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INFORMATION PROCESSING FROM CHEMORECEPTORS

by

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INTRODUCTION

In the past few years there has been a great deal of emphasis on the interdisciplinary type of approach to many problems. The primary reason for this is that most problems of today are of such magnitude and complexity that a research specialist is incapable of exploring and answering all the many facets encountered. For instance, it is not surprising to find a research team made up with mathematicians, chemists, surgeons, engineers, physiologists, and psychologists to name a few disciplines.

A major problem then encountered is one of communication between various members of the research team. To overcome this deadlock, some universities are tailored to offer a multi-disciplinary program of studies. The recent field of bio-medical engineering (also known as bio-engineering, etc.), for instance, is such an approach for it endeavors to instill in the physical scientist and the engineer the language, techniques and tools necessary to understand and explore the many problems encountered in the living system and the biosphere. Conversely, the life scientist in such a program is provided with a set of "instruments" that enable him to apply and communicate the analytical techniques available to the engineering profession. Together, they can provide a very strong component to the research team; each using his own major

professional qualifications while at the same time exploring and understanding the others' profession.

With this background in mind it should not be too surprising to find electrical engineering techniques being applied to such a diverse study as the electrophysiology of the gustatory system and the possible coding mechanism involved. The realm of electrophysiology, which is essentially concerned with the flow of ions in nervous tissue and the subsequent recording of it, has a parallel in the flow of current in electrical transmission lines with which electrical engineers are very familiar. Information processing and coding techniques are also very familiar to the electrical engineer involved in communication and information theory, and consequently one might expect these techniques to be of aid in understanding the coding mechanism of the gustatory system. The analytical techniques needed to analyse the resultant data can be readily approached with an engineering background. Finally, the sophisticated electrical instrumentation needed to carry out such a study lends itself to engineering ingenuity.

The object of this study was to investigate the gustatory system of the pigeon from an electrophysiological point of view. In particular, the afferent quality coding mechanism was investigated from both a temporal and a spatial pattern point of view. Subsidiary topics such as intensity afferent coding, individual pigeon variations to a given test solution,

the specific nerve activated by the chemical solutions, the relative effectiveness of different ions both from a single fibre and a multiple fibre viewpoint, and the electrophysiological and behavioral correlations between stimuli were also investigated.

Single fibre, few fibre and multiple fibre data were collected in carrying out the analysis. A total of 22 pigeons were used during experimentation; 12 for dissection purposes to locate and identify the nerve sensitive to chemoreception, and 10 for electrophysiological data extraction.

The pigeon was used because: (1) few studies have been concentrated on the pigeon; (2) single fibre data were easy to extract compared to mammals since very few gustatory fibres seem to be evident with the former compared to the latter species; (3) since only two basic modalities--salt and sour--seem to be conveyed by this species, it was presumed that a simple coding scheme must be involved.

Since the writer's background is essentially of electrical engineering origin rather than that of the life scientist, it was felt that a comprehensive review of the literature should be carried out. This has been done. Also, a very detailed engineering description is included in the methods, materials, and instrumentation section.

LITERATURE REVIEW

Introduction

The peripheral sensory nervous system associated with the oral cavity has been studied in four different ways: electrophysiologically, behaviorally, morphologically (both macro-and microstructurally) and from a molecular (biophysical and/or biochemical) point of view. This review focuses on the prevailing literature dealing with the electrophysiological aspects of the vertebrate oral sensory mechanism. Reviews on behavioral, morphological, and molecular approaches are considered only insofar as they aid in elucidating electrophysiological response behavior. Invertebrates are not included in this review. Furthermore, studies at the central nervous system level to various sensory stimuli are completely disregarded; only those pertaining to the peripheral nervous system are given consideration.

The review starts with the comparative aspects of the tongue peripheral nervous system. Included here are anatomical features of thermoreceptors, mechanoreceptors, and gustatory receptors along with their peripheral sensory nerves. Electrophysiology is then introduced by way of those studies describing neural activity in the taste receptors themselves, and continues on with peripheral nerve activity.

Next those studies relating to species differences to chemical stimuli, topics covering neural activity to special taste solutions, and current topics of interest on gustatory electrophysiology are covered. The final section stresses the electrophysiology of the non-gustatory nervous system.

Because of the rapid rate of data collection in this field in recent years several excellent reviews have appeared (13, 15, 20, 29, 141, 149, 191, 192, 193, 197, 200). Several symposia have taken place that cover various aspects of cutaneous sensory functions and allied topics (37, 78, 102, 103, 108, 159, 194, 196).

Some Anatomical Considerations

Specifically, the sensory receptors of interest are those subserving taste, touch or pressure, and temperature. Taste receptors are located as taste buds mainly in the moats of the papillae. Free nerve endings and organized structures form the basic units for thermoreception and mechanoreception.

Gustatory receptors and peripheral nerves

Taste buds, containing the basic gustatory unit cell, are associated with the fungiform papillae on the anterior two-thirds of the tongue, and the circumvallate papillae forming a V-shape or chevron on the back of the tongue in mammals.

Studies on chickens, by Lindenmaier and Kare (130), have shown that some taste buds are found on the posterior portion of the tongue, contiguous to the row of large non-gustatory papillae which form a chevron across the base of the tongue. They noted that while the pharynx region contained the largest number of taste organs, the anterior portion of the chicken tongue was completely devoid of them. There is a close morphological relationship between taste buds in mammals and those in chickens.

The number of taste buds varies with the species. Lindenmaier and Kare (130) found 24 taste buds in the chicken; Moore and Elliott (133) reported an average of 37 in the domestic pigeon. Each taste bud consists of a number of elongated taste cells having large nuclei. Beidler (14) states that from 4 to 8 different taste cells from a single taste bud may be innervated by the same single axon from the peripheral gustatory nervous system. Occasionally a single taste cell is doubly innervated by the same axon. Beidler estimates that the number of taste nerve fibres innervating a single human fungiform papilla is between 6 and 12. Kusano and Sato (126) suggest that one unit taste cell could be innervated by several fibres.

