ESG -DEVELOPMENT OF A NEW PSYCHOPHYSIOLOGICAL MEASURE by Martin Alvin Hing

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF ARTS (HONORS PSYCHOLOGY) in the School of Psychology

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ABSTRACT

This experiment was an exploratory attempt to develop a new psychophysiological measure called the electrosalivogram (ESG). The hypothesis that it is possible to record electrical activity originating from the activated parotid salivary gland, was supported.

Next, a descriptive analysis of the waveform revealed parameters for the following characteristics.

It was shown that the parotid salivary gland would exhibit a consistent deflection in one direction for stimulus trials but for control trials the response was variable.

Also, both the stimulus and control peak latencies were quite variable. Although control trials deviated from the mean to a slightly greater extent than did the stimulus trials.

Further, peak amplitudes for the stimulus trials were considerably larger than for control trials. Stimulus amplitudes ranged from between 40.02 microVolts to 143.405 microVolts while control amplitudes ranged between 8.46 microVolts to 53.36 microVolts.

Finally, the ESG recording exhibited slow wave activity probably due to the fact that the myoepithelial cells of the salivary gland behave much like smooth muscle cells.

These results were discussed and it was suggested that these parameters be used as the guidelines from which future research might determine the limitations of these characteristics.

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INTRODUCTION

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Salivation and physiological psychology have been linked for over eighty years, dating back to the conditioning studies carried out by Ivan Pavlov. In fact, as early as 1903 Pavlov had begun to theorize about salivation caused by "psychical" stimulation (Wells, 1956).

However long this relationship between salivation and psychophysiology may be, the area of salivation has, until recently, remained relatively obscure.

Sreebny (1987) notes that the twenty or thirty papers presented at the 1962 International Conference on the Salivary Glands comprised about one tenth of the total number of papers published that year on salivation. Compare this to the approximately 1500 papers published on the topic in 1986 and the increase is tremendous. Nonetheless, Sreebny argues that salivation has not generated the interest that the other bodily fluids such as urine or blood have, thus, the area of salivation is still a largely unexplored and developing one.

The fact that the area of salivation remains largely unexplored provides the opportunity for many new developments. Accordingly, this paper will attempt to validate a new measure of salivary activity. This new psychophysiological measure, the electrosalivogram (ESG), will attempt to record the electrical activity which occurs in the activated salivary gland.

Literature Review

<u>History</u>

Traditionally, human salivary activity has been measured from individual salivary glands by cannulation via the larger (Stensen's and Wharton's) salivary ducts (Eckhard, 1863; Oehl, 1864). Cannulation involves inserting a tube into the duct to collect the saliva, which is obviously a very intrusive measure.

More recently, variations on Carlson and Crittenden's (1910) collection device have been popular for measuring individual gland salivary rates, this is done most commonly from the parotid (Shannon and Chauncey, 1967) but also from the submaxillary and sublingual glands (Schneyer, 1954). This device and numerous subsequent derivations operate by affixing a small collection cup over the salivary duct which is drained via tubes leading outside of the mouth. However, this measure is also somewhat intrusive and very cumbersome.

Other methods for collecting saliva which are not concerned with the individual gland contribution are the whole mouth salivary measures. These are currently the most extensively used for psychophysiological research. The four most common whole mouth salivary measures are draining, spitting, suction and swab (White, 1977; Navazesh and Christensen, 1982). These will be described briefly. First, the draining method requires that the subject hang their head over a collection container and allow the saliva to drip into the container freely without swallowing any of it. The saliva can then be measured for volume, weight or composition.

Second, the spitting method requires the subject to periodically expectorate all saliva in his/her mouth into a container which can be measured.

Third, the suction method collects the saliva via a suction tube similar to the one found in dental offices.

Finally, the swab method uses gauze swabs to absorb the saliva which after a time can be weighted. The dry gauze weight is subtracted from the saliva-soaked gauze weight to provide a measure of salivation.

The parotid collection device, draining, spitting, suction and swab methods have been shown to have high intra-subject reliability although the swab method is found to be less reliable than the others (White, 1977; Navazesh and Christensen, 1982).

Although, these methods are reliable for collecting parotid and whole mouth saliva and are indispensable if a composition analysis is required, their shortcomings are that they are either intrusive, cumbersome or else not able to accurately measure the time-course of salivation.

Consequently, this experiment will attempt to validate a novel method of measuring salivary flow in humans which does not contain, the drawbacks of the previously mentioned salivary measures. This will enable a researcher to pick and choose from a greater arsenal of measurement techniques based upon the specific requirements of the saliva research to be performed.

Electrosalivograms (ESG) will be recorded using a surface electrode located directly over the parotid gland.

Theoretical Framework

Is it possible to record electrical activity from the salivary gland?

Yes, there is very good reason to believe that it is possible to record the electrical activity of the salivary gland based upon studies performed on animals. These electrophysiological studies done on animal (cat and dog) salivary glands (Lundberg, 1957; Lundberg, 1958) provide the theoretical framework for this procedure.

Animal studies

Electrophysiological experiments have been conducted on animal salivary glands and "(t)here seems little doubt that the gland electrogram is a concomitant of secretory activity in the gland cell" (Burgen and Emmelin, 1961, p. 198).

The early studies in this area were performed by Bayliss and Bradford (1885), they found that there was an electrical potential difference between two electrodes, one placed on

the convexivity of the exposed submaxillary gland and the other on the hilus, when secretion was induced by stimulating the chorda tympani.

Subsequent research showed that this potential difference was not caused by differences in blood flow, because, giving the animal atropine abolished the secretion and also caused the potential to disappear without affecting the vasodilator action. Also, it was determined that the potential was not being caused by the flow of saliva in the duct because when the duct was clamped to stop the flow of saliva the potential remained (Bayliss and Bradford, 1886; Bradford, 1887, 1888; Harreveld, 1930). These animal studies provide the theoretical support for the hypothesis that it is possible to record the electrical activity from the salivary gland. However, due to the intrusive procedures involved, which include exposing the salivary gland and innervating the appropriate nerve, these studies have not been performed on humans.

Although these animal studies suggest that stimulation of the salivary glands will result in electrical activity which can be recorded from the surface they do not provide much information on the descriptive characteristics of the waveform. In fact, Lundberg (1955) (Appendix A) shows four electrograms recording four successive innervations of the chorda from the cat submaxillary gland. It can be seen that each innervation produces a very different looking waveform, the only constant is that the initial deflection is always in the same direction (positive with respect to baseline). This suggests that the corresponding human ESG will likely not have any consistently defined waveform pattern apart from perhaps and initial response which deflects consistently in the same direction.

