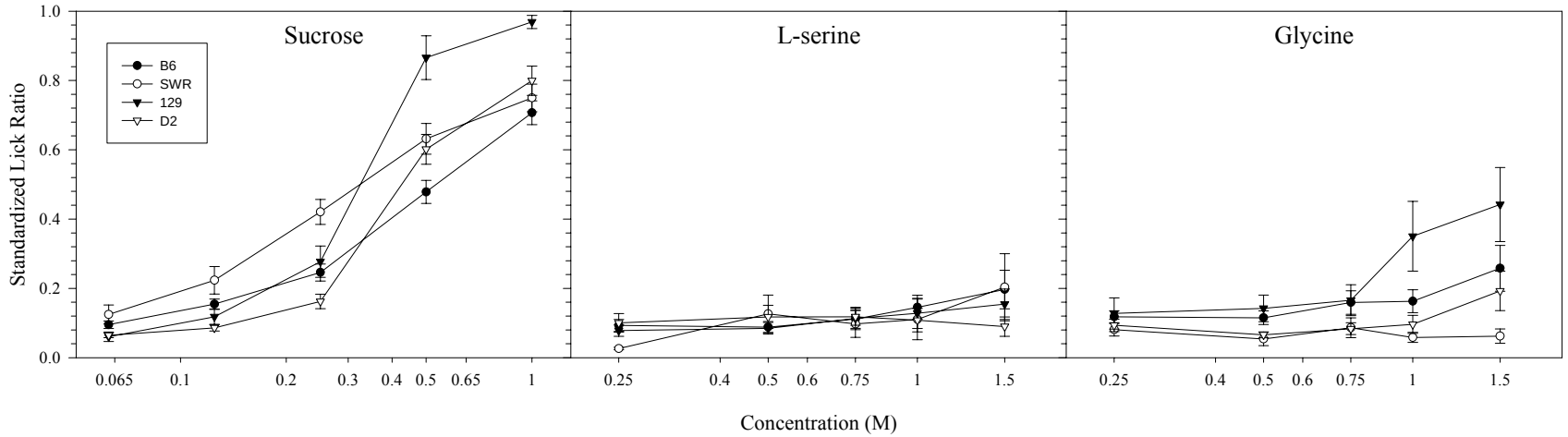

Figure 2-2: Mean (± SE) Standardized Lick Ratio as a function of sucrose, L-serine, and glycine concentration for four different inbred strains of mice. The Standardized Lick Ratio was calculated by dividing an animal's average licks to a given taste stimulus across trials by the maximum potential licks in a 5-s trial, derived from that animal's previously measured inter-lick interval distribution. This score is used for normally preferred stimuli and controls for differences in characteristic local lick rates. A score of 1.0 reflects licking to the taste stimulus that was at the maximum possible rate. These animals were tested non-deprived in 3 consecutive sessions. Only mice that had at least 1 trial at every concentration were included in the analysis of a given stimulus (sucrose: B6 [n=10], SWR [n=8], 129 [n=10], D2 [n=10]; L-serine: B6 [n=9], SWR [n=4], 129 [n=7], D2 [n=5]; and glycine: B6 [n=9], SWR [n=7], 129 [n=5], D2 [n=9]).

CHAPTER 3
EXPERIMENT 2: TASTE DISCRIMINABILITY OF L-SERINE AND VARIOUS SUGARS BY MICE

Background

Conclusions regarding the taste quality of amino acids based on data from CTA experiments suggest that a subset of amino acids is perceptually similar to sucrose and some other sugars. Consistent with this view, results from two-bottle intake experiments show that these amino acids are preferred by mice. However, although L-amino acids are thought to bind exclusively with the T1R1+3 receptor complex, preference behavior measured in the two-bottle intake test seems to depend on an anomaly in the T1R2+3 complex across strains. Moreover, data gathered in our laboratory question whether or not these compounds are actually preferred by mice at all on the basis of taste and as a result question the very nature of the taste quality evoked by these compounds. Indeed, the molecular biology of "sweetener" transduction appears to provide a neurobiological basis for behavioral discriminability (i.e., perceptual distinction). Thus, the goal of the current studies was to determine the degree to which the reported receptor specificity predicts the relative discriminability of various "sweeteners," by testing whether C57BL/6J (B6) mice can discriminate between sucrose and L-serine, as well as a variety of other sugars using an operate discrimination paradigm. To my knowledge, explicit discrimination experiments in rodents with these ligands have never been conducted.

Methodological Details

Subjects

Adult C57BL/6J (B6) male and female mice (n=12; Jackson Laboratories, Bar Harbor, Maine), ~ 8 weeks of age on arrival, served as subjects. The B6 strain was chosen because 1) it is the most common mouse strain used in taste research, 2) has been previously characterized as a "taster" strain (e.g., Capretta, 1970; Pelz *et al.*, 1973; Fuller, 1974; Lush, 1989; Capeless and Whitney, 1995; Bachmanov *et al.*, 1996), and 3) serves as a background strain in many knock-out, congenic, and transgenic manipulations (e.g., Damak *et al.*, 2003; Zhao *et al.*, 2003). The mice were housed individually in polycarbonate cages in a colony room where the lighting was controlled automatically (12:12). Testing and training took place during the lights-on phase. After arrival in the facility, subjects had free access to pellets of laboratory chow (Purina 5001, PMI Nutrition International Inc., Brentwood, MO) and purified water (Elix 10; Millipore, Billerica, MA). Seven days after arrival, mice were put on a restricted water-access schedule with fluid available Monday – Friday during testing only. Purified water was freely available on the home cage from Friday afternoon through Sunday afternoon every week. Mice that dropped below 85% of hydrated weight while on water-restriction schedule, received 1 ml of supplemental water ~2 hours after the end of the testing session. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Taste Stimuli

All solutions were prepared daily with purified water and reagent grade chemicals and were presented at room temperature. "Comparison stimuli" consisted of various concentrations of sucrose, glucose, maltose, fructose, (Fisher Scientific, Atlanta, GA) and

L-serine. In addition, sodium chloride (NaCl; Fisher Scientific, Atlanta, GA) was used as a comparison stimulus to provide a contrast that could be easily discriminated from a standard stimulus during training. Sucrose, fructose, and glucose were chosen because 1) they are prototypical sweeteners that are commonly used in taste experiments, 2) with the exception of fructose, they have been used to differentiate taster (e.g., B6) from non-taster mice in two-bottle preference and intake tests (e.g., Stockton and Whitney, 1974; Ramirez and Fuller, 1976; Lush, 1989; Bachmanov *et al.*, 1996; Bachmanov *et al.*, 1997; Bachmanov *et al.*, 2001b), as well as in an operant discrimination task (Eylam and Spector, 2004), and 3) they are thought to exclusively bind with the T1R2+3, but not the T1R1+3, receptor complex (Nelson *et al.*, 2001; Nelson *et al.*, 2002; Zhao *et al.*, 2003). L-serine was chosen because 1) there is evidence that at least in some rodents this compound shares a perceptual quality with sucrose (Kasahara *et al.*, 1987), 2) it is preferred by some strains of mice at mid-range concentrations in two-bottle preference tests (Iwasaki *et al.*, 1985), and 3) appears to bind primarily with the T1R1+3, but only poorly, if at all, with the T1R2+3 receptor complex (Nelson *et al.*, 2002; Zhao *et al.*, 2003).

Concentrations of each stimulus tested are listed in Table 3-1. These were chosen on the basis of the available behavioral data and with the intent of representing the dynamic range of responsiveness for B6 mice. The concentrations of sucrose and fructose selected encompass the dynamic range of behavioral responsiveness for B6 mice, as measured in a brief-access taste test (Dotson *et al.*, 2005; Glendinning *et al.*, 2005a; 2005b; also see Chapter 2, Figure 2-2). Glucose concentrations were chosen based on the diminished neural and behavioral sensitivity of B6 mice to glucose relative to sucrose

(Ninomiya *et al.*, 1984; Eylam and Spector, 2004). The maltose concentrations encompassed the range of behavioral preference for C57BL/6ByJ mice, as measured using a two-bottle intake taste test (Bachmanov *et al.*, 2001b). Very little data has been gathered on the behavioral responsiveness of mice to L-amino acids. Thus, the choice of concentrations for L-serine was based on the available neural data. The whole CT nerve of ddy mice responses monotonically to an increasing concentrations series of L-serine (0.01 M – 1 M, neural threshold = 0.003 - 0.01 M; Iwasaki *et al.*, 1985). Thus, those concentrations chosen for L-serine were assumed to be within the dynamic range of responsiveness for mice. NaCl concentrations represented the range of behavioral responsiveness for C57BL/6ByJ mice, as measured in a brief-access taste test (Dotson *et al.*, 2005) and were also thought to be within the dynamic range of responsiveness for the substrain of B6 mice used here.

It is important to stress that the broad range of concentrations chosen helps guarantee that there will be overlapping intensities and viscosities across the stimuli. This is important because of the need to render intensity an irrelevant cue while promoting quality as the consistent discriminable signal.

Apparatus

Animals were trained and tested in a specially designed computer-controlled gustometer modified for use with mice (Spector *et al.*, 1990; Eylam and Spector, 2002, 2003, 2004, 2005). This apparatus consisted of a modified operant chamber housed in a sound-attenuating cubicle, which had a reinforcement spout positioned on either side of a centrally positioned slot through which a mouse gained access to a sample spout. Background noise inside the sound-attenuating cubicle helped to minimize extraneous auditory cues. All stimuli and reinforcement fluids were placed in pressurized reservoirs

outside the chamber. Computer-operated solenoid valves regulated fluid delivery from these reservoirs by controlling the amount of fluid dispensed from the sample spout. This required that the sample shaft first be filled with the stimulus and then, with each subsequent lick, ~ 2.0 μL were deposited into the shaft. The volume per lick received from the reinforcement spouts was also ~ 2.0 μL. At the end of each trial, the sample spout rotated over a funnel, was rinsed with purified water, and evacuated with pressurized air. Two cue lights positioned on the ceiling of the test cage above the reinforcement spouts and a house light were turned on or off during each trial according to the programmed trial schedule to signal the beginning and/or ending of the various phases of a trial.

Experimental Design

Two groups of mice were initially tested on their ability to discriminate either sucrose or L-serine (standard stimuli) from sodium chloride (comparison stimulus). After successful completion of this discrimination test, the comparison stimulus was changed from NaCl to one of the other compounds listed in Table 3-2. When this discrimination was completed, the comparison stimulus was changed for a second time. This process was continued until all comparison stimuli had been paired with a given standard stimulus (see Table 3-2). Interposed between "test" discriminations was a series of sessions during which the animals were retested on the NaCl vs. standard stimulus discrimination to measure and maintain stimulus control. These were referred to as "stimulus-control" sessions.

Training (see Table 3-3)

a.) Spout training. Mice were trained to lick from the different spouts for fluid delivery in the gustometer by presenting the animals with only one spout each day. Water

was delivered on all 3 days of this training phase and was freely available ad libitum throughout a session.

b.) Side training. Next, mice were trained to lick from a specific reinforcement spout in response to the presentation of a mid-range concentration of one of the two compounds delivered through the sample spout by providing access only to the corresponding reinforcement spout. The access slot to the other reinforcement spout was covered. The sample solution and the matching reinforcement spout were alternated between days. In this phase, mice were allowed up to 180 s to respond after sampling (limited hold); no time-out contingency was in effect.

c.) Alternation phase. During the alternation phase, both stimuli were presented and both reinforcement spouts were available for responses. The limited hold was shortened to 15 s and a criterion number of correct responses (non-consecutively) was required for a change in the sample stimulus. The criterion, which started at four correct responses, was reduced after two sessions to two, and finally, after two more sessions to one. The time-out contingency was introduced in this phase as a punishment for incorrect responses. Initially, the time-out was set at 10 s. When the criterion reached 2, it was increased to 20 s, and finally increased to 30 s when the criterion reached 1.

d.) Discrimination training. Mice were trained to discriminate stimuli presented in randomized blocks (discrimination training). The time-out was set at 30 s. Then the two other concentrations of each stimulus were added (discrimination testing). Mice were moved from discrimination training to discrimination testing when group performance reached 85% correct responses and at least 75% for each animal. At the start of discrimination testing, the limited hold was reduced to 10 s.

Testing (see Table 3-3)

Mice were trained, as described above, to associate the taste of one stimulus with one reinforcement spout on one side of the sample spout and the taste of another with the other reinforcement spout on the other side of the sample spout (counterbalanced between animals). Session length was 25 minutes. During this time, each mouse was allowed to complete as many trials as possible. Each trial was comprised of 4 phases: (1) the sample phase, (2) the decision phase, (3) the reinforcement phase, and (4) the inter-trial interval (see Figure 3-1). The sample phase began when the mouse licked from the sample spout available in front of the slot. When initiating a trial, the mouse was required to complete an "attending response" by licking the dry sample spout twice within 250 ms to trigger stimulus delivery. The mouse was allowed 5 licks or 2 s of stimulus access, whichever came first, before the sample spout was rotated away from the slot. During this phase, the house lights were on. When the sample spout rotated away from the slot, the decision phase would begin: the house lights would be turned off and the cue lights turned on. During this phase, the mouse was required to decide which reinforcement spout to lick from. The reinforcement phase began as soon as contact was made with one of the side spouts. If a correct choice was made, the house lights were illuminated and the mouse was allowed 15 licks or 4 s of access to water reinforcement. If an incorrect choice was made or no response was initiated within 10 s (limited hold), the mouse received 30 s of time-out during which all lights were extinguished and no fluid was delivered. When 15 licks were taken, 4 s passed, or when a time-out was completed, the sample spout rotated over a funnel and was rinsed with purified water and dried with pressurized air, and then rotated back into position in front of the slot. This phase, the inter-trial interval, lasted 6 s (all lights were turned off during this phase). As mentioned above, mice were tested with

a range of stimulus concentrations. During each session, three of the reservoirs were filled with different concentrations of the standard stimulus and three others with different concentrations of the comparison stimulus (see Table 3-3). The two reservoirs connected to the reinforcement spouts were filled with purified water. Presentation order was randomized without replacement in blocks so that every concentration of the stimuli was presented exactly once before the initiation of the subsequent block.

Data Analysis

Discriminability was evaluated using the overall proportion of correct responses as the primary dependent measure. Overall performance was assessed by collapsing all trials across both stimuli and concentrations. Concentration effects were analyzed within each stimulus. These effects, as well as overall performance, were tested against chance using one-sample t tests. Performance across weeks was statistically analyzed using analyses of variance (ANOVAs). Only trials in which a response was made were used in the analyses. When technical problems with the gustometer occurred during an animal's session, the data were discarded from the analyses. Overall discriminability at 50% correct equals chance performance. Performance approaching this value indicated a failure to discriminate.

Results

As shown in Figure 3-2, both groups of mice, which were trained to discriminate a standard stimulus (e.g., sucrose or L-serine) from NaCl, learned the task. This initial phase of discrimination testing lasted for five weeks. During the last two weeks of this phase, both groups performed at ~85% accuracy on average. Overall performance ranged from 76 to 94% correct responses during the last week of testing for the group of mice

trained to discriminate L-serine from NaCl and 76 to 92% correct responses for animals trained to discriminate sucrose from NaCl.

Discrimination Testing

Table 3-4 lists the performance of the two groups during the various phases of discrimination testing. Group performance on each of the stimulus discrimination pairings is described in greater detail below.

Sucrose vs. L-serine

"Serine" group

During the first week of the second phase of discrimination testing, both groups of mice were tested for their ability to discriminate sucrose from L-serine. Overall performance dropped to levels significantly below chance for the group of mice trained to discriminate L-serine from NaCl ["serine" group; Figure 3-3; $t(5) = -4.9$; $P < .01$; null hypothesis; probability of correct response = .5]. Although the departure from chance was relatively slight (0.44), it indicates that there was a slight tendency for animals in this group to treat the novel stimulus as if it were the standard, although this propensity did not reach statistical significance.

During the second week of testing, the performance of the serine group did not significantly differ from chance [Figure 3-3; $t(5) = -0.78$; $P = .472$; null hypothesis; probability = .5] nor did it significantly differ from performance levels measured during week one [$F(1,5) = 2.8$, $P = .156$].

"Sucrose" group

For the group of mice trained to discriminate sucrose from NaCl (sucrose group), overall performance also dropped precipitously during the first week of testing. These data demonstrate that changing one of the taste compounds in this discrimination task has

the potential to substantially disrupt performance in both groups. However, these mice did perform at levels above chance, albeit poorly [Figure 3-4; $t(5) = 5.2$; $P < .01$; null hypothesis; probability = .5].

When looking at performance across all the individual concentrations of the two stimuli, mice were able to discriminate 0.4 and 0.6 M sucrose at levels above chance [Figure 3-5; both t-values ≥ 3.0; Ps $< .05$; null hypothesis; probability = .5]. However, these mice did not respond to 0.2 M sucrose, as well as all three concentrations of L-serine, at levels above chance.

During the second week of testing, performance did significantly improve relative to that measured during the first week [Figure 3-4; $F(1,5) = 11.9$, $P < .05$].

Overall performance appeared to improve, relative to week one, because mice learned to discriminate L-serine from sucrose. That is, they discriminated all concentrations of L-serine at levels above chance [Figure 3-5; all t-values ≥ 5.2; Ps $< .01$; null hypothesis; probability = .5]. Although these mice were able to discriminate 0.6 M sucrose from L-serine [Figure 3-4; $t(5) = 2.7$; $P < .05$; null hypothesis; probability = .5], they were unable to discriminate 0.2 and 0.4 M sucrose. However, their performance towards 0.4 M sucrose did approach significance ($P = .058$).