Rapuzzi and Casella (156) have electrically stimulated a single fungiform papilla in the frog and recorded the orthodromic activity from the glossopharyngeal nerve; and

conversely, stimulated the nerve trunk to record antidromic activity arriving at a papilla. They found an average of 9 glossopharyngeal nerve fibres innervating each papilla. Neural records from the fibre bundles innervating a papilla indicate that the impulse traffic propagates antidromically through collateral branches to an average of 17 neighboring papillae. Several receptor cells are therefore connected to a single afferent fibre. It was concluded that each glossopharyngeal nerve fibre divides into an average of 6 branches each going to different papillae.

Taste cells have been traditionally classified into two groups: those that are sensory in function, and those that are supportive. Recent work by Beidler (12, 14), Beidler and Smallman (23), and De Lorenzo (36) in separate laboratories has challenged this concept. They found that many cells are in a continuous state of degeneration. Simultaneously, new receptor cells growing in from epithelial cells surrounding the taste buds, supplant the degenerating old cells. Beidler (14) has reported an average turnover of about 3 to 5 days per rat taste receptor cell.

On frogs (Rana pipiens), Robbins (158) was able to show a continuous decrease in taste bud size over a 43 week period after lingual nerve denervation. At first, cell shrinkage was predominant; in later stages, total cell loss was evident. The rate of cell loss (11.6 cells per week) in denervated

frogs was comparable to the rate of cell birth (8 cells per week) in untampered animals. He concluded that the process of individual cell division is nullified through denervation. A surprising observation was that some of the denervated taste buds, after a certain time lapse, were reinervated by tiny fibrils from other non-gustatory nerve fibres.

In mammals, gustatory sensations are mediated primarily through the chorda tympani branch of the facial nerve (VII cranial nerve) and the lingual branch of the glossopharyngeal nerve (IX cranial nerve).[1] The chorda tympani nerve innervates the taste buds of the fungiform papillae. Other sensory and motor nerves are also subserved by the chorda tympani branch (14). Taste buds associated with the foliate and circumvallate papillae are innervated by the lingual branch of the glossopharyngeal nerve.

Kitchell, Ström, and Zotterman (116) found that the nerves to the tongue of pigeons and chickens arose from two independent branches of the glossopharyngeal nerve which they classified as the lingual nerve and the laryngo-lingual nerve.

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[1]Snapper (168) has indicated that the chorda tympani nerve in human beings carries the taste perception for the entire tongue area, and that there is no taste conduction by way of the glossopharyngeal nerve.

They found that the lingual nerve branches from the glossopharyngeal nerve and journeys anteriorly to the lingual artery for a short distance. It then separates and continues above the lingual bone (cornu of hyoid bone) to enter the tongue. Its main route is to the anterior portion of the tongue; although several branches innervate part of the tongue wings, part of the posterior portion of the tongue, and the lateral wall of the pharynx. The lingual nerve of the pigeon is considerably smaller than that of the chicken.

The laryngo-lingual nerve descends from the glosso-pharyngeal nerve distal to the lingual nerve origin. It activates the lateral wall of the pharynx, the surfaces of the larynx, and the posterior portion and wings of the tongue.

Recently, some attention has been paid to the microscopic anatomy and conduction velocities of taste mediated afferent nerves. Kitchell (113) has determined the number and diameter of the myelinated fibres in the chorda tympani nerve of several domestic animals. With the exception of the dog, he found that the number of myelinated fibres was directly proportional to the body mass of the animal species. The average number of fibres and the percentage of fibres having a diameter less than 6 microns for each animal is as follows: 1555, 81% for cat; 2854, 40% for dog; 3321, 49% for goat; 3423, 55% for sheep; 4366, 53% for pig; 5265, 35% for cow; 5735, 37% for horse. The percentage of myelinated

fibres larger than 10 microns in diameter are as follows: 10% for horse and cow; 3% for goat, pig, and sheep; 1% for cat. The largest diameter fibres found were in the 12 to 14 micron group. In dogs, Iruichijima and Zotterman (97) found the following average conduction velocities (in meters per second) for different taste fibres in the chorda tympani and lingual nerve: 10.2 for "salt" fibres; 8.2 for "sweet" fibres; 3.2 for "bitter" fibres; 11.6 for "acid" fibres; 8.5 for "water" fibres. The overall average conduction velocity varied from 2 to 18 meters per second. Rapuzzi and Casella (156) determined a conduction velocity range from 2 to 8 meters per second for afferent fibres from the glossopharyngeal nerve of the frog. Békésy (24) estimated a mean conduction speed of 40 meters per second along human taste nerves for different chemical solutions at 23°C. The mean value of the taste conduction velocity doubled to 80 meters per second when solutions were warmed from 23° to 38°C.

Non-gustatory receptors and peripheral nerves

Weddell (182) has stated that morphological evidence indicates two separate types of nerve endings subserving the four primary somesthetic sensations.[1] These are the

[1]The four primary somesthetic sensations are those subserved by touch, warmth, cold, and pain (38).

unencapsulated or free nerve ending and the encapsulated nerve ending. The latter are very diverse in size, shape, and structure. Weddell states that despite these morphological differences, the four primary modalities of cutaneous sensibility can be readily aroused from all sites examined.

Free nerve endings are formed by repeated division of fibres as they near their terminations. After several divisions, the fibre branches lose their myelin sheaths, and then their neurilemma, leaving only the naked axis cylinder. A specialized type of ending is known as Merkel's disc (38).

Quilliam (155) has listed the encapsulated endings that are special and found in all species. These include the Pacinian corpuscle (the largest known), the Herbst corpuscle (common in the avian), the innominate corpuscle, genital corpuscles, and the Grandry corpuscle. Others include Meissner's corpuscle, Krause end bulbs, and the Ruffini cylinder (38).

