

NON-INVASIVE MEASUREMENT OF PAROTID  
SALIVARY ACTIVITY AND STOMACH MOTILITY

by

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Non-Invasive Measurement of Parotid Salivary Activity

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### Abstract

Invasive stimulation and measurement of physiological activity are in many cases likely to interfere with a valid analysis of normal activity. The salivary and gastrointestinal system are two related areas that may be particularly sensitive to invasive stimulation and measurement. To test this assumption, a new non-invasive measurement of salivation (the test of electrosalivary activity, or ESA) was used to determine the effect of a commonly used invasive technique (the dental roll method). The use of dental rolls was shown to significantly affect salivation. A separate experiment examined the effect of non-invasive stimulation (food smells) on salivation as measured by the ESA, and gastrointestinal motility, as measured by the electrogastrogram. A significant correlation was found between salivation and gastrointestinal motility during stimulation with food smells.

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## Experiment #1

### Introduction

Salivation is involved in theories of hunger, eating disorders, introversion, and classical conditioning, as well as being important in such areas as dentistry and medicine. Yet the measurement of human salivation usually involves invasive measures which may themselves affect salivary flow. Non-invasive measurement of salivation through surface recording has been the main focus of research in the Davis laboratory at Simon Fraser University in the past few years. This pursuit of a non-invasive measure is important for obvious reasons: foreign objects in the mouth are likely to affect salivation and detract from ecological validity, and non-invasive measures are likely to be subjectively and ethically preferable to their invasive counterparts. A brief review of traditional measurement techniques is important to this discussion.

Three basic measurement techniques have been used in the study of salivation. The "whole mouth method" involves simply spitting the contents of the mouth into a receptacle and measuring by volume or weight the amount of saliva produced (e.g., Kerr, 1961). A variation involves leaving the mouth open and letting saliva constantly flow out of the mouth. A second technique is the parotid cup in which a small plastic cup is placed over Stenson's duct to collect saliva coming from the parotid glands (e.g., Lashley, 1916; Shannon, 1974). Saliva comes out of the duct into the cup and drains out through a tube to be measured by volume or weight. The third



method is the dental roll technique in which pre-weighed cotton dental rolls are put into the mouth, then taken out and weighed to measure the amount of saliva absorbed (e.g., LeGoff & Spigelman, 1987).

The whole mouth technique is simple and has been widely used (e.g., Kerr, 1961; Jenkins & Dawes, 1966; Watanabe & Dawes, 1988; Brudevold, Kashket, & Kent, 1990). No intrusive devices need be put into the mouth and little specialized equipment is needed. However, several possible confounds make this technique undesirable. Salivation rate can not be accurately measured for small time periods (Dawes & Watanabe, 1987). Kerr (1961) showed that both spitting and suction significantly increased salivation, and draining was not necessarily an effective method of removing saliva. It is possible that the amount of saliva in the mouth acts as a stimulus, and retaining or draining saliva might therefore influence flow rate. The source of salivation can not be localized, eliminating separate analysis for different glands on the basis of flow or chemical content. Long time trials cannot be used when the subject retains saliva in the mouth and constant draining restricts subject movement. Subjectively, both draining and retaining saliva are neither comfortable nor normal behaviours.

The parotid cup is perhaps the most widely used technique for measuring salivation. Variations of the parotid capsule have been used since 1916 (e.g., Lashley, 1916; Kerr, 1961; Von Knorring, Mornstad, Forsgren, & Holmgren, 1986; Shannon, 1974; Dawes & Watanabe, 1987; Bauslaugh & Davis, 1993). Variations on the basic structure have also been called the parotid capsule and the Lashley

capsule or cup. The cup is a small hollow disc, with an inner and outer chamber. The inner chamber is placed over Stenson's duct to collect saliva, which is constantly drained through a tube. The outer ring of the cup has a small negative pressure applied to it through a separate tube, which holds the cup onto the cheek. Some variations involve cannulization of the duct, with the cup designed to protect the cannula (Kerr, 1961). The saliva removed from the cup is analyzed, often in very sophisticated and complicated ways (e.g., Brown, 1970). While the parotid cup seems to be a commonly used and reliable technique, there are some major problems with its use. Though not commonly acknowledged in the literature, the capsule can be quite uncomfortable, hard to place, and prone to slipping and falling off. Too much suction applied to the outer ring can occlude the duct, and too little allows the cup to move. Locating Stenson's duct and applying the cup to the proper place can also present a problem. Apart from procedural problems, which have apparently been overcome in most cases, other confounds are not so easily dismissed. It seems likely that having a large plastic object in the mouth would interfere with normal operation of the salivary system, either by stimulating or inhibiting salivation. Specialized equipment and a certain amount of expertise are necessary to use this technique and its intrusiveness makes it undesirable for both physical and ethical reasons.

The third common technique is the dental roll method. This method has been used by LeGoff, Davis, and Bauslaugh (1994), LeGoff & Spigelman (1987), and LeGoff, Leichner, and Spigelman (1988) in relation to eating disorders and dietary restraint, two areas

of interest to this author and the Davis lab. In relation to other areas, the dental roll method has been used fairly extensively (e.g., Blundell & Freeman, 1981; Eysenck & Eysenck, 1967; Franchina & Slank, 1988). The method is simple and requires little specialized equipment. Pre-weighed cotton dental rolls (or other absorbent materials) are placed in the mouth, usually sublingually or between the cheek and gums. After the trial the rolls are removed and weighed to determine the amount of salivation. There is one main concern about this method. It is likely that the dry, absorbent dental rolls will stimulate salivation in and of themselves (Dawes, 1987; LeGoff, personal communication). In addition, since the dental rolls collect whole mouth saliva, the contribution of individual glands cannot be differentiated, and the method is not suitable for short-trial recording.

It seems likely that any of the foregoing invasive measuring techniques may affect salivation by acting as a stimulus. Because sympathetic activation leads to a decrease in salivation in humans (Young & Van Lennep, 1978; Kerr, 1961), any technique that makes the subject uncomfortable is also likely to affect salivation. As all conclusions about salivation have been drawn from the above mentioned techniques, it is important to find out the degree to which the intrusiveness of the measure affects the results. The measure of electrosalivary activity may be particularly suited to evaluating the effect of invasive procedures on salivation.

The measure of electrosalivary activity (ESA) has been validated as an accurate measure of salivary flow (Bauslaugh & Davis, 1993). The ESA uses surface electrodes above the parotid

glands to measure the electrical activity of the gland. We believe this electrical activity represents a depolarization of the secretory cells, and possibly smooth muscle activity in the basket cells of the salivary glands. A recognizable response to certain food stimuli and tentative results have been obtained relating the ESA response to time since eating, smell, visual, and taste stimuli.

Davis et al. (1994) showed a stronger response to a picture of a lemon than to a picture of a rock which served as a control. Davis et al. (1993) showed a stronger response to a lemon smell than to a pure air control in most subjects. LeGoff & Bauslaugh (1994) showed that salivation was related to time since eating. The longer subjects had gone without eating, the more they salivated to a food smell. LeGoff and Bauslaugh also demonstrated an effect for food smells over non-food smells. However, some of these results have been more descriptive than quantitative, meaning that the focus was on a description of the technique and a subjective analysis of the results. Several different methods of analysis were used in the above studies, in an attempt to quantify the results.