Overall group performance for the sucrose group was significantly above chance, which would appear to indicate that these stimuli evoke, to some degree, discriminable neural signals. However, mice in the serine group had substantially greater difficulty executing the task. Collectively, these data suggest that while potentially discriminable, the stimuli are, to some degree, qualitatively similar.

Sucrose/L-serine vs. Glucose

Serine group

During week one of L-serine vs. glucose testing, overall performance dropped to levels significantly below chance for the serine group [Figure 3-3; $t(5) = -6.4$; $p < .01$; null hypothesis; probability = .5], again indicating that when animals in this group had difficulty discriminating, there was a slight, but statistically significant tendency for them to respond to the novel stimulus as if it was the standard [i.e., L-serine; $t(5) = -5.1$; $P < .01$; null hypothesis; probability = .5).

Their performance, however, did significantly improve during week two [Figure 3-3; $F(1,5) = 50.5$, $P < .01$]. This improvement, however, did not yield performance that was significantly greater than chance [$t(5) = 2.5$; $P = .052$; null hypothesis; probability = .5].

These mice were tested for an additional week to see if group performance would improve. Overall performance during week three differed significantly from that measured during week one [$F(1,5) = 25.2$, $P < .01$] but not from levels observed during week two. After three weeks of testing with glucose (and two earlier weeks with sucrose), performance finally reached levels significantly higher than chance [Figure 3-3; $t(5) = 3.0$; $P < .05$; null hypothesis; probability = .5]. However, individual performance during this week was highly variable. Indeed, three out of the six mice in this group appeared to have substantial difficulty performing the discrimination (Figure 3-3). Overall performance ranged from 51 to 67% correct responses during week three.

Mean performance appeared to improve, relative to week one, because mice stopped responding to glucose as if it were L-serine. During week one, mice responded to all concentrations of glucose at levels significantly below chance [Figure 3-6; all *t*-

values ≤ -2.6; *P*s < .05; null hypothesis; probability = .5]. That is to say, mice were responding disproportionately on the "L-serine" spout. During week three, mice corrected this bias and responded to all three concentrations of glucose at chance levels.

During the last week of testing with glucose, mean overall group performance for the serine group was significantly above chance, which would appear to indicate that these stimuli evoke, to some degree, discriminable neural signals. However, as detailed above, group performance was at best mediocre and individual performance was highly variable (see Figure 3-3). As with sucrose vs. L-serine testing, these data suggest that while potentially discriminable, L-serine and glucose are, to some degree, qualitatively similar.

Sucrose group

As expected, mice in the sucrose group had great difficulty discriminating sucrose from glucose. Their overall performance never differed significantly from chance [week one or week two; Figure 3-4; both *t*-values ≤ 1.6; *P*s ≥ .179; null hypothesis; probability = .5]. In addition, performance levels observed during week two did not significantly differ from that measured during week one [$F(1,5) = 0.05$, $P = .832$]. We did not run these mice for a third week for fear of losing stimulus control. These data are consistent with the notion that sucrose and glucose activate the same transduction pathways (e.g., Zhao *et al.*, 2003; see discussion below for elaboration).

Mice tended to respond best to sucrose at its highest concentration (0.6 M) and best to glucose at its lowest concentration (0.5 M), although this pattern did not reach statistical significance (see Figure 3-7). In any event, this response strategy did not lead to overall performance levels that were greater than chance (however, see Sucrose vs. Fructose below).

Sucrose/L-serine vs. Maltose

Serine group

During the first week of L-serine vs. maltose testing, overall performance was significantly higher than chance [Figure 3-3; $t(5) = 3.5$; $P < .05$; null hypothesis; probability = .5]. Mice were able to discriminate 0.4 and 0.6 M L-serine at levels above chance [Figure 3-8; both t-values ≥ 4.8; Ps $< .01$; null hypothesis; probability = .5]. However, these mice did not respond to 1.0 M L-serine, as well as all three concentrations of maltose, at above chance levels.

Oddly, overall performance for these mice, although above chance during week one, was not maintained at these levels during the last two weeks of testing. This despite the fact that mean performance increased each week during this phase of testing (see Figure 3-3). During the last two weeks of testing, performance did not significantly differ from chance [Figure 3-3; both t-values ≤ 2.4; Ps $\geq .06$; null hypothesis; probability = .5], nor did the values significantly differ from performance levels measured during week one.

As with L-serine vs. glucose testing, individual performance was highly variable. Two mice substantially affected the amount of variability observed during L-serine vs. maltose testing, considerably pulling down group performance (Figure 3-3). These same mice performed at ~chance levels during L-serine vs. glucose testing (Figure 3-3). Overall performance ranged from 46 to 75% correct responses during week three.

Sucrose group

Unlike the failure of these mice to discriminate sucrose from glucose, they did display some reliable discrimination between sucrose and maltose. During the first week of sucrose vs. maltose testing, overall performance was significantly above chance

[Figure 3-4; $t(5) = 4.2$; $P < .01$; null hypothesis; probability = .5]. During week one, mice were able to discriminate 0.2 M sucrose at levels above chance [Figure 3-9; $t(5) = 3.2$; $p < .05$; null hypothesis; probability = .5]. However, these mice did not respond to 0.4 and 0.6 M sucrose, as well as all three concentrations of maltose, at above chance levels. Performance did not significantly improve during weeks two or three. However, by week three, mice were able to discriminate 0.2 M maltose and all concentrations of sucrose at levels above chance [Figure 3-9; all t-values ≤ 2.6; Ps $< .05$; null hypothesis; probability = .5].

The ability of mice to discriminate sucrose from maltose, detailed above, suggests that maltose is qualitatively distinctive from sucrose and the pattern of responsiveness across concentrations suggest that 0.2 M maltose is more distinguishable from sucrose relative to the higher maltose concentrations tested (i.e., 0.4 and 0.6 M maltose).

Sucrose vs. L-serine II

Serine group

We conducted a second phase of sucrose vs. L-serine testing (Sucrose vs. L-serine II) to ascertain whether the performance of the serine group would improve on this discrimination as a function of experience. During the first week of testing, performance did not significantly differ from chance [Figure 3-3; $t(5) = 2.3$; $P = .07$; null hypothesis; probability = .5].

During the next week, however, performance did reach levels significantly higher than chance [Figure 3-3; $t(5) = 2.9$; $P < .05$; null hypothesis; probability = .5]. Yet these levels were not significantly different from those measured during week one.

Mice were then tested for an additional week to see if group performance would improve. During this final week of testing, overall performance did differ significantly

from levels measured during week one [$F(1,5) = 17.0$, $P < .01$] but not from that observed during week two. Mice were able to discriminate all concentrations of L-serine at levels above chance during all three weeks of testing with these stimuli [Figure 3-10; all t-values ≥ 2.7; Ps $< .05$; null hypothesis; probability = .5]. However, during this time, they were unable to discriminate any of the sucrose concentrations at above chance levels [Figure 3-10; all t-values ≤ 2.1; Ps $\geq .09$; null hypothesis; probability = .5]. This response pattern is consistent with what was seen in the last week of L-serine vs. glucose testing. That is, mice in the serine group did not respond to any concentration of sucrose or glucose at levels above chance.

As with L-serine vs. glucose and maltose testing, individual performance was highly variable. The two mice that performed at levels substantially below the other animals during glucose and maltose testing continued to do so during this phase of testing (Figure 3-3). Overall performance during week three ranged from 46 to 75% correct responses.

The data presented above suggest that mice in the serine group can indeed discriminate both sucrose and glucose from L-serine. That said, this discrimination appeared to be exceedingly difficult for these animals.

Sucrose group

As in the first sucrose vs. L-serine discrimination task, the sucrose group did perform at levels above chance [Figure 3-4; $t(5) = 9.7$; $P < .001$; null hypothesis; probability = .5]. The performance during the first week of sucrose vs. L-serine II testing did not significantly differ from the last week of the first phase of sucrose vs. L-serine testing [69 and 72 % group performance, respectively].

Mice were able to discriminate 0.4 and 0.6 M sucrose and all three concentrations of L-serine at levels above chance [Figure 3-11; all *t*-values ≥ 4.1; *P*s < .01; null hypothesis; probability = .5]. As in the first phase of sucrose vs. serine testing, mice did not respond to 0.2 M sucrose at above chance levels.

Overall performance significantly improved during week two when compared to that seen during week one [Figure 3-3; $F(3,36) = 23.7$, $P < .01$]. During this week, mice were able to discriminate all concentrations of both stimuli at levels above chance [Figure 3-11; all *t*-values ≥ 4.7; *P*s < .01; null hypothesis; probability = .5].

Performance appeared to reach asymptotical levels during week two. Overall performance levels, by session, did not significantly improve during week two [sessions 6-10: 0.79, 0.83, 0.77, 0.77, 0.75, respectively; $F(4,8 = 0.336$, $P = .85$]. As a result, mice were not tested for a third week.

Sucrose/L-serine vs. Fructose

Serine group

During week one, the overall performance of the serine group did not significantly differ from chance [Figure 3-3; $t(5) = 1.8$; $P = .144$; null hypothesis; probability = .5]. During the next week, however, performance did reach levels significantly higher than chance [Figure 3-3; $t(5) = 4.7$; $P < .01$; null hypothesis; probability = .5]. Yet these levels were not significantly different from these measured during week one. During week two, mice were able to discriminate 1.0 M L-serine, as well as 0.6 and 1.0 M fructose, at levels above chance [Figure 3-12; all *t*-values ≥ 3.1; *P*s < .05; null hypothesis; probability = .5].

Mice were then tested for an additional week to see if performance would improve. During this week, overall performance was again significantly above chance

[Figure 3-3; $t(5) = 4.6$; $P < .05$; null hypothesis; probability = .5]. Performance did not differ significantly from that measured during week one or two. During this last week, mice were able to discriminate 0.6 and 1.0 M L-serine and 1.0 M fructose at levels above chance [Figure 3-12; all t-values ≥ 3.2; Ps $< .05$; null hypothesis; probability = .5].

During this phases of discrimination testing, individual performance variability decreased somewhat. The two mice that depressed performance throughout testing appeared to finally improve ever so slightly, relative to their behavior measured in preceding phases (see Figure 3-3). Overall performance during week three ranged from 57 to 76% correct responses. Mice in the serine group, for the most part, demonstrated that, after some experience, they can discriminate all of the sugars, including fructose from their standard stimulus.

Sucrose group

During the first week of sucrose vs. fructose testing, overall performance was significantly greater than chance [Figure 3-4; $t(5) = 4.4$; $P < .01$; null hypothesis; probability = .5]. Mice were able to discriminate the high concentrations of sucrose (0.4 and 0.6 M) at levels above chance [Figure 3-13; $t(5) = 3.2$; $p < .05$; null hypothesis; probability = .5]. These mice, however, did not respond 0.3 and 0.6 M fructose at above chance levels. Interestingly, mice responded to 1.0 M fructose at levels significantly below chance [Figure 3-13; $t(5) = -23.0$; $P < .001$;null hypothesis; probability = .5]. During week two, overall performance was again significantly greater than chance [Figure 3-4; $t(5) = 6.9$; $P < .01$; null hypothesis; probability = .5], but not significantly different than performance levels measured during week one.

Mice were tested for an additional week to see if group performance would improve relative to that measured during weeks one and two. During this final week of

testing, performance again was significantly higher than chance [Figure 3-4; $t(5) = 2.8$; $P < .05$; null hypothesis; probability = .5]. However, performance did not differ significantly from that observed during week one or two.

Although no significant changes in the overall percentage of correct responses were observed over the three weeks of testing, the pattern of responsiveness towards the various concentrations of the two stimuli did differ slightly from week one to week three. By week three, the mice only responded to the highest concentration of sucrose and the lowest concentration of fructose at above chance levels. This pattern of responsiveness is consistent with animals using "intensity" cues to guide the discrimination (Figure 3-13; see discussion below).

In summary, when mice in the sucrose group were tested on the ability to discriminate sucrose from fructose, these animals showed that they could do so at levels significantly greater than chance. However, as detailed above, this ability was very limited (~55% correct during the last week of testing) and appeared to result from mice responding on the basis of intensity, and not to taste quality, per se.

Stimulus Control Sessions

Figure 3-14 shows the performance of both groups on the first and last day of a given stimulus-control session, as well data for the subsequent discrimination task. The number of stimulus-control sessions required to reach criterion performance (i.e., 85% group performance for one week or for two consecutive sessions at the end of a week and individual performance ≥ 75%) is listed in Table 3-5. As can be seen, after stimulus-control - I, the number of sessions required to regain criterion performance dropped dramatically. Collectively, these data demonstrate that stimulus control can be quickly

reestablished in experienced mice even after a substantial period in which animals are presented with difficult discrimination tasks.

Discussion

Mice had difficulty, depending on the stimulus and the training history, discriminating sucrose from L-serine, maltose, fructose and glucose. Indeed, when concentration effects are taken into consideration, it appears that mice are unable to discriminate sucrose from glucose or fructose, suggesting that these three sugars generate a unitary percept. "Monogeusia," or the indiscriminability of a "class" of compounds, has been demonstrated in humans with natural sweeteners (e.g., Breslin *et al.*, 1996) and in rats with "bitter" tastants (Spector and Kopka, 2002). To my knowledge, monogeusia for natural sweeteners has never been demonstrated in rodents.

Monogeusia

All mice trained to discriminate sucrose from NaCl (i.e., sucrose group) were entirely unable to discriminate between sucrose and glucose as assessed by their overall performance during both weeks of testing. During the first week of testing, mice did not respond to any concentration of either stimulus at levels above chance. Indeed, they responded to 2.0 M glucose at levels significantly below chance. This indicates that the mice tended to treat the highest concentration of glucose as if it were the standard (i.e., sucrose). Although not significant, this tendency persisted in week two. Indeed, mice appeared to be responding on the basis of stimulus intensity. A broad concentration range was used for all compounds so that intensities and viscosities would overlap across stimuli. This manipulation was important so that non-qualitative cues would be rendered less redundant, causing quality to be the only consistent discriminable signal. However, if the concentration ranges did not sufficiently overlap, then stimulus intensity could be

used as a discriminable cue, albeit not very successfully. Theoretically, if these mice were responding to two qualitatively similar stimuli on the basis of perceived intensity, then they would be expected to respond disproportionately to all "weak" stimuli on one reinforcement spout and all "strong" stimuli on the other. Accordingly, as the concentration of the stimulus perceived to be of higher intensity **increases**, performance levels would also **increase**. This is because, as the concentration of the "stronger" stimulus increases, it would become more distinctive relative to the perceived intensity levels of the "weaker" stimulus. However, as the concentration of the lower intensity stimulus **increases**, performance levels would **decrease**. This is because, as the concentration of "weaker" stimulus increases, it overlaps, to a greater degree, with the perceived intensity levels of the "stronger" stimulus. It is this opposing concentration dependency that is the telltale sign of a stimulus intensity based taste discrimination.

With a response strategy such as this, animals could successfully discriminate two qualitatively identical stimuli, the concentration ranges of which did not sufficiently overlap, at levels greater than chance. Mice appeared to try this very response strategy when attempting to discriminate sucrose from glucose. As can be seen in Figure 3-7, mice responded to sucrose as if it were the "stronger" of the two stimuli being discriminated (i.e., as the concentration **increased**, performance levels also **increased)** and to glucose as if it were the "weaker" stimulus (i.e., as the concentration **increased**, performance levels **decreased**). Indeed, by week two, mice were able to discriminate the lowest glucose concentration at levels above chance. However, this feat, in and of itself, did not lead to overall performance levels that were greater than chance.

When mice were tested for the ability to discriminate sucrose from fructose, these animals showed that they could do so at levels above chance. However, this ability appeared to result from the mice using intensity cues. During the first week of testing with fructose, mice did not respond to any concentration of the stimulus at above chance levels. Indeed, they responded to 1.0 M fructose at levels significantly below chance. This indicates that there was a tendency for animals to treat the highest concentration of fructose as if it were the standard (i.e., as the concentration **increased**, performance levels **decreased)**. This response profile is analogous to that observed during the first week of testing with glucose, referred to above. By week three, mice only responded to the **highest** concentration of sucrose and the **lowest** concentration of fructose at levels above chance. Moreover, animals tended to respond to the highest concentration of fructose and the lowest concentration of sucrose as if they were the "other" stimulus, but these tendencies did not meet statistical significance standards. As can be seen in Figure 3-13, throughout testing with these stimuli, the opposing concentration dependency referred to above is plainly apparent. This response pattern is exactly what would be predicted if these animals were responding on the basis of perceived intensity. Thus, it is likely that the concentrations of fructose and sucrose chosen did not sufficiently overlap in intensity.

Nevertheless, these results strongly suggest that B6 mice cannot distinguish perceptually between the tastes of fructose, glucose, or sucrose. Because these are negative findings, we cannot conclusively rule out that some discriminative ability exists on the basis of quality. However, if B6 mice can distinguish between these compounds, they do so only poorly at best. Thus, it appears clear that in B6 mice, as in humans, all of

these sugars possess a similar taste quality that humans have described as "sweet" (Breslin *et al.*, 1996). The phenomenon of a class of compounds generating a unitary qualitative percept has been termed "monogeusia" (Breslin *et al.*, 1996).

Monogeusia for sugars likely results from an indiscriminable neural signal that originates from the stimulation of a common receptor(s). As mentioned in Chapter 1, sucrose and fructose were both shown, *in vitro*, to stimulate the T1R2+3 receptor complex. In a separate experiment, again, alluded to in Chapter 1, it was reported that T1R2+3 knock-out mice show no neural or behavioral responsiveness to sucrose or glucose. Thus, the aforementioned behavioral data reported here support the contention that all of these sugars activate the same receptor, and based on the molecular biology of "sweet" ligand transduction, the likely candidate is the T1R2+3 receptor complex.