In the tongue the four primary sensations are subserved by afferent fibres from the chorda tympani nerve, the lingual nerve, and the trigeminal nerve (V cranial nerve). Iruichijima and Zotterman (97) have quoted average conduction rates for afferent fibres in the chorda tympani and/or lingual nerve of the dog for the following fibre types: (1) warm fibres -- 9.1 meters per second in chorda tympani; (2) cold fibres -- 9.3 meters per second in lingual nerve; (3) touch fibres --

11.1 meters per second in chorda tympani, 11.6 meters per second in lingual nerve; (4) pressure fibres -- 4.3 meters per second in chorda tympani, 3.4 meters per second in lingual nerve. Zotterman has judged cold fibres to be Aδ fibres, warm fibres to be slightly larger than Aδ fibres, and touch fibres to be A fibres. All fibres were medullated (97,199).

The depth of cold receptors in the cat has been determined by Hensel, Ström, and Zotterman (86; summarized in 197) to be approximately 0.18 millimeters below the tongue surface. Dodt (42) has shown that thermal sensations are due to direct activation of thermal receptors within the sensory end organ. Direct stimulation of the myelinated nerve fibre innervating the receptor seldom produced a response.

Electrophysiology of the Gustatory Peripheral Nervous System

Electrophysiological studies of the mechanism underlying impulse conduction in gustatory fibres has been investigated in four different ways: the single taste cell approach, the single papilla approach, the single or few nerve fibre approach, and the multiple fibre or nerve bundle approach. Some of the more important findings to each of these approaches are outlined in the following paragraphs.

Single taste cell studies

The first comprehensive study of the depolarizing action of the taste cell to various taste solutions was undertaken by Kimura and Beidler (111, 112; summarized in 12, 14, 15, 20) on rats and hamsters. With KCl-filled microelectrodes, they were able to record a resting potential which varied from -30 to -50 millivolts. The magnitude of the resulting electrical depolarization was taken as an index of the response of the taste cell to the applied stimulus. The resulting response-concentration curves, representing the four basic taste qualities,[1] indicated that each receptor cell is responsive to one or more stimuli and is not specialized exclusively to but one type of chemical stimulus. There was also variability in overall sensitivity between different taste cells to a series of select stimuli of varying concentration. For instance, many taste cells do not respond to sucrose, but others do. Some respond to sucrose as well as acid and salt. They concluded that their results were similar to those found from single fibres of the chorda tympani nerve. Further studies by Tateda and Beidler (178), in which they designated the potential as a receptor potential, supported these conclusions.

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[1]The four basic taste qualities are considered to be salty, sweet, acid, and bitter tastes.

Single papilla approach

By stimulating a single papilla with a suitable stimulus, neural data can be collected from fibres in the glossopharyngeal and chorda tympani nerves, and an understanding of the type and number of fibres innervating a single papilla can be realized. This approach differs from the single and multiple fibre approach in which the total tongue is bathed by an appropriate solution, thus eliciting responses from several papillae.

Pfaffmann, Fisher, and Frank (152), noting that other investigators had difficulty in stimulating the glossopharyngeal nerve receptive field in the rat with conventional techniques, devised a glass pipette, of diameter 0.1 to 0.2 millimeters, which could be easily inserted into the moat of a single circumvallate papilla. Solutions comprising the four basic taste modalities were made to flow through the pipette into the papilla and drained out via a wick such that other papillae were not subjected to stimulation. This technique enabled them to determine that quinine HCl was the most effective stimulus, in terms of overall magnitude, for the glossopharyngeal nerve receptive field. Other modalities gave similar response magnitudes in both nerves using the two techniques mentioned above (22, 144).

Earlier, Kusano and Sato (126) stimulated a single fungiform papilla in the frog (Rana nigromaculata) to

mechanical stimuli (touch and pressure), distilled water, and the four fundamental modalities of taste. Finding responses to all stimuli, they concluded that discrimination to different sapid solutions was not a function of the number of taste receptors employed.

Subjective tests on human observers enabled Békésy (25, 26, 27) to state unequivocally that only four distinct tastes are produced when single papilla are stimulated either electrically or chemically. He observed that chemical and electrical excitation of a single papilla produced very similar sensations qualitatively. Chemical stimulation of a large receptive field gave a noticeably different quality from that of the single papilla approach. His major contention was that the nervous discharge pattern does not play a role in the discrimination of simple chemical stimuli; but that this pattern becomes important for complex taste analysis primarily because delays are introduced from stimulating different kinds of taste buds as the solution moves over the tongue surface.

Single or few gustatory nerve fibre studies

A single nerve fibre may innervate several taste cells, hence the question arises as to how specific are the single nerve fibres. Various research units have attempted to investigate this question. Pumphrey (154), in 1935 stated that "There is evidence that a single taste bud responds to substances in one only of these categories" (sweet, sour,

bitter, and salt). His "evidence" was based on single fibre responses to salt and acid solutions on the tongue of the frog. No responses were recorded from sweet and bitter substances.

Single fibre studies on cats by Pfaffmann (143, 148) revealed three main fibre types in the chorda tympani nerve: (1) those responding only to acid; (2) those responding to salt and acid; (3) those responding to bitter and acid. No single fibres responding to sugar were specifically found (although it was acknowledged that multiple fibres do sometimes respond to sugars). Pfaffmann (144) observed that the thresholds for any one substance are different from one fibre to another as related to chorda tympani elements in the rat, cat, and rabbit. He noted that the frequency of discharge during the first second approximates a sigmoid function of the logarithm of the stimulus concentration. Each of his single fibre preparations was characterized by a different pattern of sensitivity to the four basic taste stimuli. Furthermore, each fibre responded to more than one of the four basic taste stimuli, but to varying degrees. No simple classification of receptor types was obvious.