Since the ESA is a relatively new measure, it is unclear what the important components of the ESA response are. The most distinctive characteristic demonstrated so far is a fast negative or positive change immediately following (within five seconds) a strong stimulus, followed by a slow return to baseline (Bauslaugh & Davis, 1993). Other findings (LeGoff & Bauslaugh, 1994) have shown a significant average positive increase over baseline on stimulus trials, and others (Davis et al., 1994) have found differences at certain points after presentation of the stimulus. These observations have

been made with different types of stimulus; Bauslaugh and Davis (1993) used a lemon juice stimulus delivered to the mouth while LeGoff and Bauslaugh (1994) used food smells. LeGoff has argued that these two types of stimuli are qualitatively different and will produce different types of responses (LeGoff, personal communication). Therefore, they may also require different types of analysis.

The ESA has several significant advantages over other methods. Most importantly, it is non-invasive and requires little from the subject in terms of training, restrictions in swallowing, position, or movement. The non-invasive nature of the technique allows long periods of recording which is not possible, or is uncomfortable, with other methods. It produces analog data which may be valid at short intervals. The ESA may also be able to differentiate between different glands, enabling analysis not only of the parotid, but the sublingual and submaxillary (also known as the maxillary and the submandibular) glands as well. While the ESA may also have some drawbacks (e.g., interference from muscle activity), it is likely to provide information not obtainable with other methods.

The main purpose of this study was to test the hypothesis that intrusive salivary measures, like the ones described above, artificially affect the rate of salivation. Since the dental roll technique suggests the most obvious chance of this kind of salivary stimulation and has been used in relevant research, it was chosen to test this hypothesis. Dental rolls were used as a stimulus and the whole mouth method and ESA were used to measure the effect of that stimulation. Both lemon juice and dental rolls should increase

salivation compared to water and no stimulus, respectively. An interaction between stimuli is not expected.

## Method

### Subjects

22 university undergraduates (14 female, 8 male) participated in the experiment. Subjects were recruited through the psychology subject pool and participated for course credit. All subjects appeared to be in their late teens or early twenties. Subjects were fully briefed about all relevant aspects of the procedure and informed consent obtained..

### Materials/Apparatus

Lemon juice has been shown to produce a strong salivary response (e.g., Bauslaugh & Davis, 1993; Kerr, 1961). Lemon juice was chosen as one stimulus, with water being used as a control. These stimuli were delivered to the subjects on different trials through separate tubes placed in the left side of the mouth. To ensure equal 1.5ml volumes for the stimuli, Compet bottle top dispensers were used to measure and deliver the liquids.

Two locally made phazo-amplifiers (Gabert, 1983) were used to amplify the ESA signal prior to recording. Hem Data Corporation's Snap Series Software (© 1990) was used to digitize and store the data. The computer system consisted of two Zenith 386-based computers.

Dental rolls were used as a second stimulus on half of the trials. An electronic scale (accurate within 0.1g) was used to weigh the dental rolls. On average, the dry rolls weighed approximately 0.6g per pair.

Five Beckman Ag/AgCl electrodes were used to record the ESA. One was placed above each parotid gland, one on each mastoid, and one ground electrode on the left wrist. The parotid electrodes were placed just above and forward from the back corner of the jaw (See Figure 1).

## Procedure

Subjects arrived at either 11:00 am or 1:00 pm. All subjects had fasted for at least four hours prior to arriving. After a thorough briefing (including a detailed description of the entire procedure) subjects were seated and the necessary electrodes were attached. The experimenter sat behind the subject. Each trial lasted sixty seconds, with a break of approximately sixty seconds between each trial. Each trial consisted of a stimulus of lemon juice or water, with or without cotton dental rolls placed in the mouth, giving four possible combinations of stimuli. A total of eight trials were given; two of each combination. The order was randomized in two blocks (each of the four combinations was presented in random order, then each was presented again in random order).

The dental rolls were inserted by the subjects, one between the cheek and gums on each side of the mouth, immediately before each trial began. The lemon juice and water were squirted into the left

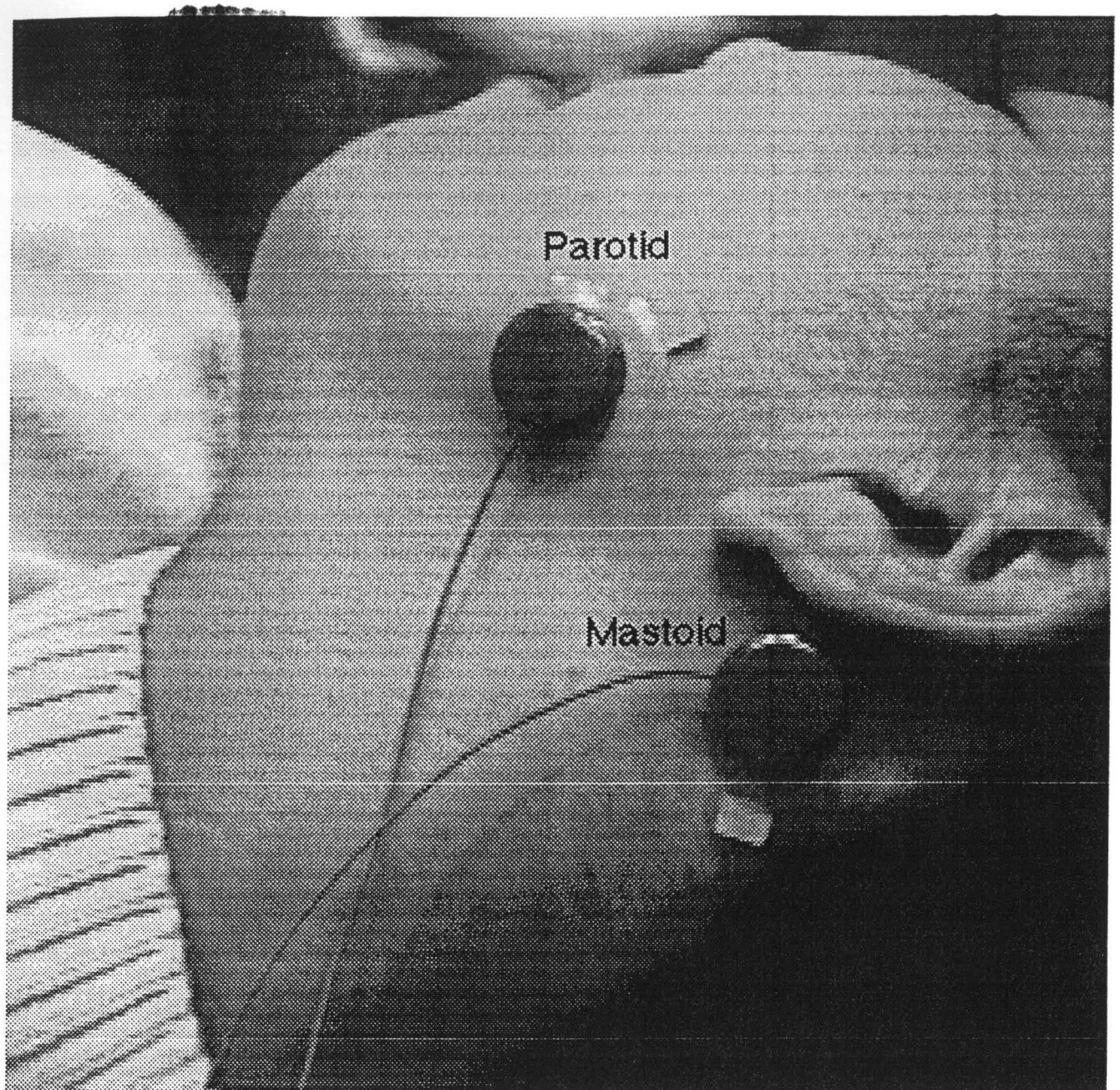


Figure 1. Placement of electrodes for measuring electrosalivary activity.



side of the mouth fifteen seconds into each trial. Subjects were instructed not to swallow during the trial. Before each trial subjects were given a pre-weighed Styrofoam cup and dental roll on half of the trials. At the end of each trial subjects were to spit the contents of their mouths (dental rolls, saliva) into the cup. The cup was re-weighed and then disposed of. Subjects were given a cup of water that they could drink from between trials.