It should be mentioned, however, that the only index used to assess the neural responsiveness of T1R2+3 knock-out mice was whole nerve electrophysiological recording from the CT. Yet, it is likely that detection of these stimuli is based on more than just input from the CT. Moreover, brief-access testing, which was used to evaluate the behavioral responsiveness of these knock-out mice, only assesses the motivational properties of a tastant, not the relative detectability of that stimulus, per se. As mentioned in Chapter 1, taste stimulus detection is best assessed from tasks in which taste serves as a cue for some other event (e.g., reinforcement or punishment) that will generate a trained directed response regardless of the hedonic characteristics of the taste stimulus.

Indeed, recent data, gathered using such procedures, call into question the notion that the T1R2+3 receptor complex is exclusively responsible for the activation of TBCs responsible for the detection of sugars. These results suggest that at least one sugar can

be transduced independently of at least one of the members of the T1R2+3 receptor complex. It was reported that mutant mice, lacking the receptor T1R3, show absolutely no deficit in their ability to detect the presence of sucrose (Delay *et al.*, 2006). It should be mentioned, however, that these data are somewhat controversial and have yet to be replicated. That said, it does appear possible for the T1R2 receptor to bind with taste ligands in the absence of T1R3 (e.g., Nie *et al.*, 2005; Temussi, 2006), although this remains to be demonstrated *in vivo*.

Maltose, however, appears to generate a distinctive taste quality relative to sucrose, depending on concentration. Although mice could only discriminate the lowest concentration of maltose at levels above chance, they were able to discriminate all three concentrations of sucrose successfully during the last week of testing. This response pattern is quite different from that observed when these mice were attempting to discriminate sucrose from either glucose or fructose. Therefore, these data suggest that, although qualitatively similar, sucrose and maltose must generate a discriminable neural signal at the periphery. Surprisingly, however, it is the lowest concentration of maltose that appears to be the most distinctive, suggesting that as the concentration increases, maltose becomes qualitatively more similar to sucrose. The discriminability of maltose and sucrose has been demonstrated, in rodents and humans, by a variety of other researchers (e.g., Ninomiya *et al.*, 1984; Spector and Grill, 1988; Breslin *et al.*, 1996; Spector *et al.*, 1997).

In addition to sucrose and glucose, T1R2+3 knock-out mice also show no neural or behavioral responsiveness to maltose. If these data are correct, and the T1R2+3 receptor complex mediates the transduction of both sucrose and maltose, then some other factor

must be influencing the nature of the signal arising from the periphery. Possibilities include ligand binding/TBC activation characteristics that lead to differential signaling (e.g., rise & decay). It is also possible that other undiscovered members of the T1R family of receptors or other mechanisms of ligand transduction exist that generate a discriminable neural signal. It would be informative to see if T1R2+3 knock-out mice could detect the presence of maltose in a task explicitly designed to assess taste thresholds (e.g., an operate detection task). If so, data such as these would definitively prove that maltose could be transduced independently of the receptors T1R2 and/or T1R3.

Sugars vs. L-serine

All mice in the sucrose group learned to discriminate sucrose and L-serine. After many weeks of experience with sucrose as a standard, they were able to discriminate sucrose from L-serine ~80% of the time during the last week of testing with these stimuli. As a group, these mice responded to all concentrations of both stimuli at levels above chance. Mice in the serine group were able to discriminate all the concentrations of L-serine from sucrose. These mice were also able to discriminate L-serine from glucose, fructose, and maltose at levels above chance.

The data presented above suggest that sucrose and L-serine are distinguishable. On the other hand, the data also suggest that the two stimuli share some qualitative features. A comparison of the performance of animals when they were discriminating their respective standard stimulus from NaCl during the stimulus-control sessions (i.e., ~85 - 90% correct responses) to that observed when they were attempting to discriminate sucrose from L-serine indicates that there was some degree of perceptual confusion (see Figures 3-3 and 3-4).

In addition to maintaining and providing a measure of stimulus control, the stimulus-control sessions also provided a performance standard based on a discrimination task that was "easy" for the animals to accomplish. Because these stimuli (i.e., sucrose/L-serine vs. NaCl) are thought to be independently coded at the periphery (i.e., independently transduced; e.g., Zhang *et al.*, 2003), it was postulated that mice could perform this discrimination task without a great deal of difficulty. It was assumed that deviation from 100% correct responding during these sessions was the result of non-sensory factors (e.g., motivational state, task difficulty, etc). Thus, during the various discrimination testing phases, it was believed that deviation from the performance standards observed during the stimulus-control sessions indicated increasing perceptual confusion. This "uncertainty" likely resulted from comparison stimuli evoking neural signals that are more similar to one another relative to those generated by sucrose/L-serine and NaCl.

Indeed, despite five weeks of testing with these stimuli, mice trained to discriminate L-serine from NaCl (i.e., serine group) never responded to any concentration of sucrose at levels above chance. During the first phase of sucrose vs. L-serine testing, animals in the sucrose group were unable to discriminate 0.2 M sucrose from L-serine. It took until the second week of the second phase before they were able to learn the discrimination. These findings imply that 0.2 M sucrose tastes more similar to L-serine than do the higher concentrations of sucrose. Accordingly, L-serine, across the range of concentrations tested, likely evokes a mild "sweetness" relative to that elicited by 0.4 and 0.6 M sucrose. This fact might contribute to the lack of appetitive behavior displayed towards this stimulus detailed in Chapter 1.

Collectively, these data suggest that at least some of the signals generated by the receptor(s) responsible for the transduction of these stimuli converge somewhere alone the gustatory neuraxis. Might this convergence be at the initial site of stimulus transduction (e.g., the TBCs themselves)? As mentioned in Chapter 1, Kim *et al.*, 2003 reported, contrary to previous studies, that there exists a population of TBCs that co-express all three T1R receptors. Perhaps these cells mediate the signals that are responsible for the qualitative similarity between these stimuli. It should be said, however, that even if they do exist, it remains unclear what the response properties of such a cell would be. Another possibility is that "sweet-tasting" amino acids achieve their qualitative similarity with sucrose by binding with the same receptor(s) (e.g., T1R2+3). Indeed, as mentioned in Chapter 2, the putative "sweet-tasting" amino acid glycine appears able to bind with the T1R2+3 and the T1R1+3 receptor complex. This hypothesis is consistent with the fact, as mentioned in Chapter 2, that two-bottle preference for "sweet-tasting" L-amino acids and glycine appears to depend on the "taster" status of the mouse strain based on testing with sugars. Perhaps L-serine, like glycine, has the ability to activate transduction pathways also activated by sucrose. However, it should be mentioned that in an operant discrimination task designed to assess taste stimulus thresholds, values for glycine in mice did not distinguish taster and non-taster strains in straightforward a manner (Eylam and Spector, 2004). That said, glycine detectability may be unaffected by the polymorphism in the T1R3 receptor. As mentioned in Chapter 2, this polymorphism selectively affects the functionality of the T1R2+3 receptor complex. Thus, taste input from remaining functional receptors (e.g., T1R1+3) could be sufficient to maintain task performance. However, the loss of taste

input from the T1R2+3 receptor complex, which mediates, at least in part, the affective potency of highly appetitive nature sweeteners such as sucrose, would likely impact upon the relative affective valence of glycine, if not upon its threshold levels.

Training history had a huge impact on the ability of mice to discriminate all of the stimuli, particularly L-serine from sucrose. Mice, in the serine group, appeared to have more difficulty discriminating L-serine from sucrose relative to animals in the sucrose group. Indeed, two mice in the serine group were unable to discriminate L-serine from all of the sugars. This training history asymmetry may have resulted from a relative difference in the efficacy of sucrose and L-serine as "standards" (i.e., compounds to which all comparison stimuli are discriminated against). This difference in efficacy likely results from the degree of qualitative "purity" elicited by sucrose and L-serine, which is discussed in detail in the following chapters.

Table 3-1: Stimulus concentrations

	Concentrations
Sucrose	0.2, 0.4, 0.6 M
Glucose	0.5, 1.0, 2.0 M
Maltose	0.2, 0.4, 0.6 M
Fructose	0.3, 0.6, 1.0 M
L-serine	0.4, 0.6, 1.0 M
NaCl	0.2, 0.4, 0.6 M

Table 3-2: Order of stimulus discrimination pairings

Group	**Standard Stimulus**	Comparison Stimuli					
1	**Sucrose**	NaCl	L-serine	Glucose	Maltose	L-serine	Fructose
2	**L-serine**	NaCl	Sucrose	Glucose	Maltose	Sucrose	Fructose

Table 3-3: Representative training and testing parameters for the 2 discrimination groups

Phase	Stimuli	Limited Hold	Sample Licks/time(s)	Reinf. Licks/time(s)	Timeout(s)	Presentation Schedule
Spout Training	H_2O	None	–	–	None	Constant
Side Training	Middle Conc. of Comparison Stimulus 1 and Middle Conc. of Standard Stimulus	180	5/2	15/30	0	Constant
Alternation	Middle Conc. of Comparison Stimulus 1 and Middle Conc. of Standard Stimulus	15	5/2	15/30	10, 20, or 30	Criterion (4-1)
Discrimination Training	Middle Conc. of Comparison Stimulus 1 and Middle Conc. of Standard Stimulus	15	5/2	15/4	30	Randomized blocks
Discrimination Testing	All Conc. Comparison Stimulus 1 and All Conc. Standard Stimulus	10	5/2	15/4	30	Randomized blocks
Discrimination Testing	All Conc. Comparison Stimulus 2 and All Conc. Standard Stimulus	10	5/2	15/4	30	Randomized blocks
Discrimination Testing	All Conc. Comparison Stimulus 3 and All Conc. Standard Stimulus	10	5/2	15/4	30	Randomized blocks
Discrimination Testing	All Conc. Comparison Stimulus ***1*** and All Conc. Standard Stimulus	10	5/2	15/4	30	Randomized blocks
Discrimination Testing	All Conc. Comparison Stimulus 4 and All Conc. Standard Stimulus	10	5/2	15/4	30	Randomized blocks

Table 3-4: Overall percentage correct during the last week of testing for the stimulus discrimination pairings.

	Standard Stimulus	Comparison Stimuli									
	Sucrose	NaCl	L-serine	SCI	Glucose	SCII	Maltose	SCIII	L-serine	SCIV	Fructose
Overall % Correct		85%	69%	85%	52%	82%	63%	84%	79%	89%	56%
	L-serine	NaCl	Sucrose	SCI	Glucose	SCII	Maltose	SCIII	Sucrose	SCIV	Fructose
Overall % Correct		85%	48%	86%	58%	83%	60%	85%	65%	83%	65%

SC = stimulus-control sessions

Table 3-5: Number of stimulus-control sessions required to criterion performance.

	Stimulus Control Sessions			
	SCI	SCII	SCIII	SCIV
Sucrose	15	5	5	5
L-serine	20	5	7	5

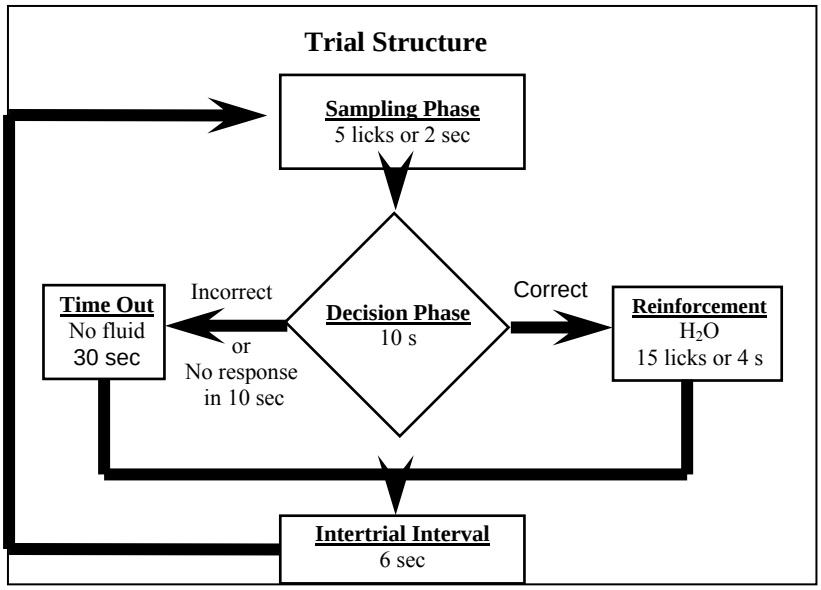

Figure 3-1: Trial structure (see text for more details).

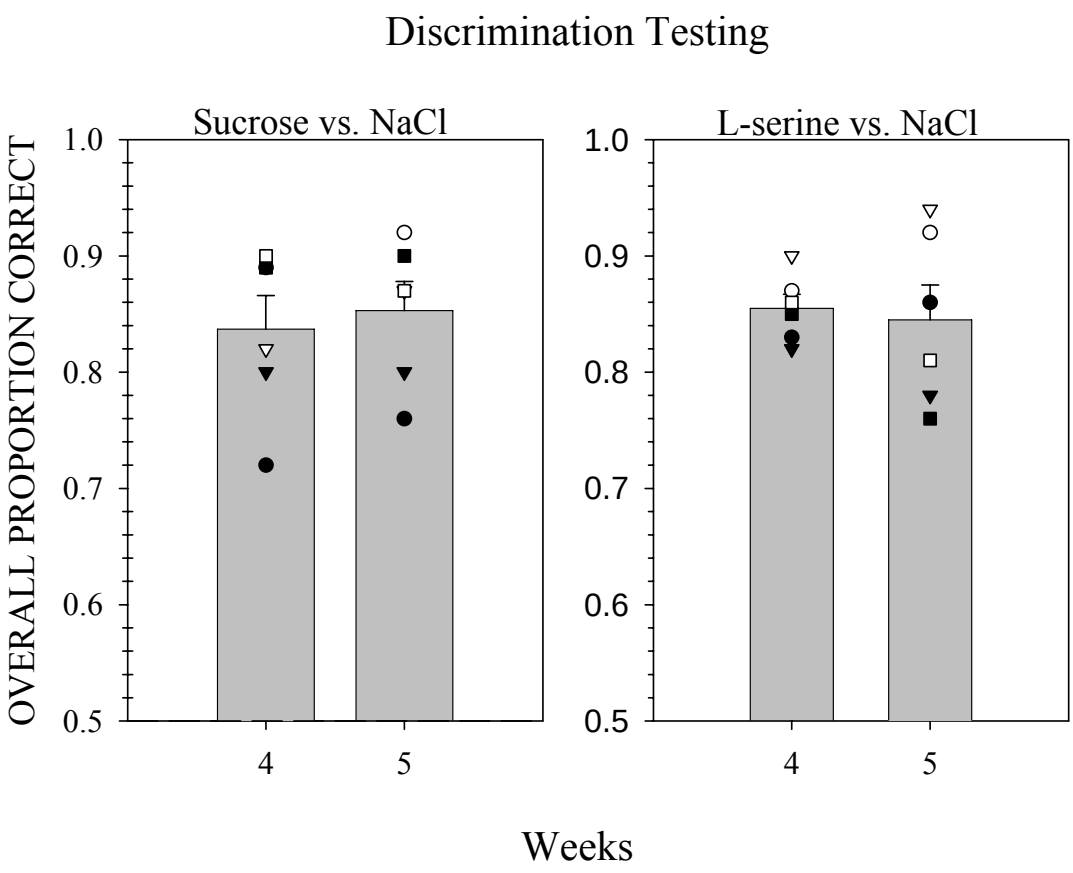

Figure 3-2: Individual animal (symbols) and group mean (± SEM; grey bars) data for mice trained to discriminate either sucrose or L-serine from NaCl. Performance on all trials with a lever press is depicted collapsed across all stimuli during a week. Chance performance equaled 0.5.