A single chorda tympani fibre analysis on the cat by Cohen, Hagiwara, and Zotterman (35) indicated that there were receptor units of mixed and multiple sensitivity. For instance, several single fibre preparations responded

specifically to either distilled water, or very low concentrations of quinine HCl, and mineral acids such as HCl. This fibre type was referred to as a "water" fibre. Another fibre type was referred to as a "salt" fibre. In addition to responding to various salts above 0.1 molar (M) concentration, it was also stimulated by acid. A third fibre type, the "quinine" fibre responded primarily to quinine HCl and seldom to strong acids. It was not stimulated by hypertonic salt solutions or by distilled water. The final type of fibre isolated, the "acid" fibre responded only to acids below a pH of 2.5.

Fishman (61) showed that even though individual single fibres differ in sensitivity to each of the four primary taste qualities, the response of the whole chorda tympani nerve, as indicated by integrator studies, is characteristic, constant and reproducible within a given species. By appropriately summing single fibre responses, he was able to produce curves similar to the integrated whole nerve response.

Andersen, Funakoshi, and Zotterman (4) took electrophysiological recordings to determine the interaction between sweet and salty stimuli. They were able to classify single fibres into five different types, namely: (1) specific "sweet" fibres; (2) fibres responding to salt and sugar with the sugar response > salt response; (3) as in (2) but sugar

response < salt response; (4) as in (2) but sugar response = salt response; (5) specific salt fibres.

Records from few fibre and single fibre preparations taken from the chorda tympani nerve of the Macacus Rhesus monkey, by Gordon, Kitchell, Ström, and Zotterman (66), indicated that the monkey possessed specific fibres that responded only to sweet substances, others specifically to salt, and some that responded only to acid.

Kusano and Sato (125) have suggested that two distinct receptor mechanisms are responsible for initiating taste impulses. The first mechanism is produced by ion movement across the receptor mechanism and applies to monovalent salts such as KCl and NaCl; the second mechanism occurs through chemical reactions on the receptor surface, and is representative of divalent salts such as $MgCl_2$ and $CaCl_2$, sucrose, quinine HCl, and acetic acid. To investigate these mechanisms, Kusano (124) isolated single fibre units from the glossopharyngeal nerve of the Japanese common frog (Rana nigromaculata) and studied their responses to solutions of distilled water, NaCl, KCl, $MgCl_2$, sucrose, quinine HCl, and acetic acid. Of 105 units studied he was able to identify 3 units which responded to all taste solutions employed, 11 units which responded to only one kind of taste solution, 67 units that responded to divalent salts, 9 units responding to quinine HCl, and 7 examples of units responding mainly to

acetic acid. After Sato and Kusano (163), he was able to divide these receptor units into four main types: (1) D-units, which responded to divalent salts, sucrose, and water; (2) M-units, which responded primarily to monovalent salts; (3) Q-units, or those units primarily sensitive to quinine HCl; (4) A-units, which gave a response mainly to acetic acid. No receptor units specific to sucrose were found. Previously, Nejad (136) had isolated two main fibre types in Rana pipiens glossopharyngeal nerves. These were tentatively classified as small fibres and large fibres. Small fibres were more responsive to salts than large fibres.

According to Konishi and Zotterman (122, 123), neural activity from single fibres (isolated from the palatine branch of the carp glossopharyngeal nerve) could be classified on the basis of phasic activity or persistent activity. In addition, they noted that some fibres displayed a group discharge rather than a continuous discharge. Of 114 units, they were able to categorize fibre types according to seven classifications: (1) 37 which responded to many taste substances; e.g. NaCl, glycol, and human saliva, but not to quinine HCl; (2) 21 fibres which responded in different degrees to many kinds of taste stimuli, including quinine HCl and showing a large variety of individual responses; (3) 21 fibres characterized by their high sensitivity to sweet substances; (4) 16 fibres with high sensitivity to salt substances; (5) 13 fibre units

responding to quinine HCl and acid; (6) 3 units which responded only to saliva; (7) 3 units which responded only to weak acetic acid. Types (1) and (2) were identified as the most unspecific taste fibres in the glossopharyngeal nerve of the carp. Types (4), (5), and (7) were never stimulated by saliva.

A recent study by Iggo and Leek (96) on single fibres from the chorda tympani and glossopharyngeal nerves of sheep has indicated that the five basic types of units, as defined by Cohen et al. (35) and Pfaffmann (143) could be applied to their analyses. Of a total of 45 single fibre units, 3 could be classified as "salt" fibres, 6 as "acid" fibres, 23 as "salt/acid" fibres, 8 as "sweet" fibres and 5 as "bitter" fibres. Fibres from the chorda tympani nerve were sensitive only to salt, acid, and salt/acid stimuli, whereas those from the glossopharyngeal nerve could be classified according to all five basic unit types.

Konishi, Uchida, and Mori (120) recorded electrical impulses from few fibre or single fibre facial nerve preparations of the sea catfish (Platosus anguillaris) to various sapid substances which were dissolved in sea water and distilled water. They concluded that the sea catfish is highly responsive to hypertonic NaCl dissolved in seawater, to quinine HCl and to acid, but not to sugar. Of a total of 28 nerve preparations, 26 were found to respond to extracts

of marine worm, indicating the possibility of specialization for so-called "tasty" substances.

Tateda (174, 177) has shown that the majority of single fibres in the barbels of the catfish (Parasilusus asotus) responded to acid and salt, but not to sucrose and quinine. He found it impossible to classify fibre types, as the pattern for relative effectiveness of chemical stimuli used varied from fibre to fibre.

Multiple fibre approach

Recordings taken from the whole nerve bundle make up the greater majority of studies on electrophysiological data from gustatory fibres. In recent years a popular device for interpreting the results from whole nerve bundles has been the electronic summator or peak integrator initiated by Beidler (19), which quantifies the neural activity by cumulatively summing all electrical activity picked up by the recording electrodes, i.e. it rectifies and then smooths the neural impulses. The summator time constant determines the rate of decay of the rectified impulses or the degree of smoothness of the integrated response.

