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The effect of phenol denervation of the hepatic portal vein nerves on taste aversion learning

Deborah Jane Hooks

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THE EFFECT OF PHENOL DENERVATION OF THE HEPATIC PORTAL VEIN NERVES ON TASTE AVERSIONS LEARNING

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology

by
Deborah Jane Hooks
Spring 1993
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May 7, 1993
ABSTRACT

The origin of the illness signal in taste aversion learning is unknown. Coil, Rogers, Garcia and Novin (1978) found that subdiaphragmatic vagotomy blocks CuSO₄-induced taste aversion learning, demonstrating that the illness signal originates from the gut. However, which organ in the gut elicits this signal has not yet been determined. As the liver is the primary organ for detoxification of the blood and it is the first organ to receive nutrients from the gut, it is the most likely organ. The present study is a partial replication of the study by Coil, et al. (1978) in which the liver is neurologically isolated from the stomach. It was hypothesized that chemical denervation of the nerves along the hepatic portal vein would block taste aversion learning, and that this would vary as a function of emetic and method of administration. After denervation, the rats were presented with salty wet mash and then made ill with LiCl or CuSO₄ injections administered either intragastrically or intraperitoneally. They were then tested for a taste aversion over four trials. No difference was found between denervated animals and controls on the first or fourth extinction trials. Promising, however, are the trends in the data, which indicate that differences may exist. Results are discussed in terms of how the illness signal may be transmitted between the liver and the stomach, and ultimately to the brain.
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Introduction

Traditional learning theorists explain the modification of behavior using the classical and instrumental learning models. In the 1950s and 1960s these learning theorists studied behavior strictly in empirical terms, with the idea that general laws of behavior could be discovered by pairing specific arbitrary events with specific outcomes. A number of assumptions were inherent in theories during this time, but some more recent discoveries have led to questions regarding their central tenets. This paper discusses one of those discoveries, the phenomenon of taste aversion learning (TAL); when presented with a novel taste followed by illness, an organism will avoid consumption of the ingesta if presented with it again. The animal has learned an association between the taste and subsequent illness.

TAL does not fall exclusively into either the classical or instrumental models of learning. Over the past 25 years, the recognition of TAL has challenged scientists to reevaluate, or at least to redefine, these models as the only means by which learning occurs. Some psychologists believe that TAL is separate and distinct from classical and instrumental learning, while others believe that it still falls within the realm of these traditional learning models, and that it is the models themselves which must be modified (Bolles, 1985; Rescorla, 1988; Seligman, 1970).
Research has examined TAL from a variety of viewpoints in order to understand its mechanism and function more clearly. The phenomenon of TAL has been found to occur in all of the wide variety of species studied, and only a few stimuli have been found to be relevant cues for this type of learning. The question of what is the underlying physiological mechanism of taste aversion learning, however, remains largely unanswered, and precisely how the association is established has not been clearly defined.

The history of taste aversion research is summarized in the first section of this paper, emphasizing the particular characteristics of TAL and how it is different from other forms of learning. Also discussed are the theories which have been applied to TAL and how the research on TAL has expanded in the field of psychology. The physiology of the organism, and how the illness signal may be generated during TAL will be addressed in the second section. In this section the physiology and mechanisms of feeding behavior, including the role of the organs of the gut in feeding, and more specifically, how the liver may be the organ responsible for sending the illness signal to the brain will also be reviewed. Finally, the current study will be presented, incorporating previous theories of TAL with new findings in physiology. The liver's role in TAL will be examined with respect to its interaction with the stomach.
History

In the traditional classical learning model, a stimulus which normally produces a response is paired with an unrelated stimulus many times until the unrelated stimulus by itself is capable of producing the desired response. Pavlov (1927) presented dogs with food (unconditioned stimulus, US) contiguously with the clicking sound of a bell (conditioned stimulus, CS) resulting in an unconditioned response (UR) of salivation. Over several pairings the dogs learned to associate the sound of the bell with food, and would salivate to the sound of the bell. Psychologists were intrigued by Pavlov's findings. Decades of research followed, analyzing the possibility that by pairing a specific arbitrary event with another in the artificial setting of the laboratory, general laws of learning could be determined. While Pavlov continued to study salivation in dogs, Thorndike (1911) studied the ability of cats to learn to pull a string to escape from a puzzle box. Because the cats learned this behavior only gradually, he determined that animal learning is by trial and error. During the early and into the mid-1900s, as evident in both Pavlov's and Thorndike's laboratories, there existed a basic premise of animal learning; that given an artificial situation and the application of an arbitrary stimulus or event, such as the sound of a metronome or the presence of a string, general laws of learning and behavior.
would emerge.

In the mid 1950's John Garcia and his colleagues found that animals exposed to radiation formed aversions to foods they ate prior to their radiation treatments (Garcia, Kimeldorf, & Hunt, 1961; Garcia, Kimeldorf, & Koelling, 1955). They demonstrated that when a novel taste such as saccharin was paired with radiation-induced illness, the animals would associate the taste with the illness, and would later avoid the ingesta. Garcia used Pavlov's classical conditioning model to describe TAL. The CS was the taste of the ingesta, the emetic was the US which would produce the UR of illness or nausea, and avoidance of the taste was the CR.

The scientific community paid scant attention to Garcia's work in taste aversion until Garcia and Koelling published a paper now known as "Cue-to-Consequence" in 1966. In this paper they presented two groups of rats with a novel tasting solution contiguously with a light and a noise. Following consumption one group was made ill and the other group was shocked. To test for aversions, half of the illness-induced group was presented with flavored water and the other half was presented with plain water with the noise and light. This procedure was repeated for the group that was shocked. It was found that the animals who were made ill did not drink the flavored water, but did drink plain water in the presence of the light and noise; whereas, the subjects who were shocked
averted to the light and noise, but not to the flavored water. This suggested to the researchers that animals are biologically predisposed to associate only certain stimuli with certain consequences.

When Garcia uncovered this new phenomenon, most psychologists believed that behavior followed either the classical conditioning or instrumental conditioning models of learning. General process learning theory (Seligman, 1970) tied these two models together with four well established principles: 1) all stimuli and responses have an equal potential of being associated (principle of equipotentiality), 2) learning requires many trials of training, 3) the CS-US, or response-reinforcer interval must be short (principle of contiguity), and 4) species are equal in their ability to make simple associations. However, Garcia and Koelling's 1966 study changed the way psychologists looked at learning. In their cue-to-consequence experiment, they demonstrated that the principle of equipotentiality did not hold up, not all stimuli were equally associable. Garcia's previous studies (Garcia, et al. 1961; Garcia, et al. 1955) found that rats learned to associate illness with taste in only one pairing, demonstrating that repeated pairings were not necessary with TAL. These findings were not easily accepted by the scientific community because they implied that the central tenets of the general process model of learning were seriously
flawed. Furthermore, the principle of contiguity was questioned when Etscorn and Stephens (1973) and Smith and Roll (1967) demonstrated aversions after taste-illness delays of up to 24 hours.

A flurry of research followed the publication of the cue-to-consequence experiment. Scientists sought to confirm Garcia and Koelling's results and to seek out the parameters of TAL. Domjan and Wilson (1972) slightly modified the cue-to-consequence method and obtained similar results. In addition, Garcia, Ervin, and Koelling (1967) gave rats serum from irradiated donors and induced a taste aversion, demonstrating that there is a blood-borne component to TAL. Furthermore, taste aversions were found in a wide variety of species, such as chickens (Capretta, 1961), codfish (Mackey, 1974), monkeys (Johnson, Beaton, & Hall, 1975), coyotes (Ellins & Catalano, 1980) and pigeons (Lett, 1984), indicating that TAL occurred across many species. Roll and Smith (1972) found that rats learned aversions while under general anesthesia. No other kind of learning has been demonstrated while the subject is under anesthesia (Kalat, 1977).

As evidence for TAL increased, the implications of these findings were examined. Barker, Best, and Domjan (1977) point out that Garcia's work brought to psychology Darwin's theory of evolution through adaptation. An omnivore, such as a rat, must be able to quickly discriminate a safe from an unsafe
food. The ability to quickly associate internal distress with internal cues is imperative, yet an equal ability to associate external cues, such as light and noise, with external distress is likewise imperative to the survival of the organism. The animal which can detect a predator before it finds him is more likely to elude capture. External cues such as sight, sound and odor thus become extremely important for survival (Garcia, et al., 1974; Hankins, Rusiniak, & Garcia 1976). A learned aversion to the specific cue of taste is therefore expected because this would enable the organism to more easily learn from and survive experiences with toxic substances (Rozin & Kalat, 1971). An animal who can not learn to associate basic cues with consequences would not be likely to survive and reproduce.