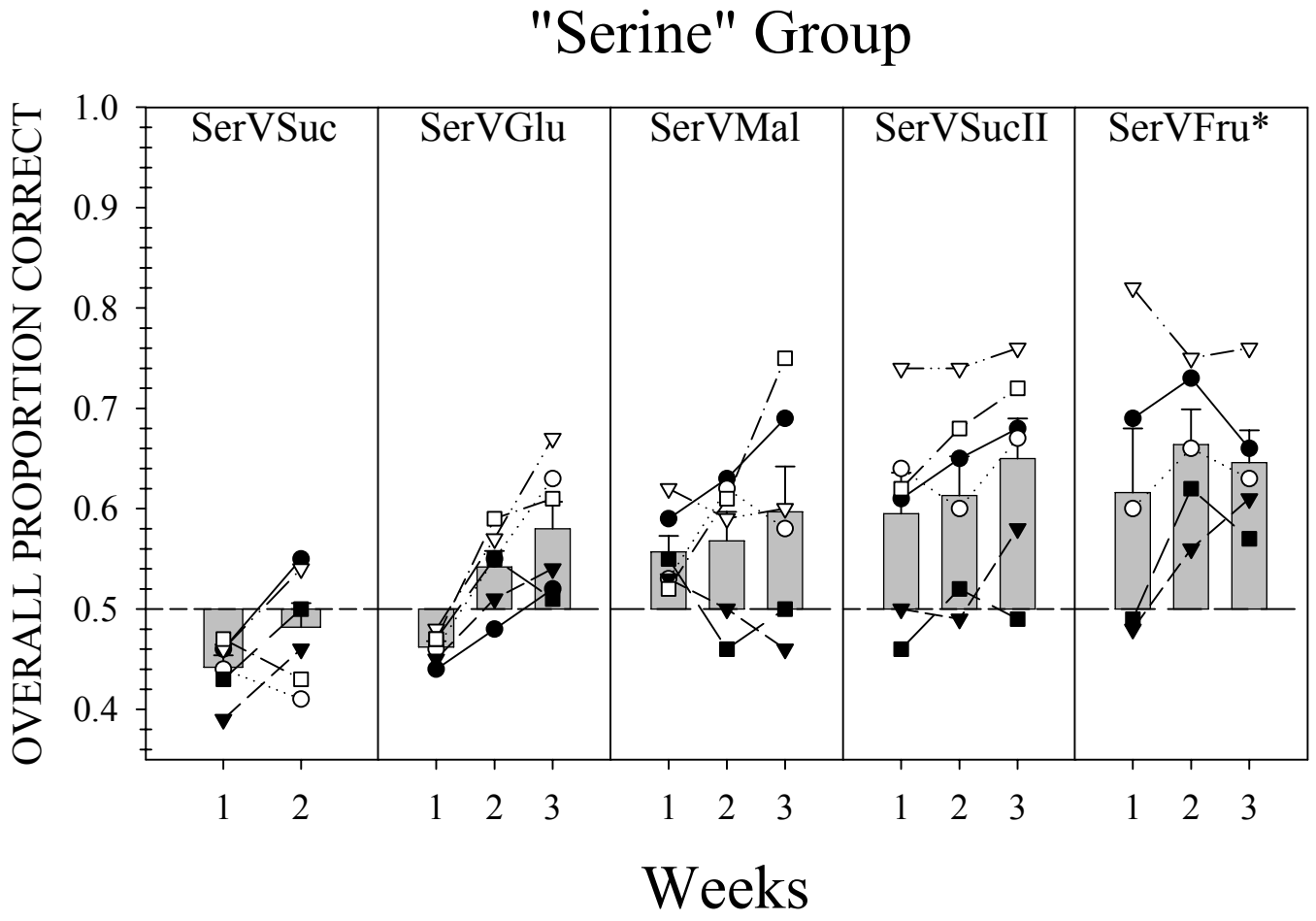

Figure 3-3: Individual animal (symbols) and group mean (± SEM; grey bars) data are plotted across all test phases for mice initially trained to discriminate L-serine from NaCl. Performance on all trials with a lever press is depicted collapsed across all stimuli during a week. Chance performance equaled 0.5. (*) One mouse was removed from L-serine vs. fructose testing because of illness.

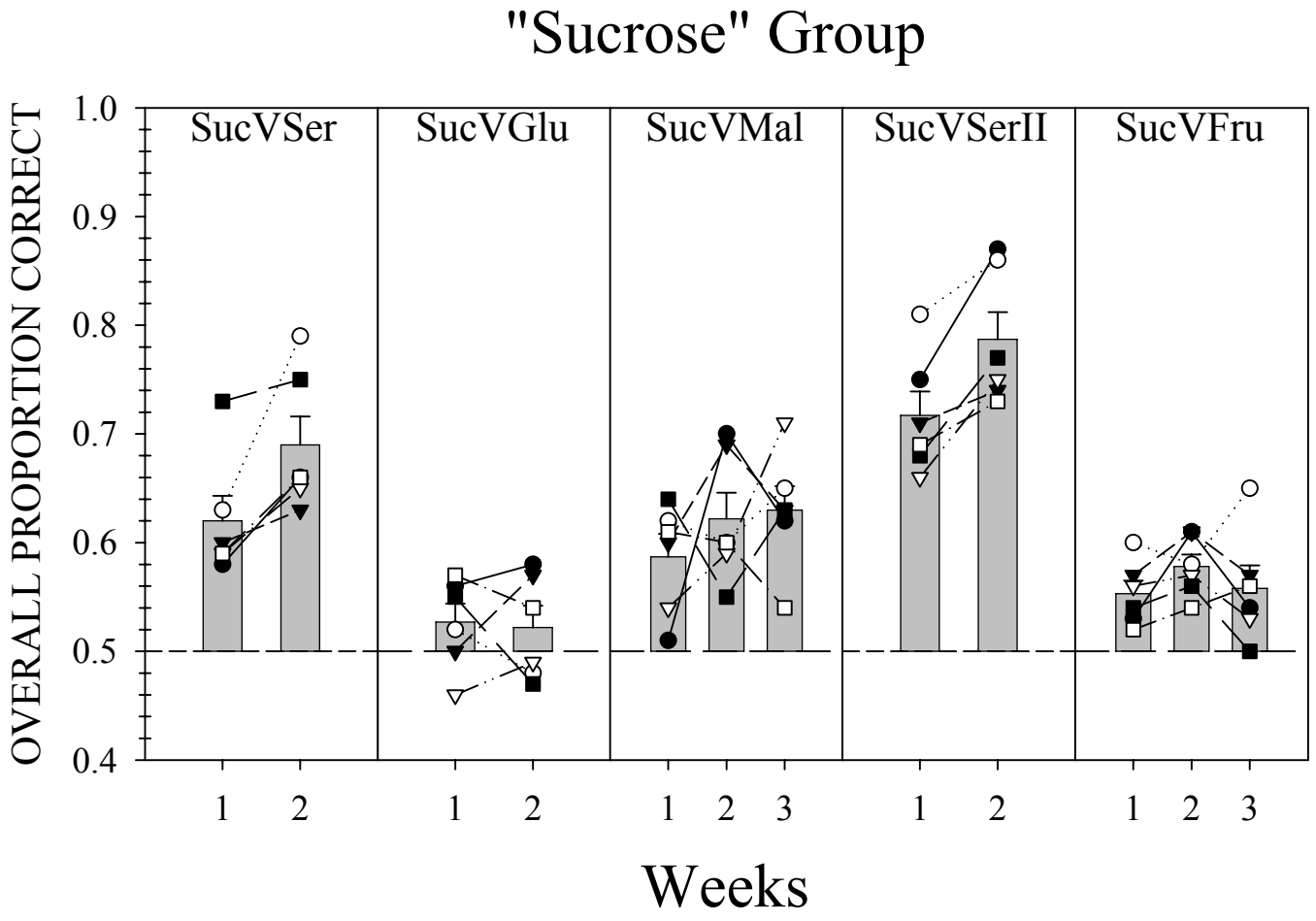

Figure 3-4: Individual animal (symbols) and group mean (± SEM; grey bars) data are plotted across all test phases for mice initially trained to discriminate sucrose from NaCl. Performance on all trials with a lever press is depicted collapsed across all stimuli during a week. Chance performance equaled 0.5.

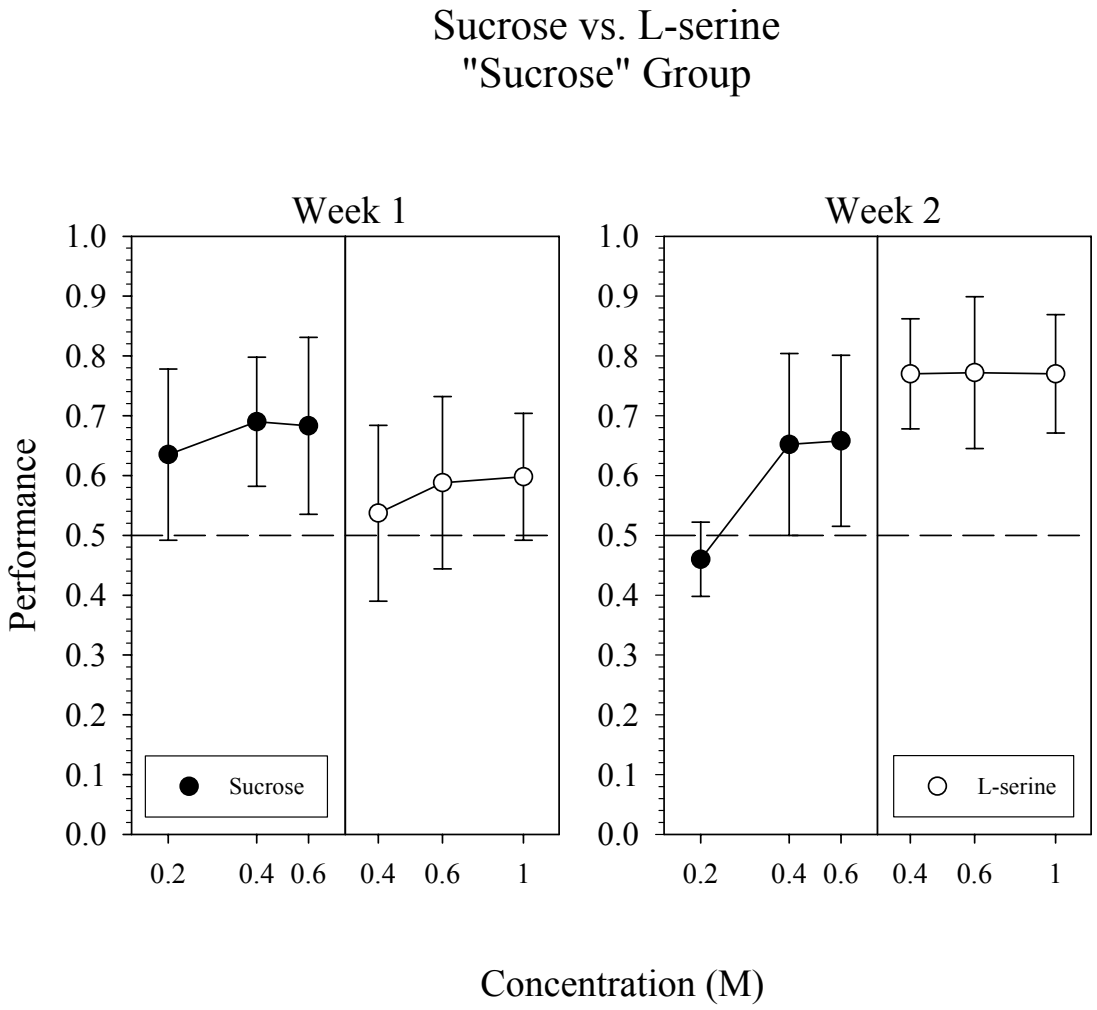

Figure 3-5: Mean (± SD) data for mice attempting to discriminate L-serine from sucrose. These mice were initially trained to discriminate sucrose from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

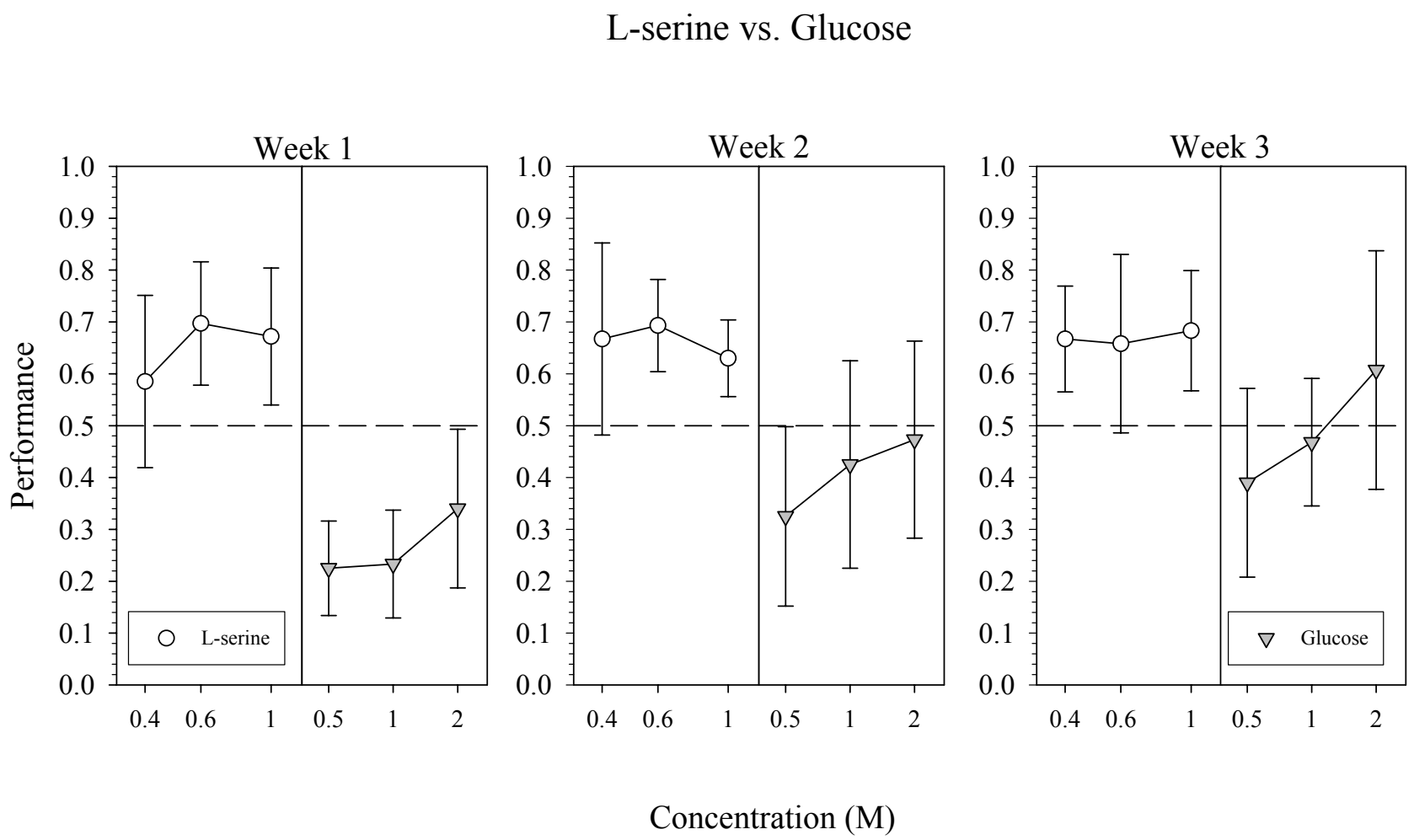

Figure 3-6: Mean (± SD) data for mice attempting to discriminate L-serine from glucose. These mice were initially trained to discriminate L-serine from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

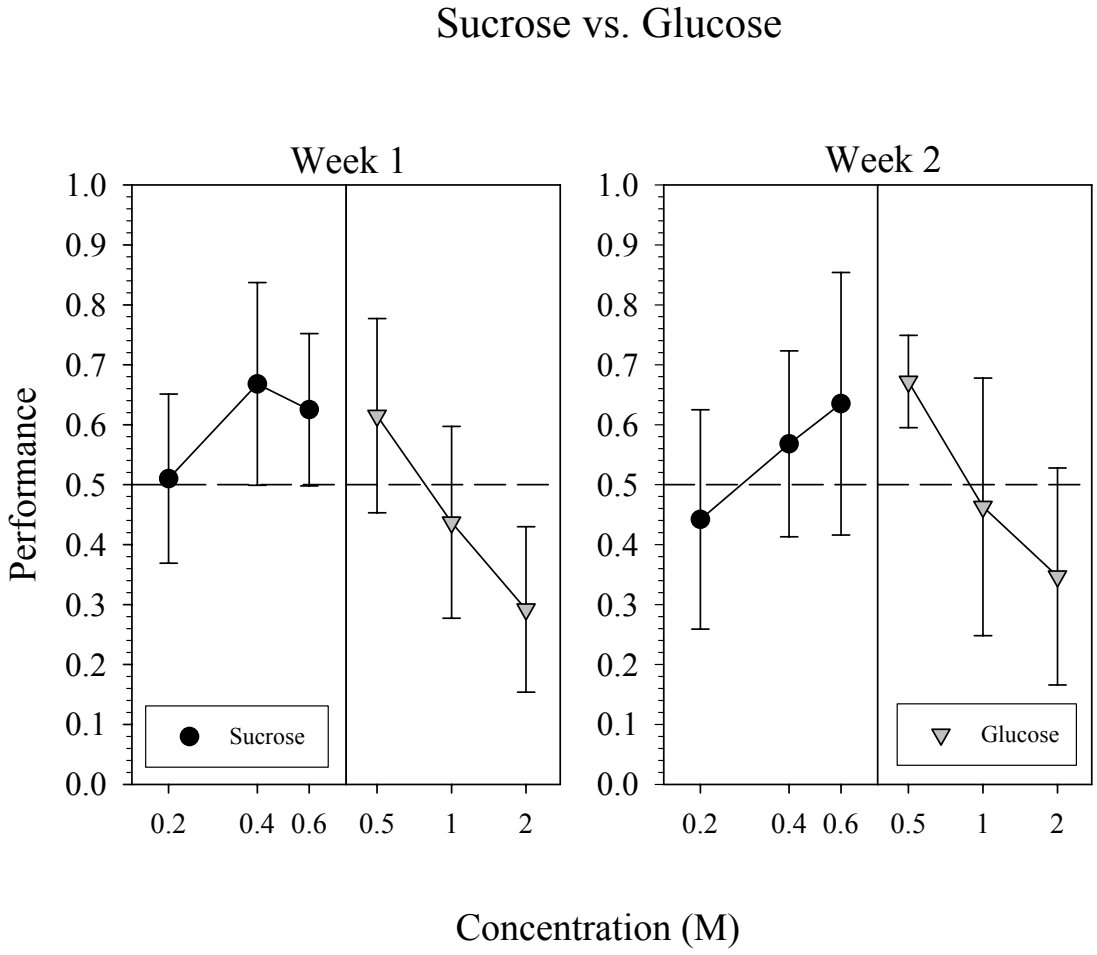

Figure 3-7: Mean (± SD) data for mice attempting to discriminate sucrose from glucose. These mice were initially trained to discriminate sucrose from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