The summator output will vary according to the number of active fibres, their frequency of firing, the magnitude and shape of the action potentials recorded, the overlap of these spikes, the summator time constant, the sensitivity of the summator, the type and placement of the electrodes, plus

whether recording is done monophasically or biphasically (114, 141). Kitchell (114) and Iggo (95) have cautioned in the proper use of the summator method. Kitchell states that by studying the output of the summator alone, one cannot differentiate between a group of fibres transmitting neural pulses at a specific frequency and a smaller number of fibres transmitting at a different frequency. Furthermore, a few fibres conducting at a given frequency and having a large action potential amplitude (large fibres) cannot be distinguished from a large number of fibres transmitting at the same frequency with a small amplitude (small fibres). Iggo adds to this by saying that the higher frequency of discharge in large fibres as compared to that of smaller fibres can lead to questionable interpretation of the data if improperly handled, particularly with regard to cutaneous afferent fibres since their diameters cover a wide range from 0.25 to 16 microns. Beidler (14) defends his use of the summator by stating that since the smaller fibres display a smaller action potential height but a greater width than comparable action potentials in large fibres, the disparity in summator response between large and small fibres is not as great as expected. He indicates, however, that the summator process is not a useful technique for interpretating the physiological basis of taste quality.

Whole nerve recordings first appeared in the literature in the 1930's. Hoagland (93) in 1933 recorded impulses from

the facial nerve complex to acetic acid, NaCl, and meat juice bathing the barbels and lips of the catfish (Ameirus nebulosus). Very small and barely detectable impulses were recorded. Pumphrey (154) in 1935 was able to obtain responses from afferent mouth fibres of the frog to salt and acid solutions but not to sweet or bitter solutions. The work of Zotterman (190), also in 1935, extended the results to include gustatory impulses from afferent fibres in the glossopharyngeal and chorda tympani nerves of the domestic cat. In 1936, Barron (10) found impulses transmitted in the chorda tympani nerve of cats, rabbits, and rats to various chemical stimuli. Both Barron, and Zotterman before him, noted the negligible activity associated with sucrose solutions. Later Pfaffmann (143) denoted the lines of demarcation on the cat's tongue to the four basic taste qualities based on multiple fibre recordings from the chorda tympani and glossopharyngeal nerves. The tip and anterior lateral margins of the tongue were found to be most sensitive to salt, the base and posterior margins to quinine HCl, and all regions except the mid-dorsum were sensitive to acid. Since responses to sugar rarely occurred, no definite region could be localized.

Since these early pioneering studies, the list of vertebrates has grown to include teleosts, amphibians, birds, and mammals. Some of the major findings in the literature to each of these vertebrates are outlined below.

Teleosts As stated previously Hoagland (93) first recorded nerve discharges from gustatory fibres of the catfish. Much later, Konishi and Zotterman (122, 123) recorded from the glossopharyngeal nerve which innervates the palatal organ of the Swedish carp (Cyprinus carpio). They found that the summated response to acid and sucrose was particularly strong, whereas that to NaCl and quinine HCl was very weak. Human saliva, earthworm, and milk, gave notable responses. Tap water did not elicit a response. In comparing Swedish carp to Japanese carp, they found that the former displayed a large summated response to sugar and acid, and a low response to quinine HCl, while the latter indicated a low response magnitude to sugar, but a large response to bitter substances; clearly an individual variability. Konishi and Niwa (119) found the summated response-concentration curves for various electrolytes and nonelectrolytes in the carp to be complex discontinuous nonmonotonic functions. Nevertheless, they noted that although the response-concentration curves may differ in shape, a given solution (whether electrolyte or nonelectrolyte) always reproduces its specific response peak(s) at definite values of concentration.

Amphibians The only available literature on multiple fibre gustatory activity in amphibians is that related to the frog and toad. Pumphrey was the first to record action potentials from the glossopharyngeal nerve of frogs. His main

conclusions have been presented elsewhere (see page 23), and will not be repeated here. Since then, many other studies have been done on neural responses from the frog's tongue but very few of these have been specifically concerned with multiple fibre analysis to gustatory stimuli.

Yamashita (187) used the summator method to evaluate the stimulating effectiveness of cations and anions on the Japanese common frog (Rana nigromaculata). His records, taken from the glossopharyngeal nerve trunk, showed that relative effectiveness variations are larger with the cation series than with the anion series, i.e. the cations have a greater effect in stimulating the taste receptors. The order of effectiveness is $NH_4^+ > K^+ > Cs^+ > Na^+ > Li^+$ for monovalent cations of 0.5M concentration and $Ca^{++} > Sr^{++} > Mg^{++} > Ba^{++}$ for divalent cations of 0.016M concentration. The integrated response magnitude to sodium salts was found to be similar to the sum of the single fibre activity obtained by Kusano (124) (see page 18) on the same type of frog. Kusano and Sato (125) used a modified anion-Ringer solution in which all Cl^- was substituted for various other anions. Little change in threshold and response magnitude to various taste stimuli occurred immediately; however, after a certain time lapse all sensitivities were reduced or eliminated. Anion effectiveness was in the order of $SCN^- > NO_3^- > Br^-$.

Andrew (7) recorded afferent impulses from the glossopharyngeal nerve of the toad. The chemoreceptors appeared to be of a single type responding to salt solutions. Isotonic salt solutions did not evoke any responses while hypertonic solutions above a certain threshold did.