This Darwinian view was in sharp contrast to the majority of the thinking in psychology in the early and mid 1900s. Scientists began the study of learning with the premise that in creating a contrived environment of levers, feeders, and metronomes to study arbitrary behaviors, they would be able to find general laws of learning. This approach did not take Darwin's law of natural selection into account when studying behavior. The search for mechanism and universal laws are the main focus of general process theorists. Adaptive change and function are of interest to the Darwinian theorist, with the study of the organism's predisposition to associate events of
more importance than the search for universal laws, which may actually only apply to certain species under certain conditions. TAL demonstrates that not all stimuli are created equal; some stimuli are more easily associated with consequent events than other stimuli.

Shortly after Garcia and Koelling published their cue-to-consequence findings, other learning phenomena which were inconsistent with traditional learning models, such as instinctive drift (Breland & Breland, 1961), autoshaping (Brown & Jenkins, 1968), and species-specific defense reactions (Bolles, 1970), surfaced in the literature. These additional findings gave further support to the notion of inherent biological constraints on learning certain associations. To accommodate natural selection within the framework of general process theory, Seligman (1970) suggested modifications to the theory. He pointed out that an organism brings to any experiment its own genetic predispositions to learning; that through evolution, each organism is more or less prepared to associate a certain events, and that the laws of learning may vary with the preparedness of the organism to make these associations. Seligman suggests that through arbitrarily contrived experiments, the general process theory has only elucidated those learning phenomena in which the stimuli are equally associable. He suggests that the preparedness of the organism to learn an association is on a
continuum which has three basic regions, prepared, unprepared and contraprepared, and that an animal's preparedness can be measured by the number of trials required for it make the desired response reliably. An organism who requires only a few pairings of the CS with the US demonstrates that it is prepared to learn this association. If an organism requires many pairings, then it is relatively unprepared to learn this association. If extensive pairings are required, or the behavior does not occur at all, then the organism would be considered contraprepared to learn this association. Ethologists concentrate on the prepared region of the continuum, which would include TAL scientists, whereas general process theorists concentrate on the unprepared region. Garcia and Koelling's (1966) experiment of cue-to-consequence demonstrated all three parts of this continuum. The rats were able to quickly associate illness with taste, demonstrating that they were prepared to associate internal cues with internal distress. They required several pairings to learn the association between light and noise with shock, demonstrating that they were relatively less prepared to learn these associations. However, the rats were contraprepared to associate light and noise with illness, and taste with shock.

In summary, Garcia has developed a multidisciplinary approach to learning while conducting taste aversion research. He has combined biology, general process theory, evolutionary
theory, and other fields to create a paradigm utilizing all of these resources (Garcia, Lasiter, Bermudez-Rattoni, & Deems, 1985). As a result, interest in TAL has spread over the last 25 years to other scientific areas. Workers in fields such as physiology (Kiefer, 1985), behavioral ecology (Bronstein, 1985), pharmacology (Revusky, 1985), medicine (Bernstein, 1985), and predation control (Ellins & Catalano, 1980) are interested in understanding taste aversion learning is, how an aversion becomes learned, and what underlying mechanisms are involved in learning this aversion.

Physiology of the Illness Signal

Much is known now about the phenomenon of taste aversion learning, but little is known about its underlying physiology and anatomy. TAL is unique in that it is one of the few phenomena which provides a system of learning that has a known biological function. Thus, one of the interesting aspects and advantages of taste aversion learning is that it enables the study of the biology of a learning system. The relevant stimuli can be manipulated, the illness-producing signal and the organ from which it is generated can be determined, and the paths the illness signal takes to the brain can be elucidated. Thus, we can understand how taste aversions are learned by studying the underlying physiology.

Within the vast literature on TAL there are results which seem to contradict Garcia's TAL paradigm. A variety of drugs
may induce a taste aversion, yet not all of these drugs produce overt signs of illness. Thus, many researchers maintain that emesis alone is not an adequate predictor of aversion. Still, there are some details which are currently known and generally accepted about the physiology of TAL. These can be broken down into three categories; taste, emesis, and neural integration (Kiefer, 1985). See Figure 1 for a diagram of the relevant organs, tissues, and neural structures that are involved in this system.

Taste. An aversion can be induced to any taste, even if the taste stimulus is a preferred one, such as sucrose (Garcia, et al., 1955). Flavor is a combination of virtually all taste and odor compounds. Hankins, Rusiniak and Garcia (1976) found that when taste and odor were separated, taste was a strong cue for taste aversion learning while odor without taste was ineffective. They demonstrated also that odor was a good cue for shock, while taste alone was ineffective. Furthermore, they found that the stronger the flavor/odor compound, and the greater the shock, the more the animals relied on odor cues. Rusiniak, Hankins, Garcia and Brett (1979) also found that odor alone was a weak cue for illness, yet when a taste and an odor were conditioned together, the taste potentiated the odor as a cue for illness, and odor became a powerful cue for avoidance of the toxin. This is consistent with Darwinian views because learning to
Figure 1. Anatomy of a rat and the possible neural and vascular routes of feedback (diagram prepared by Bruce Clemens).
avoid a toxic substance by merely relying on its odor reduces an animal's risk and greatly enhances its chances of survival.

To test an animal's hedonic response to a taste, Grill and Norgren (1978a) proposed a taste reactivity test. An animal's lingual, masticatory, and facial muscle responses to a taste were recorded using a facial mirror and a video camera. They demonstrated two different fixed-action patterns to a taste, one which is characteristic of a hedonically positive taste and one which is characteristic of a hedonically negative taste. They presented animals with sucrose which resulted in the ingestion sequence of tongue protrusions, rhythmic mouth movements, and paw licking. Based on these responses, sucrose was categorized as a hedonically positive substance. Yet when the taste of sucrose was paired with illness, the fixed-action pattern changed. The animals then exhibited an aversion sequence of gapes, chin rubs, head shakes and paw rubs, indicating that the taste of the sucrose was now hedonically negative. Garcia, Hankins, and Rusiniak (1974) coined this phenomenon as a "hedonic shift" in the quality of the taste.

The ability to distinguish different tastes has been found to be a brainstem reflexive behavior. When decerebrate rats undergo taste aversion learning (Grill & Norgren, 1978b) they still exhibit ingestive and aversive sequences, demonstrating that higher cortical functioning is unnecessary,
and that the primary taste/emic center is located in the brainstem.

**Emesis.** Emesis is also a brainstem reflexive behavior. Borison and Wang (1953) demonstrated that the vomiting center is located in the brain in an area lateral to the reticular formation and adjacent to and overlapping the nucleus of the solitary tract (NST). Stimulation of this area produces the emetic responses of nausea, retching and vomiting. Emetic receptors can be found in both the peripheral and central nervous systems. The majority of receptors in the peripheral system are located in the gastrointestinal tract and information is transmitted along the vagus nerve and sympathetic afferent fibers to the NST. Copper sulfate (CuSO$_4$) is a drug which has been found to have its emetic effects through local gastric irritation when presented intragastrically or intraperitoneally. Its emetic response is significantly reduced by vagotomy, but not by sympathectomy alone (Wang & Borison, 1951a), yet sympathetic afferents are involved in emesis. When vagotomy and sympathectomy are combined, emesis is blocked to all but very high doses of CuSO$_4$ (Wang & Borison, 1952). However, vagotomized animals are able to learn a taste aversion when injected intraperitoneally with LiCl (Martin, Cheng, & Novin, 1978), a blood-borne emetic. Thus, the vagus nerve appears to be the primary means of transmission of gastric irritation to the
brain's emetic center. Other peripheral receptors are located in the inner ear (Wang & Chinn, 1956).

Kiefer, Rusiniak, Garcia, and Coil (1981) taste averted intact rats to saccharin using two different emetics; CuSO₄, a neurally transmitted emetic, and apomorphine, a blood-borne emetic. They then performed subdiaphragmatic vagotomies and found that the rats which had received CuSO₄ did not avert to the target taste; those that received apomorphine demonstrated normal aversions, yet they extinguished these aversions faster than controls. With this study they demonstrated that the vagus nerve not only plays a role in the formation of a taste aversion, but in its maintenance as well.

On the floor of the fourth ventricle in the brain lies the area postrema, a highly vascularized structure, which contains the emetic chemoreceptor trigger zone. The weakness of the blood-brain barrier at the area postrema enables it to detect toxic chemicals in the blood. The area postrema and the nucleus of the solitary tract (NST) have reciprocal neural connections (Morest, 1960; 1967), thus blood-borne information may also be transmitted to the NST (Borison, 1974). Borison and Wang (1953) found that when the area postrema was lesioned, blood-borne toxins no longer induced vomiting. Ritter, McGlone, and Kelley (1980) found that lesions of the area postrema disrupted TAL when LiCl was administered intraperitoneally. Furthermore, they found that area postrema
lesions blocked aversions induced by intraperitoneal injections of LiCl and scopolamine, a local irritant which does not cross the blood-brain barrier, but amphetamine did not affect taste aversion learning in the area postrema lesioned rats. Thus, the area postrema was shown to be at least partially responsible for emesis.