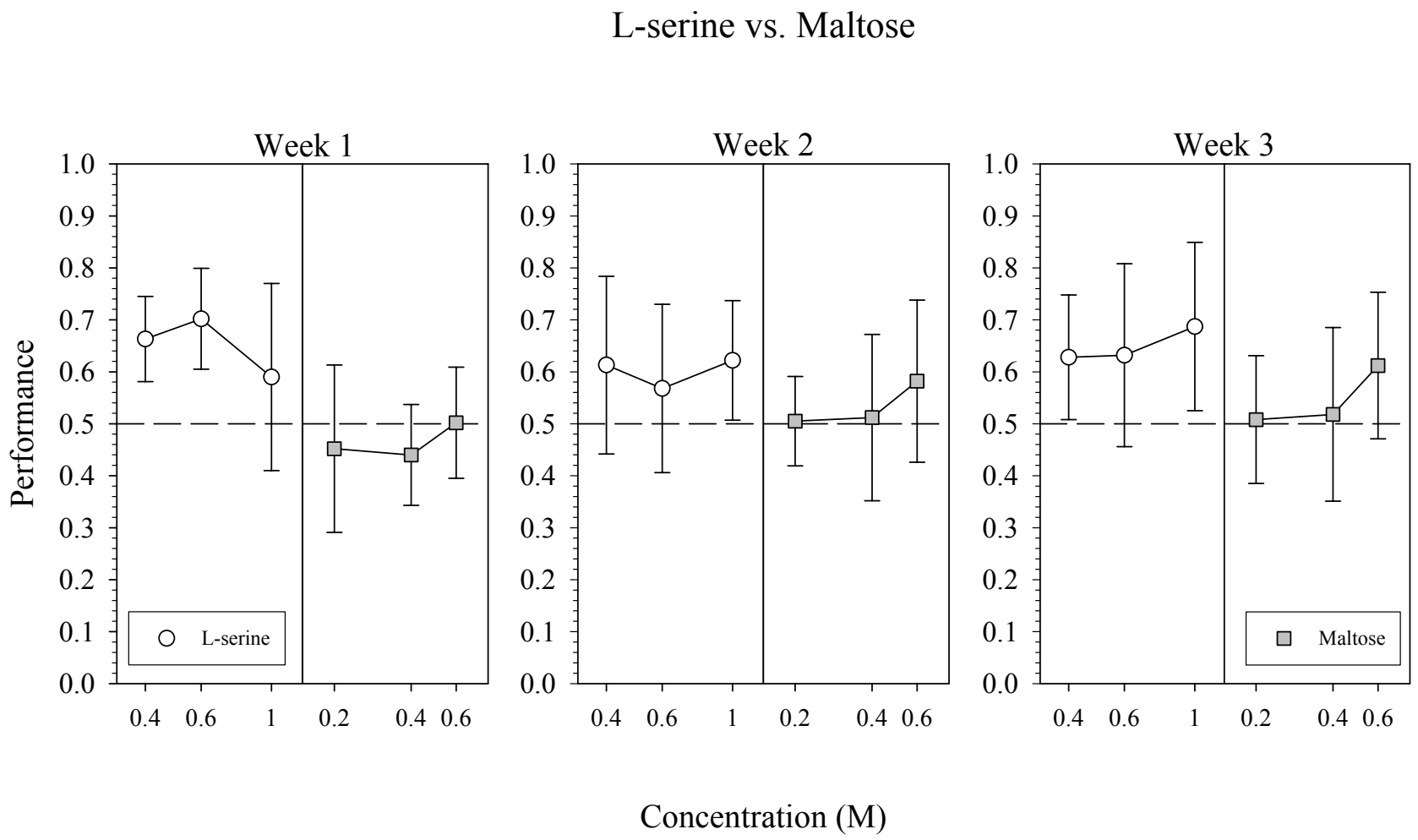

Figure 3-8: Mean (± SD) data for mice attempting to discriminate L-serine from maltose. These mice were initially trained to discriminate L-serine from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

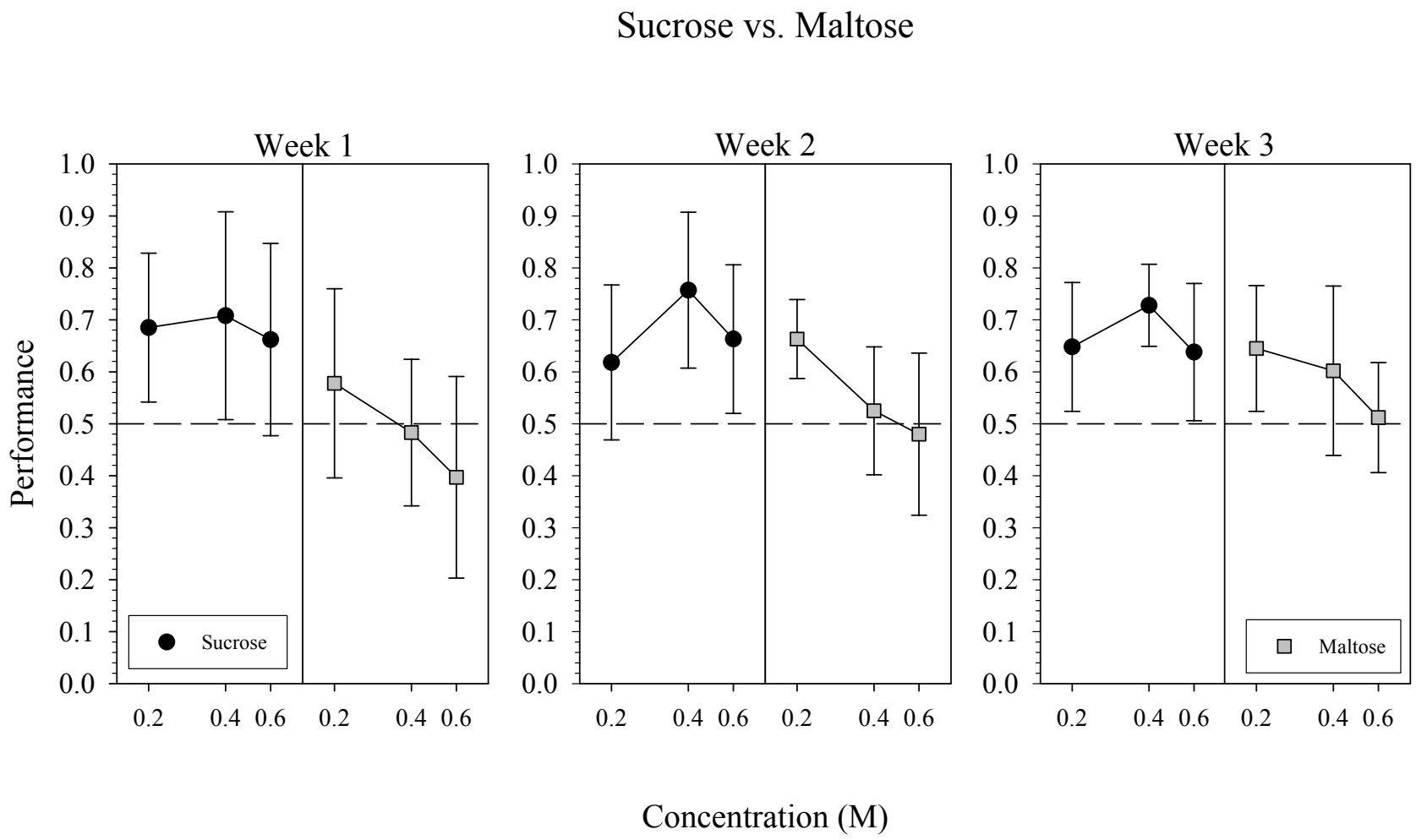

Figure 3-9: Mean (± SD) data for mice attempting to discriminate sucrose from maltose. These mice were initially trained to discriminate sucrose from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

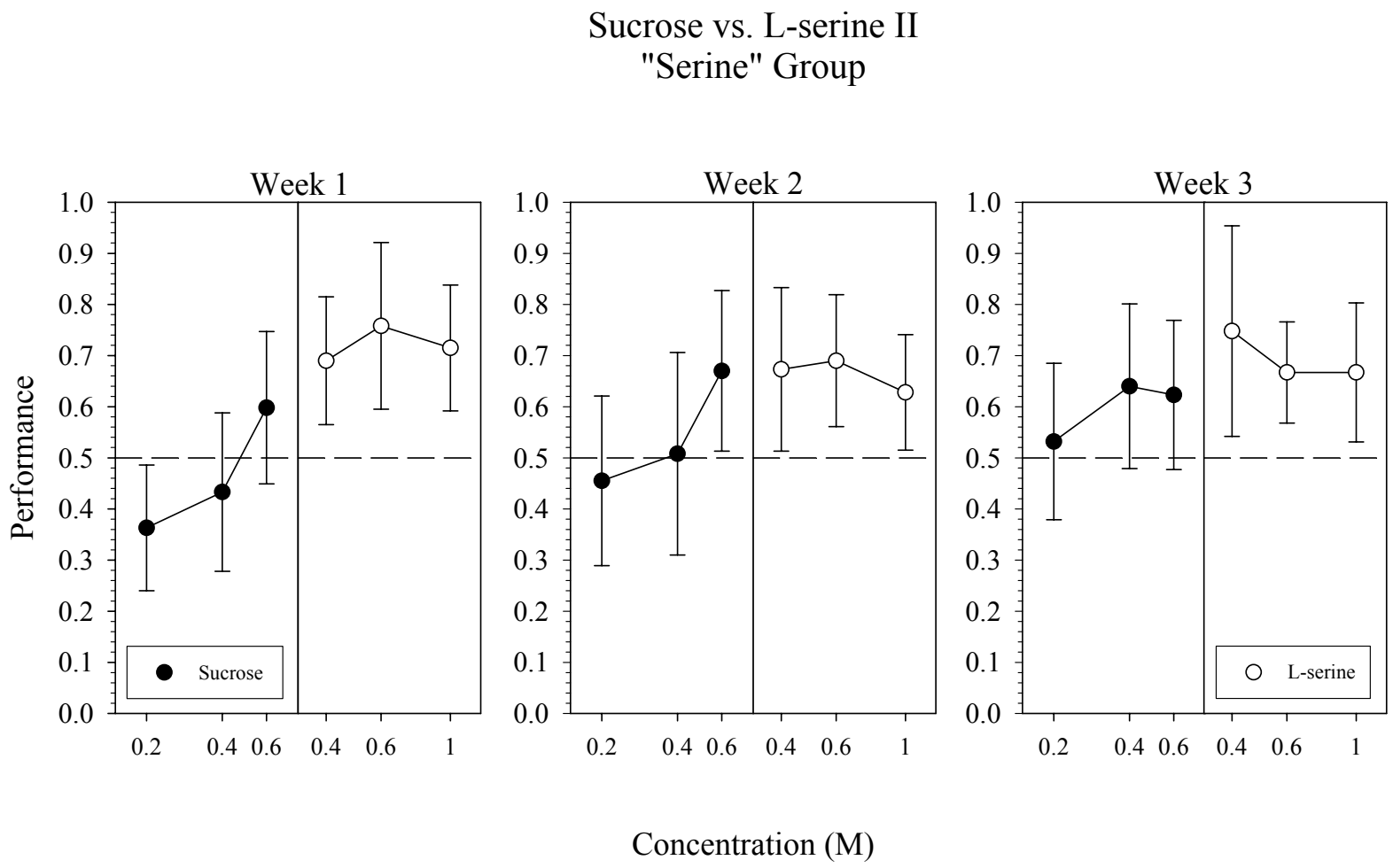

Figure 3-10: Mean (± SD) data for mice attempting to discriminate L-serine from sucrose for a second time. These mice were initially trained to discriminate L-serine from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

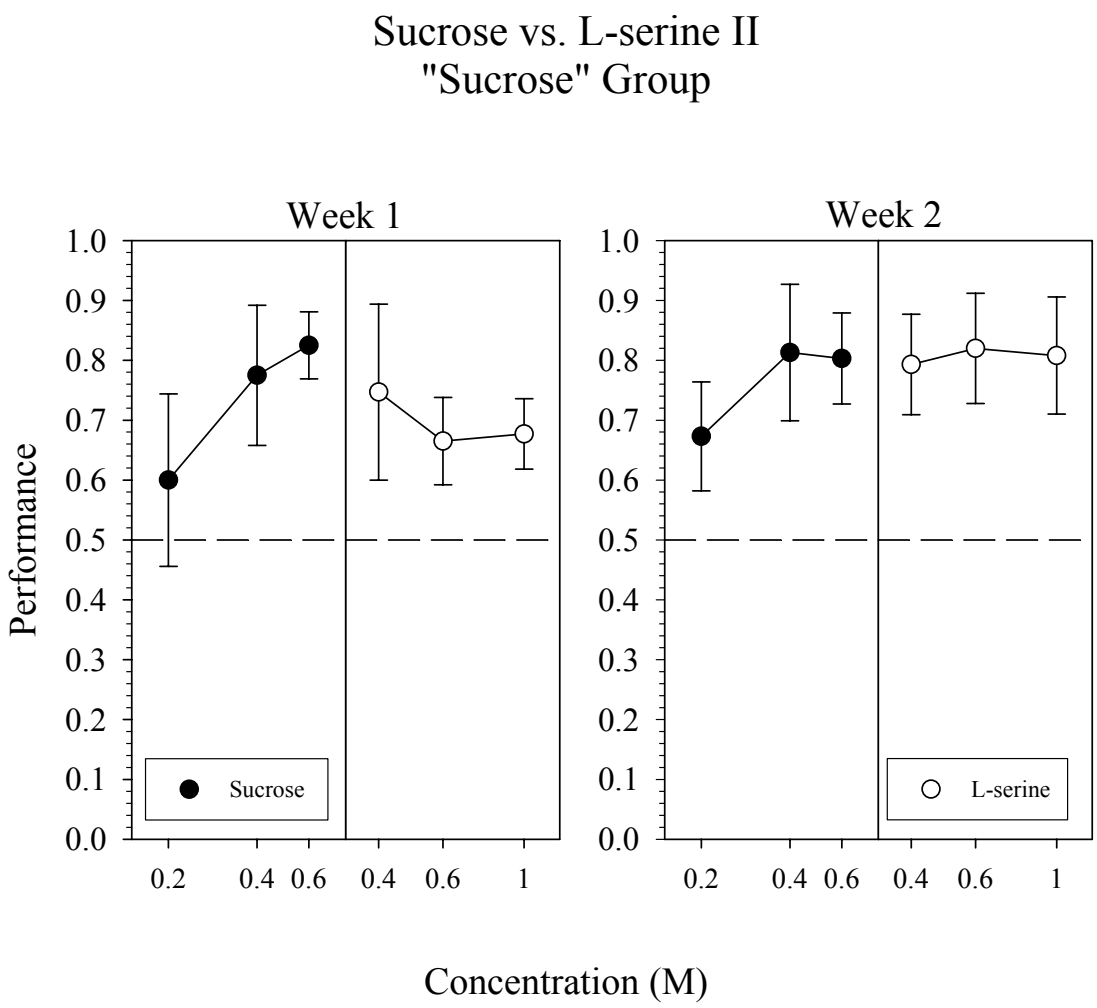

Figure 3-11: Mean (± SD) data for mice attempting to discriminate L-serine from sucrose for a second time. These mice were initially trained to discriminate sucrose from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

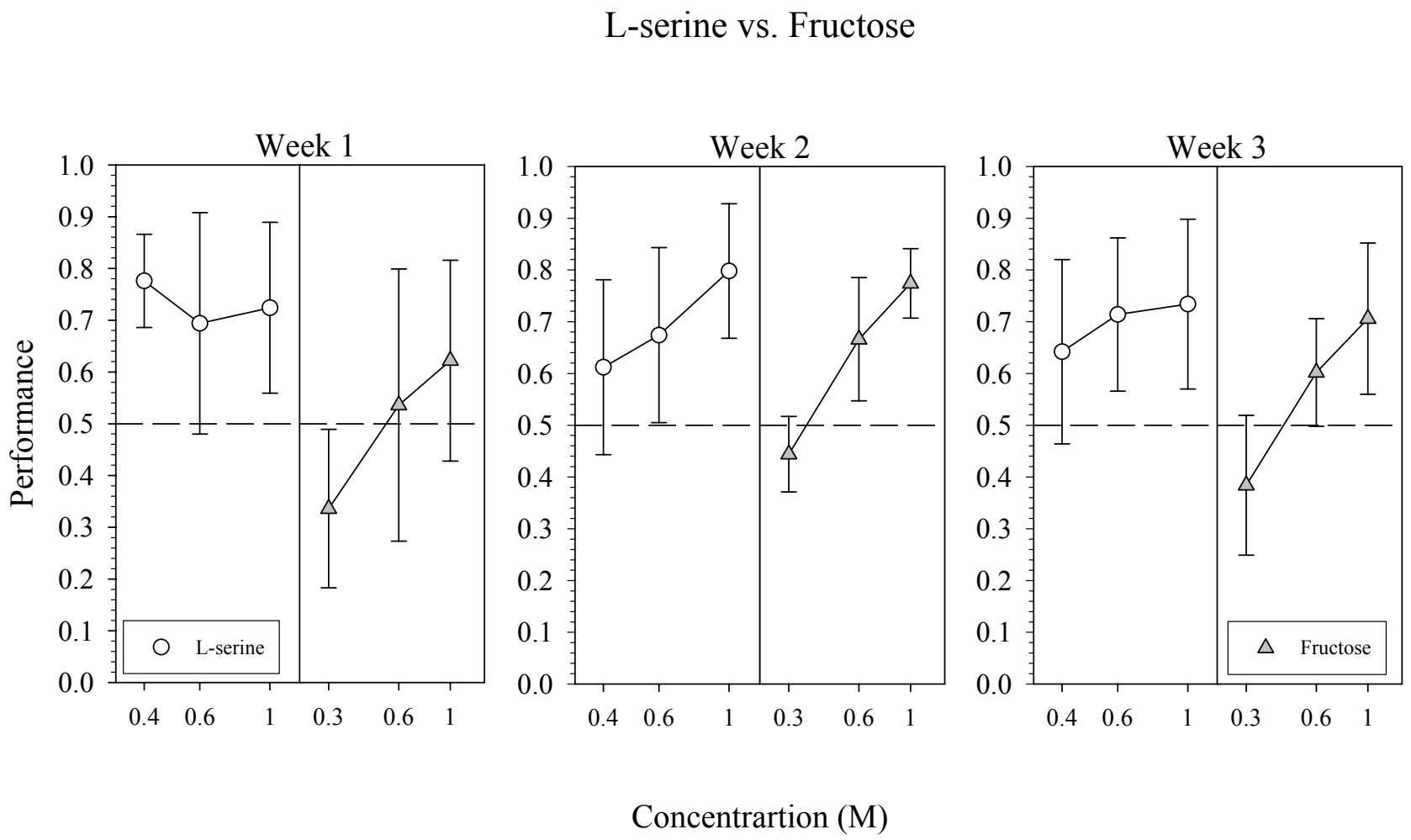

Figure 3-12: Mean (± SD) data for mice attempting to discriminate L-serine from fructose. These mice were initially trained to discriminate L-serine from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