Also, there are two procedural differences between Lundberg's animal studies and the human ESG measure which will likely alter the results obtained.

First, the size of the waveform is not likely to rival those obtained by Lundberg, which as can be seen in electrogram D (Appendix A), may approach nearly 2mV. This is because Lundberg is able to place an electrode directly on the exposed gland, whereas the

human ESG measure is taken from the skin surface. Lundberg's data however, may provide a helpful clue towards the size of the ESG activity, which will likely be 2mV or less.

Second, by stimulating the nerve Lundberg is able to consistently obtain a response on the electrogram with an onset latency of between 0.2 and 0.6 seconds. However, because the ESG measure uses a natural stimulus (lemon juice) administered to the mouth, there will likely be a latency of responding greater than Lundberg's results. This is because the time involved to react to the natural stimulus may involve a number of unknown factors, such as, when exactly does the lemon stimulus leave the administration tube and more importantly when does it come in contact with a taste receptor in the mouth. Also, the afferent nerve impulses must conduct the signal centrally before a response can be made. These and other factors imply that the latency of the ESG measure will likely be greater than that achieved by Lundberg's experiment and more importantly, will vary somewhat from stimulus to stimulus due to the unknown factors discussed above.

These rather vague implications from Lundberg's study do not tell a great deal about the electrical response which can be predicted for the human ESG measure. However, this is currently the best predictive information available and it does provide ballpark estimates of what to expect ahead of time. Namely, a consistent initial deflection, a signal likely 2mV or smaller and a variable latency greater than 0.2 to 0.6 seconds.

Physiology

Structure and function of the parotid gland

Anatomically and physiologically what is responsible for producing the electrical activity the ESG is attempting to record?

Anatomically, pre-formed saliva is stored within acinar secretory cells which resemble small globular sacks containing the saliva. Surrounding these acini are the myoepithelial cells

which have a cell body containing the nucleus and cytoplasmic organelles and long slender processes which embrace or grasp the acini much like a hand would grasp a ball.

Physiologically, it is the contractile function of the myoepithelial cells, which behave much like smooth muscle cells, that results in the electrical activity required for the ESG. This contraction by the myoepithelial cells essentially squeezes the saliva out of the acini and into the ducts. The myoepithelial cells work together and the synchronous contraction of a large number of these cells will cause a spurt of saliva into the mouth via the ducts (Sreebny, 1987).

The fact that myoepithelial cells act much like smooth muscle cells is an important finding. This is because smooth muscle cells like those recorded during electrogastrograms (EGG's) have a distinctively slow wave form (in the order of seconds). If this is true of the myoepithelial cells in the salivary glands then, a further clue to the characteristic of the ESG waveform is obtained. That is, the ESG waveform should show a relatively slow waveform (measured in seconds) in response to stimulation. This is very useful information because it suggests that much of the artifactual activity in the ESG can be easily recognized and discounted because much of the artifactual activity tends to be relatively fast activity such as muscle or quick (saccadic) eye movements.

The possible artifactual activity which might obscure or interfere with the ESG measure was dealt with during pre-testing trials. These results will now be discussed.

Pre-test results

ESG -Accounting for possible artifact

In order to support the theorized electrosalivogram (ESG) measure, it is necessary to account for possible artifactual causes of ESG activity. This has been done in previous testing , trials (Davis, 1988).

These previous trials focused upon systematically accounting for competing explanations of ESG activity by examining the likely contributors. These were electroencephalogralphy (EEG), electromyography (EMG), electrodermal response (EDR), electrooculography (EOG) and electrogastography (EGG). The EEG and EGG measures were found to have very inconsistent or else, non-existant correlations with the ESG measure and as a result have been discarded. However, EMG, EDR and EOG have shown fairly consistent correlations and thus will now be discussed.

Electromyography (EMG)

A problematic finding with regards to EMG appears to be with jaw clenching. There did appear to be some slow wave responses on the ESG channel in response to jaw clenches.

However, this slow wave activity is always accompanied by very fast, large amplitude responses which are easily distinguishable on the ESG recording. Thus, subject instruction to relax the facial muscles as much as possible has proven to be an effective safeguard against such EMG artifact. This is because once the subject is generally relaxed, smaller muscle activity (i.e., swallowing) has not been shown to significantly influence the ESG recording.

Electrodermal Response (EDR)

Another possible source of artifactual ESG recordings was thought to be electrodermal response (EDR). Thus, in order to record EDR, an ESG electrode was attached to one side (the skin had been cleaned and abraded) and and EDR electrode was attached to the corresponding point on the contralateral side (without skin preparation). Thus, it was proposed that the two records should covary to a large extent except that the amplitude of the ESG waves should be greater, because, the ESG should be producing a cleaner recording of salivary activity due to abrasion. However, in light of the fact that bilateral dominance of salivary glands does exist (Korchin and Winsor, 1940) the EDR measure will be relocated to the the subjects palm. This is because due to bilateral dominance we would now predict the

possibility of obtaining different recordings from the two parotid glands.

Electrooculography (EOG)

The measurement of electrooculography (EOG) in conjuction with ESG, has shown that eye movement (both horizontal and vertical) undoubtedly results in heavy interference of the ESG recording. Therefore, the subject will be instructed to fixate upon a point on the wall and not to move her eyes. This is one of the reasons that one trained subject was selected for use in this experiment because pre-test results indicate that new subjects asked to fixate upon a point on the wall still exhibit a great deal of eye movement. However, the single trained and experienced subject exhibited remarkably little eyemovement during experimentation.

In order to account for errant eye movements, electrodes will be attached to record both vertical (VEOG) and horizontal (HEOG) electrooculography.

Pre-test methodology

Based upon pre-test trials done by Brown, Hing and Aranoff (1988) there is reason to believe that an ESG measure is feasible. Although pre-test results have been inconclusive this may be due largely to problems of methodology and/or physiology which were not controlled for. These problems have been taken into account and the controls have been implemented in this experiment. The problems of methodology and/or physiology will now be discussed.

Problems of metholology and/or physiology

One problem not accounted for during pre-testing was the possible differences in size, structure or innervation of glands within an individual (Brown, 1970). These possible anatomical or physiological differences within gland pairs may account for the already mentioned finding that bilateral dominance occurs in the majority of subjests tested. Korchin and Winsor (1940) measured (parotid) glandular dominance in 78 subjects and found 44% were right gland dominant and 41% of the subjects were left gland dominant.