The entire procedure took between 30 and 60 minutes. All materials that came in contact with subjects were disposed of, with the exception of the electrodes, which were washed between subjects and reused.

Subjects were debriefed afterwards and any questions they had were answered.

## Analysis

Weight measures of salivation were taken by simply subtracting pre-trial weights of cup (and rolls) from post trial weights. The weights recorded include the 1.5 ml of liquid squirted into the mouth in each trial.

ESA data were summarized in the following way. Data from the 10 seconds immediately prior to stimulus presentation were averaged to represent the pre-stimulus level. 10 seconds of data, starting 5 seconds after the stimulus, were averaged to represent the post-stimulus level of response. The pre-stimulus level was then subtracted from the post-stimulus level. Since the electrodes had a tendency to drift, a correction for drift was introduced into the

calculation. Since the drift was roughly linear, one fourth of the total drift (first value subtracted from final value in the trial) was subtracted from the pre-stimulus post-stimulus difference. Finally, the absolute values were taken. This was done on the basis of past research in this lab showing inconsistent directional changes, and research done by Lundberg (1955), showing similar inconsistent directional response to direct stimulation and recording of the electrical activity of cat parotid glands.

Saliva weights were obtained for all trials from all subjects. In all, 44 trials of each kind were recorded. ESA was recorded bilaterally, producing a possible 2x2x2 ESA analysis. Usable ESA records were obtained on between 38 and 41 trials of each kind. ESA trials were rejected on the basis of technical difficulties only, including excessive drift and computer errors.

## Results

Data were averaged across subjects. Average saliva weights for each type of trial are as follows: lemon/dental roll 5.51g (sd=1.77), lemon/no roll 4.87g (sd=1.4), water/dental roll 2.92g (sd=.8), and water/ no roll 2.29g (sd=.65). Results are summarized in Figure 2 . Significant differences were found between lemon and water trials  $F(1,83)=93.523$ ,  $p<.001$ . Significant differences were found between dental roll and no dental roll trials  $F(1,83)=5.447$ ,  $p=.022$ .

Average ESA results for each type of trial are as follows: lemon/dental roll (left side) .17mV (sd=.172), lemon/dental roll (right side) .162mV (sd=.14), lemon/no roll (left side) .202mV

(sd=.196), lemon/no roll (right side) .202mV (sd=.25), water/dental roll (left side) .13mV (sd=.12), water/dental roll (right side) .108mV (sd=.152), water/no roll (left side) .138mV (sd=.162), and water/no roll (right side) .168mV (sd=.095). **Left and right sides were averaged and summarized in Figure 2.** Significant differences were found between lemon and water trials  $F(1,299)=5.502$ ,  $p=.02$ . Differences between dental roll and non-dental roll trials were found to be marginally significant.  $F(1,299)=3.133$   $p=.078$ . No difference was found for side, and no interactions approached significance.

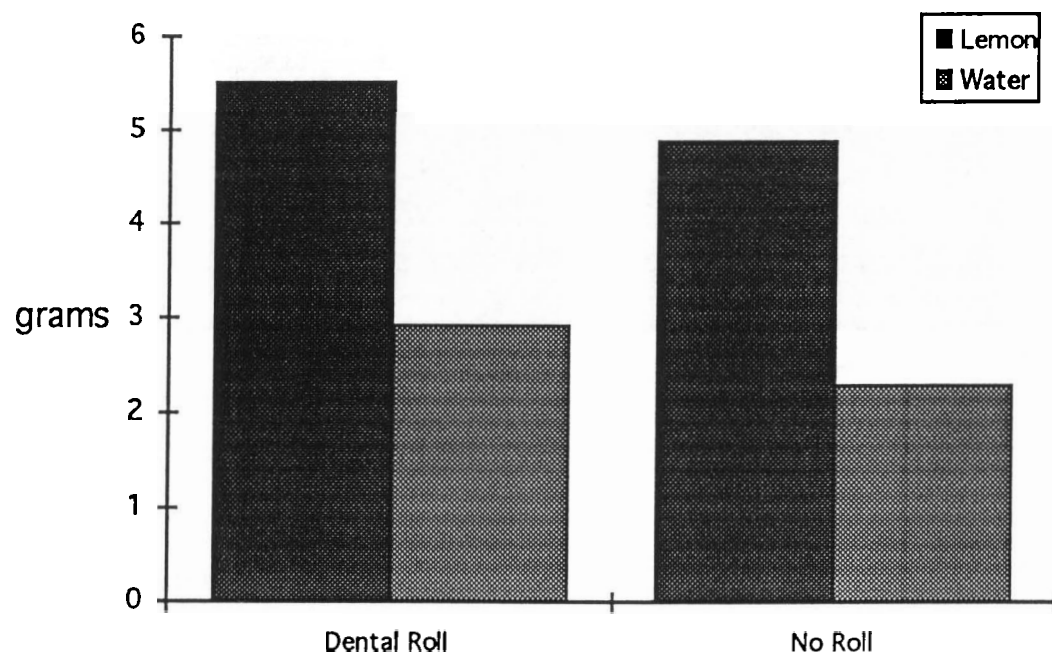


Figure 2. Average Saliva Weights.

Different results were found when absolute values were not taken for the ESA data. When direction as well as magnitude of change is taken into account, there was a significant difference between left and right side  $F(1,299)=5.164$ ,  $p=.024$ . **The right side**

was consistently more negative than the left, although the magnitude was similar (as can be seen from the absolute values). See Figure 4 for an example of an ESA recording showing opposite direction of change. Note that voltage is relative to the start of the trial.

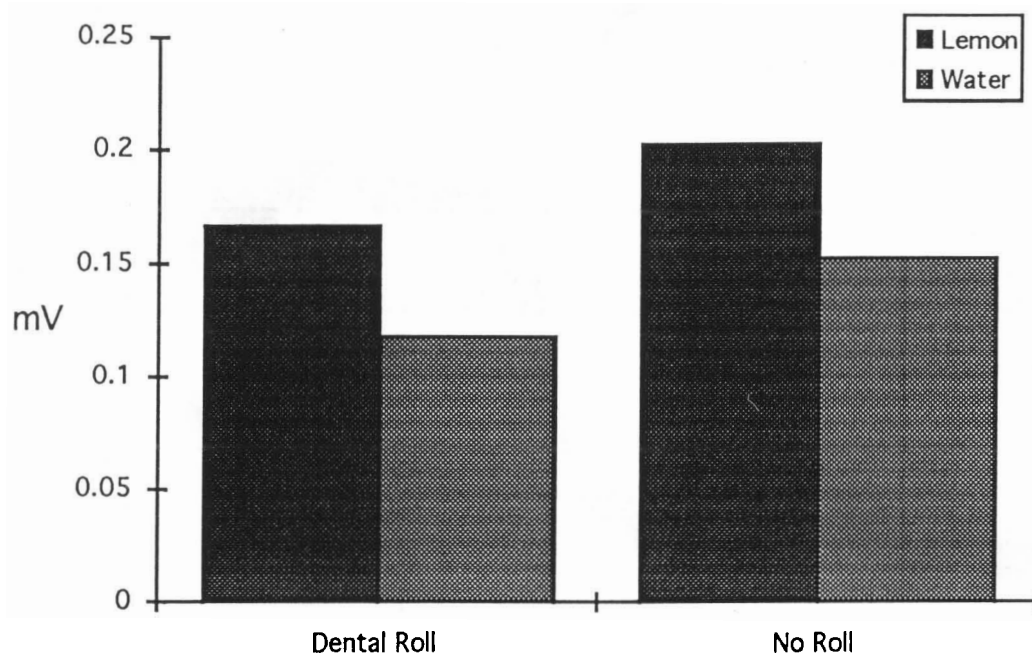


Figure 3. Average ESA Values.