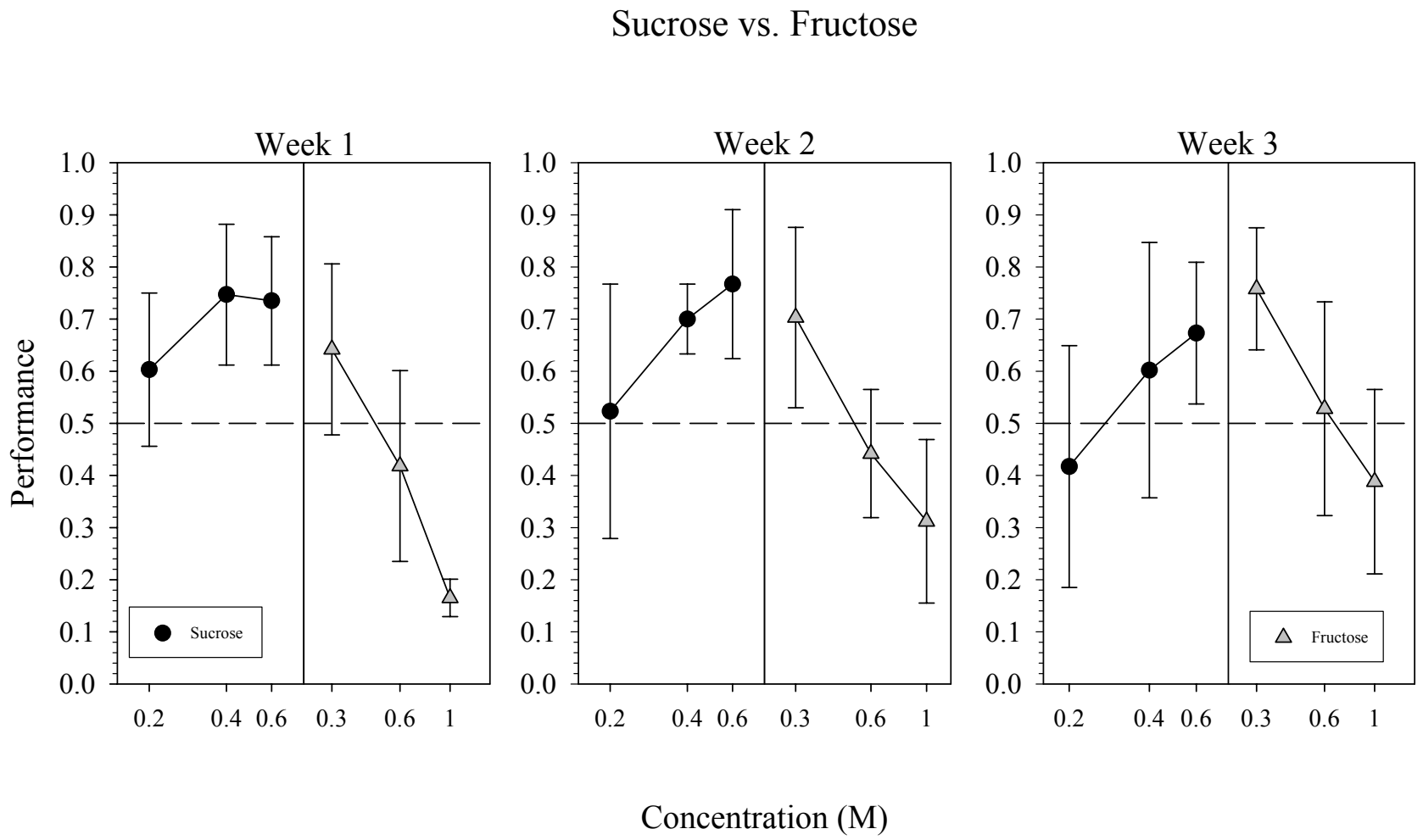

Figure 3-13: Mean (± SD) data for mice attempting to discriminate sucrose from fructose. These mice were initially trained to discriminate sucrose from NaCl. Performance, by concentration, on all trials with a lever press is depicted collapsed across a week. Chance performance equaled 0.5.

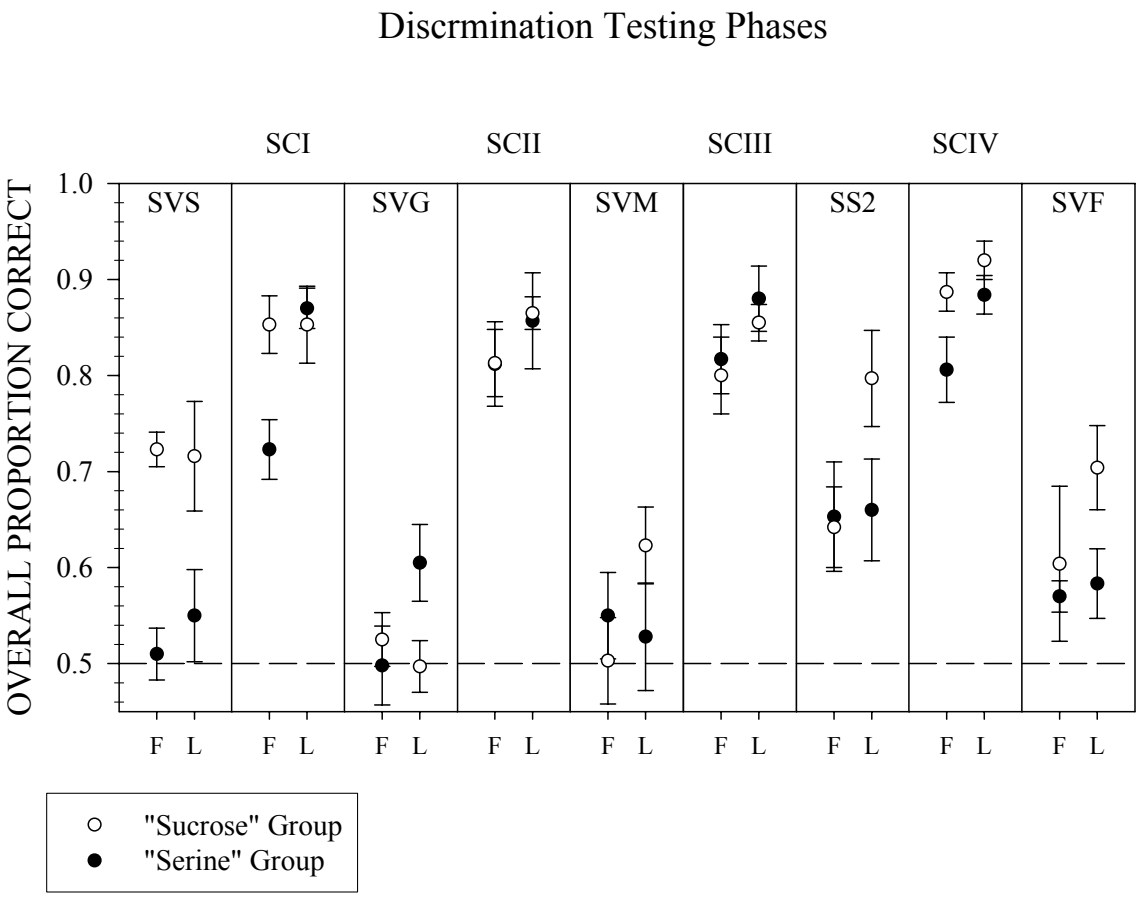

Figure 3-14: Mean (± SEM) data for both groups of mice are plotted across all test phases. Performance on all trials with a lever press is depicted averaged across all stimuli in a session. Only the first (F) and last (L) day of each phase are shown. The "sucrose" group was initially trained to discriminate sucrose from NaCl. The "serine" group was initially trained to discriminate L-serine from NaCl. Chance performance equaled 0.5 (SVS = Sucrose vs. L-serine; SVG = Sucrose/L-serine vs. Glucose; SVM = Sucrose/L-serine vs. Maltose; SS2 = Sucrose vs. L-serine II; SVF = Sucrose/L-serine vs. Fructose; SC = stimulus-control sessions).

CHAPTER 4
EXPERIMENT 3: PERCEIVED SIMILARITY BETWEEN L-SERINE, L-THREONINE AND CHEMICAL COMPOUNDS REPRESENTATIVE OF THE FOUR BASIC TASTE QUALITIES

Background

The results presented in Chapter 3, as well as those from CTA generalization studies, suggest that a subset of L-amino acids, including L-serine, share a qualitative similarity with the taste of sucrose. However, data detailed in Chapter 2 show that L-serine appears to be unlike sucrose in its ability to generate licking in mice. Thus, while it would appear that L-serine might share some qualitative characteristics with sucrose, this amino acid does not share the affective potency of this sugar. One possible explanation for this finding is that L-serine generates additional qualities that impact upon its affective value. For example, saccharin is both "sweet" and "bitter" tasting to humans depending on concentration (e.g., Bartoshuk, 1979; Schiffman *et al.*, 1979). Although, saccharin was initially thought to only activate the T1R2+3 receptor complex, researchers has recently demonstrated, using an *in vitro* preparation, that it also activates the human "bitter" receptors hTAS2R43 and hTAS2R44 at concentrations known to elicit a "bitter-taste" perception in humans (Kuhn *et al.*, 2004). It is quite possible that at least some L-amino acids also activate separate transduction pathways that lead to different taste perceptions. Although all 20 common L-amino acids, including L-serine, were shown to interact with the mouse T1R1+3 receptor complex (Nelson *et al.*, 2002), behavioral studies in rodents and humans suggest that the taste qualities evoked by L-amino acids are varied (e.g., Ninomiya *et al.*, 1984; Shallenberger, 1993). These data

suggest that L-amino acids may activate other taste transduction pathways independent of the T1R1+3 receptor complex.

To my knowledge, the only CTA generalization study that directly addressed the taste quality(ies) evoked by L-serine in mice was conducted by Kasahara and colleagues (1987). These researchers showed that when conditioned to avoid 0.2 M sucrose, ddy mice generalized the aversion to 0.2 M L-serine. However, these researchers did not use L-serine as a conditioning stimulus (only as a test stimulus). If also used as a conditioning stimulus, then perhaps mice conditioned to avoid L-serine would have generalized an aversion to one or more of the other tested compounds thought to represent other basic tastes. Thus, to investigate the taste qualities evoked by L-serine, CTA generalization tests were conducted in the current investigation. In addition, the similarity of the L-amino acid, threonine, to compounds representative of the four basic tastes was also investigated. I am unaware of any prior CTA generalization experiments that have explicitly examined the qualitative similarity of L-threonine and compounds representative of the basic taste qualities.

Methodological Details

Subjects

Adult C57BL/6J (B6) male mice (n=44; Jackson Laboratories, Bar Harbor, Maine), ~ 10 weeks of age on arrival, served as subjects. The use of B6 mice allowed for a comparison of results with those obtained in Experiment 2. The mice were housed individually in polycarbonate cages in a colony room where the lighting was controlled automatically (12:12). Testing and training took place during the lights-on phase. After arrival in the facility, subjects had free access to pellets of laboratory chow (Purina 5001, PMI Nutrition International Inc., Brentwood, MO) and purified water (Elix 10; Millipore,

Billerica, MA) for six days before training and testing took place. All procedures, described below, were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Taste Stimuli

All solutions were prepared daily with purified water and reagent grade chemicals and presented at room temperature. The compounds that served as conditioned stimuli (CSs) were sucrose, L-serine, and L-threonine. The logic of choosing sucrose and L-serine as CSs was the same as that used to justify their inclusion in the discrimination experiment. That logic was described in detail in Chapter 3. The choice of L-threonine as a CS was based on the fact that it reportedly gives rise to a sweet taste in humans (e.g., Shallenberger, 1993) and is preferred, at certain concentrations, by rodents (Pritchard and Scott, 1982a; Iwasaki *et al.*, 1985). The panel of test stimuli (TSs) was composed of various concentrations of sucrose, L-serine, L-threonine, NaCl, citric acid (Fisher Scientific, Atlanta, GA), and quinine hydrochloride (QHCl; Sigma-Aldrich, St Louis, MO). Sucrose, NaCl, citric acid, and QHCl were chosen as TSs because they are frequently used as prototypical representatives of compounds that elicit a sweet, salty, sour, and bitter taste, respectively (Schiffman and Erickson, 1980). L-serine, L-threonine, and sucrose were also used as TSs to allow for the assessment of any generalization asymmetries. Asymmetrical relationships can arise when the conditioning and test stimuli, albeit similar, are not qualitatively identical. Yet, generalization, or the lack thereof, can also occur based on stimulus characteristics other than quality (i.e., stimulus intensity; see Nowlis, 1974). As a result, if the conditioning and test stimuli evoke a qualitatively identical percept, but the TS is of a low intensity relative to the CS, then expression of the learned aversion may be weak or non-existent. Thus, the use of

more than one concentration increases ones confidence that a learned aversion towards a CS will generalize to at least one of the concentrations of a TS (Spector and Grill, 1988; Spector, 2003). In view of this, two concentrations of each compound were included in the test stimulus arrays. These concentrations are listed in Table 4-1. The concentrations chosen for L-serine, L-threonine, and sucrose were the same as those used in the discrimination experiment described in Chapter 3. For QHCl, citric acid, and NaCl, an attempt was made to choose concentrations that would produce comparable sensation magnitudes. Stimulus concentrations that produced ~equivalent degrees of lick avoidance (Tastant/Water Lick Ratio: taste stimulus licks/water licks) were chosen from the dynamic range of behavioral responsiveness for C57BL/By6J mice, as measured in a brief-access taste test (Dotson *et al.*, 2005). For the high concentration, stimuli that produced a ~50% decrease in the lick rate of animals, relative to water, were chosen. For the low concentration, those that produced a ~25% decrease were chosen. These values were chosen in an attempt to elicit, in non-conditioned mice, lick rates to these normally avoided stimuli that were sufficiently high enough to allow any differences between conditioned and non-conditioned mice to be fully discernible.

Apparatus

Lickometer training and testing took place in an apparatus commonly referred to as the Davis rig (Davis MS-160, DiLog Instruments, Tallahassee, FL; see Smith, 2001). This device allows a mouse access to a single sipper tube containing a stimulus. Animals can also be restricted to licking in brief trials (5 s) by offering access to the different tubes via a motorized table and shutter. An 8-s inter-presentation interval was interposed between stimulus presentations. The test array for each mouse included the two different concentrations of each test stimulus detailed above, the CS, and purified water all of

which were contained in separate bottles (i.e., fourteen stimulus sipper tubes; in addition to the stimulus tubes, a non-stimulus "rinse" sipper tube was also included in the array – see details below). A given trial started upon the first lick. Each lick on a sipper tube was registered by a contact circuit. These responses were recorded by computer for later analysis.

During the conditioning phase, intake tests were conducted in the home cages. Fluids were presented in 25 ml graduated pipettes fitted with stainless steel sipper tubes on one end. Pipettes were secured to the shelf above with cable clips to reduce spillage. Intake was measured to the nearest 0.1 ml during this phase.

Experimental Design

Davis rig training

The mice were trained under a restricted water-access schedule. Water bottles were removed from the home cages the day before the start of the training phase. The mice were first trained for 2 daily consecutive 30-min sessions in the Davis rig with a stationary sipper tube containing purified water positioned in front of the access slot. The mice were allowed to take as many licks as possible within a 30-min session. Next, the mice were trained for 3 days in the Davis rig with a brief-access paradigm, in which access to water was available in 5-s trials from fourteen different sipper tubes. A water rinse (5 lick maximum) presentation was interposed between all trials. This was done so that "stimulus" presentation was consistent with that during the Davis rig test session (see below). Presentation order was randomized without replacement in blocks. The mice were allowed to complete as many trials as possible within the 25-min session.

Conditioning phase (see Table 4-2)

Water bottles were removed the day before the start of the conditioning phase. During this phase, starting at 0900, each mouse received water from a drinking spout in its home cage for 15 min at the same time each day (the start of the trial for animals was staggered by 5 min to allow time for intake measurements and injections). Approximately 5 h after the start of each animal's morning session, the mice were given access to purified water for 1 h to allow for rehydration. After three days of one-bottle water testing, the animals were divided into six groups (n = 8 mice per group) according to the CS (L-serine, L-threonine, or sucrose) and unconditioned stimulus (US; LiCl or NaCl) they would receive. Mice were assigned to groups on the basis of their body weight, mean water intake during the first three days of the conditioning phase, mean licks/trial, and mean number of trials during the last three days of Davis rig training. There were no significant differences between the groups regarding these parameters.