Further, the sex of the subject is another factor which may affect bilateral glandular dominance. Korchin and Winsor (1940) found that males were more likely to be left glanded than females, and females were more likely to be right glanded than males.

These bilateral differences were not controlled for in the pre-test trials and consequently may have introduced unnecessary variability into the results.

This experiment will attempt to limit this potential source of variability by consistently recording from over only one parotid gland. Also, because the single subject is female, the right parotid gland was chosen as the site for the recording in order to maximize the odds of obtaining the greatest salivary activity possible, based upon Korchin and Winsor's (1940) findings described above.

A second potential source of variability is that a stimulus applied to one side of a subject (i.e., taste stimulus to one side of the mouth) will cause the ipsilateral parotid gland to secrete more than the contralateral one (Lashley, 1916a). This also was not accounted for during pre-test experimentaiton, as the stimulus was placed on the centre of the tongue.

Again, this experiment will attempt to limit this source of variablility by always presenting the stimulus to the same (ipsilateral) side of the mouth from which the ESG recording is taken (the right side).

Another aspect of the experiment which will be more tightly controlled is the time of day that the subject is tested. This is because Dawes and Ong (1973) and Dawes (1972) found that human salivary glands follow circadian rhythms in which parotid saliva flow rates peak at around 4 pm.

In pre-test trials the time of experimentation was not controlled for and subjects were run at any time throughout the day.

In this experiment although it was logistically not practical to begin each experimental block at exactly 4 pm. The times of the 10 experimental blocks (each consisting of 10 trials -5 stimulus and 5 control) all commenced within plus or minus two hours of 4 pm. That is the earliest block commenced at 2 pm and the latest block commenced at 5:30 pm. The mean start time of the 10 blocks was 3:34 pm (Appendix B).

The first three problems discussed, bilateral dominance, increased saliva flow rate to ipsilateral stimulation and circadian rhythms are not fatal to the ESG experiment. That is, by not controlling for these problems only the variability in the magnitude of the response is likely to increase. In other words, the amplitude of the ESG recordings will likely vary greatly. For example, using the circadian rhythm findings, if the subject was tested at 11 pm it is likely that the amplitude of the ESG measure will be smaller than if the subject was tested at 4 pm. However, for a more persuasive experiment it is desirable to keep variablility to a minimum, thus, these potential confounds will be controlled for as previously stated.

There are two further problems of methodology, of greater consequence, which are the likely causes of the inconclusive results obtained in pre-test experimentation. These are the method of stimulus administration and the pre-mature use of a between subjects design.

First, in pre-test experimentation the method of stimulus administration was for the subject to open his/her mouth and a ground (to powdery consistency) stimulus was dumped onto the tongue from a small plastic container. However, when the subject opens his/her mouth prior to and during administration of the stimulus, it introduces artifacts such as eye movement and muscle activity into the ESG recording which tend to obscure any potential ESG activity. Also, accurate and consistent stimulus placement is also a problem using this method.

In the current ESG experiment the method of stimulus administration is a liquid stimulus (lemon juice) introduced via plastic tubing placed into the mouth. Thus, no active

participation is required by the subject prior to or during stimulus administration, consequently, the artifacts present in pre-test experimentation are eliminated. Also, because the plastic tubing remains in one location throughout the experimental block of ten trials, accurate and consistent stimulus placement is ensured.

Further justification for switching to a liquid lemon juice stimulus is that sour lemon drops (SLD) are the conventional stimulus for salivation research (Dawes, 1984).

The second problem of methodology during pre-test experimentation was that a between-subjects experimental design was pre-maturely used. A between-subjects design might more appropriately be used after the waveform has been identified, for the purposes of testing the generalizability of the results. However, by using a different subject for each experimental block (during pre-test experimentation) too much uncontrollable variability was introduced into the experiment. This is based upon the findings that there are large inter-subject differences in salivation (Lashley, 1916a; Spealman, 1943; White, 1977; Navazesh and Christensen, 1982).

Consequently, this ESG experiment will utilize a within-subject design using only one subject to minimize variability. One subject is deemed sufficient because the aim of this experiment is to validate the measure (that is, whether or not it is possible to record ESG activity), not to generalize the results to a broader population or even to assess its usefulness. By controlling as much of the variability as possible through rigid experimental procedures, this experiment should allow any differences between stimulus and control trials the opportunity to become apparent.

Further considerations

Other considerations to be taken into account are as follows.

It is of interest to know the percentage contribution of each of the major salivary glands. Schneyer and Levin (1955) discovered that under resting conditions the submaxillary glands contribute approximately 69%, the parotid glands about 26% and the sublingual glands contribute approximately 5% of total salivation collected from the three gland pairs.

One previously suggested problem was that if the subject is seeing, thinking or hearing about food, salivation may occur and cause spurious results. In fact, some pre-test experiments were designed to determine if salivation could be elicited when the subject saw pictures of food or thought about food.

However, review of the literature indicates that "there is to present time little incontrovertable evidence that anything short of actual taste or smell of food can produce salivation in the human" (Brown, 1970, p.75).

Another potential problem to consider is that the water used for rinsing may affect salivation because it is ingested. However, studies on the effects of dehydration and water loading have shown that, although dehydration will reduce salivation, water loading of the subject has no effect on salivation (Winsor, 1930a, 1930b). Therefore, the small amounts of water ingested during the experiment will not affect salivation.

Finally, one very important consideration is the possibility of the salivary reflex habituating. However, Lashley (1916b) found that the unconditioned salivary reflex is habituated very slowly if at all.

Logic of the experiment

Due to the fact that this is an exploratory validation study there exist many unknowns. For instance, the shape, latency and amplitude of the waveform remain unknown although, certain clues do exist as detailed in the "Theoretical framework" section.

Consequently, certain logical assumptions and inferences must be proposed in order to best design the experiment. The logic of the experiment is as follows. IF it is possible to record electrical activity originating from the activated parotid salivary gland <u>AND</u>, there are no artifacts interfering with the ESG recording <u>AND</u>, the subject is in a non-stimulated state prior to stimulus administration <u>AND</u>, the recording equipment is appropriately placed and sensitive enough <u>THEN</u>, there will necessarily be an amplitude response to the stimulus trials on the ESG measure which significantly exceeds that of the control trials.