Different results between left and right ESA were also found when ESA was correlated with weight. For all trials combined, left ESA was slightly but significantly correlated with weight ( $r=.224$ ,  $p=.006$ ). For lemon trials (with and without rolls) and dental roll trials (lemon and water combined), left ESA and weight were significantly correlated ( $r=.243$ ,  $p=.037$ , and  $r=.313$ ,  $p=.007$ , respectively). **Right side ESA did not correlate significantly with weight with any set of stimuli.** Left side ESA was not significantly correlated with weight on water trials, and was not correlated with

weight on non-roll trials. Thus, only left side ESA correlated with weight, and only on trials with lemon and/or gauze stimuli.

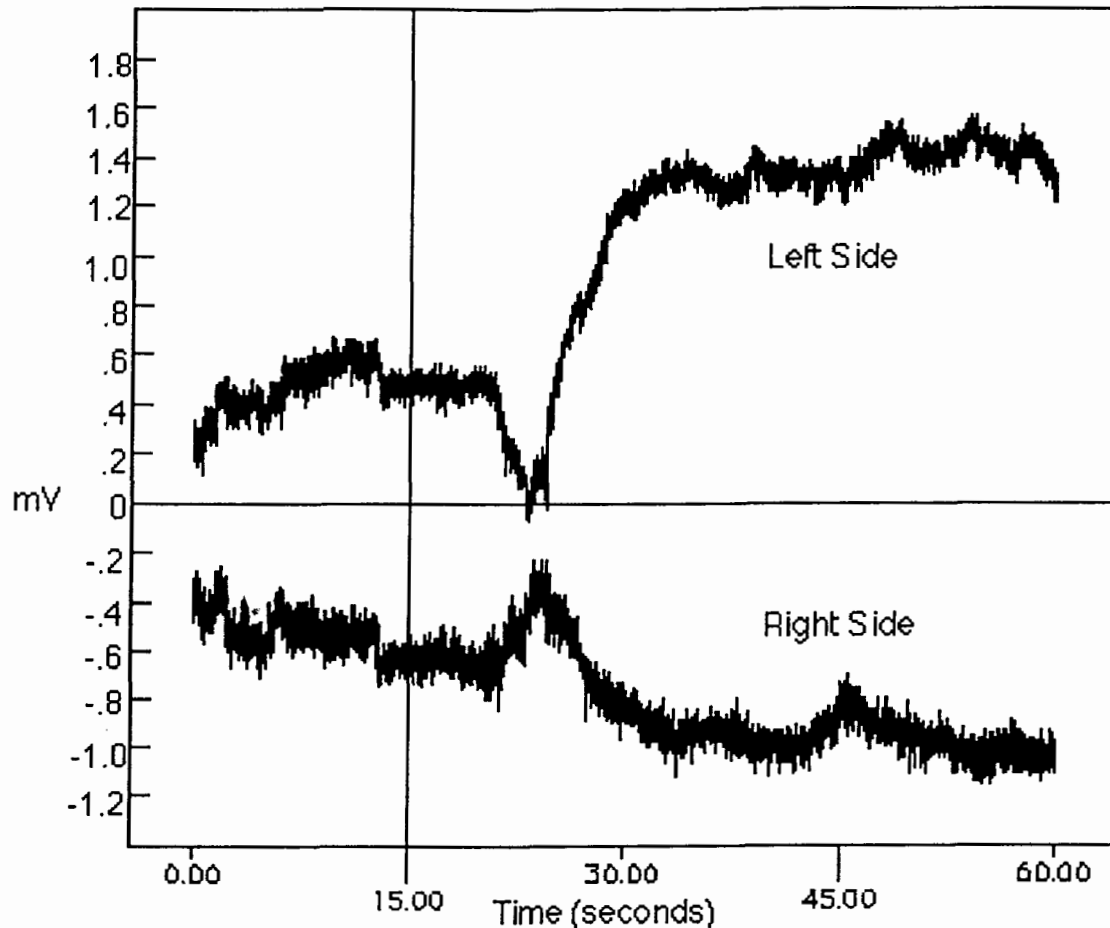


Figure 4. Example of an ESA recording. (Note the opposite direction in the left and right channels).

### Discussion

Both the weight and ESA methods showed significant differences between lemon and water trials, in the expected direction of an increase in salivation on lemon trials. Both techniques showed a difference between dental roll and no dental roll trials, although

the ESA method only approached significance. Dental Rolls increased saliva weight, but decreased ESA. Neither method showed any hint of an interaction between the two types of stimulation.

The direction of effect for dental rolls was different for the two methods. Dental rolls were associated with an increase in saliva weights compared to control trials, but a decrease in ESA. Dental rolls increased salivation, but decreased the ESA recorded. This decrease is probably due to the time at which the dental rolls were placed in the mouth. The rolls were inserted before the trial began, producing the strongest response either before the trial began or during the baseline period at the beginning of the trial. The saliva from that response would be retained until the end of the trial, but the ESA response at that time would be incorporated into the baseline. Thus, increased salivary activity as a response to the dental roll, before the trial began, would tend to decrease the recorded ESA. It would be interesting to put the dental rolls in during the trial; however, any ESA activity might be confounded by muscle activity associated with opening and closing the mouth.

The method of analysis of the ESA was based on the experimenter's experience with this type of recording. As it is a new area of research, it is difficult to find the ideal method of interpreting results without excessive post-hoc decision making. Even with the somewhat arbitrary analysis, significant and logical results were obtained. With further research and identification of the critical components of the ESA, it seems likely such analysis can be refined further.

The bilateral recording of ESA allowed a comparison of left vs. right side activity. While there was no difference between sides for magnitude of change, there was a significant directional component. Right side averages were consistently lower (smaller or more negative). The right side response tended to be the inverse of the left side. When the absolute value of the ESA change was not taken (i.e., direction of change was retained) a significant difference was found between left and right side ESA. Stimuli (lemon juice and water) were presented to the left side only, leading to a possible conclusion about laterality of response.

Only the left-side ESA was correlated with saliva weight, and only on trials with a significant stimulus, either lemon, dental roll, or both. This finding makes sense, as random noise should make up most of the data when there is little response due to lack of a stimulus. The fact that only the left side correlated is important, because the stimulus of lemon juice or water was delivered to the left side only. This again indicates a possible laterality of response. However, gauze stimuli were presented bilaterally, and right side ESA did not correlate on those trials either.