The assimilation of these neurological and behavioral studies led to the idea that the UCS signal in TAL is emesis which results in the activation of the emetic response and consequent avoidance of taste (Garcia, 1985). Furthermore, these results suggest that the mechanisms which control vomiting also contribute to the formation of TAL. Aversions which are induced by blood-borne toxins, such as ethanol or LiCl (Kiefer, Cabral, Rusiniak, & Garcia, 1980; Martin, et al. 1978, respectively) are not affected by vagotomy. However, subdiaphragmatic vagotomy has also been found to be effective in producing a taste aversion itself (Bernstein & Goehler, 1983). This incongruity is difficult to explain, as the same procedure which is used to attenuate aversions can also be used to induce them.

Neural Integration. Upon consumption of food, molecules are dissolved in saliva and stimulate the taste buds along the tongue and soft palate (Carlson, 1986). Taste information is then transmitted along two cranial nerves; the facial (VII) and the glossopharyngeal (IV). The vagus nerve (X) also makes
a small contribution to the transmission of taste (Kiefer, 1985). These fibers converge at the NST in the brainstem. The subdiaphragmatic vagus nerve also transmits information from the gut and synapses with the NST. From the NST, fibers project rostrally to the parabrachial nucleus (PBN) of the pons (Norgren, 1978). From here, fibers project out to various areas of the brain. One set of fibers project to the amygdala, hypothalamus, and substantia innominata, while a second set of fibers project to the thalamus and then on to the gustatory neocortex (Carlson, 1986 p. 278). See Figure 2 for a diagram of the neural routes.

Even though both taste discrimination and emesis are brainstem reflexive behaviors, their association can not be established by the brainstem alone. Decerebrate rats exhibit ingestive or aversive sequences as do normal rats, but show no change in these sequences after the taste has been paired with illness, even after many pairings (Grill & Norgren, 1978b).

Emesis and TAL are closely tied together for a number of reasons. Both gustatory and vagal afferent nerves meet at the NST. Borison and Wang (1953) found that the emetic center is located in an area adjacent to and overlapping the NST. Furthermore, the chemoreceptor trigger zone (CTZ) found in the area postrema is linked to the emetic center by the NST. Mechanisms which mediate taste information, visceral

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Figure 2. Schematic drawing of the gustatory and visceral pathways in the rat. Olfactory afferents are also included. Abbreviations: (AM) amygdala, (CB) cerebellum, (CTZ) chemoreceptor trigger zone, (XC) cortex, (GNC) gustatory neocortex, (IC) internal capsule, (LH) lateral hypothalamus, (LRF) lateral reticular formation, (ML) medial lemniscus, (NTS) solitary nucleus, (OB) olfactory bulb, (OFC) orbitofrontal neocortex, (PP) prepyriform cortex, (PTA pontine taste area, (SI) substantia innominata, (ST) subthalamic nucleus, (VB) ventrobasal thalamic complex (Kiefer, 1985).
information and emesis are all located in proximity to one another within the brainstem.

Thus, it appears that higher cortical functions are necessary for the association of taste and illness. The hypothalamus, hippocampus, amygdala and neocortex have all been determined to be involved in TAL. The most important of these appears to be the gustatory neocortex, located in the anterolateral portion of the forebrain (Kiefer, 1985). Subjects which have undergone gustatory neocortex lesions exhibit two characteristics: an inability to learn taste aversions to both preferred and non-preferred tastes, and the tendency to generalize these aversions to other non-target tastes (Kiefer & Braun, 1979). Furthermore, when subjects are previously averted to a taste and then undergo gustatory neocortex lesions, they no longer exhibit that taste aversion (Kiefer, Leach, and Braun, 1984). Thus in some way, the gustatory neocortex plays a part in the memory of a taste aversion.

Establishment of Taste Aversions. The emetic/UCS hypothesis does not explain all conditions in which taste aversions are learned. Three main contradictions exist in the literature which challenge Garcia's paradigm: (1) certain drugs that are known to be toxic, such as cyanide and strychnine, which produce extreme symptoms of toxicosis, are ineffective in producing a taste aversion (Ionescu & Buresova, 19
1977; Nachman & Hartley, 1976); (2) self-administered drugs such as amphetamine, which serve as reinforcers of motor behavior, are also capable of inducing taste aversions (Wise, Yokel, & deWit, 1976); (3) some drugs that elicit no overt signs of illness, such as radiation, also induce taste aversions (Garcia, Kimeldorf, & Hunt, 1961).

Numerous explanations have been proposed to respond to these problems. TAL has been shown to be dosage dependent (Riley & Tuck, 1985). LiCl, for example, is ineffective at dosages less than .60mEq. .15M (Nachman & Ashe, 1973). Many of the toxins that do not induce taste aversions were examined at only one dose level (gallamine, cyanide, warfarin) which may have been below the threshold dosage required to induce taste aversions (Riley & Tuck, 1985).

A drug's dose, duration and number of learning trials may all affect its effectiveness in producing an aversion, but Garcia, et al. (1974) suggested that it is the physiology of the organism that dictates whether or not a substance will induce a taste aversion. They found that there are two systems of defense in the body, skin-defense and gut-defense, or milieu externe and milieu interne. An animal must use visual, auditory and olfactory senses to prevent predators from inflicting damage upon its skin. Using the same receptors, he may locate food and potential mates and guide motor responses accordingly. Reward and punishment in
external coping mechanisms are immediate and dependent upon peripheral cues. Internal coping mechanisms are dependent upon different criteria to determine whether a food is safe and nourishing, or if it is toxic, and the animal selectively associates taste with illness. Because internal reward or punishment may take hours to take effect, long delayed learning is necessary, and specific cues must be paired with that reward or punishment.

Olfactory and visual pathways belong to neither system in particular, and they provide information to both areas (Garcia, et al., 1985). Thus, toxins which induce peripheral pain, such as gallamine and cyanide, may not be capable of inducing internal distress signals in order to elicit a taste aversion. Pain is more easily associated with external stimuli, and according to the milieu interne hypothesis, would not easily be associated with taste. Lett (1985) has produced evidence establishing just this point. Gallamine, a motor response inhibitor, is known to induce a place avoidance, but not a taste aversion, while LiCl is known to induce a taste aversion but not a place avoidance. This demonstrates that there are two separate defense systems within a biological system. One system defends the animal against external distress (milieu externe), and the other against internal distress (milieu interne). These two systems do not interact with one another, thus a cue for external distress does not
easily elicit a taste aversion.

Ionizing radiation is another treatment which can induce a learned taste aversion. Garcia, Kimeldorf and Hunt (1955) discovered serendipitously that rats given radiation treatments would no longer drink the sweet water that they had consumed prior to the treatment. Radiation of low intensity gamma, X-rays or neutrons can produce a taste aversion above and below the emetic threshold (Garcia et al. 1955; Garcia & Koelling, 1957; Garcia & Koelling, 1960). Radiation-induced aversions appear to be mediated by the area postrema. Lesions of the area postrema result in disruption of learned taste aversions in cats (Rabin, Hunt, Chedester, & Lee, 1986) and in rats (Ritter, McGlone & Kelley, 1980). Motion has also been observed to induce taste aversions. Hartley (1977) dissociated the vestibular apparatus from the brain via bilateral labyrinthectomy and found that motion was no longer capable of inducing a taste aversion. This demonstrated that nausea can be induced through motion, and this nausea is reduced when the apparatus the organism uses to measure motion is disconnected from the brain.

**Generation of the Illness Message in TAL.** Many treatments are capable of producing a learned taste aversion, however, the origin of the illness message has not been specifically determined. Coil, Rogers, Garcia and Novin (1978) performed subdiaphragmatic vagotomies followed by taste
aversion learning using CuSO₄ and three different methods of administration: intragastric (ig) infusions, intraperitoneal (ip) or intravenous (iv) injections. They found that animals who received the CuSO₄-ig and CuSO₄-ip injections developed weak or attenuated aversions demonstrating that an intact vagus nerve was critical in sending the illness signal to the brain, while those rats who received CuSO₄-iv injections developed strong aversions, very likely affecting the area postrema directly. But the CuSO₄-iv rats extinguished their aversions more quickly than the CuSO₄-iv controls. Furthermore, Kiefer et al. (1981) found that subdiaphragmatic vagotomy attenuated previously learned taste aversions.