Of course this is an ideal state which is never completely obtainable. However, by careful experimental design and appropriate operational definitions the possible confounds (the <u>AND's</u>) may be effectively minimized. If the <u>AND's</u> can be controlled effectively then it will be possible to test the hypothesis that; It is possible to record electrical activity originating from the activated parotid salivary gland via an appropriately placed electrode.

How this experiment will control the possible confounds (the AND's) will now be discussed.

Controlling the AND's

In order to test the hypothesis that it is possible to record the electrical activity originating from the activated parotid salivary gland via an appropriately placed electrode, it is first necessary to minimize the confounds (or the <u>AND's</u> in the logic statement).

First, although it is never possible to control for all potential artifact based upon the information detailed in the section "ESG –accounting for possible artifact" the following control measures were taken. 1) Perhaps the most important control is that a trained and experienced subject was used, and was instructed to fixate her eyes on a point on the wall and to relax her facial muscles as much as possible. 2) Electrooculography (VEOG and HEOG) electrodes were attached to determine whether eye movement may be accounting for any of the ESG activity. 3) Electrodermal response (EDR) electrodes were attached to the right hand to

determine any resulting artifact on the ESG channel.

Second, the experiment requires that the subject be in a non-stimulated state prior to stimulus onset. This is because if significant salivation is occuring prior to or during stimulus administration the results will be confounded. In order to ensure that the subject was in a non-stimulated state prior to each administration the following precautions were taken.

Following each rinse period (the subject was permitted to take as long as she wished to thoroughly rinse her mouth after each administration) the experimenter would wait until the subject had ceased all overt movements and audible sounds (i.e., throat clearing) and then allowed a minimum of fifteen (15) seconds to pass (the recovery period) before administering the next trial. The trained subject was effectively able to stabilize just about all waveform activity within this time period during informal testing.

Finally, the appropriate placement of the electrode was determined from the diagram in Appendix C so that the electrode placement would lie directly over the bulk of the parotid gland. Also, the sensitivity of the equipment is an uncontrollable factor, however, the equipment used is felt to be sufficiently sensitive.

Two-part results

The results of this experiment will be analyzed in two parts.

Initially, the results will deal with the validation of the electrosalivogram. That is, I will attempt to determine whether or not it is possible to record electrical activity originating from the activated parotid salivary gland via an appropriately placed electrode. Due to the fact that much of the variability has been controlled for within the experimental design, the statistical analysis for the validation will simply be a one-tailed t-test of the control and stimulus means (an amplitude measure).

The second part will be a descriptive analysis of the waveform with respect to deflection, latency, amplitude and waveform pattern. The analysis will consist of pictorial plots which depict the distinctive features of the waveform, such as, grand averages of control and stimulus trials.

A summary of the hypothesis and research question follows.

Hypothesis:

1) It is possible to record electrical activity originating from the activated parotid salivary gland using an appropriately placed electrode.

Research Question

1) Using a one-tailed t-test is the mean of the amplitude measure for the 50 stimulus trials significantly greater than the corresponding mean for the 50 control trials?

Definitions

1) Artifact will be operationally defined as any deflection on the ESG recording which can be directly identified as having been caused by a known source, i.e., eye movement or muscle activity.

2) Non-stimulated state will be defined as the point on the ESG recording which corresponds to stimulus onset.

3) An appropriately placed electrode will be defined as an electrode placed 2 1/2 cm caudally from the ear canal and 1 1/2 cm towards the nose in line with the tip of the nose.

4) The electrosalivogram (ESG) measure, is defined as the electrophysiological recording obtained from an appropriately placed electrode on the abraded skin surface.

5) The amplitude measure will be defined as the greatest absolute deflection (in millimeters) occuring in the ESG channel within ten (10) seconds of stimulus onset measured from baseline.

6) Baseline will be defined as a horizontal line drawn to intersect the point in the ESG channel which corresponds to stimulus onset.

METHOD

Subject

The subject was a 21 year old female. She is a friend of the experimenter and volunteered her time. The time involvement was between 1 and 1 1/2 hours per block of ten trials, thus the total time over the ten blocks was between ten and fifteen hours over a four day period.

Apparatus

The apparatus used was Dr. Christopher Davis' psychophysiological laboratory and equipment consisting of; 1) a Data General Nova System 3 computer; 2) a DP-11 Complot plotter; 3) custom designed software packages for collection, analysis and plotting created by the Simon Fraser University psychology department support staff; 4) the phazoamplifiers, filters, and recording equipment hardware were all locally built by Simon Fraser University electrical engineers for the psychology department; 5) a subject room containing a relaxing chair, and an electrode box and signal box both of which are connected to the control room hardware; 6) a calibration signal box; and 7) and ohm meter for checking impedances.

Supplies

The supplies were; 1) silver-silver chloride surface electrodes; 2) Redux electrode paste; 3) gauze pads; 4) a water bottle; 5) ethyl alcohol; 6) electrode collars; 7) two 30cc plastic syringes; 8) plastic tubing 93.5 cm long; 9) masking tape; 10) a ruler; 11) a screw; 12) the lemon juice stimulus, Realemon (commercial brand name); and 13) a wristwatch.

Procedure

The experimental design is a within subject repeated measures design. The 100 trials (50 stimulus and 50 control) were broken down into 10 blocks of 10 trials each for convenience. Each block of 10 trials contained 5 stimulus and 5 control trials which were randomly ordered (see Table 1–1, Appendix B).

Initially, the subject was briefed as to the procedure and purpose of the experiment and asked to sign a release form. Copies of the three forms given to the subject are included in the Appendices (Appendix D, E & F).

Prior to each block of ten trials the computer data file was configured to collect the data and the stimulus/control trial order was determined by the flip of a coin (heads=stimulus, tails=control) until all five stimulus and five control trials have been randomly ordered. The subject at no time knew whether a control or stimulus trial would be administered next.

The subject was then seated comfortably in the reclining chair and the electrode sites were located and prepared. The electrode placements were; 1) on the right mastoid process, this was used as the reference electrode for the ESG measure; 2) the ESG electrode –located over the right parotid gland as determined by measuring 2 1/2 cm caudally and 1 1/2 cm towards the nose, in line with the tip of the nose; 3&4) an electrode was placed at the outer edge of each eye (outer canthi) in order to take bipolar recordings of horizontal eye movement or horizontal electooculography (HEOG); 5&6) similarly, electrodes were placed above and below the left eye in order to record vertical electrooculography (VEOG); 7&8) electrodes were placed on the palm and the back of the hand, in order to record electrode right is to the right electrode to the right e

ankle.