There could be several explanations for the apparent laterality of response. The right side ESA could be measuring unilateral left side activity through volume conduction. The result might be an inverse view of the same activity. Alternately, laterality of response has been reported in whole mouth stimulation (e.g., Davis et al., 1993; Kerr, 1961). Laterality of salivation may be natural, like handedness, with some subjects being predominantly left parotid responders and some right side.. The results could also represent

some inadequacies in some component of the research. It would seem likely that the lemon juice would eventually disperse over the entire mouth, eliminating the initial laterality of stimulation. An identical right-side response later in the trial could cause the right side ESA early in the trial to be more negative. So while the results clearly show some laterality of response, the meaning is not clear. Using stimuli on either side and in the middle (on different trials) might help resolve the issue.

The results from both methods show that the dental rolls do indeed act as a stimulus for salivation. This may call into question the ecological validity of some of the studies done on salivation in the past. It is clearly undesirable for a measurement technique to affect what it is measuring. Fortunately, the effect seems to be additive with other stimulation, with no evidence of an interaction. However, the effect cannot be additive across all levels of stimulation and may be subject to ceiling effects at higher levels.

## Experiment #2

### Introduction

Gastrointestinal motility refers to a number of actions of the stomach and intestinal systems, including electrical pacemaker activity and the mechanical actions of digestion. The actions of the digestive system are of importance in such areas as medicine and eating disorders (Dubois, Gross, Ebert, & Castell, 1979) and may be useful in understanding hunger, appetite, and dietary restraint.



Measurements of this motility can be used to diagnose a number of disorders, including systemic sclerosis (Rees, Leigh, Christofides, Bloom, & Turnberg, 1982) and ulcers (Itoh & Sekiguchi, 1981). Gastrointestinal activity may be correlated with emotion (Coddington, Sours, & Bruch, 1964), menstruation (Wald, Van Thiel, Hoechstetter, Gavaler, Egler, Verm Scott, & Lester, 1981), and, of course, with feeding.

Gastrointestinal motility and salivation are related to a number of the same areas, including eating disorders, hunger, emotion, dietary restraint, and other related areas. It is likely, based on their similar role in the body and their parallel relations to external factors, that motility and salivation will be correlated. The Davis laboratory at Simon Fraser University has researched each of these areas independently. This experiment represents the first attempt to examine them together.

The dominant stomach rhythm is a three-cycle-per-minute (3cpm) rhythm that entrains stomach muscle activity. Superimposed on this rhythm is spiking activity, generally regarded as contractile stomach activity (Torsoli & Corazziari, 1982; Stoddard, 1978; Schang & Devroede, 1983, Connell, 1978). Many techniques have been used to examine these two types of stomach activity. Inflated balloons are used to detect changes in pressure, internal electrodes (both swallowed and surgically implanted) record electrical activity, and various radiology and ultrasound techniques view the physical activity of the system (Connell, 1978). Another method, electrogastrography (EGG) uses surface electrodes placed on the abdomen, above the stomach, to record stomach activity non-

invasively. Results from the different techniques have yielded similar data and can therefore be reviewed in general.

The stomach has a 3cpm rhythm that entrains muscle activity. Its function seems to be to time the contractile activity of the stomach so that the stomach contracts in rings. This rhythm is locally generated (Stoddard, 1978). Fourier analysis has been used to show that the 3cpm rhythm gets stronger after feeding (Stern, 1985; Grashuis, Van Der Schee & Geldof, 1985). This rhythm is a pacemaker, giving a constant cyclic rhythm that entrains sporadic contractile stomach activity.

Superimposed on the 3cpm rhythm is a faster spiking activity. This activity has been generally accepted as contractile activity of the stomach and intestines. This spiking activity may represent the contractile activity entrained by the 3cpm rhythm. Four cyclic phases of spiking activity have been identified (Torsoli & Corazziari, 1982). Phase one has no spiking activity, phase two shows sporadic and increasing spiking activity, phase three shows periodic spiking phase locked with the slow wave (3cpm) activity, and phase four (only sometimes reported) involves a rapid reduction in activity. Interesting results have shown that feeding immediately (within a maximum of 60 second latency being reported by [Sinar & Charles, 1983]) affects stomach spiking activity. However, the exact nature of this effect is not clear. Sinar & Charles (1983) report an immediate increase in spiking activity in the intestine and a change from periodic to irregular spiking. Others (Steinbach & Code, 1980; Defilippi & Valenzuela, 1981) have reported a significant delay in the onset of phase three activity, meaning that activity immediately

after feeding should remain about the same as before feeding, for some period of time. Feeding has also been shown to abolish the 3cpm for a period of time (Holt, McDisken, Anderson, Stewart, & Heading, 1980; Lorber, Komarov, & Shay, 1950), or alternately has been shown to speed it up (Funch-Jansen, Kraglund, Oster, & Thommeson, 1982).

The delay in spiking activity is thought to allow time for the meal to be consumed, so that spiking activity during the meal does not empty the stomach prematurely. The cyclic activity periodically empties the stomach in a sort of housecleaning routine, and feeding resets the system. Some studies have pointed to the chemical properties of the meal as being critical to the strength of the effect. Sinar and Charles (1983) point to carbohydrates as being the critical factor, while others (Sinar & Charles, 1983) have pointed to glucose as being important. However, sham feeding (chewing and spitting out) in humans and dogs (Lorber, Komarov, & Shay, 1950; Defilippi & Valenzuela, 1981; Steinbach & Code, 1980) has produced the same effect, as has direct stimulation of rat brains (Lee, 1982). The fact that indirect stimulation can produce changes in motility calls into question the importance of any chemical properties other than taste or smell.

Electrical activity can be recorded from the surface of the abdomen through a technique called the electrogastrogram. The EGG has been around for about seventy years (Stern, 1985) but it has never gained the status of other techniques and has never been the dominant method of measuring stomach rhythms. Since the data obtained from internal and external electrodes seem to be similar

(Abell, Tucker, Malagelada, 1985) and, in general, all the techniques seem to yield similar types of data (personal review of research), this preference is difficult to understand. Physiologically, there should not be any major advantage to recording from inside the stomach over recording externally from the abdomen: both electrodes are separated from the source of the signal by some barrier (skin or stomach lining). Numerous problems can arise because of the difficulty of ensuring contact and location internally to mention nothing of the unpleasantness for the subject. Stomach balloons and all manner of probes used internally would have to be unpleasant. Wenham (1979) found that cannulating the system interfered with its normal functioning. Surgically implanted electrodes are used primarily in animals and, again, seem to give similar data, except for the difference between human and animal rhythms (dogs have a 5 cycle per minute dominant rhythm). Radiology has the problem of exposing the subjects to unnecessary radiation. All of the arguments in favour of using a non-invasive technique for measuring salivation apply to the measurement of stomach activity: invasive procedures are unpleasant for the subject and likely to affect what they are supposed to measure.

The critical components of the EGG are the 3cpm rhythm and the spiking activity. Analysis of these components should be used to evaluate changes in the EGG. Research indicates either an increase in amplitude of the Fourier strength of the 3cpm activity, an abolition of the 3cpm for a short period of time (with an associated decrease in spiking activity), or a increase in speed of the 3cpm activity. Feeding may change the amount or kind of spiking activity.

Using non-invasive stimuli may be just as important as using non-invasive measurements. Since sham feeding, including just the sight and smell of food, has been shown to be an effective stimulus (Steinbach & Code, 1980) and since smell has been shown to be effective in producing salivary activity (LeGoff & Bauslaugh, 1994), smell may be an effective stimulus for gastric motility.