These studies suggest that by isolating the gut from the brain, subdiaphragmatic vagotomy blocks the illness signal. However, this surgical procedure isolates all of the organs of the gut from the brain, and does not indicate which organ may be contributing to the illness signal. In order to determine which organ or organs may be responsible for sending the illness signal, it is necessary to examine the function of these organs. Within the gut are several organs which are involved in the processing of food. The first is the stomach which is responsible for churning and preparing the food for digestion. The gall bladder aids digestion by releasing bile, a substance produced by the liver, into the stomach to emulsify fats. The pancreas aids in digestion by secreting
enzymes which break down proteins, lipids, starch and nucleic acids. The pancreas also secretes bicarbonate into the intestine in order to neutralize stomach acid as the food is absorbed into the bloodstream. The small intestine further processes the ingesta, utilizing the enzymes from the stomach and the pancreas. The nutrients are taken up into capillaries in between the villi of the duodenum and into the bloodstream of the hepatic portal vein where it is taken to the liver. The liver is responsible for maintaining homeostasis within the body through energy storage and release, hormone inactivation, and detoxification of the blood (Friedman & Stricker, 1976). Within the liver, sugars are oxidized for immediate energy to the body, or they are stored as glycogen, or they are converted into lipids and transported to adipose tissue for storage. As the liver is the first organ to receive nutrients from the stomach, the liver's sensory neurons may form an "early warning" signal to the brain which will react to toxins that it finds (Sawchenko & Friedman, 1979). Furthermore, sensory information from the liver reaches the hypothalamus (Schmitt, 1973). These findings, combined with the analysis of its strategic location and direct involvement with the metabolism of nutrients, suggest that the liver is the most likely candidate for eliciting an illness signal from the gut to the brain.

Some experimental evidence supports this idea. Ellins
and Costantino (1987) performed a partial hepatectomy (75% removal of the liver), leaving the vagus nerve intact, to determine its effect on taste aversion learning. Rats will regenerate their liver within 21 days post-surgery (Higgins & Anderson, 1931). After 13 days post-surgery it was discovered that, although all of the animals averted to novel sweet water, the partially hepatectomized group averted less to the water than did the sham controls, and extinguished their aversions at a much faster rate than the controls. Following full regeneration of their liver, subjects were again illness conditioned. Subjects previously partially hepatectomized performed as naive subjects. Costantino, Duva, Hooks, Van Norman, and Ellins (1990) conducted a partial replication of this study to establish the necessary control groups, with one exception. The same molarity of LiCl was used, but the method of administration was ip injection instead of ig infusion. The partially hepatectomized subjects demonstrated a faster extinction of their aversion to the sweet water than did controls, however, the hepatectomized rats averted to the taste similarly to the sham controls. This finding supported Martin et al.'s (1978) study in which subdiaphragmatic vagotomized rats presented a novel taste followed by LiCl-ip, averted to the novel taste. Thus, LiCl is a vascular drug and is not a good emetic to determine neural models of TAL, where LiCl is used to examine neural transmissions between the liver.
and the brain.

Other research questions the role of the liver in feeding behavior. Louis-Sylvestre, Servant, Molimard, and Le Magnen (1980) examined the change in feeding of rats following bilateral hepatic vagus denervation. They found that although blood glucose levels were significantly reduced in vagotomized rats, hepatic afferents or efferents were not found to be important in food intake. This would appear to contradict findings that subdiaphragmatic vagotomy influences food intake in TAL studies. Yet their research did not include an examination of taste aversion learning, which may differ from normal feeding patterns. Their results did indicate that feeding is not adversely affected by the surgery. Snowden and Wampler (1979) found that vagotomized rats reduced their liquid consumption, and that animals maintained their weight when given pellet diets following surgery rather than wet mash. This suggested that maintaining a pellet diet would be advisable for vagotomized rats.

A review of these results suggests that the liver mediates TAL in some way and that partial hepatectomy interferes with the sending of that illness signal. To further determine the possible implication of the liver in TAL, the effect of regeneration was examined using partial hepatectomy (Duva, 1990). Animals were tested at 1, 2, 3, 4, 5, 6, and 7 days post-surgery. In addition, a 13 day
The post-hepatectomy group was included to reference earlier work of Ellins and Costantino (1987) and Costantino et al. (1990). It was discovered that the subjects were able to demonstrate a TAL before the liver had regenerated, suggesting that the liver was fully functional prior to full regeneration.

The liver is innervated by the hepatic branch of the vagus nerve (Lautt, 1983) through two major plexuses, the anterior plexus and the posterior plexus. The anterior plexus forms a sheath around the hepatic portal vein and can be isolated for stimulation or denervation quite easily. The posterior plexus wraps around the bile duct and portal vein and has connections to the anterior plexus. As shown in Figure 3, both plexuses wrap around the hepatic portal vein on their way to the liver. At Site 2 lies an ideal place for denervation, however the hepatic portal vein is extremely delicate, and it is common to tear or cut the vein in the process during the ligation. Furthermore, the anatomy of the hepatic vagus nerve varies greatly within each animal (Lautt, 1983). Lautt and his colleagues developed an alternate technique to alleviate this problem, opening new avenues of research. High concentrations of phenol causes protein denaturation and necrosis which is specific to all nerve fibers (Schaumberg, et al. 1970), and can block nervous transmissions with either reversible or irreversible effects (Nathan & Sears, 1960). Lautt and Carrol (1984) found that
Figure 3. Site of liver denervation (Lautt, 1983).
85% phenol painted with a cotton swab along the hepatic portal vein of cats at Site 2 results in complete denervation within 20 min of application and remains complete 1-2 weeks later.

Statement of the Problem

This experiment was designed to examine the neural transmissions between the liver and the stomach. If the illness message is neurally transmitted between the liver and the stomach, then neurologically isolating the liver from the stomach in rats, and then illness training them, should block the illness signal. No aversions should be found for the treatment group. Different drug treatments may also help to shed further light on the route of the illness signal.

This study was done in two steps, the initial experiment (Experiment #1) and the follow-up experiment (Experiment #2). Experiment #1 examined the liver's role in taste aversion learning by neurologically isolating the liver from the stomach by disrupting the information passing along the hepatic portal nerve using the chemical phenol, which was painted along the hepatic portal vein, while a sham control group was painted with NaCl (see Table 1). Following recovery from surgery, subjects were presented a novel tasting wet mash. All subjects were divided into illness groups of LiCl or CuSO₄ emetics, and there was a phenol denervated NaCl group to control for the phenol actually inducing aversions. The method of administration of emetic was also varied. Half of
the groups were given intraperitoneal (ip) injections, and half were given intragastric (ig) infusions with the exception of the illness control group of NaCl which received only ig infusions. The subjects were then tested for consequent aversions two days later following recuperation from illness. It was hypothesized that the phenol-treated groups would vary their attenuation depending on their illness conditions. Because LiCl is believed to be a vascular drug, the phenol-treated LiCl-ip group should demonstrate an aversion the same as the sham-treated LiCl-ip group because the drug would be taken up by the intestines and travel to the liver via the hepatic portal vein, bypassing the neural block. The same should be true of the phenol-treated LiCl-ig group. It is believed that CuSO₄ is a neural emetic, thus presenting this emetic intragastrically should stimulate the feedback loop. CuSO₄ should be taken up by the tissues and its effect should be blocked along the neural pathway to the liver. Therefore it is hypothesized that no aversion will be found. However, if CuSO₄ takes the quickest route to the brain via the vagus nerve branching directly from the stomach, then these animals will demonstrate an aversion.

Experiment #2 was performed one month following the completion of Experiment #1. The subjects underwent taste aversion learning again to determine whether the surgical treatment had a permanent effect on the hepatic nerves. If
there is an illness message being sent between the liver and the stomach, then a taste aversion should not be found in one of the phenol-treated groups in Experiment #1. The most likely group to find no significant aversion should be one of the phenol-treated CuSO₄ groups, as it is a known gastric irritant, whereas LiCl is a known vascular drug. Since the chemical denervation technique being used produces temporary, not permanent, nervous disruption, those groups which did not avert to the wet mash in Experiment #1 should form aversions in Experiment #2.
Experiment 1

Method

Subjects and Housing

The subjects were 96 male Sprague-Dawley rats (Harlan Labs), three months of age at the start of the experiment. They were individually housed in 18 x 21 x 24-cm stainless steel cages and were kept on a 12-hr light/dark cycle. They were run during their dark cycle. They received ad lib tap water and Purina Laboratory Rat Chow except where noted. The room was maintained at 21°C. A protocol for the use of these animals was approved by the University's Institutional Animal Care and Use Committee.