Preparation of the electrode sites was as follows.

The mastoid and ESG electrodes were first cleaned with alcohol then abraded with redux paste until the subject felt it was sufficient. A screw was used to make a temporary indentation identifying the precise spot for the appropriate placement of the ESG electrode. Both electrodes were then attached and this pairing (mastoid/ESG) of electrodes was then tested on the ohm meter to ensure that the impedance was below 2000 ohms in order to minimize any interference in the recording. In most blocks the impedance was well below 1000 ohms.

For the four electrode sites recording eye movement HEOG and VEOG all the sites were cleaned with water (alcohol fumes burns the eyes) and abraded with redux paste. These were not abraded to the extent of the mastoid or ESG electrode sites because EOG activity is a very large waveform and heavy abrasion is unnecessary and irritating to the subject.

The EDR electrodes were set up to record monopolar recordings thus the inactive electrode (back of the hand) was abraded but the active electrode (palm) was not abraded (Stern, Ray and Davis, 1980).

Finally, the ground electrode (ankle) was cleaned with alcohol but not abraded.

The electrodes were then connected to the electrode box in the following manner.

Both the ESG and mastoid electrodes were connected to split leads and the signal was split and sent to both channels 1 and 2. This permitted the same ESG signal to be treated differently at the amplifiers. That is, channel 1 was basically unfiltered with the low pass (L.P.) filter set at 1000 Hz and the high pass (H.P.) filter set at 0.1 Hz with a gain (or amplification) of 1000. Channel 2 on the other hand was set at L.P.=50 Hz, H.P.=0.1 Hz and a gain of 2000. This was constant for all 10 blocks.

The HEOG electrodes were then connected to channel 3 on the electrode box. The HEOG filter settings were L.P.=1000 Hz, H.P.=10 Hz and the gain for the first 3 blocks was 500 which was later reduced to 200 for the remaining 7 blocks due to an unacceptable rate of resets.

The VEOG electrodes were connected to channel 4 and the filters were set at L.P.=1000 Hz, H.P.=10 Hz. However, during the first three blocks channel 4 was malfunctioning and the gain setting was turned down to 20 in order to avoid resets. In the remaining seven blocks the gain was then turned back up to 200.

The two EDR electrodes were plugged into channel 5 and the filters were set at L.P.=1000 Hz, H.P.=0.1 Hz and the gain=100.

The ground electrode was attached to the terminal input on the electrode box.

The stimulus and control syringes were prepared as follows.

One syringe was filled with the lemon stimulus and the other was filled with the tap water control. Once filled they were turned upside down (ejection nozzle facing up) to allow any air bubbles to rise to the top, the plastic tubing was then attached. The liquid was then injected the length of the tubing, pushing any air bubbles ahead of it. The 30cc syringe was large enough to contain sufficient liquid to fill the length of the tube and yet have the 15cc's required for the 5 trials per syringe (at 3cc each trial) remaining in the chamber. The two plastic tubes (stimulus and control) were then taped together approximately 2 1/2 inches from their ends so that they would remain together during the experiment.

The ends of the tubes were then placed in the lower right hand side of the subjects mouth, between the teeth and the cheek and towards the rear molars. The subject may have varied the position slightly from block to block, as the subject was instructed to place the tubes where she felt salivation was most likely to be elicited. The tubes were then attached

with masking tape placed horizontally across the lower mandible, this proved to be a very convenient and secure method of attachment. Liquid was then slowly injected through each tube until the subject tasted the slightest bit of each liquid (lemon and water). This ensured that the lemon stimulus and the water control were immediately available at the tube ends upon commencement of the first trials. The subject was then allowed to rinse any lemon from her mouth.

The subject then fixated her eyes on one point on the wall (either a tack or a piece of tape which was adjusted as the subject desired). The experimenter went to the control room to scan the data being received and asked the subject to perform some horizontal and vertical eye movements and jaw clenches in order to ensure all channels were functional and correctly set. The subject was then asked to relax, and when all but very small waveform activity had ceased on all channels the experimenter began collection of the data and re-entered the subject room.

The experimenter would then take his seat situated to the left and rear of the subject (outside of the subjects peripheral vision). The two syringes were placed on the experimenters lap and the stimulus/control sequence sheet, the wristwatch and the digital signal box were all located on a table within reach of the experimenter. After ensuring that the subject was motionless and quiet the experimenter waited the 15 second minimum recovery time and then administered the first trial, 3cc of either the stimulus or control. The syringe was operated with the left hand and the digital signal button was pressed with the right hand. The signal button was pressed just as the syringe was injected and the signal button was then held down until the 3cc had been administered, thus, the digital signal (channel 0) is a fairly accurate account of the onset, duration and offset of the injection of the liquids, probably accurate to within one second.

After the administration of the stimulus or control, the subject was left with the liquid in her mouth for one minute and fifteen seconds, after which the experimenter said, "rinse"

or "okay, rinse". At this time the subject would swallow and then take the water bottle resting on her lap and (with her left hand, the one without the EDR electrodes) rinse her mouth. The subject was instructed prior to the experiment to take as long as necessary in rinsing. Accordingly, this rinsing time varied from about 10 seconds after some control trials where the subject did not use the water bottle but only swallowed and cleared her throat, right up to 40 seconds, after some stimulus trials where the subject tried to rinse the lemon taste from her mouth.

Following this rinse period, once the subject had ceased any overt movements or audible sounds the fifteen second recovery period commenced, after which the next trial began. This was repeated until all ten trials were completed. The average time to complete a block of ten trials was 19 minutes and 57 seconds (average of the ten blocks) with the range from 18 minutes and 35 seconds to 21 minutes and 10 seconds.

It should be noted that the atmosphere in the subject room during the period of experimentation was very hushed with the subject remaining quiet and relaxed while the experimenter attempted to remain as quiet as possible while manipulating the necessary equipment.

RESULTS

Part I -Validation

A one-tailed t-test showed that the mean of the amplitude measure for the 50 stimulus trials was significantly greater than the corresponding mean for the 50 control trials (t=13.328, p=.005). Thus, the hypothesis that it is possible to record electrical activity originating from the activated parotid salivary gland using an appropriately placed electrode is supported by this data.