Birds Multiple fibre studies on birds have been featured in two major independent investigations. The first study was done on chickens (White Leghorn) and pigeons (of mixed breeds) by Kitchell, Ström, and Zotterman (116; summarized in 113, 114, 192, 193, 200, 201). In the chicken they found summated responses to the application of salt (NaCl), bitter (quinine HCl), acid (acetic acid), and water. They were unable to obtain any definite responses to sucrose or saccharine, yet were able to produce pronounced responses to ethylene glycol and glycerine, both of which taste sweet to humans. The pigeon was found to have distinct electrophysiological responses to water, salt, and acid, yet no response to bitter and a questionable response to sugar. As with the chicken, ethylene glycol and glycerine evoked responses; but unlike the chicken positive responses to saccharine were obtained in one-half the birds tested. It is interesting to note that Duncan (49) was unable to record any activity in the lingual branch of the glossopharyngeal nerve of feral pigeons (Columba livia) to test solutions other than 0.5% acetic acid. He ascribed the lack of activity to the small number of taste buds in the bird.

Whole nerve recordings by Halpern (71; summarized in 75) on chickens (Rhode Island Red-Barred Plymouth Rock cross-breeds) gave evidence that individual differences were very prominent in the 10 birds investigated. For instance, in one-half the chickens examined, sucrose and NaCl gave larger responses than in the other one-half. Lingual nerve responses were obtained to low concentrations of $FeCl_3$ and sucrose octa-acetate, NaCl, KCl, xylose, sucrose, glucose, glycerine, ethylene, glycol, HCl, acetic acid, Na saccharine, and quinine sulphate. Variability in response magnitude depended on the temperature of the solution, its concentration, and whether the solvent used was distilled water on Ringer's solution, and also according to which one-half grouping the chickens were placed. In general, small and consistent responses were recorded to 1.0M sucrose and glucose, and 0.5M xylose. Also, 0.1M acetic acid in Ringer's solution gave larger responses than did 0.1M KCl, or 1.0M sucrose or glycerine in Ringer's solution. The records given indicate that the largest responses were recorded for 0.001M $FeCl_3$ and NaCl at high concentrations.

Mammals The three mammalian species studied most thoroughly are the cat, rat, and dog. A few studies have concentrated on the guinea pig, hamster, opossum, raccoon, rabbit, sheep, goat, pig, calf, bat, monkey, squirrel, fox, and man.

Appelberg (8) compared the chorda tympani response to the glossopharyngeal response for the monkey, cat, dog, rabbit and rat to different taste modalities and found that the two nerves convey mainly the same type of afferent fibre responses. Salt, bitter, and acid taste were found in all animals. Pfaffmann (144), working on the rat, cat and rabbit, found that NaCl and HCl responses were of larger magnitude than those to quinine HCl and sucrose. The basic taste stimuli, in order of threshold were ranked as follows: quinine HCl (lowest), HCl, NaCl, and sucrose (highest). During the same time period, Beidler, Fishman, and Hardiman (22) studied the magnitude of the gustatory response in cats, guinea pigs, rabbits, cats, dogs, hamsters, and raccoons. They found it very difficult to assess differences in magnitude of response to salt, sour, bitter, and sweet substances. They felt that the summated response method proved to be an excellent preliminary technique in deciding which species to choose from, for a given taste solution, in order to conduct single fibre analyses.

Beidler (19) in a previous paper, in which he had introduced the summator technique, found that the summated responses from the chorda tympani branch of the rat could be classified into three general groupings. The first group responded to acids and salts, the second group responded to sugars, and the third group to quinine, ethyl alcohol and other sapid

solutions. This classification was based on the degree of activity and response magnitude recorded with the summator; i.e. large responses, and rapid activity were associated with the first group; modest activity and smaller response activities were indicative of the second group; minimal activity and negligible response amplitude dominated the third group. In passing, it should be pointed out that the artificial sweetner, saccharine, belonged to the first group rather than the second group.

Bell and Kitchell (28; summarized in 9, 113) recorded action potentials in the chorda tympani branch and glossopharyngeal nerves of the goat, sheep and calf. Summated responses from all three species were obtained with salt, sweet, bitter, and sour solutions in addition to such solutions as sodium bicarbonate ($NaHCO_3$), ethylene glycol, glycerine, and saccharine. They also found that chorda tympani responses were more prominent with salt and acid solutions; glossopharyngeal responses with sugars, quinine, and acids. Glossopharyngeal taste responses under conditions of mechanical stimulation have been recorded in the dog and pig, by Kitchell (113), and Kitchell and Hartke (115). With both species, responses were obtained to the four basic taste types.

Kitchell (113) summarizes the results obtained for the dog, pig, sheep, goat, and calf to various taste solutions initiating responses in the chorda tympani branch and

glossopharyngeal nerve. He states that the summated response to 0.2M acetic acid was greater in most species than that to other substances (specifically 0.5M NaCl, 0.46M sucrose, distilled water, and 0.02M quinine HCl). In the calf, sheep and goat, 0.2M acetic acid gave responses comparable to those elicited by 0.5M NaCl. Soltuions of 0.46M sucrose gave low magnitude responses of long duration in all three animal species. In the dog and pig larger responses were recorded to 0.46M sucrose than 0.5M NaCl. A larger response to 0.02M quinine HCl was noted in pigs and dogs than in calves, sheep, and goats. In general larger magnitude responses were obtained from the glossopharyngeal nerve than from the chorda tympani nerve branch. Glossopharyngeal nerve responses from the pig were smaller to 0.2M acetic acid, and 0.5M NaCl than to 0.46M sucrose, and 0.02M quinine HCl.

The utility of the summator method aided Bernard (30) in localizing the chorda tympani receptive field of the calf into two specific regions, which he called the anterior and posterior fields respectively. The anterior field was found to respond to NaCl and acetic acid (both starting at 0.001M), and KCl and propionic acid (both starting at 0.01M). Sugars such as fructose, glucose, xylose, and sucrose had higher thresholds than equimolar salt concentrations but were less effective in both fields. Also, quinine sulphate, Na saccharine, and distilled water were relatively ineffective

in both fields. On the posterior field acetic acid and propionic acid were more effective relative to equimolar NaCl concentrations than they were on the anterior field. Small responses to chemical stimulation were noted from the glossopharyngeal nerve despite mechanical movement of circumvallate papillae on the tongue.