Apparatus

The behavioral apparatus included five sound attenuated isolation chambers (Coulbourn Instruments E10-1020), each containing a low wattage light and a small ventilation fan. Within each isolation chamber was a cage that was 27.5 x 18 x 18 cm-high, built of 3-mm thick clear Plexiglas. On one side of the box was a sliding door 25 x 12.5 cm-high permitting access to the inside of the box. A hole was cut in the adjacent side of the box to fit a clear 3-mm thick Plexiglas feeding tray 5.08 x 5.08 x 1.8-cm high. This feeding tray was set on a platform that could be slid through the hole and locked into place. Each feeding tray was fitted with a clear Plexiglas lid which had a 3.5 cm diameter hole cut in the
center to permit the subjects access to the food, but to reduce foraging behavior. The floor was constructed of a stainless steel grid 15 x 13 cm-wide with grid spacings of approximately 1.5 cm. A foam brush was used at each cage to wipe up the food that was spilled onto the floor.

During the surgical procedure described below it was necessary to stimulate the hepatic vagus nerve to determine whether or not the chemical denervation technique had any effect. To measure the effectiveness of phenol denervation, an electrical pulse was applied above the denervated area and picked up below, using a Stoelting Stimulator, model # 58019 with gold tipped probes. The voltage was set for 5.5 V with a duration of 50 ms. A single continuous pulse was sent at 1 pulse per second for a duration of 40 ms. To record the signal transmission, a hardware package distributed by Coulbourn Instruments Inc. (1980) was used which included a software package by Dataq. The hardware equipment converted the pulse from an analog to a digital signal, amplified it to better view the signal and filtered out the background noise. The sampling rate was set for 4000 samples per second, representing the resolution of the signal. From here the signal was sent to an AST Bravo/286 16 MHz IBM compatible computer, recorded using Dataq's software package CODAS, and saved onto 3-1/2" floppy diskettes.
Procedure

Assignments. All subjects were randomly assigned to one of five isolation chambers and to order of running, with the subject's assigned chamber and order remaining constant for the duration of the two experiments. The isolation chamber assignment determined their surgery day. A pipeline method was used throughout the experiment to balance the surgery days. Following habituation, all subjects were divided into two groups. One group was painted with phenol around their hepatic portal veins, resulting in hepatic vagotomy. The other group underwent the same procedure, but their hepatic portal veins were painted with NaCl instead of phenol. Nine rats were selected to be in a phenol-treated illness control group (PNa-ig) in which these animals were denervated, but given only saline as their illness. See Table 1 for a definition of the groups and a final breakdown of sample sizes for each group. It was anticipated that some animals would be lost during surgery, therefore, five rats were added to the phenol group to ensure adequate sample size. Thus, the total phenol treatment group contained 53 animals and the sham treatment contained 39. During the course of surgeries, some animals were lost, and on the last day of surgeries, three animals assigned to phenol treatment died. Now, to balance the two groups, four animals originally assigned to receive the sham treatment on the last day of surgeries were treated.
with phenol instead. This provided the phenol group a total of 43 subjects, and 33 in the sham group, for a total of 76 subjects by the end of Experiment 1.

Three days following the surgical procedure, all subjects went through taste aversion learning. There were four emetic groups and one control group. The emetics consisted of either .15 M LiCl or .15 M CuSO₄ injected either intraperitoneally (ip) into the abdomen, or infused intragastrically (ig) directly into the stomach. Unfortunately, it was found that .15 M CuSO₄ was lethal when injected intraperitoneally, and four subjects died on the first day of taste aversion learning. The remaining subjects in this group were randomly reassigned to other emetic conditioning groups. Intragastric NaCl was used as a control for the possibility of the phenol itself inducing taste aversions.

**Habituation.** Fourteen days prior to their surgical day, the subjects were habituated to eating in the isolation chambers. The animals were food-deprived for 12-hrs prior to the start of their habituation. Each animal received one 10-min habituation session per day for seven days. After each day of habituation, the animals were provided the equivalent of two full pellets of Purina Laboratory Rat Chow.

**Surgical Procedure.** Food was removed from the home cages of the subjects scheduled for surgery three to four hr prior to surgery. Water was removed from home cages just before
surgery. All of the subjects were anesthetized with a 50 mg/kg dose of Nembutal in pairs 20-min prior to their surgery. Boosters of .05 cc Nembutal were provided as needed. Using aseptic technique, the surgical area was then shaved and cleansed with betadine solution. Each animal was then laid on its back with its tail toward the researcher. A midline ventral abdominal skin incision was made from the xiphoid to the umbilicus. The skin was retracted and a similar incision was made in the body wall. The body wall was then clamped with hemostats and retracted, exposing the peritoneal cavity. A small cotton bolster was placed underneath the thorax causing the liver to slightly fall away from the diaphragm. Suspensory ligaments attaching the liver to the diaphragm were cut. A piece of gauze dampened with sterile isotonic saline was placed above the incision. The investigator retracted the liver from the peritoneal cavity by placing both hands around the incision and pushing the gut just posterior to the liver forwards and upwards in a concave semicircle with a light compression of the abdominal cavity (Waynforth, 1982). The median and left lateral lobes of the liver were laid onto the dampened gauze. Two other suspensory ligaments that attahed the liver to the peritoneal cavity were exposed and cut. The internal viscera and liver were irrigated throughout the surgical procedure to prevent oxidation of the liver and adhesions of the internal organs. The stomach was then
retracted by looping 0 suture around the esophagus, and clamping the suture away from the body cavity. To lift the site of denervation away from the viscera, a glass stirring rod was gently inserted beneath the hepatic portal vein, being careful not to rupture the tissue. For further support, an additional rod was placed alongside the first. The denervation site was determined during a previous pilot study by staining the hepatic portal vein with methylene blue and subsequently analyzed by light microscopy. The site was determined to be approximately 1.5-cm from the liver as is previously depicted in Figure 3. Once this site was isolated, an electrical test was performed to determine baseline electrical activity. Electrodes were placed above and below the denervation site, and a pulse sent through the electrical probe. This pulse was picked up by the other electrode. The baseline of general tissue was found by measuring conductivity across the stomach tissue. The resulting pulse was measured and recorded (see Figure 4a).

Using Lautt's (1983) chemical denervation technique, a sterile swab was dabbed in 90% phenol and then dabbed on gauze to remove the excess. The phenol was then swabbed onto the denervation site, making certain that the phenol was placed all around the hepatic portal vein. The surgical site was then covered, and the subject was set aside. After 5-min, the denervation procedure was repeated and then tested again with
Figure 4. Electrical denervation test for (A) and after (B) chemical denervation. Recordings made with CODAS software application.
the electrical pulse to be certain that the area was indeed
denervated (see Figure 4b). A 75% drop in conductivity was
found to be the baseline of normal tissue, and therefore was
the verification of denervation. The sham group was treated
exactly the same as the phenol group, except that instead of
being swabbed with phenol, this group was swabbed with sterile
isotonic saline.

Following the procedure, all subjects were irrigated with
sterile saline heated to body temperature, their organs gently
irrigated and replaced into the body cavity. The body wall
incisions were closed with 000 nondissolving silk suture, or
with regular silk thread, doubled up. Stainless steel
Autoclips were then applied to the skin to close the incision
and encourage healing. Water was returned to the animals as
they came out of anesthesia, and food was returned four hrs.
after that. Overall surgery survival rate was about 87%.
Total experimental survival rate was about 82%.

Illness Training. Following three days of recovery from
surgery, the animals were again food-deprived for 12 hr, then
placed in their previously assigned isolation chamber, and
their water bottles were removed. The subjects were presented
with a novel salty (2-g NaCl in 50-ml powdered Purina Rat
chow) wet mash for 10 min, and the amount of food eaten was
measured as the difference in weight between the food tray
before and after the trial. To be certain that all the food
was being measured, the bottom of the tray was swept out after each trial, and the remaining food was also measured. These data were used as a baseline consumption measurement.

The animals then received their illness condition of one of the following: Li-ip, Li-ig, Cu-ig, or Na-ig. All intraperitoneal injections were administered using 26 gauge x 1/2 in. needles attached to a 5-cc plastic syringe. All intragastric infusions were administered using an infant feeding tube (Monojet 3-1/2) attached to a 5-cc plastic syringe. Ad lib water and food were returned three to four hr following presentation of the illness condition to prevent the illness from being associated with non-target tastes.

Within 30-min of receiving the emetics, the subjects exhibited piloerection and a decrease in overall activity, indicating gastrointestinal distress. The subjects which received the saline infusions displayed no overt signs of illness. Following recovery from illness, the subjects were provided with ad lib food and water for three days.