The mean amplitude measure for the 50 stimulus trials was 11.6 mm (\underline{SD} =3.864) and for the 50 control trials it was 4.91 (\underline{SD} =1.268).

The histogram in figure 1-1 displays the frequency distribution of the amplitude measure for all 100 trials (50 stimulus and 50 control).

Part II - Descriptive Analysis

The next step after achieving a significantly greater amplitude response during stimulus trials will be to attempt to define the waveform.

Pictorial Information

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Sometimes, the most persuasive argument available in the field of electrophysiology is to simply look at the recordings. This is certainly the place to begin.

Figure 2-1 shows a 51 second average (beginning 10 seconds before stimulus onset) of all non-resetting stimulus trials taken from channel 2. Figure 2-2 shows the same information for the control trials.

The reason that all trials containing resets have been eliminated from these grand averages is due to equipment limitation. The computer is not currently able to compensate for the resets, consequently a large response during a stimulus trial or DC electrode drift may cause the channel to reset. Although this is not a problem when scoring the data by hand, for the purposes of computer averaging 10 of the 100 trials will be eliminated from the grand average (9 stimulus and 1 control trial(s)). The consequences of omitting these trials are not significant as long as the grand average is used for descriptive purposes only.

These two grand averages, figure 2-1 (stimulus) and figure 2-2 (control), clearly illustrate the significantly greater amplitude measure in the stimulus trials. Now that all random fluctuations have been averaged across 41 stimulus trials (figure 2-1) and 49 control





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trials (figure 2-2) a consistent and distinctive waveform emerges in the grand average of the stimulus trial.

The waveform in figure 2-1 has a large positive (with respect to baseline) deflection, it begins, then peaks then returns towards baseline (although it does not cross the baseline for the duration of the average) within 10 seconds of stimulus onset and it is a relatively slow wave (in the order of seconds). These characteristics will now be explored.

Consistent Deflection

As described in the Theoretical Framework section, Lundberg's (1955) cat electrogram (Appendix A) shows a consistent initial deflection in one direction (positive with respect to baseline). Consequently, it was predicted that the ESG measure would show a similar, consistent initial deflection on stimulus trials, whereas there should be no consistent initial deflection on control trials.

However, the recordings did not prove to be conducive to an initial deflection measure because artifact such as slight DC electrode drift in the ESG channel at stimulus onset would have resulted in obviously spurious results. Also, Lundberg was able to receive an immediate response (within 0.6 seconds) because he had innervated the nerve, whereas, the stimulus used in this experiment is a natural one (lemon juice) which will provide more variability.

As a result, the deflection measure used (positive or negative with respect to baseline) was taken from the largest deflection within 10 seconds of stimulus onset. The stimulus trials were very consistent as forty-eight (48) trials had a positive deflection and only two (2) had a negative deflection. The two trials with the negative deflection appeared to have a higher than usual baseline due to activity prior to stimulus onset, thus, these two contradictory trials may effectively be explained.

The control trials conversely showed no consistent deflection with twenty-five (25) trials showing a positive deflection and twenty-five (25) trials showing a negative deflection. These results are consistent with a random fluctuation explanation, that is, on any trial where the salivary gland is inactive one would expect a fairly even random fluctuation between positive and negative deflections.

Latency

The latency from stimulus onset was measured at greatest amplitude (greatest absolute deflection) because of a problem with adequately defining wave onset. For measurement purposes the first of two or more peaks tied in amplitude, in any one trial, was used for the latency calculation.

The latency of the stimulus trials ranged from 1.6 seconds to 9.8 seconds with a mean latency of 6.22 seconds and a standard deviation of 1.85 seconds. The latency of the control trials ranged from 1.8 seconds to 10 seconds with a mean latency of 6.61 seconds and a standard deviation of 2.36 seconds.

Figure 2-3 shows a histogram of the latency distributions.

Amplitude

As stated in Part I the mean amplitude of the stimulus trials (in millimeters) was 11.6 mm with a standard deviation of 3.864 mm and a range of 6 mm to 21.5 mm. This converts to a mean amplitude of 77.37 microVolts with a standard deviation of 25.77 microVolts and a range of 40.02 to 143.405 microVolts.

The mean amplitude of the control trials was 4.91 mm which converts to 32.75 microVolts with a standard deviation of 1.268 mm or 8.46 microVolts and a range of 20.01 to 53.36 microVolts (3 mm and 8 mm respectively).



DISCUSSION

Validation

The finding that there is a significantly greater amplitude response during stimulus trials, supports the stated hypothesis that it is possible to record electrical activity originating from the activated parotid salivary gland using an appropriately placed electrode.

Not only was the stated hypothesis supported, but also some of the predictions describing the characteristics of the waveform. These findings will now be discussed.

Waveform Characteristics

The waveform depicted in the grand average of all non-reset stimulus trials (figure 2-1) resembles the waveform from a prototypical individual stimulus trial (figure 3-1).

These waveform characteristics are:

1) A consistent largest peak deflection (positive with respect to baseline) as described in the Results section.

2) A significantly greater peak amplitude measure was found in the stimulus trials.

It was predicted that the peak amplitude of the waveform was not expected to be greater than 2 mV because, Lundberg's (1955) recordings from the exposed cat submaxillary glands showed maximum amplitudes of around 2 mV. This prediction was in fact supported as the range of amplitudes in the stimulus trials was between 40.02 and 143.405 microVolts.

3) Another prediction from Lundberg's data provided a minimum latency period of between 0.2 and 0.6 seconds. It was expected that the latency of the ESG activity would exceed these predicted times because a natural stimulus (lemon juice) was used (as opposed to nerve innervation). However, due to problems in defining wave onset this method of analysis was





abandoned and instead peak latency was determined.

An interesting finding was obtained from this peak latency measure. Although the ranges were very similar between control and stimulus trials the standard deviation was greater in control trials than in stimulus trials (2.36 and 1.85 seconds respectively). This greater variability which is seen in the control trials may be a result of the peak latencies being randomly distributed over the 10 second measurement period, based upon the assumption that the tap water control does not elicit salivation and that any peaks are a result of random fluctuations around the baseline. However, this assumption may not be correct and the fact that the standard deviation (although larger) is only larger by 0.51 seconds provides some support for this alternative assumption.

Another expected latency finding was that the latencies of the stimulus trials should be quite variable because of many unknown factors influencing the exact time of gland activation. Some of the possible unknown factors which might cause variable latencies when using a natural stimulus were discussed in the Theoretical Framework section. Accordingly, this may be a contributing factor in the large range of latencies found in the data, from 1.6 to 9.8 seconds.