Summated responses from the entire chorda tympani branch of the Rhesus monkey indicated to Gordon, Kitchell, Ström and Zotterman (66; summarized in 114, 192, 193, 200, 201) that this animal is sensitive to such diverse solutions as distilled water, salt, sucrose, saccharine, quinine, acid, glycerol, and ethylene glycol. They felt that the Rhesus monkey has as large a response range to sapid solutions as any mammal studied.

Diamant, Funakoshi, Ström, and Zotterman (39; summarized in 192, 193, 201, 202) successfully recorded neural impulses from the whole chorda tympani nerve from human beings. They found an adequate response to 0.5M NaCl, 15% sucrose, 0.04% saccharine, 0.02M quinine sulphate, and 0.2M acetic acid.

The glossopharyngeal nerve of the rat, cat and rabbit was the subject of an electronic summator investigation by Yamada (183, 184, 185) to the four basic taste modalities. He observed that the quinine response was greater than the comparison salt response in all species. This was in contrast to the

findings of Beidler et al. (22) who recorded the chorda tympani nerve trains in the same species. Yamada noted that the response magnitude to salt increased with the logarithm of NaCl concentration from 1 to 4M.

Much of the material covering such mammals as the guinea pig, hamster, opossum, raccoon, bat, monkey, squirrel and fox are covered in the next section (see Species Differences to Chemical Stimuli) and will not be given here.

Species Differences to Chemical Stimuli

Chorda tympani nerve responses

Barron (10) was the first researcher on record to study action potentials from gustatory stimuli on different species. He noted that no individual differences appeared between cats, rats, and rabbits when recording from the whole chorda tympani nerve. Pfaffmann (144, 147), recording from multiple fibres, noted that the relative effectiveness of HCl, KCl, NaCl, sucrose, and quinine HCl in suprathreshold concentrations was different in the same three species. For the rat, HCl = NaCl>KCl; for the cat, HCl>KCl>NaCl; for the rabbit, HCl> KCl>>NaCl. For sucrose and quinine he found: cat, quinine > sucrose; rat, quinine = sucrose; rabbit, sucrose > quinine. Single fibre recordings indicated that the order of effectiveness of HCl, KCl and NaCl did not always correspond

to that found for the total nerve response within the same species as well as between species. Each single fibre preparation responded differently to more than one of the four basic taste stimuli.

Whole and single nerve fibre preparations were used by Beidler, Fishman, and Hardiman (22; summarized in 12) in analyzing responses from rats, guinea pigs, rabbits, cats, dogs, hamsters, and raccoons. They found significantly smaller relative response magnitudes to salts in rabbits, dogs, and cats compared to those found in rats, hamsters, and guinea pigs. They found that the rodents tested respond better to NaCl than to KCl, whereas the reverse was found with carnivores. Specifically the Na/K values found for six species were: 0.74 for raccoon; 0.67 for cat; 0.44 for dog; 2.0 for rat; 2.8 for hamster; 2.6 for guinea pig. In a later paper Tamar (172, 173; summarized in 13, 15, 62) recorded a specific value of Na/K = 0.50 for the opossum (Didelphys virginiana) and indicated a large value for the insectivorous little brown bat. The relative magnitude of sucrose (relative to NaCl) was found to vary from very small in the cat, to moderate in the dog and rat, to quite large in the hamster, guinea pig, and rabbit (22; summarized in 12, 13, 15).

Fishman (59, 60, 62) has recently completed a study, which encompasses such diverse animals as the bat, squirrel, monkey, and fox. Specifically, three species of bat -- a

sanguivorous bat (Desmodus youngi), a frugivorous bat (Artibeus jamaicensis), and an insectivorous species (Molossus aterniricans) -- four species of squirrel -- the ground squirrel (Spermophilus tridecemilineatus), the flying squirrel (Glaucomys valvous), the Arizona gray squirrel (Sciurus arizonensis), and the fox squirrel (Sciurus niger) -- the white face ringtail monkey (Cebus capucinius) and the red fox (Vulpes folva fulva) were used.

With the three bat species, Fishman found that the relative responses (relative to NaCl) to the four basic modalities were very different. For instance, he found that the response to sugar was relatively higher with Desmodus than that of the other two species; although, all three species showed a sugar response below that of the comparison NaCl. Acid response was higher than NaCl for Desmodus, Molossus, and Myotis [extracted from Tamar's work (172, 173)] and lower than NaCl in Artibeus. The bitter response was higher than NaCl in Desmodus and Molossus, and lower than NaCl in Artibeus and Myotis. Fishman indicates that the acid response in Desmodus represented the highest acid response magnitude obtained from any species of animal that he had studied (see references 22, 59, 60, 62, 172 for species covered). Na/K values for each of the three species are as follows: 1.4 for Desmodus; 2.2 for Artibeus; 0.73 for Molossus.

Bitter responses were of very low magnitude with all squirrels tested by Fishman. NaCl response-concentration curves for the gray, flying, and ground squirrels were very similar to that of the rat, whereas that of the fox squirrel is very different. Relative to NaCl, the acid response of the fox squirrel was higher, that of the gray squirrel somewhat lower, and that of the flying and ground squirrel considerably lower. Fishman found the relative magnitude of the steady state sugar response of the fox and ground squirrel to be constant within species and somewhat less than the NaCl response, while that of the flying squirrel was considerably lower than the comparable NaCl response. He found that the sugar response of the gray squirrel was not constant but varied over a range of 0.75 to 0.90 of the NaCl value. Na/K values were 3.3 for the fox squirrel, 2.0 for the gray squirrel, 10.0 for the flying squirrel, and 4.0 for the ground squirrel.