**Testing.** After three days, subjects were again food-deprived for 12-hrs. The animals were then placed in their isolation chamber for 10-min, and permitted to eat a salty wet mash. The amount the animals consumed during this trial was measured. Following each test trial, two food pellets were placed in the animal's cage. This procedure was repeated for a total of four consecutive days.
Results

Experiment 1 examined the effect of denervation on taste aversion learning. The data were transformed to a percent of baseline by dividing the amount the subject ate at each trial by the amount eaten at baseline, where the rat was first exposed to the novel taste prior to illness. Transforming to a percent of baseline was to control for the variance found when analyzing individual raw scores. The mean consumption in the initial experiment is presented in grams as a percent of baseline for all groups, and is seen in Figure 5. The cell means and standard deviations for Trials 1 through 4 for Experiment 1 can be found in Table 2. Data are shown as a percent of baseline.

A 2 x 3 x 4 (Surgery condition x illness treatment x extinction trials) mixed analysis of variance (ANOVA) was performed on the data for both experiments. See Table 4 for the sums of squares of the analysis for Experiment 1. In Experiment 1, no main effect was found for the surgery condition, indicating that surgery had no effect on feeding behavior, F(1, 58) = .01, p > .05. Also, no main effect was found for the illness treatment, indicating that type of emetic had no effect on feeding behavior, F(2, 58) = 1.30, p > .05. Furthermore, no interaction was found between surgery conditions and illness treatments, F(2, 58) = .55, p > .05.

In analyzing the main effects for within-subjects
Figure 5. Means as a percent of baseline in Experiment 1 for Li-ip (A), Li-ig (B), and Cu-ig (C) for phenol, sham and control subjects across four extinction trials.
differences, a trials effect was observed, indicating that rats consumed different amounts across trials, $F(3, 174) = 33.23, p < .000$. As a result of this trials effect in Experiment 1, Fisher's least significant difference (LSD) tests were performed for each illness group between Baseline and Trial 1 to test for the presence of an aversion, and between Trials 1 and 4 to test for extinction of aversion. Only the PLi-ig and SCu-ig groups averted to the salty mash at Trial 1, $p < .05$ (see Table 6). For all groups except the PCu-ig group there was an increase in consumption across test trials in spite of the fact that subjects did not demonstrate a taste aversion, $p < .05$ (see Table 6).

No differences were found between surgery conditions and trials, $F(3, 174) = .16, p > .93$. A difference was found between illness treatment and trials, indicating that different emetics exerted different effects across trials, $F(6, 174) = 2.47, p < .05$. However, surgery conditions did not affect illness treatment across feeding trials, $F(6, 174) = .31, p > .05$. 

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Experiment 2

At the end of Experiment 1, two phenol animals and one sham animal were pulled from the study due to abnormal feeding and drinking. This left 41 animals in the phenol group and 32 animals in the sham group. One month following completion of Experiment 1, the remaining subjects underwent their previous illness training schedule as before. Animals were presented salty food in their same isolation chambers, then given their same illness condition, permitted to recuperate, and tested over four consecutive days for the presence of a taste aversion.

Results

Experiment 2 examined the possible long term effect of chemical denervation on taste aversion learning. The mean consumption for Experiment 2 is presented in grams as a percent of baseline for all groups, and can be found in Figure 6. The cell means and standard deviations for Trials 1 through 4 for Experiment 2 can be found in Table 3. Data are shown as a percent of baseline.

A 2 x 3 x 4 (Surgery condition x illness treatment x extinction trials) mixed analysis of variance (ANOVA) was performed on the data for both experiments (see Table 5). In Experiment 2, again no main effect was found for the surgery conditions, indicating that surgery had no effect on feeding behavior, $F(1, 58) = .38, p > .05$. Also, no main
Figure 6. Means as a percent of baseline in Experiment 2 for Li-ip (A), Li-ig (B), and Cu-ig (C) for phenol, sham and control subjects across four extinction trials.
effect was found for the illness treatments, indicating that type of emetic had no effect on feeding behavior, $F(2, 58) = .46, p > .05$. Furthermore, no interaction was found between surgery conditions and illness treatment, $F(2, 58) = .10, p > .05$.

Differences across trials were again found, $F(3, 174) = 15.57, p < .05$. Tests for the presence of aversions and consequent extinctions were repeated using Fisher's LSD, examining differences between Baseline and Trial 1, and differences between Trials 1 and 4, respectively. All six illness groups averted to the salty mash, $p < .05$ (see Table 7). Extinction of aversions were found in all but the SLi-ip and SLi-ig groups, $p < .05$ (see Table 7).

Surgery conditions were not found to affect feeding across trials, $F(3, 174) = .96, p > .05$. Also, illness treatment was not found to affect feeding across trials, $F(6, 174) = .40, p > .05$. Surgery conditions were not found to affect illness conditions across trials, $F(6, 174) = .61, p > .05$.

Taste aversions have been found to be a universal phenomenon. Yet in this experiment, no statistical significance was found between the phenol and sham groups in either Experiment 1 or Experiment 2. It was decided to run Fisher's LSD tests in spite of this, as the lack of an aversion seemed not to be possible. No statistical
significance was found in any of the three illness groups in either Experiment 1 or Experiment 2 at either Trial 1 or Trial 4, $p > .05$. 
Discussion

The findings of the present study failed to determine that a mechanism exists by which signals are transferred between the liver and stomach to contribute to the formation of a learned taste aversion. It was expected that since LiCl is considered to be a vascular drug, all of the animals given LiCl would demonstrate aversions at Trial 1, whether they were denervated or not. Regardless of the method of administration, it was thought that LiCl would enter the bloodstream, bypass any neural feedback mechanisms between the liver and the stomach, and directly affect the area postrema, resulting in illness. According to the design of the experiment, it is not possible to determine whether or not the LiCl went directly to the area postrema. CuSO₄ is believed to be a local irritant. It activates the nucleus of the solitary tract by stimulating the nervous system. When injected intragastrically, the CuSO₄ would have irritated the stomach lining, resulting in a neural message passing either directly to the liver, or directly to the brain. If it passed directly to the liver, no aversion would have been observed. If it went directly to the brain, an aversion would have been evident. Because all of the CuSO₄ animals appeared to have averted to the wet mash, the results indicate that the illness message did go directly to the brain. Although some results from this study were promising, overall the results did not
demonstrate any effect of hepatic vagus denervation on taste aversion learning.

TAL has been demonstrated in virtually all animals tested. Under the conditions of the present experiment, researchers have demonstrated taste aversions in intact rats using LiCl (Nachman & Ashe, 1972) and CuSO₄ (Kiefer, et al., 1981). Therefore, aversions should have been found at least in the sham control animals. In fact, in Experiment 1, as in Experiment 2, all of the means were found to be in the right direction. The overall pattern of results, although not significant, was suggestive. This implies that the procedures were accurate, and that the problem resides in the statistics. Although the size of the means would indicate that differences between the phenol and sham groups exist, the small sample size (N = 10 or 11) in each of these groups possibly did not contribute sufficient statistical power to the experiment. The experiment started with low power as a result of low sample size, and this problem became progressively worse with the loss of animals across the course of the experiment. In addition to this, error variance was high. In some cases, the variance for a group was greater than its mean. This variance may be attributed to various factors. A pilot study suggested that rats recuperated from the surgery within five days. To be certain that the hepatic vagus nerve was still denervated when the behavioral testing was underway, it was decided to
run the rats three days following the surgical procedure.
However, the rate of recuperation may have been different in
different animals. If this were the case, the subjects may
have been tested while they were still ill from the surgery.
This would increase overall nausea in some animals, causing
differential consumption across animals. Thus low sample size
and illness due to the surgical procedure may have contributed
to the inability of this experiment to find differences
between the groups.

Some results were encouraging. Differences were found
between illness conditions and trials, indicating that there
was a difference in the rate of extinction between these
groups. The rate of extinction may reflect the strength of
learning; groups which have a slower rate of extinction are
probably more strongly averted to the salty wet mash than the
other groups due to a stronger illness effect. Thus the
CuSO₄ group may have been more ill than the LiCl groups,
resulting in a slower extinction of aversions.

This difference may also have been dependent upon the
method of administration of the emetic. The route of
administration of the emetic has been found to be an important
factor when examining the mechanisms of taste aversion
learning. There are four common methods of emetic
administration: intraperitoneal injection, intragastric
infusion, intravenous injection, and subcutaneous injection.
Different disruptive procedures do not necessarily result in attenuated aversions with all administrations of the emetic. For example, rats which undergo subdiaphragmatic vagotomy do not exhibit taste aversions when CuSO₄ is administered intragastrically or intraperitoneally, but they do exhibit taste aversions when CuSO₄ is administered intravenously, although these animals attenuate their aversions much faster than controls (Coil, et al. 1978). The effect of the vagus nerve on TAL appears to vary by drug. Martin, Cheng, and Novin (1978) performed subdiaphragmatic vagotomies on rats and found that this did not affect TAL when the emetic was LiCl injected intraperitoneally. These studies indicated that there are two main routes of the illness signal; a neural route which passes along the vagus nerve, and a vascular route in which the blood-borne emetic is detected by the area postrema.