4) Finally, one waveform characteristic based upon the physiological finding that myoepithelial cells behave much like smooth muscle cells, predicted that the waveform would be a relatively slow wave (in seconds) similar to EGG recordings. This prediction does appear to be supported by the ESG recording of the stimulus grand average (figure 2–1) as well as individual stimulus trials (figure 3–1). This slow waveform also exhibited a slight positive skew with a quick onset and slower offset.

To summarize, the stated hypothesis was supported and some characteristics of the waveform were defined. That is, the ESG waveform has a consistent peak amplitude deflection, a peak amplitude ranging between 40.02 and 143.405 microVolts, a variable peak

latency ranging between 1.6 and 9.8 seconds and, the waveform has slow wave characteristics.

Other Findings

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There was an unpredicted finding which may be interesting to explore in future research. At approximately three seconds after stimulus onset there is a negative deflection in the ESG recording. This negative deflection can be seen occuring on both stimulus and control grand averages (figures 2–1 and 2–2). These deflections are not readily apparent on many individual trials and were thus thought to be random fluctuations prior to creating the grand averages. The fact that the negative deflection appears in both control and stimulus averages (at exactly the same latency) leads to the proposition that the introduction of any form of stimulus (whether lemon juice of tap water) results in a consistent negative deflection on the ESG recording. It may be that this negative deflection is a component of the salivary waveform whose characteristics have been described. However, if this is a salivary response component due to salivation to the tap water on the control trials then, it is difficult to explain why there is not a positive deflection immediately following it on the control average, (see figure 2–2) similar to the one found on the stimulus average (figure 2–1). This finding is not easily explainable from the available data and thus is a source of future study.

Another finding that was briefly explored was that tongue movements may provide another uncontrolled for source of artifact. Thus, the subject's ESG measure was recorded while the subject was instructed to "move your tongue as much as you have during experimentation", the results are shown in figure 3-2.

Next, the subject was instructed to move her tongue "more than you have during experimentation". These results are shown in figure 3-3.

These results are inconclusive but do not appear to be the cause of the ESG activity obtained, although, they certainly may be contributing factors. The reason that tongue movement is not considered to be a confound to the results, is because the amplitude of



even the large tongue movements (figure 3-3) rarely exceed 50 microVolts. Whereas, ESG activity ranges from 40.02 to 143.405 microVolts. Further, the waveform duration of the tongue movements is considerably shorter than the stimulus average (figure 2-1) or the prototypical stimulus trial (figure 3-1).

Procedural Comments

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The overall analysis of the data did not take into account any of the artifactual controls (HEOG, VEOG and EDR). This was because a preview of the data (individual trial recordings) led to the conclusion that the trained and experienced subject was very good at fixating her eyes (HEOG and VEOG) and thus did not provide much artifact. Also, whatever artifact was produced by eye movements did not appear to occur consistently at any one period during experimentation thus, it was felt that the results of this random eye movement would cancel out in the overall analysis.

However, HEOG and VEOG although not used in the data analysis was used for explanatory purposes to explain some of the divergent results. An example is shown in figure 3-4.

Palmar electrodermal response (EDR) which had not been pre-tested, provided no apparent artifact in the ESG recording and thus it's usefulness must be questioned in any further ESG research.

The exploratory nature of this project must be emphasized. Due to the fact that this is an attempt to develop a new psychophysiological measure none of the characteristics were known beforehand thus, it was not possible to develop rigorous procedures which would test for specific characteristics such as latency and amplitude. Consequently, after the initial validation of the measure the data was previewed for consistent patterns in order to provide some characteristic trends in the data which might be tested more concisely in future research.



It should also be acknowledged that the measurement template used for the validation analysis was based upon logically analyzing the potential and actual waveform, in order to determine the most suitable form of measurement. For example, defining the baseline as the point in the ESG channel which corresponds to stimulus onset was seen as a reasonable method for determining the baseline, because theoretically the subject should have returned to a "normal" non-salivating state by this time. However, there are reasonable and logical arguments for deriving a different baseline from this one which may be just as, or even more, valid. Consequently, future research may provide more efficient or accurate measurement templates for the ESG measure once the limits of it's proposed characteristics have been defined.

Future Research

If future research of the ESG measure is to prove productive, two short term research goals must be considered in further detail. They are, inter-subject reliability and the practicality of the ESG measure.

It is possible that the ESG measures may still prove to be useful even if it is shown to be unreliable for a between-subjects measure. Possible uses might include measuring fluctuations in salivary activity, within an individual, when presented with certain food choices or smells (perhaps in market research).

However, the ability to test across a wide range of subjects would clearly result in a more powerful measure which may eventually provide the clinician with further diagnostic tools for such things as stress, or perhaps, a number of other cognitive or physiological ailments may be been correlated with salivation.

Therefore, although it was initially necessary to minimize variability and use only one subject, future research must clearly tackle this problem of inter-subjects reliability.

Finally, the practicality of the ESG measure must be determined. Recall, the initial justification given for the development of the ESG measure. Namely, a measure which is neither cumbersome, intrusive and is able to accurately measure the time-course of salivation. Undoubtedly, if ESG activity can be significantly correlated with saliva flow in further validation studies, it would be one of the most precise measures of the time-course of salivation available.

However, just how non-intrusive and non-cumbersome is it to ask a subject to stare at a point on the wall for an extended period of time without significantly moving their head or facial muscles. This in fact is a very boring and monotonous chore and as the subject stated after three consecutive blocks in one afternoon (30 trials), "if I had to go another minute I would have gone crazy!".

Also, in animal studies Burgen and Emmelin (1961, p.199) state that, "despite valiant attempts, analysis of the external salivary electrogram has not yielded results useful in explaining secretory activity but the presence of such electrical activity has encouraged the development of more sensitive methods of study".

Although, with human subjects who can say how they are feeling, thinking or sensing, psychophysical data can be correlated with ESG's in order to unveil perhaps unknown relationships.

Summary

This experiment was exploratory research which attempted to validate the electrical recording of salivary activity.

First, an overview of the area of salivation and the current state of salivary measurements were covered.

Second, the theoretical framework was described and, based upon animal studies and the physiology of the salivary glands some rough predictions for deflection, latency, amplitude and waveform were made.