This study, therefore, investigated the effect of smell stimuli on salivation and gastric motility, and the relationship between the two. Included within this broad topic is an investigation into what methods can best be used to analyze the data from both measures and what critical components can best distinguish different groups.

## Method

### Subjects

21 subjects participated: 6 women and 15 men. Of these, most were in their mid-twenties. Subjects were all friends of the experimenter, and most had some knowledge of the area of research. Informed consent was obtained from all subjects.

### Materials/Apparatus

Two locally-made phazo-amplifiers (Gabert, 1983) were used to amplify the ESA and EGG signals prior to recording. Hem Data Corporation's Snap Series Software (© 1990) was used to digitize and

store the data. The computer system consisted of two Zenith 386-based computers.

A total of five Ag/AgCl electrodes were used: one on the cheek and one on the mastoid in normal ESA configuration, one ground electrode on the left wrist, one on the abdomen over the stomach, and one reference electrode on the left wrist.

Five different food smells were prepared by finely grinding the stimuli and placing them in identical jars. The five foods were: salt and vinegar potato chips, nacho chips, glazed doughnut, cinnamon bun, and chocolate. The grinding produced a stronger smell than the original stimuli. These foods and technique were used by LeGoff and Bauslaugh(1994) in a previous study. A sixth control jar contained no food stimulus.

## Procedure

Before starting the experiment, all subjects were fully briefed on all relevant aspects of the procedure and informed consent was obtained.

The cheek electrode was placed over the parotid gland, slightly above and forward of the back corner of the jaw. This is the standard position used in the Davis lab. The abdominal electrode was placed approximately two inches to the right of the midline, on a horizontal line with the bottom on the ribcage (See Figure. 4 for EGG electrode placement). This position was chosen, based on pilot tests that showed it to be a reliable site, and on the observations of Davis, McIntosh, and Murray (1985) which cited this as the most active of

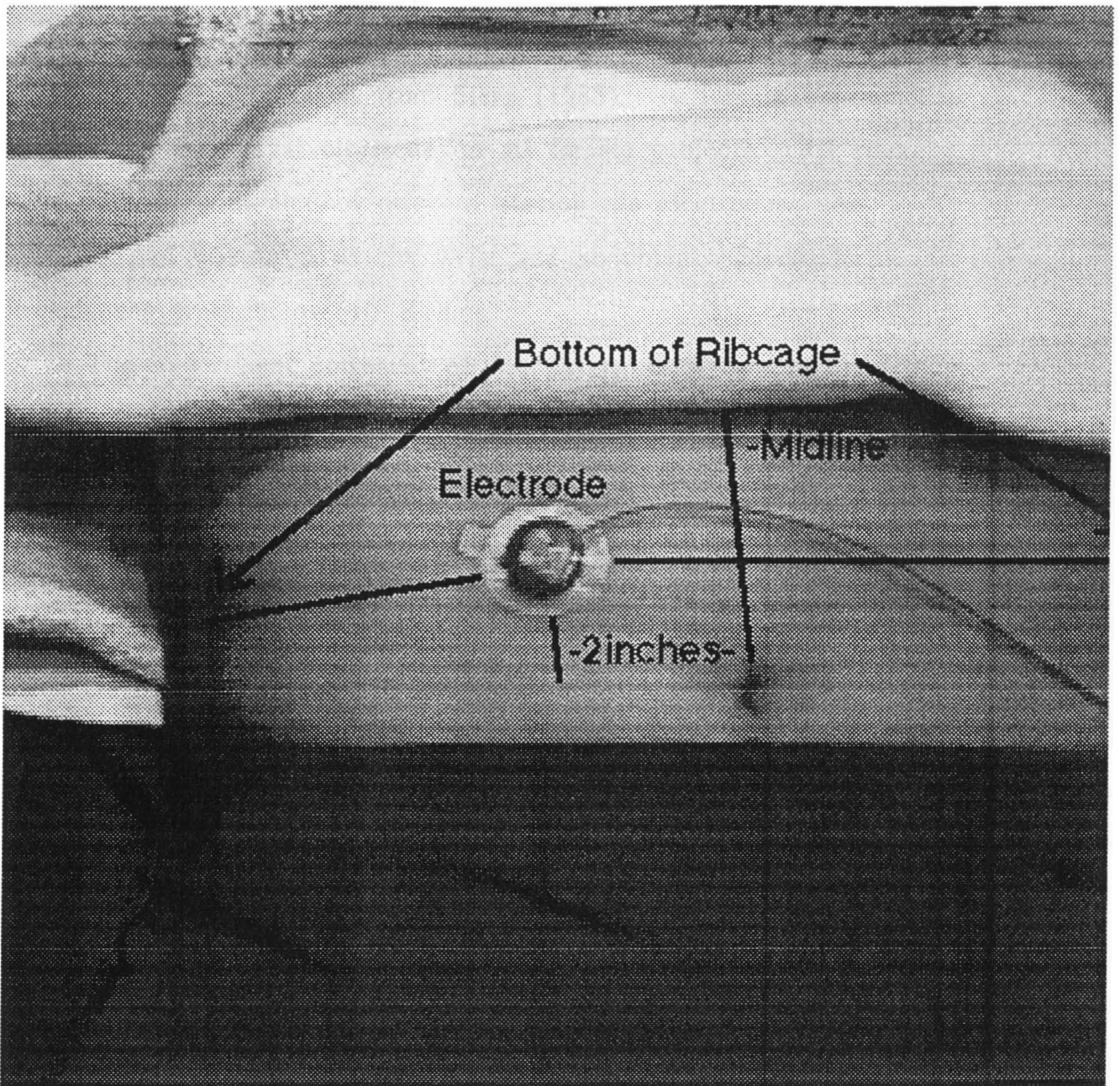


Figure 5. Placement of electrodes for measuring electrogastrography.

the eight sites from which they recorded. The general procedure for EGG recording was taken from Stern (1985). A female experimenter applied the abdominal electrode on all female subjects. All sites were cleaned with alcohol prior to placing the electrodes. An abrasive pad was used on the wrist and abdomen. Electrode impedances were below 10 Kohms.

The experiment consisted of four trials, each two minutes long, with approximately one minute between each trial. On each trial, the subject received a fixed series of smells, either food odours (in the order salt and vinegar chips, nacho chips, doughnut, cinnamon bun, chocolate), or a series of five empty jars. The order of trials was counterbalanced across subjects.

During the experiment the subject lay on a bed to ensure proper placement and recording of the EGG. The experimenter sat just behind the subject. During each trial, the subject was asked to relax, keep movements to a minimum, close his or her eyes, and breathe normally. Each jar was presented for approximately twenty seconds, with the first presentation starting at twelve seconds. The jars were held approximately one inch away from the subject's nose. The series of empty jars (the non-food trials) was presented in the same way as the food-odour series.

The subjects were told that they would be asked at the end of the experiment to identify the smells, and that they should try to identify (to themselves) the smells during the trials. This was done to ensure that the subjects could actually smell the stimuli during the experiment, and identify them as food smells. At the end of the experiment, the subjects were asked to identify the smells and were



rated by the experimenter as either being able or not able to identify each of them. Subjects were rated as being able to identify the smells if they were able to correctly identify at least three smells. In fact, there was a clear difference between subjects on this variable, with a few being unable to identify any smells and the rest being able to closely identify at least four smells.