Subsequently, five conditioning trials followed in which mice were presented with the appropriate CS for 15 min, immediately followed by an intraperitoneal (i.p.) injection (3.0 mEq/kg body wt) of either 0.15 M LiCl[1] or 0.15 M NaCl. The purpose of the LiCl injection was to induce visceral malaise. Mice that drank less than 0.1 mL of their respective CS, had ~0.1 mL infused in their oral cavity with a syringe before receiving the US injection. Water bottles were returned to the home cages ~5 hours after a conditioning trial. The following day, water bottles were removed from the cages, starting at 0915, on the same staggered schedule used on the previous conditioning phase

[1] The 1st US injections for all three experimental groups were carried out with LiCl dissolved in 0.15 M NaCl. All subsequent injections were completed with LiCl dissolved in purified water. Also, four mice died after the 1st injection for unknown reasons. As a result, n = 6 – 8 mice per group.

day. Approximately 5 h after the removal of each animal's water bottle, the mice were again given access to purified water for 1 h to re-establish the 18-h schedule of water restriction. The next conditioning trial was separated by one more day of the restricted water-access schedule. After the fifth conditioning trial, water bottles were replaced on the home cages for one day.

Davis rig testing phase

Water bottles were removed ~23.5 h before each animal's brief-access testing phase began. On the first day of testing, the mice were presented with 5-s water trials from fourteen sipper tubes, as described above for brief-access training. This water testing was intended to reacquaint the mice to the task in the Davis rig and to increase their motivation for licking on the following test day. On the next day, the mice were presented with 5-s trials of their specific "test stimulus" array. A water rinse (5-lick maximum) presentation was interposed between the test trials for all stimuli to help control for potential carryover effects (see St John *et al.*, 1994; Boughter *et al.*, 2002). Presentation order was randomized without replacement in blocks so that every stimulus and water was presented exactly once before the initiation of the subsequent block. The mice were allowed to complete as many trials as possible within the 25-min session.

Data Analysis

A CS-suppression ratio was derived by dividing the CS intake before the first conditioning trial by the CS intake before the fifth (final) trial for each mouse. A ratio of 1.0 signifies equal intake between the first and last conditioning trials, while a ratio less than or greater than 1.0 signifies decreased or increased intake, respectively, in the last conditioning trial relative to the first trial.

In the brief-access test, Tastant/Water Lick Ratios were calculated for each mouse representing the mean number of test stimulus licks per trial divided by the mean number of water stimulus licks per trial (water rinse trials were not included in the analysis). All of these data were analyzed with analyses of variance (ANOVAs) and t-tests. The statistical rejection criterion was set at the conventional value of .05. Bonferroni adjustments were also performed to control for the use of multiple comparison on the same data set. This extremely conservative standard is reported for the benefit of the reader (see Figures 4-2, 4-3, and 4-4). However, the design was based on testing the response of the control and experimental mice to each stimulus. Thus, we chose to base our interpretation on the unadjusted values which are also detailed.

Results

Conditioning Phase

All of the LiCl-injected groups demonstrated convincing evidence of an aversion acquired to their respective CS during the conditioning phase. T-tests revealed a significant difference from a value of 1.0 in the CS suppression ratio for all three LiCl injected groups [Figure 4-1; all t-values $\leq$ -8.4, $P < .001$]. This indicates that there was a significant decrease in CS intake between the first and fifth conditioning trial for these groups. There was also a significant decrease in CS intake for the saline injected "serine" group on the fifth conditioning day relative to the first [$t(6) = 3.0$, $P < .05$]. However, this decrease was slight and, as a result, interpretively insignificant. Indeed, the CS suppression ratio for each LiCl-injected group was significantly lower than its corresponding control group [Figure 4-1; all t-values $\leq$ 5.0, $P < .001$]. Collectively, these data confirm the effectiveness of the conditioning procedures. However, it is well known that, relative to rats, mice have a greater resistance to CTA acquisition procedures

(Ninomiya *et al.*, 1984; Kasahara *et al.*, 1987; Welzl *et al.*, 2001). Thus, of mice in the three LiCl conditioning groups, only those that consumed less than 0.5 ml during the fifth and final conditioning session were included in the subsequent Brief-Access Testing Phase. Only two mice (out of 8 in the sucrose conditioning group) consumed more than 0.5 ml and thus were discarded from all analyses reported here. The fact that sucrose conditioned mice were the most resistant to the procedures is consistent with at least one published report on the use of sugars as CSs, albeit in the rat (Smith, 1971).

Brief-Access Testing Phase

Sucrose CS group

T-tests indicted that, relative to controls, mice conditioned to avoid 0.4 M sucrose showed strong suppression to all three concentrations of sucrose [Figure 4-2; all *t*-values ≥ 3.6, *P*s < .01]. A one-way ANOVA indicated that the Tastant/Water Lick Ratios of these concentrations did not significantly differ. Relative to controls, sucrose conditioned mice did not avoid any other stimulus in the test stimulus array.

L-serine CS group

T-tests revealed that, relative to controls, mice conditioned to avoid 0.6 M L-serine also showed strong suppression to all three concentrations of L-serine [Figure 4-3; all *t*-values ≥ 2.3, *P*s < .05]. A one-way ANOVA indicated that the Tastant/Water Lick Ratios of these concentrations did not significantly differ. Relative to controls, these mice also avoided both concentrations of sucrose [Figure 4-3; both *t*-values ≥ 3.4, *P*s < .01] and L-threonine [Figure 4-3; both *t*-values ≥ 2.4, *P*s < .05]. The Tastant/Water Lick Ratios of the two sucrose concentrations did not significantly differ. However, a paired t-test revealed that the conditioned mice avoided 0.7 M L- threonine significantly more than 0.175 M L-threonine [$t(7) = 4.0$, $P < .01$]. Surprisingly, in addition to sucrose and L-

threonine, L-serine conditioned mice also avoided 0.625 mM QHCl to a significantly greater degree than did control mice [Figure 4-3; $t(13) = 2.5$, $P < .05$]. These animals did not avoid any other stimulus in the test stimulus array.

L-threonine CS group

Relative to animals in the control group, mice conditioned to avoid 0.35 M L-threonine, similar to the other two conditioned groups, showed strong suppression to all three concentrations of their CS [Figure 4-4; all t-values ≥ 2.5, Ps $< .05$]. A one-way ANOVA indicated that the Tastant/Water Lick Ratios of these concentrations did not significantly differ. Relative to controls, these mice also avoided 0.6 M sucrose [Figure 4-4; $t(11) = 2.5$, $P < .05$] and 1.0 M L-serine [Figure 4-4; $t(11) = 3.2$, $P < .01$]. These animals did not avoid any other stimulus in the test stimulus array.

Discussion

The results from Experiment 3 demonstrated that animals trained to avoid 0.6 M L-serine subsequently avoided both concentrations of sucrose. These data suggest that L-serine possesses a sucrose-like taste quality to B6 mice. This finding is consistent with the only CTA experiment on the "taste" of L-serine in rodents, as well as the large body of human psychophysical studies and the data reported in the preceding chapter. In addition, L-threonine also appears to possess a sucrose-like taste quality to these animals. This has never been explicitly demonstrated in rodents. Collectively, data from Experiments 2 and 3 suggest that at least some of the signals generated by the receptor(s) responsible for the transduction of these stimuli (i.e., L-amino acid sweeteners and sucrose) converge somewhere in the gustatory system. The mechanisms potentially underlying this qualitative similarity were discussed in detail in Chapter 3.

Interestingly, the generalization seen towards sucrose by mice trained to avoid L-serine was not "reversible." That is, mice trained to avoid 0.4 M sucrose did not avoid either of the L-serine concentrations tested or, for that matter, either concentration of L-threonine. Yet, L-serine did reversibly generalize with L-threonine. These data, as well as those gathered in Experiment 2, suggest that "sweet-tasting" L-amino acids, while more similar to sucrose than to other prototypical representatives of the basic taste qualities, possess discriminable taste characteristics. The source of the discriminable cue may lie in the pattern of receptor expression. TBCs that express receptor(s) which respond to L-serine (e.g., T1R1+3) may generate discrete neural signals that remain distinguishable as the information ascends through the nervous system. However, another possibility is suggested by the generalization pattern observed in Experiment 3. The results from this experiment suggest that to B6 mice, L-serine may be perceived as both "sweet" and "bitter." At least one published study has reported that humans perceive the taste of L-serine crystals as sweet, with a bitter/sour aftertaste (Haefeli and Glaser, 1990). Humans also state that the artificial sweetener, saccharin, gives rise to a similar taste perception (i.e., sweet, with a bitter aftertaste; e.g., Bartoshuk, 1979; Schiffman *et al.*, 1979). The putative "bitter-taste" evoked by this stimulus would appear to provide a plausible explanation for the difference in the affective potency of L-serine and sucrose.

Because of the inherent floor effect involved with measuring conditioned avoidance to naturally aversive compounds in a CTA design, the fact that animals trained to avoid L-serine significantly suppressed their licking of quinine relative to control mice raises our confidence that this amino acid possesses a quinine-like taste component. The

response of control animals to a bitter TS such as quinine is naturally suppressed, making further avoidance difficult to discern (see Smith and Theodore, 1984 for an example). Nevertheless, mice conditioned to avoid L-serine suppressed their lick responses to quinine above and beyond these factors. That said, it would be instructive to repeat Experiment 3 using a taste assessment procedure in which the affective characteristic of the stimuli are rendered irrelevant (e.g., Grobe and Spector, 2006). Such a procedure would not be plagued by the problems mentioned above and as a result, would likely be more sensitive to any "bitter-taste" potentially elicited by the CSs (e.g., L-threonine).

It would also be informative to repeat Experiment 3 with mice lacking one or more of the T1R receptors. This would reduce or eliminate the neural input from TBCs that express the family of receptors that are likely responsible for the relative similarity of L-serine and sucrose. These manipulations would allow for a more focused evaluation of the "bitter" taste-evoking potential of L-serine. I would predict that one or more of these groups would generalize an aversion learned to L-serine more strongly to quinine than would wild-type mice. Based on responses of T1R knock-out mice reported in Zhao *et al.*, 2003, in at least one group of these animals (e.g., T1R1 and/or T1R3 knock-out mice; see Table 1-1 or Zhao *et al.*, 2003), L-serine would not evoke T1R peripheral neural input, and as a result, it would potentially taste more purely "bitter."

Interestingly, as detailed in Chapter 1, 66% of the T2R-expressing cells in the circumvallate papilla also expressed T1R1, which, in combination with T1R3, is thought to bind with L-amino acids, including L-serine. It has been postulated by some, that rodents will reject amino acids in proportion to their toxicity (Pritchard and Scott, 1982a). However, it is not known whether taste input has a role in this rejection. The discovery

of a “bitter” receptor responsible for L-amino acid rejection would allow for a conformation that this behavior is based, to a certain extent, in peripheral taste physiology. As covered earlier in this chapter, two bitter receptors were recently shown to be responsible for mediating the “bitter” aftertaste elicited by saccharin. As detailed in Chapter 2, water-restricted D2 mice avoided L-serine in a concentration dependent manner. This is consistent with the fact that L-serine appears to evoke a bitter taste perception. However, in this same experiment, B6 mice did not avoid the stimulus. It is well known that these two strains differ in their sensitivity to various “bitter” tasting compounds (e.g., B6 mice are more sensitive to quinine and D2 mice are more sensitive to raffinose undecaacetate; Boughter *et al.*, 2005; Nelson *et al.*, 2005). These behavioral phenotypes can be exploited to help identify any potential polymorphisms (e.g., in genes encoding for T2R receptors) that contribute to the observed behavioral divergence (i.e., L-serine avoidance).

Table 4-1: Test Stimulus Arrays for the Three Conditioning Groups

	Test Stimuli														
	Sucrose			L-Threonine			L-Serine			QHCl		Citric Acid		NaCl	
	CS[#]	High	Low	**CS**[#]	High	Low	**CS**[#]	High	Low	High	Low	High	Low	High	Low
Group 1	0.4 M	0.6 M	0.2 M	-	0.7 M	0.175 M	-	1.0 M	0.4 M	0.625 mM	0.325 mM	13.25 mM	6.25 mM	0.375 M	0.175 M
Group 2	-	0.6 M	0.2 M	-	0.7 M	0.175 M	0.6 M	1.0 M	0.4 M	0.625 mM	0.325 mM	13.25 mM	6.25 mM	0.375 M	0.175 M
Group 3	-	0.6 M	0.2 M	0.35 M	0.7 M	0.175 M	-	1.0 M	0.4 M	0.625 mM	0.325 mM	13.25 mM	6.25 mM	0.375 M	0.175 M

*High = ~50% of the lick rate relative to water; low = ~75% of the lick rate relative to water. See text for more details.

[#]These were concentrations used in Experiment 2

Table 4-2: Conditioning phase schedule

Conditioning phase														
AM	15-m water access	15-m CS access ↓ US injection	Water bottles removed	15-m water access	15-m CS access ↓ US injection	Water bottles removed	15-m water access	15-m CS access ↓ US injection	Water bottles removed	15-m water access	15-m CS access ↓ US injection	Water bottles removed	15-m water access	15-m CS access ↓ US injection
PM	1-h water access	Water bottles returned 5-h after injection	1-h water access	1-h water access	Water bottles returned 5-h after injection	1-h water access	1-h water access	Water bottles returned 5-h after injection	1-h water access	1-h water access	Water bottles returned 5-h after injection	1-h water access	1-h water access	Water bottles returned 5-h after injection
	3 days	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*	*1 day*

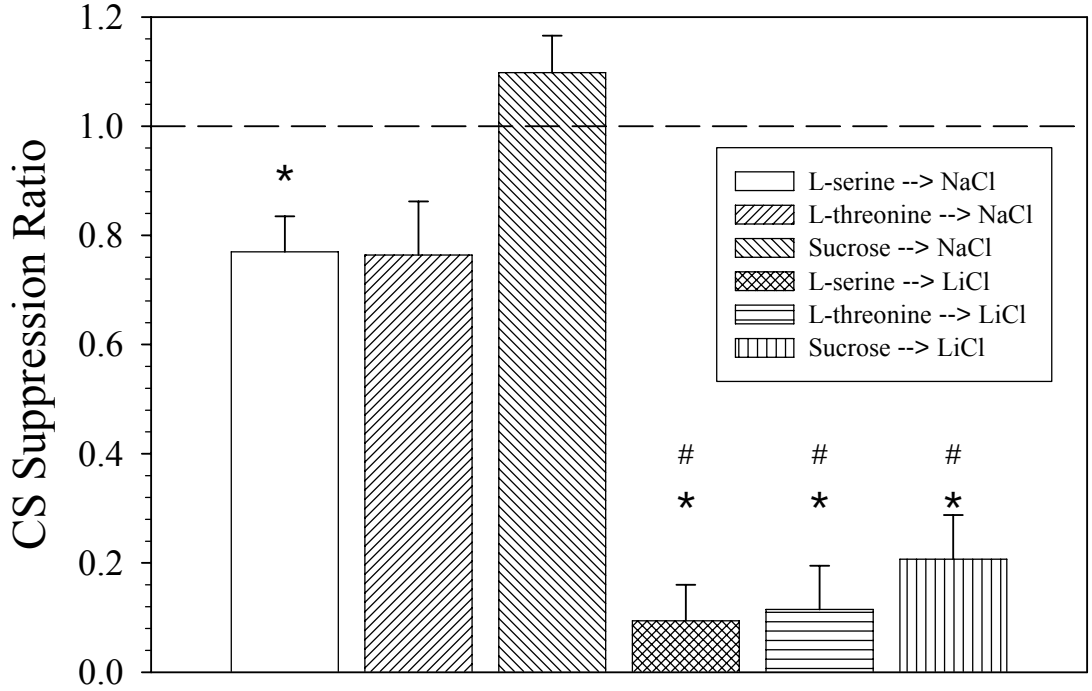

Figure 4-1: Mean (± SEM) of the CS Suppression Ratio for each conditioned stimulus (CS). A ratio of 1.0 signifies equal intake between the first and last conditioning trials, while a ratio less than or greater than 1.0 signifies decreased or increased intake, respectively, in the last conditioning trial relative to the first trial. An asterisk (*) denotes a significant difference from a ratio value of 1.0 while a pound (#) indicates a CS Suppression Ratio for each LiCl-injected group that was significantly lower than its corresponding control group.