The area postrema is a highly vascularized structure at the base of the brain. Lesions of this structure block taste aversions when CuSO₄ is administered intravenously, yet when the emetic is administered intragastrically, robust taste aversions result (Coil & Norgren, 1981). Thus, chemoreceptors in the area postrema must be intact to detect toxins circulating in the blood, but when the emetic affects the nervous system, the illness signal travels to another organ via the subdiaphragmatic vagus nerve. It would appear that
hepatic portal vein denervation might be effective in blocking a taste aversion when CuSO₄ is administered intraperitoneally, but not very effective when the emetic is administered intragastrically. Thus, examining the effect of intraperitoneal injections using various emetics more thoroughly may be an important step in further elucidating the role of the liver in TAL. Emetics which may be useful in demonstrating TAL may include lithium, copper sulfate and ethanol.

**Experiment 1**

Notwithstanding the lack of statistical significance, trends appeared in the data which indicated an effect was present. In order to explain some of these trends, the results will be discussed with respect to their percent of baseline means.

In surveying the means, when lithium chloride was administered intraperitoneally it is found that the phenol-treated rats consumed nearly 100% of their baseline level compared to slightly over 65% for the corresponding sham group, inferring that the PLi-ip group did not avert to the salty mash. This trend supports the findings of Costantino, et al. (1990) and Duva (1990) who found that when LiCl was administered intraperitoneally to partially hepatectomized rats, a different surgical procedure, the rats did not avert to the sweetened water. As no lesions of the area postrema
occurred to affect LiCl-induced taste aversion learning, it is possible that neural signals from the liver are involved in the acquisition of taste aversions. Since the liver is the organ responsible for detoxification (Carlson, 1986), it would be likely that the signal is emanating from the liver.

In the LiCl groups, eating behavior seemed to vary as a function of the method of administration. When LiCl was administered intragastrically to denervated rats, both the phenol-treated and sham-treated rats averted to the salty mash. This observation is in contrast to the results of Ellins and Costantino (1987) who found that partially hepatectomized rats, administered lithium chloride intragastrically, did not avert to the sweet water on the first extinction trial. From the results presented here, it would appear that LiCl administered intragastrically travels vascularity from the stomach to the liver through the hepatic portal vein, and no neural signals along the hepatic portal vein are necessary to elicit the illness signal. Since LiCl is not a local irritant, we would not expect a neurally mediated signal emanating from the stomach to be sent to the liver or ultimately to the brain via the liver. Thus, it was expected that aversions would be found in the denervated subjects which received LiCl as their emetic.

It was evident that although both groups received LiCl as their emetic, the Li-ip and Li-ig groups ate differently from
one another. This difference by may be due to the process of LiCl illness. When LiCl is injected intraperitoneally, it is taken up by the small intestine which delivers the toxin in a gradual process to the liver where some may be detoxified and an illness signal sent. When administered intragastrically, the toxin is concentrated in one place, and taken up by the vascular route directly to the liver. The liver may be overwhelmed with LiCl, and can not process the large volume of toxin, permitting the LiCl to enter the bloodstream and affect the area postrema directly, signalling toxicity of the food. This would explain the apparent stronger aversion on Trial 1 for Li-ig group compared to the Li-ip group.

Another interesting trend is apparent in the data which suggests that the phenol animals in the Li-ig group averted more strongly than the animals in the sham Li-ig group. This presented a puzzle. Why would the phenol-treated rats consume less, on average, than their corresponding sham-treated group? Several explanations have been postulated. Nachman and Asche (1973) demonstrated that the strength of an aversion is directly related to the magnitude of the UCS. Subjects who receive a stronger dose of an emetic develop stronger aversions than another group who receive a weaker dose. Thus, it may be that the phenol, combined with the LiCl, made the PLi-ig animals more ill than the SLi-ig animals. If this was the case, then the other denervated animals, such as the PLi-
ip and PCu-ig should also be more ill than the corresponding sham animals. Under this premise, the phenol and sham groups would tend to consume similarly, and it would become increasingly difficult to find differences between these groups.

A second explanation of this trend in the Li-ig group examined the condition of the animals which were in the denervated LiCl-ig group. To be certain of denervation, the amount of phenol application increased slightly over surgery days. In reviewing the raw data, more Surgery Day 5 denervated animals were found in the LiCl-ig illness group than in any other. This strengthened the above argument that the phenol itself induced a stronger illness and elicited a stronger aversion.

A third possible explanation involves the concept of homeostasis. It is possible that the surgical procedure disrupted the animals' first line of defense in detecting a toxic substance, a neural signal from the stomach to the liver. Thus the liver, not preconditioned to encounter the toxin, becomes hypersensitive and elicits a stronger aversion signal. This may be supported by the results from the Cu-ig group. The rats in the phenol denervated CuSO₄ group appear to extinguish their aversion more slowly, indicating that this emetic has some effect on retaining a taste aversion. This implies a neural aspect to taste aversion learning since CuSO₄
is a local irritant.

The phenol denervated rats given Cu-ig, a local irritant, formed normal taste aversions, suggesting that no neural illness message is passed between the liver and the gut. However, Coil, et al. (1978) found that subdiaphragmatic vagotomy block taste aversion learning. Thus, it would appear that CuSO₄ facilitates an illness message which is directly transmitted to the brain from the stomach via the vagus nerve.

Experiment 2

The trends in the follow-up experiment demonstrated that phenol does not have a lasting effect on an animal's ability to learn an aversion. Rats appeared to have consumed more in Experiment 2 than they did in Experiment 1. However, the control group consumed an average of 100% of its baseline level throughout Experiment 2, indicating that the control animals were at their ceiling level at their baseline trial, and could not consume significantly more food in the time given them. The Li-ip group consumed approximately 40% of its baseline level at Trial 1, comparable to the consumption of the other groups in Experiment 1. Furthermore, the PLi-ip group extinguished its aversion at a faster rate than any other group, which did not extinguish their aversions. This suggested that the PLi-ip group was undergoing a first extinction trial.

Furthermore, all groups with the exception of the PLi-ip
group consumed 60% of their baseline level at their first extinction trial, suggesting that they were not as averted to the wet mash as the PLi-ip group. These rats were undergoing a second taste aversion learning, where they could be exhibiting one of two diametric effects; they may be either more resistant to averting to the taste due to previous exposure without the illness, or they may be more sensitive to averting to the taste due to the initial illness. If they were more resistant to averting to the taste, they would have consumed more of their percent of baseline and would have had a higher extinction curve. If they were more sensitive to the learned taste aversion, the groups would have consumed less of their percent of baseline and would have resisted extinguishing their aversions. The data indicate the latter. The rats may have actually consumed very little at their baseline level in the second experiment, preventing them from eating much less than that in their first trial, resulting in a floor effect. Furthermore, their extinction curves are fairly flat, suggesting a resistance to extinction.

Future research

This experiment revealed trends that the liver may be involved in taste aversion learning via a feedback loop between the liver and the stomach. In order to further define the role of the liver in learning, the possible neural connection between the liver and the brain should be examined.
Future research should focus on isolating the liver from both the gut and the brain. This could be accomplished by combining hepatic portal vein nerve denervation with hepatic vagus nerve denervation, using Lautt's chemical denervation technique. In addition, a complete analysis of emetics and method of administration and their effects on taste aversion learning with respect to the liver involvement is important. Thus, denervation of the hepatic portal vein, coupled with hepatic vagus surgical or chemical denervation should be used to compare and contrast a variety of methods of administration and emetics, such as intragastric infusion, intraperitoneal injection and intravenous injection of LiCl and CuSO₄, to provide a wealth of information.

Other ways to verify denervation techniques may be tried in order to reduce increased phenol application and consequent illness; techniques such as hypoglycemic shock and electrical stimulation to measure increases of hepatic portal pressure (Lautt, 1984; Louis-Sylvestre, Servant, Molimard, & Le Magnen, 1980).

Furthermore, in order to determine if the phenol itself may be inducing increased aversions in the subjects, it will be necessary to include a sham-treated saline illness group with the phenol-treated saline illness group. This may have helped to explain the trends evident in the LiCl-ig group.