## Analysis

ESA data were analyzed in the same fashion as in experiment one. An average of the ten seconds immediately before the first stimulus was subtracted from a ten second average beginning five seconds after the onset of the first stimulus. One eighth of the total drift was then subtracted from that difference. Because this limited analysis to the first stimulus in the series (salt and vinegar chips), another analysis was performed, taking into account the whole trial. In a method similar to analysis performed by LeGoff and Bauslaugh (1994), an average of the whole post-stimulus part of the trial was compared to the pre-stimulus part of the trial. ESA was recorded and analyzed unfiltered, as it was felt that the averaging used in this analysis would compensate for any random noise in the data.

EGG data was filtered using a software 0.1Hz low-pass Butterworth filter to eliminate ECG and other noise and artifacts. The filtered trials were then analyzed using a fast Fourier transformation to determine the strength of the three cycle per minute rhythm. Information was also recorded for frequencies up to 4.5 cpm to account for variations in the speed on the rhythm, such as reported

in Davis et al. (1985). Although Davis reported rates from one cpm to four cpm, rates slower than three cpm were not recorded as they tended to be obscured by their position close to the fundamental frequency, and it was felt these values would most likely not be valid. It is possible that the 0.1Hz cutoff on the low pass filter may have attenuated some of the higher range of frequency recorded (.075Hz), but analysis of the data using a more liberal cutoff (0.2Hz) yielded essentially the same results. All analyses were performed using Hem Data Corporation's Snap Series Software (c 1990).

ANOVAs were performed on the ESA data and the EGG data. Pearson correlations were then computed between the ESA and EGG data.

To summarize, a change between pre-stimulus and post-stimulus levels of ESA was used to represent salivary activity. Stomach motility was summarized as the Fourier strength of the 3cpm rhythm in the EGG over the entire trial. Thus, each measure resulted in one number that summarized activity for that trial. Note that the ESA activity level is based only on a period of 25 seconds during the trial, while the EGG level is based on the entire trial. A correlation will indicate that activity in the salivary glands and stomach occur at roughly the same time and to similar stimuli.

## Results

See Figure 6 for an example of the filtered EGG signal.

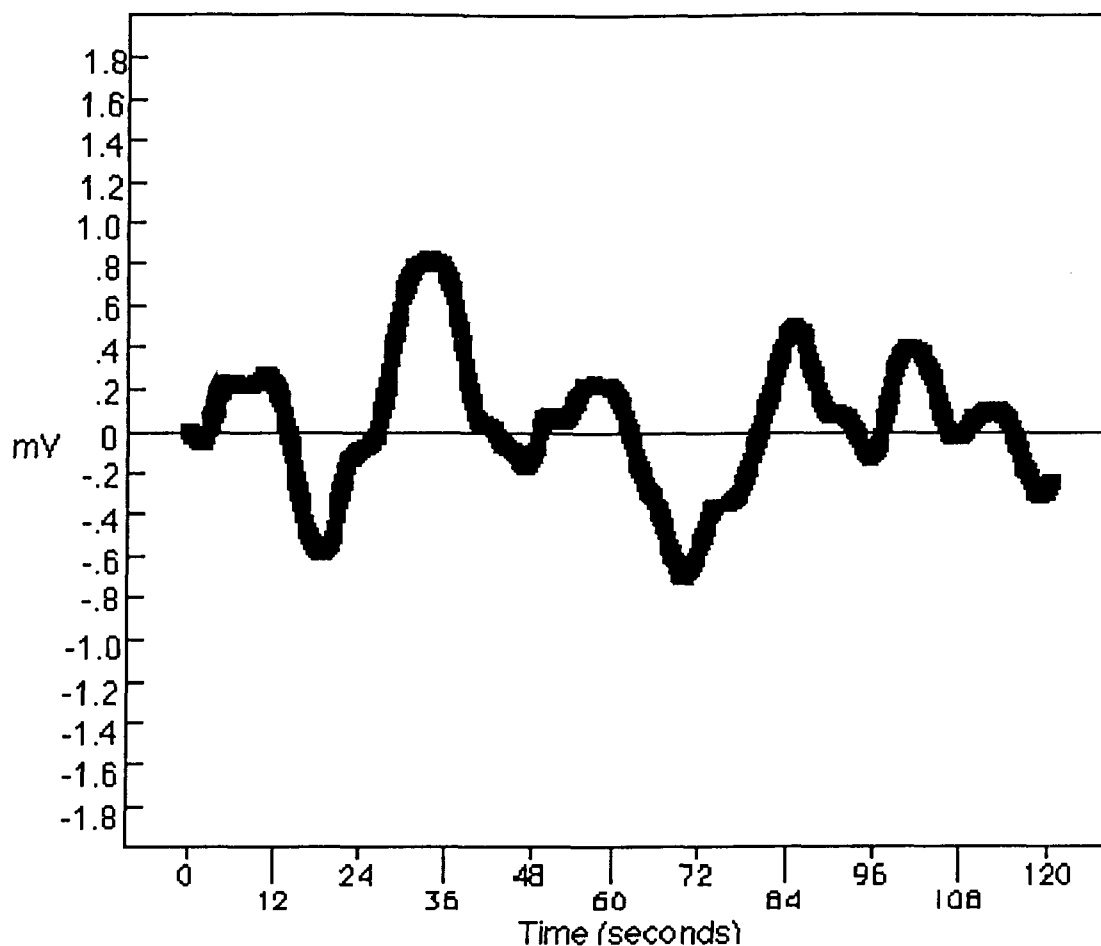


Figure 6. Example of filtered EGG.

An ANOVA performed on the ESA yielded no significant difference for stimulus ( $p=.761$ ) or order ( $p=.369$ ). No interaction was noted ( $p=.629$ ).

An ANOVA performed on the EGG yielded no significant differences for stimulus ( $p=.978$ ) or order ( $p=.613$ ). No interaction was noted ( $p=.209$ ).

ANOVAs were performed comparing the first trial of subjects who received food smells first to those that received no smell first. There were no significant differences for ESA ( $p=.399$ ) or EGG ( $p=.334$ ).

Using the second method of analyzing ESA data, that is subtracting the baseline average from the average post stimulus level for the rest of the trial, did not improve upon the above results. In addition, taking an average of the whole post stimulus interval decreased the number of viable trials, as more needed to be rejected because of DC electrode drift.

A significant correlation was found between ESA and EGG on food trials ( $r=.377$ ,  $p=.023$ ). No significant correlation was found between ESA and EGG on non-food trials ( $r=.056$ ,  $p=.754$ ).

Only the 0.05Hz (3cpm) fft data were used in this analysis. An average of the range of 0.05Hz to 0.075Hz was tried instead of the 0.05Hz alone, but this method did not improve upon the 0.05Hz data alone.

Of the 21 subjects, 3 were unable to identify more than one of the smells, even when allowed to smell the stimuli again after the experiment. This number was too small for a separate meaningful analysis. It is not known if they were unable to smell the stimuli, or simply unable to identify them.

No spikes (other than ECG) were detected in the EGG before the data was filtered.

## Discussion

The most interesting finding in this experiment is the significant correlation between salivation and gastric motility, as measured by the ESA and EGG, respectively. The positive correlation indicates that salivation and gastric motility may both respond in

similar, measurable ways to certain stimuli. In this case, in the presence of food stimuli, salivation and gastric motility seem to have behaved in a related way. This correlation is similar to the correlations noted in experiment one. ESA was correlated to whole mouth saliva only on stimulus trials. In both cases, baseline measures of activity were not related, whereas stimulated activity was related.