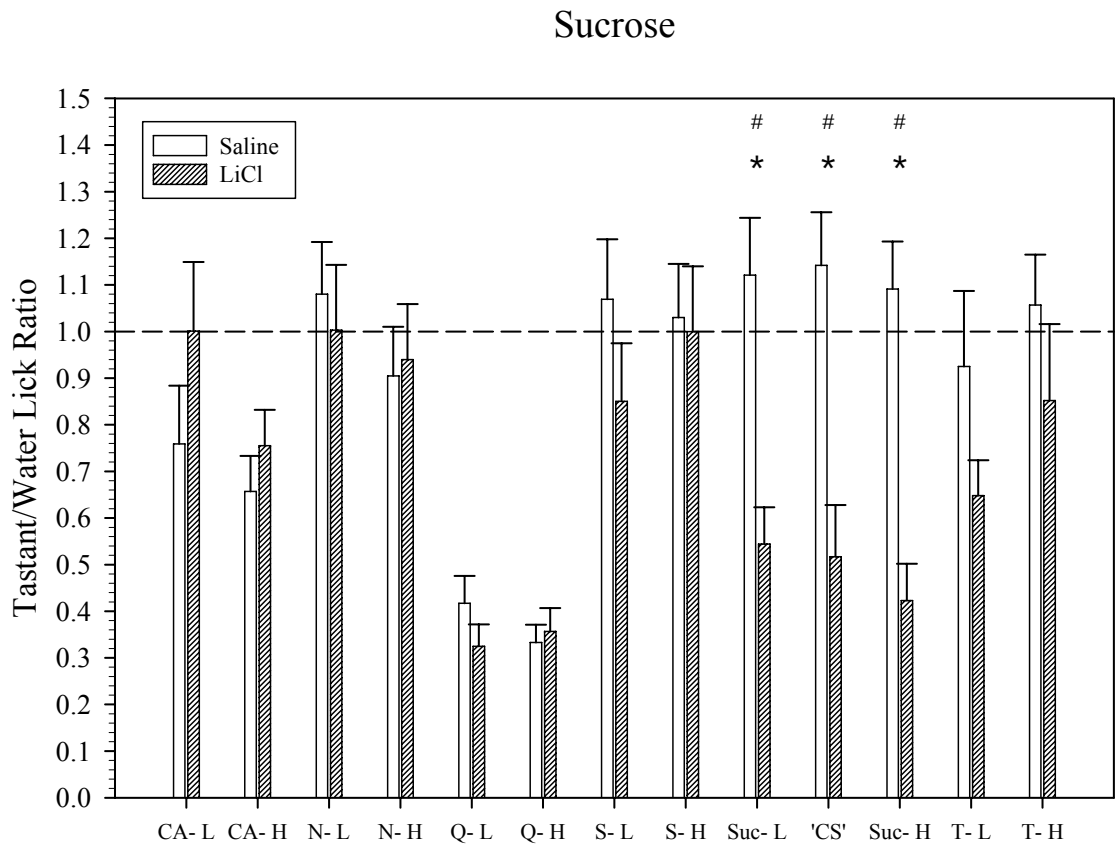

Figure 4-2: Mean (± SEM) Tastant/Water Lick Ratios for the sucrose CS group for all test stimuli. The Tastant/Water Lick Ratio was calculated by dividing an animal's average licks to a given taste stimulus across trials by the average licks to water. The dashed line on the graph represents a Tastant/Water Lick Ratio of 1.0, which indicates licking to the taste stimulus was equivalent to licking to water. An asterisk (*) denotes a significant difference between the Tastant/Water Lick Ratios of the control and the conditioned groups. A pound (#) denotes a significant difference that survives the Bonferroni alpha adjustment. (CA- L = 6.25 mM citric acid, CA- H = 13.25 mM citric acid; N- L = 0.175 M NaCl, N- H = 0.375 M NaCl; Q- L = 0.325 mM QHCl, Q- H = 0.625 mM QHCl; S- L = 0.4 M L-serine, S- H = 1.0 M L-serine; Suc- L = 0.2 M sucrose, Suc- H = 0.6 M Sucrose; T- L = .0.175 M L-threonine, T- H = 0.7 M L- threonine; CS = 0.4 M sucrose).

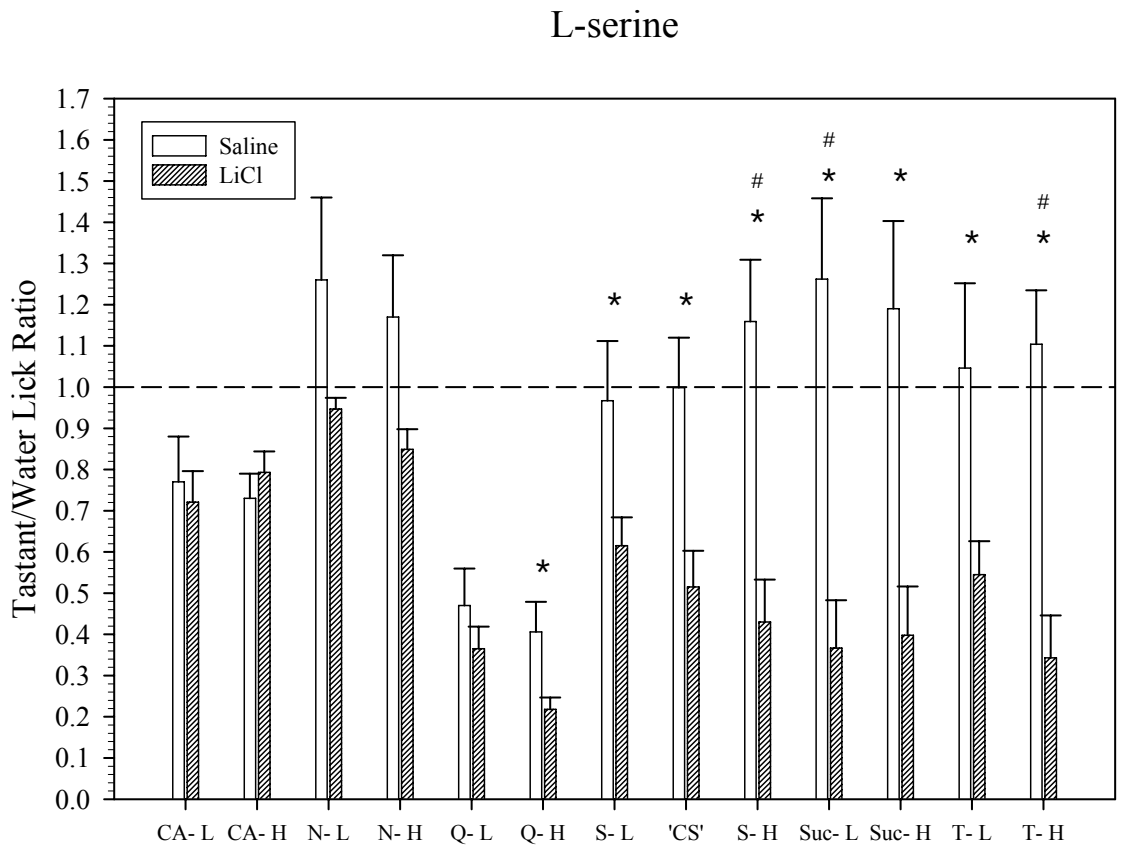

Figure 4-3: Mean (± SEM) Tastant/Water Lick Ratios for the L-serine CS group for all test stimuli. The Tastant/Water Lick Ratio was calculated by dividing an animal's average licks to a given taste stimulus across trials by the average licks to water. The dashed line on the graph represents a Tastant/Water Lick Ratio of 1.0, which indicates licking to the taste stimulus was equivalent to licking to water. An asterisk (*) denotes a significant difference between the Tastant/Water Lick Ratios of the control and the conditioned groups. A pound (#) denotes a significant difference that survives the Bonferroni alpha adjustment. (CA- L = 6.25 mM citric acid, CA- H = 13.25 mM citric acid; N- L = 0.175 M NaCl, N- H = 0.375 M NaCl; Q- L = 0.325 mM QHCl, Q- H = 0.625 mM QHCl; S- L = 0.4 M L-serine, S- H = 1.0 M L-serine; Suc- L = 0.2 M sucrose, Suc- H = 0.6 M Sucrose; T- L = .0.175 M L-threonine, T- H = 0.7 M L- threonine; CS = 0.6 M L-serine).

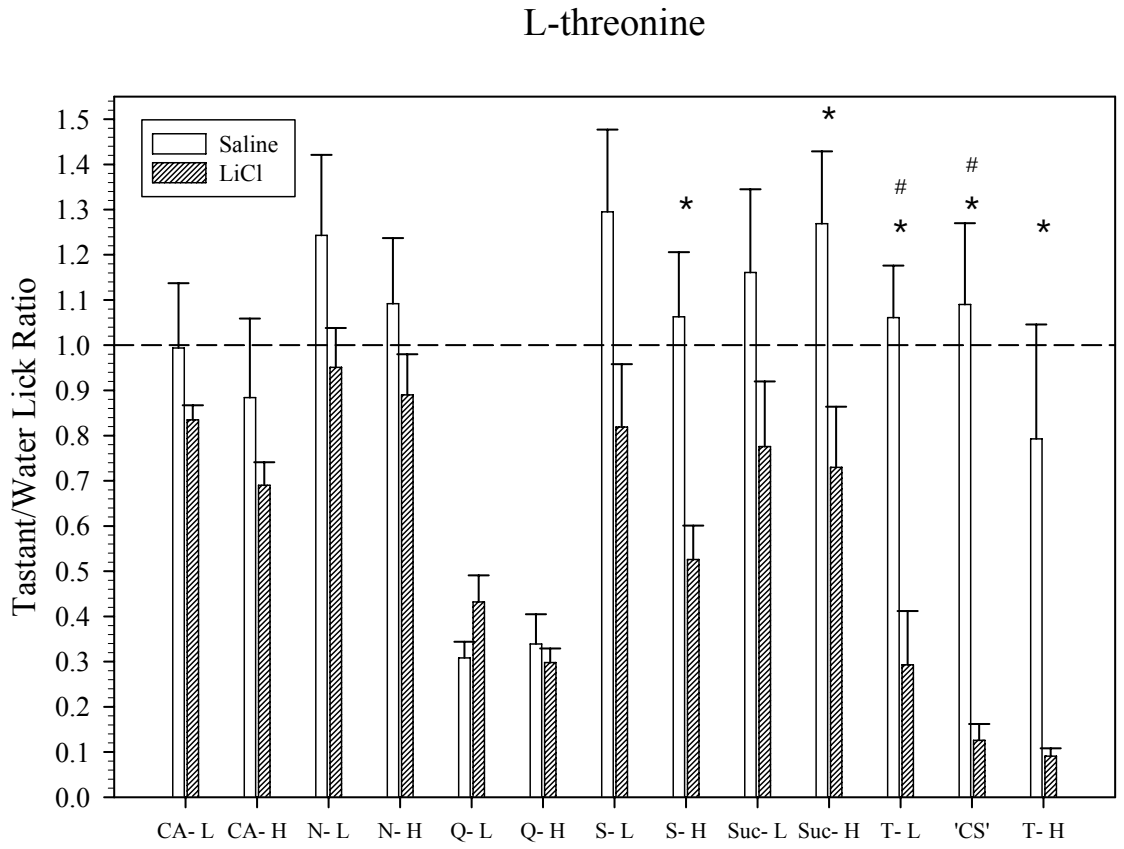

Figure 4-4: Mean (± SEM) Tastant/Water Lick Ratios for the L-threonine CS group for all test stimuli. The Tastant/Water Lick Ratio was calculated by dividing an animal's average licks to a given taste stimulus across trials by the average licks to water. The dashed line on the graph represents a Tastant/Water Lick Ratio of 1.0, which indicates licking to the taste stimulus was equivalent to licking to water. An asterisk (*) denotes a significant difference between the Tastant/Water Lick Ratios of the control and the conditioned groups. A pound (#) denotes a significant difference that survives the Bonferroni alpha adjustment. (CA- L = 6.25 mM citric acid, CA- H = 13.25 mM citric acid; N- L = 0.175 M NaCl, N- H = 0.375 M NaCl; Q- L = 0.325 mM QHCl, Q- H = 0.625 mM QHCl; S- L = 0.4 M L-serine, S- H = 1.0 M L-serine; Suc- L = 0.2 M sucrose, Suc- H = 0.6 M Sucrose; T- L = .0.175 M L-threonine, T- H = 0.7 M L- threonine; CS = 0.35 M L- threonine).

CHAPTER 5
GENERAL DISCUSSION

The first experiment presented is this dissertation was designed to assess the taste-related affective potency of glycine, L-serine, and sucrose. As a result of the complete lack of appetitive behavior observed during testing with the putative sweetener L-serine, we explicitly tested the ability of mice to distinguish between L-serine and various sugars in an operant taste discrimination task. Additionally, we examined the ability of mice to distinguish between an assortment of natural sweeteners. Finally, CTA generalization tests were conducted to make inferences regarding the taste quality(ies) evoked by some putative "sweet-tasting" L-amino acids. The results of these studies were consistent with the notion that all of these compounds share some qualitative similarities. Mice had difficulty, depending on the stimulus and the training history, discriminating sucrose from L-serine, maltose, fructose and glucose. However, despite the apparent qualitative similarity of L-serine and the natural sweeteners tested in the current report, mice appeared to possess a limited ability to distinguish between them. The results of CTA generalization experiments imply that L-serine evokes a complex taste perception that includes a sucrose-like taste quality. Indeed, this complexity, like the taste of saccharin to humans, appears to result from, at least in part, the generation of both a "sweet" and "bitter" taste, and may provide the cue that distinguishes L-serine from sucrose, as well as from other sugars. Along with the relatively weak "sweet-taste" evoked by L-serine postulated in Chapter 3, its ability to generate a "bitter-taste" would appear to provide an

additional plausible explanation for the difference in the affective potency of L-serine and sucrose.

It should be pointed out that some of the concentrations of the amino acids tested, although within the range of that commonly used to assess the behavioral responsiveness of rodents in other experiments (e.g., Pritchard and Scott, 1982a; Iwasaki *et al.*, 1985; Zhang *et al.*, 2003; Zhao *et al*., 2003), were relatively high. Since stimuli at these concentrations would not likely be encountered in nature, the response of the gustatory system to these stimuli is of questionable biological relevance. That said, based on electrophysiological data, both the chorda tympani (Pritchard and Scott, 1982a; Iwasaki *et al.*, 1985; Inoue, *et al.*, 2001; Danilova and Hellekant, 2003) and glossopharyngeal nerves (Danilova and Hellekant, 2003) display monotonically increasing responses across a high concentration range of L-amino acids including L-serine and L-threonine. These results suggest that investigating the responses of rodents to a broad concentration array of various amino acids may yet provide ecologically relevant information. In any event, studying ligands that robustly active the gustatory system could prove useful as we attempt to uncover the principles governing the functional organization of taste in the periphery.

During Experiment 2, training history had a huge impact on the ability of mice to discriminate all of the stimuli, particularly L-serine from sucrose. Mice, trained to discriminate L-serine from NaCl (i.e., serine group), appeared to have more difficulty discriminating L-serine from sucrose relative to animals in the sucrose group. Indeed, two mice in the serine group were unable to discriminating L-serine from all of the sugars. Although there are other possible explanations for this training history

asymmetry, one likely explanation arises from the apparent qualitative complexity of L-serine. In Experiment 3, mice did not generalize an aversion learned to sucrose to any other stimulus in the TS array. Thus, sucrose appears to be more unitary in its perceptual quality. As a result, mice trained with the more complex stimulus array (i.e., L-serine) may have had a greater expectation of the qualitative variability of their standard stimulus (e.g., pure "sweetness" at low concentrations and "sweetness" and "bitterness" at higher concentrations) than did animals in the sucrose group (pure "sweetness" across the range of concentrations). If so, mice in the sucrose group would have an easier time discriminating a complex comparison stimulus from their qualitatively "pure" standard stimulus. Mice in the serine group, on the other hand, would have more difficulty discriminating a "pure" comparison stimulus, which, depending on concentration, appears to evoke qualities similar to those elicited by their "complex" standard, again depending on concentration. This was exemplified by the fact that during the first week of L-serine vs. sucrose or L-serine vs. glucose testing, mice had a tendency to respond to the comparison stimulus as if it were the standard. Mice in the sucrose group never responded in such a way.

To test this hypothesis, two groups of mice could be trained, as in Experiment 2, to discriminate a standard stimulus from an easily discriminable comparison stimulus (NaCl). One group would be given a qualitatively pure standard (sucrose), while the other would be given an artificially complex standard (e.g., a sucrose/quinine mixture). As in Experiment 2, once the animals were trained in the task, the comparison stimulus (NaCl) could be replaced with the standard stimulus from the other group, so that both sets of animals would be discriminating sucrose from a sucrose/quinine mixture. Based

on the hypothesis mentioned above, I would predict that B6 mice trained to discriminate an artificially complex standard from NaCl would have more difficultly with this discrimination task.

In summary, the findings here suggest that all of the putative "sweet" tasting L – amino acids and sugars tested in the current report share some qualitative features. However, the results of these experiments also imply that "sweet-tasting" L-amino acids, such as L-serine, also possess distinguishable taste characteristics. To my knowledge, the qualitative complexity elicited by L-serine, which includes a "bitter" taste, has never been previously demonstrated before in rodents. Yet, the ability of L-serine to evoke multiple taste percepts would explain much of the behavioral results observed in this dissertation (i.e., the efficacy of L-serine as a "standard"/training stimulus, its lack of affective potency, and its discriminability from sugars).

In addition, "monogeusia" for sucrose, glucose, fructose, and to a lesser extent, maltose has been demonstrated here for the first time in rodents. However, we cannot conclusively rule out the existence of some discriminative competency if these animals were tested under different experimental procedures. That said, if B6 mice can discriminate between these sugars, our results at least imply an extremely limited ability. Thus, these data suggest that the peripheral input evoked by these sugars is channeled into a single neural pathway as it ascends through the nervous system. This convergence likely occurs in the TBCs themselves (i.e., stimulation of a common receptor(s) (e.g., T1R2+3 receptor complex.).

Lastly, these data provide a sorely need functional context in which to interpret the neurobiology of "sweet" taste. This behavioral framework will allow researchers to

evaluate the taste-related consequences of manipulation of the gustatory system, assumed to affect "sweet-taste" perception (e.g., taste receptor knock-outs), with the knowledge of how "normal" mice react to these stimuli. Previously experiments have been beset by unconfirmed assumptions regarding the taste quality(ies) elicited by these stimuli and the subsequent behavioral responses evoke by their ingestion (e.g., if rodents generalize a sucrose aversion to a TS in a CTA experiment, then that TS will be unconditionally preferred). These findings also generate many questions to answer. For example, which T1R receptors, if any, contribute to the qualitative similarity of sucrose and L-serine? As mentioned in Chapter 4, the pattern of behavioral generalization would be expected to vary greatly between various T1R knock-out mice.

By providing a better understanding of the function of chemosensory systems, studies such as these can lead to improvements in the diagnosis and treatment of smell and taste disorders and contribute to our knowledge of how sensory systems operate and how their signals are integrated in the brain, as well as add to our understanding of the controls of food selection and intake.

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BIOGRAPHICAL SKETCH

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