The purpose of this experiment was to examine one of the
possible roles of the liver in taste aversion learning. Specifically, is there a feedback loop between the liver and the gut? This was accomplished through the use of the chemical phenol to denervate the nerves leading between the liver and the stomach. Statistical significance was not found due to low sample sizes combined with high variance, yet trends indicated that differences do exist. The phenol-treated LiCl-ip group consumed at 100% of its baseline feeding on the first extinction trial compared with the other groups which mostly ate at 35-40% of their baseline feeding. This indicates that an effect may well exist, but the variance is clouding the data. Future research would do well to reexamine this question, using higher sample sizes and more control groups.
References


Garcia, J., Lasiter, P. S., Bermudez-Rattoni, F., & Deems, D.


Lett, B. T. (1984). Extinction of taste aversion does not eliminate taste potentiation of odor aversion in rats or...


Rusiniak, K. W., Palmerino, C. C., Rice, A. G., Forthman, D.


Footnotes

SPSS was used to run the statistical analysis. It was discovered that a Sham Na-ig group was necessary to run the analysis. Because this group was not created, the Phenol Na-ig group was not included in the statistical analysis. All test trial consumption data were transformed to a percent of baseline. Experiment 1 and Experiment 2 data were analyzed separately.
Table 1.
Sample Sizes and Groups for Experiment 1 and Experiment 2

<table>
<thead>
<tr>
<th>Illness Treatments</th>
<th>Surgical Treatments</th>
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<tbody>
<tr>
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</tr>
<tr>
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<td></td>
<td>11</td>
</tr>
<tr>
<td>Li-ig</td>
<td>11</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Cu-ig</td>
<td>10</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Na-ig</td>
<td>9</td>
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<td></td>
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</table>

Note. Illness treatment groups are defined as: Li-ip = LiCl injected intraperitoneally, Li-ig = LiCl infused intragastrically, Cu-ig = CuSO₄ infused intragastrically, and Na-ig = NaCl infused intragastrically.
<table>
<thead>
<tr>
<th>Group</th>
<th>Trials</th>
<th></th>
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<th></th>
<th></th>
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<td>1</td>
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<td>114</td>
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<td></td>
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<td>140</td>
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<tr>
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<td>SD</td>
<td>38.7</td>
<td>43.7</td>
<td>104</td>
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<tr>
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<td>72.5</td>
<td>109</td>
<td>104</td>
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</tbody>
</table>

**Note.** Group abbreviations are: P = Phenylated animals, S = Sham animals, Li-ip = lithium administered intraperitoneally, Li-ig = lithium administered intragastrically, and Cu-ig = copper sulfate administered intragastrically.
Table 3.

**Cell Means and Standard Deviations of Consumption as a Percent of Baseline for Trials 1 Through 4 for Experiment 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Trials</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
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<td>60.1</td>
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<tr>
<td></td>
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<td>82.2</td>
<td>66.8</td>
<td>95.2</td>
<td>89.8</td>
</tr>
<tr>
<td>PLi-ig</td>
<td>M</td>
<td>57.5</td>
<td>34.0</td>
<td>76.2</td>
<td>78.3</td>
</tr>
<tr>
<td></td>
<td>SD</td>
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<td>43.1</td>
<td>84.8</td>
<td>59.5</td>
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<tr>
<td>PCu-ig</td>
<td>M</td>
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<td>42.8</td>
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<tr>
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<td>SD</td>
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<td>39.2</td>
<td>84.2</td>
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<tr>
<td>SLi-ip</td>
<td>M</td>
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<td>18.7</td>
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<tr>
<td></td>
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<td>32.6</td>
<td>66.3</td>
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</tr>
<tr>
<td>SLi-ig</td>
<td>M</td>
<td>57.4</td>
<td>23.5</td>
<td>60.2</td>
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<tr>
<td></td>
<td>SD</td>
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<td>23.7</td>
<td>74.5</td>
<td>38.3</td>
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<td>26.7</td>
<td>69.2</td>
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<tr>
<td></td>
<td>SD</td>
<td>93.6</td>
<td>48.1</td>
<td>87.3</td>
<td>36.8</td>
</tr>
</tbody>
</table>

**Note.** Group abbreviations are: P = Phenylated animals, S = Sham animals, Li-ip = lithium administered intraperitoneally, Li-ig = lithium administered intragastrically, and Cu-ig = copper sulfate administered intragastrically.
<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Sig of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within cells</td>
<td>206.52</td>
<td>58</td>
<td>3.56</td>
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</tr>
<tr>
<td>Surgery condition</td>
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<td>1</td>
<td>0.04</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>Illness condition</td>
<td>9.24</td>
<td>2</td>
<td>4.62</td>
<td>1.30</td>
<td>0.28</td>
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<tr>
<td>Surgery by Illness condition</td>
<td>3.93</td>
<td>2</td>
<td>1.96</td>
<td>0.55</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Table 5.
Statistical Summary Table for Experiment 2

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Sig of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within cells</td>
<td>32.15</td>
<td>58</td>
<td>0.55</td>
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<tr>
<td>Surgery condition</td>
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<td>0.21</td>
<td>0.38</td>
<td>0.54</td>
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<tr>
<td>Illness condition</td>
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<tr>
<td>Surgery by Illness condition</td>
<td>0.11</td>
<td>2</td>
<td>0.05</td>
<td>0.10</td>
<td>0.91</td>
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</table>
Table 6.
Fisher's LSD Test of Within Group Differences in Experiment 1

<table>
<thead>
<tr>
<th>Baseline vs. Trial 1</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
<td>Difference</td>
<td>p &lt; .05</td>
</tr>
<tr>
<td>PLi-ip</td>
<td>11</td>
<td>.006</td>
<td>*</td>
</tr>
<tr>
<td>PLi-ig</td>
<td>11</td>
<td>.636</td>
<td>*</td>
</tr>
<tr>
<td>PCu-ig</td>
<td>10</td>
<td>.370</td>
<td>*</td>
</tr>
<tr>
<td>SLi-ip</td>
<td>11</td>
<td>.332</td>
<td>*</td>
</tr>
<tr>
<td>SLi-ig</td>
<td>10</td>
<td>.456</td>
<td>*</td>
</tr>
<tr>
<td>SCu-ig</td>
<td>11</td>
<td>.565</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trial 1 vs. Trial 4</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
<td>Difference</td>
<td>p &lt; .05</td>
</tr>
<tr>
<td>PLi-ip</td>
<td>11</td>
<td>1.004</td>
<td>*</td>
</tr>
<tr>
<td>PLi-ig</td>
<td>11</td>
<td>1.72</td>
<td>*</td>
</tr>
<tr>
<td>PCu-ig</td>
<td>10</td>
<td>.450</td>
<td>*</td>
</tr>
<tr>
<td>SLi-ip</td>
<td>11</td>
<td>.912</td>
<td></td>
</tr>
<tr>
<td>SLi-ig</td>
<td>10</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>SCu-ig</td>
<td>11</td>
<td>.965</td>
<td>*</td>
</tr>
</tbody>
</table>

Note. $t_{.05/2,(174)} = \sqrt{\frac{(2)(.08)}{n}}$
Table 7.
Fisher's LSD Test of Within Group Differences in Experiment 2

### Baseline vs. Trial 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Difference</th>
<th>p &lt; .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLi-ip</td>
<td>11</td>
<td>.570</td>
<td>*</td>
</tr>
<tr>
<td>PLi-ig</td>
<td>11</td>
<td>.425</td>
<td>*</td>
</tr>
<tr>
<td>PCu-ig</td>
<td>10</td>
<td>.410</td>
<td>*</td>
</tr>
<tr>
<td>SLi-ip</td>
<td>11</td>
<td>.503</td>
<td>*</td>
</tr>
<tr>
<td>SLi-ig</td>
<td>10</td>
<td>.426</td>
<td>*</td>
</tr>
<tr>
<td>SCu-ig</td>
<td>11</td>
<td>.458</td>
<td>*</td>
</tr>
</tbody>
</table>

### Trial 1 vs. Trial 4

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Difference</th>
<th>p &lt; .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLi-ip</td>
<td>11</td>
<td>.522</td>
<td>*</td>
</tr>
<tr>
<td>PLi-ig</td>
<td>11</td>
<td>.273</td>
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<tr>
<td>PCu-ig</td>
<td>10</td>
<td>.252</td>
<td>*</td>
</tr>
<tr>
<td>SLi-ip</td>
<td>11</td>
<td>.166</td>
<td></td>
</tr>
<tr>
<td>SLi-ig</td>
<td>10</td>
<td>.171</td>
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<tr>
<td>SCu-ig</td>
<td>11</td>
<td>.331</td>
<td></td>
</tr>
</tbody>
</table>

Note. \( t_{0.05/2, (174)} \sqrt{\frac{(2)(.08)}{n}} \)