There are some significant problems with the correlation between ESA and EGG found in this experiment. The correlation, found only during stimulation, would seem to indicate that activity was different between stimulation with food smells and without. This was not the case. The ANOVAs showed no significant effects (nor even any suggestive results) for a difference between stimulated and control trials. If there is a correlation during stimulation and no correlation during control trials, there should be a measurable difference in activity between stimulated and control trials.

Because of the different methods of analysis, there is little chance that volume conduction or some third variable (a different source) could be responsible for the correlation. For instance, the 3cpm stomach rhythm can be recorded from nearly any location on the body, but EGG picked up by the ESA electrodes would not cause a correlated change in the ESA. The ESA data in this experiment are determined by a change in potential at a specific time, whereas the EGG data are determined by the strength of a continuous rhythm; it is not time dependent as is the ESA.

The correlation is especially interesting because it is based on two independently derived measures of activity. The ESA measure is based on the previous experiment, whereas the EGG measure is based largely on analyses in Stern (1985), Davis et al (1985) and Martin, Murat, Nicolov, and Masson (1985). Both measures were chosen prior to analysis with no post hoc decision making. The two measures represent two summaries of activity derived in different ways.

Because of the absence of any effect for food smells compared to the control condition, the correlation noted above should be taken somewhat tentatively. However, because few tests were performed and the correlation of ESA and EGG was a primary goal of this experiment, the result cannot be dismissed.

The correlation has a probable neural basis. The gastrointestinal and the salivary system have parasympathetic cholinergic stimulatory neural control. The parotid gland is innervated by the inferior salivary nucleus via the glossopharyngeal nerve (cranial nerve IX), located in the medulla. Other salivary glands are innervated by the superior salivary nucleus via the facial nerve (cranial nerve VII). The gastrointestinal tract is innervated by the dorsal motor vagus nucleus via the vagus nerve (cranial nerve X), also located in the medulla (Dodd & Role, 1991). The position of these nuclei and the columnar organization of nuclei in the brain stem suggest a similar function (Role & Kelly, 1991). Both systems also have sympathetic innervation which generally acts in an oppositional manner. Activity in the gastrointestinal system is also mediated by local sensory input.

Parasympathetic arousal may underlie any correlation found between salivary and gastric activity. A possible system responsible for control of both salivary and gastric function lies in the medulla. The solitary nucleus receives taste input via the facial and glossopharyngeal nerves, as well as input from the vagus nerve (Role & Kelly, 1991). The solitary nucleus then innervates the vagus motor nucleus which acts on the gastrointestinal tract, as well as the heart and several other autonomic systems (Dodd & Role, 1991). Because of the interconnection of the nuclei in the reticular formation, it is probable that the salivary nuclei are also innervated by the solitary nucleus. Taste and gastrointestinal stimulation affect the solitary nucleus, which innervates the salivary nuclei and the dorsal motor vagus nucleus, which then control the salivary glands and the gastrointestinal tract. However, many other factors may mediate these systems, including the higher areas of the brain and various hormonal and chemical influences.

Sympathetic and parasympathetic innervation of the salivary glands do not always have opposite affects. Both types of stimulation can lead to increases in salivation, with parasympathetic yielding a serous secretion and sympathetic yielding a viscous secretion (Dodd & Role, 1991). The ESA probably could not differentiate between these two different types of activity. Sympathetic innervation in the stomach would decrease activity. It is possible that this difference could have resulted in the absence of a correlation on non-stimulus trials.

The negative results of the ANOVAs show no effect for food smells over control trials, as measured by ESA and EGG. Because of

the position of the subjects and the jars it is possible that the subjects could not smell the stimuli adequately; however, most subjects were able to identify the stimuli without smelling them again after the experiment. Of the subjects that reported being unable to identify the smells, none improved by sitting up and trying the smells again after the experiment. In addition, the experimenter was usually able to smell the stimuli from arm's length, indicating that the smells were fairly strong.

Because of the apparent failure to generate a response, food smells cannot be recommended as a non-invasive alternative to direct taste stimulation. Research on the topic of smell and vision have been fairly equally divided as to whether they can (Pangborn, 1968; Pangborn, Witherly, & Jones, 1979; LeGoff & Bauslaugh, 1994; Jenkins & Dawes, 1966) or cannot increase salivation (Birnbbaum, Steiner, Karmeli, & Ilsar, 1974; Shannon, 1974). Some replications (e.g., Shannon's replication of Pangborn) have found results opposite to the original. The difference between the present research and that of LeGoff and Bauslaugh (1994) is of particular interest because of the similarity of the procedures used.

The procedure used in LeGoff and Bauslaugh (1994) was very similar to the present procedure. Both authors were present during both of the experiments, although different experimenters were responsible for running subjects in each. Smells were presented in similar ways, although this experiment substituted an empty jar for non-food smells used by LeGoff. It was felt that some of the smells used in the non-food condition (pine, tobacco, alcohol, soap and dirt) could be easily confused with some food smells. It possible that the



non-food smells had their own effect on salivation which may have amplified the difference between food and non-food smells. The non-food smells may have been able to 'cleanse the palate' between food trials, allowing the food smells to regain the impact of the first trial. This position, however, is not compatible with the results from this experiment showing no difference between food smells and controls on first trials. This result shows that factors such as time between trials, number of trials, and order of trials are not responsible for the difference between the two experiments.

The subjects were positioned differently in the two experiments, with subjects in this experiment lying down and subjects in LeGoff and Bauslaugh (1994) sitting up. This may have affected the strength of the stimuli. While the subjects were typically able to identify the smells in this experiment, it is possible that sitting up in a more natural position and holding the jars in a better position would give the subject a stronger, more immediate smell. Other factors that subject position might affect are the deepness and regularity of breathing, attentiveness of the subject, and position of saliva in the mouth (possibly affecting feedback for salivation and swallowing). Kerr (1961) found that salivation was diminished when subjects were seated in a reclined position. That effect may be stronger with the subject lying down. Unfortunately, the lying position is important for good recording of the EGG.

The time frame chosen for the trials in this experiment was a compromise based on technical requirements and the known response characteristics of the ESA and the EGG. The EGG is usually recorded in sessions lasting up to an hour or more (e.g., Jones and

Jones, 1985; Davis et al., 1985), but software limitations only allowed Fourier analysis on trials up to two minutes long. Two minutes was more than enough time to record the relatively fast changes in the ESA. Even with this short time interval, DC electrode drift was a significant problem on many of the trials.

The 3cpm rhythm was clearly present in the EGG recording of many subjects. Fourier analysis of the EGG revealed that the 3cpm tended to be the strongest frequency other than the ECG frequency and its harmonics. The strength of this rhythm was apparently unaffected by the stimuli. A test to determine whether the rhythm changed in frequency also produced negative results. Davis et al. (1985) reported that the frequency of EGG could change rapidly from approximately 1cpm to 4.5 cpm. The Fourier analysis may not have been capable of detecting such a change, or the direction of the change may not be uniform across subjects.

The absence of EGG spiking is interesting. Early analysis of the data showed some spiking which, upon closer examination, turned out to be ECG. Spiking activity seems to be reported more with other methods of recording, which may have to do with filtering or some other characteristic of the recording. Because research on EGG has generally focused on the 3cpm rhythm, many researchers have used conservative filtering eliminating higher frequencies (e.g., Davis et al., 1985). This experiment, however, recorded EGG unfiltered and still found no evidence of spiking activity. The EGG may not be capable of recording the spiking activity, it may be disguised in the EGG signal, or the spiking may be an artifact of other recording procedures.

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