Third, pre-test results were described which took into account possible artifacts which might interfere with or obscure ESG activity. Then the problems of methodology and/or physiology which had not been accounted for during pre-testing were first discussed and then controls were suggested and implemented for this experiment.

Next, the logic behind the experimental design was stated and the possible confounds to the logic were discussed and controls were again implemented.

The hypothesis stated that, it is possible to record electrical activity originating from the activated parotid salivary gland using an appropriately placed electrode. This was then defined as a research question. The procedure was then described in detail because the experiment was designed to keep all conditions equal except for the different administrations of lemon juice or tap water.

It was found that the mean amplitude of the stimulus trials was significantly greater (p=.005) than the mean amplitude of the control trials. This supports the stated hypothesis.

After validation, a descriptive analysis of the waveform was performed in order to delineate the waveform characteristics. They were; a consistent deflection, a variable latency, a large amplitude measure (compared to controls) and a slow waveform.

These results were discussed, and it was concluded that further experimentation would be necessary in order to determine the limitations of these characteristics.

Finally, it was pointed out that future research, with the immediate goals of determining inter-subject reliability and the issue of practicality would be necessary in order to judge the feasibility of pursuing this ESG measure.

A Hppendix



Four consecutive electrograms recorded from the cat submaxillary gland. Illustrating a consistent initial deflection and variability of the waveform (Lundberg, 1955).

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TABLE 1-1. Table of experimental design.

	Approximate		_	Trial Number	
Date	Starting Time(hrs)	Block #	Tape Filename	Stimulus	Control
4/22/89	14:00	1	MT0:08	#1,2,4,9,10	#3,5,6,7,8
4/22/89	15:00	2	MT0:09	3,4,7,8,9	1,2,5,6,10
4/22/89	15:45	3	MT0:10	2,3,4,8,10	1,5,6,7,9
4/23/89	14:15	4	MT0:12	3,5,8,9,10	1,2,4,6,7
4/23/89	15:30	5	MT0:13	1,3,4,6,9	2,5,7,8,10
4/25/89	14:30	6	MT0:15	2,3,4,6,8,	1,5,7,9,10
4/25/89	15:40	7	MT0:16	1,2,4,9,10	3,5,6,7,8
4/25/89	16:00	8	MT0:17	4,6,7,9,10	1,2,3,5,8
4/26/89	16:50	9	MT0:19	1,3,7,8,9,	2,4,5,6,10
4/26/89	17:30	10	MT0:20	1,3,4,6,9	2,5,7,8,10

<u>Appendix C</u>



endix

Simon Fraser University

INFORMED CONSENT BY SUBJECTS TO PARTICIPATE IN A RESEARCH PROJECT OR EXPERIMENT

Note: The University and those conducting this project subscribe to the ethical conduct of research and to the protection at all times of the interests, comfort, and safety of subjects. This form and the information it contains are given to you for your own protection and full understanding of the procedures, risks and benefits involved. Your signature on this form will signify that you have received the document described below regarding this project. that you have received an adequate opportunity to consider the information in the document, and that you voluntarily agree to participate in the project.

Having been asked by MARTIN HING

of the

PSYCHOLOGY Faculty/School/ Department of Simon Fraser University to participate in a research project experiment, I have read the procedures specified in the document entitled:

ELECTROSALIVOGRAMA (ESG) SURJECT FAMILIARIZATION

I understand the procedures to be used on this experiment and the personal risks to me in taking part.

I understand that I may withdraw my participation in this experiment at any time.

I also understand that I may register any complaint I might have about the experiment with the chief researcher named above or with

ROGER BLACKMAN NP.

Dean/Director/Chairman of PSYCHOLOGY Simon Fraser University.

Copies of the results of this study, upon its completion, may be obtained by contacting:

MARTIN HING

I agree to participate by PERMITTING REICKAING ELECTRODES TO RE ATTICHED

TO ME . FIVATING MY EYES ON A POINT ON THE WALL RELAXING MY

EASTAL MUSCLES AND INGESTING A AWAGAGA STIMULUS.

(state what the subject will do)

as described in the document referred to above, during the period:

at <u>ASYCHOLOGY AEPT. - SFU</u> <u>RM 4211</u> (place where procedures will be carried out)

NAME (Please print):	
ADDRESS:	
SIGNATURE:	WITNESS:
DATE:	

Once signed, a copy of this consent form and a subject feedback form should be provided to you.

SFU RESEARCH ETHICS REVIEW COMMITTEE

sendix

Form #4

SUBJECT FEEDBACK FORM

Completion of this form is optional, and is not a requirement of participation in the project. However, if you have served as a subject in a project and would care to comment on the procedures involved, you may complete the following form and send it to the Chairman, University Research Ethics Review Committee. All information received will be treated in a strictly confidential manner.

Name of Principal Investigator:
Title of Project: <u>ELECTRUSALIVOGRAM</u> (ESG) - <u>NEUELCIPALENT</u> OF
A NEW PSYCHOPHYSIOLOGICIAL MIEASURE
Department: <u>PSYCHOLOGY</u>
Did you sign an Informed Consent Form before participating in the project?
Were you given a copy of the Consent Form?
Were there significant deviations from the originally stated procedures?
I wish to comment on my involvement in the above project which took place:
(Date) (Place) (Time)
Comments:
Completion of this section is optional
Your name:
Address: Telephone:

This form should be sent to the Chairman, University Ethics Review Committee, c/o Vice-President, Research and Information Systems, Simon Fraser University, Burnaby, B.C., V5A 1S6.



Information Sheet for Subjects

Form #5 Simon Fraser University Psychology Department

ESG SUBJECT FAMILIARIZATION

This experiment has been designed to measure salivation via recording electrodes placed on the surface of the skin. Electrodes will be placed on your face, hand and ankle. At no time during the experiment will any electricity pass through the electrodes except that which your body generates itself (very small amounts).

During the experiment you will be comfortably seated, asked to relax and fixate your eyes on a point on the wall this will help in obtaining accurate recordings. Also, please refrain from speaking during this period.

Subsequently, you will be given a number of trials where you will either receive a lemon juice stimulus or else, a tap water control via plastic tubing placed in your mouth. Tubing is disposed of after each subject.

This experiment will take approximately 1 and 1/2 hours, so please notify the experimenter of any time constraints you may have prior to commencing the experiment. Also, you may withdraw from participating in this experiment at any time should you wish to do so. Thank you for your assistance and cooperation.

Martin Hing

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