Fishman found that the ringtail monkey exhibits a good relative response (relative to NH_4Cl) to sweet and acid tastes, and a fair response to the bitter taste. A good response to NH_4Cl was observed whereas that to NaCl was considerably lower. With the squirrel monkey (Saimiri sciureus) Pfaffmann (145) observed a higher response to NaCl than to NH_4Cl at comparable concentrations, whereas a small response was noted for sugar relative to NH_4Cl. Gordon, Kitchell, Ström and Zotterman (66) found that the Rhesus monkey responds well to salt, acid, quinine and sugar. They discovered that

single fibre preparations responding well to sugars inevitably respond well to saccharine also. This is not obvious with other animals. Fishman noted the Na/K value of the ringtail monkey to be 0.43. This is more representative of that found in carnivores than in rodents.

The red fox was found to fit well within the carnivore group, having a Na/K ratio of 0.52. As with other carnivores, Fishman found that NH_4Cl was a better stimulus than NaCl and was, therefore used as the reference modality. Acid and bitter responses were relatively high and considerably higher than those found in a species belonging to the same family -- the dog. The initial response to sugar was found to be extremely high after which it dropped off rapidly to a low steady state level.

Glossopharyngeal nerve responses

One of the first serious attempts to quantitate species differences in glossopharyngeal nerve responses was done by Appelberg (8). His study on cats, dogs, rabbits, rats, and a Rhesus monkey showed that salt, bitter, and acid solutions evoke responses in all animals. With the exception of the cat, all other animals responded to sweet-tasting solutions. Only the monkey, however, responds to saccharine.

Species differences have also been noted by the following observers: Kitchell, Ström and Zotterman (116; summarized

in 113, 114, 192, 193, 200, 201) who found that bitter solutions evoked responses in chickens but not in pigeons; Yamada (183, 184, 185), who found the following Na/K values: 0.69 for rat; 0.52 for rabbit; 0.59 for cat; Nejad (136), who determined that frog responses to chloride salts are similar to those of cats and different from those of rabbits; Tateda (176) who found that response magnitudes to different acids at equimolar concentration varied with molecular size as follows: increased for catfish (Parasilurus asotus), decreased for toad (Bufo vulgaris), no change for frog (Rana nigromaculata).

Neural Studies Using Special Taste Solutions

Several laboratories have been concerned with collecting neural data from peripheral nerves under conditions in which the tongue bathing solutions differed immeasurably from the standard four modalities of sweet, sour, bitter, and acid. Specific studies incorporated the following types of chemical stimuli: (1) distilled water; (2) mono-and di-saccharides; (3) taste enhancers; (4) amino acids; (5) ethyl alcohol; (6) alkaline substances. Details of the results are covered below.

Nerve fibres responding specifically to water

In 1949, Zotterman (195; summarized in 192, 193, 200, 201) observed a massive volley of impulses in the large fibres of

the glossopharyngeal nerve of the common Swedish frog (Rana temporaria) to the application of common tap or distilled water to the tongue. This water effect or so-called "water response" increased very rapidly and continued on for over a minute with slowly decreasing frequency. With the application of Ringer's solution, NaCl and other isotonic salt solutions the water effect could be abolished. Isotonic sucrose solutions, however, did not abolish the "water response." In a later paper Andersson and Zotterman (6) stated that the water effect appeared to be mediated by specific nerve fibres of large diameter. Moncrieff (132) feels that these results should be interpreted in that any solution which is less salty (distilled water) or more salty than the neutral solution, saliva, should initiate neural activity and therefore elicit a sensation of taste.

The detailed work of several Japanese investigators also deserves some attention. Kôketsu (117) and later Kusano and Sato (126) have indicated that common monovalent salts such as NaCl and KCl, and uncommon salts such as sodium acetate and sodium saccharine have a depressing effect on the "water response," whereas this does not occur with $CaCl_2$ and $MgCl_2$. Kusano and Sato have proposed that the "water response" is initiated by the withdrawal of cations, possibly potassium ions, from the cell interior. They further state that if one assumes that calcium and magnesium ions are not permeable

to the receptor membrane as with the membrane of other excitable cells, then the non-depressing action of $MgCl_2$ and $CaCl_2$ becomes evident. Then Kusano (124), using single fibre preparations, reported that receptors sensitive to water almost always respond to divalent salts. Finally Nomura and Sakada (138) indicated that the so-called "water response" of the frog's tongue is really the response to calcium ions. They advanced this opinion after recording nerve impulses to solutions of tap water, distilled water, and $CaCl_2$. No responses were observed to calcium-free distilled water, whereas the response magnitude to tap water was very close to that of $CaCl_2$ solutions having similar calcium concentrations as tap water.

In an investigation on single taste fibres from the chorda tympani nerve of the cat, Cohen, Hagiwara, and Zotterman (35; summarized in 192, 193, 201) found that the activity of fibres giving the water effect was depressed by such common salt solutions as NaCl, KCl, $CaCl_2$, etc., whereas salt solutions such as sodium acetate and sodium formate had little depressing action (cf. findings of Kôketsu, Kusano and Sato, page 38). They proposed that the outward flow of ions across the receptor membrance stimulates the "water response" while an inward flow inhibits this response. Cohen et al. found that some of the "water" fibres could be stimulated by quinine HCl of concentration less than 0.009M as well as

mineral acids of pH = 2.5 and below. Liljestrand and Zotterman (129; summarized in 192, 200, 201) were able to find mammalian nerve fibres responding to distilled water and not to 0.5M NaCl solutions in addition to preparations that responded to 0.5M NaCl but not to distilled water.

Since Zotterman's initial report (195) many animal species have been tested. "Water responses" have been found or confirmed in the following species by the researchers cited: cat, Cohen, Hagiwara, Liljestrand, and Zotterman (35, 129, 192, 198, 200, 201); dog, Liljestrand, Zotterman, and Kitchell (113, 115, 129, 192, 200); rabbit, Zotterman, Beidler, Fishman, and Hardiman (22, 198); chicken, Kitchell, Ström, Zotterman, Halpern, and Kare (71, 75, 113, 114, 116, 192, 193, 200, 201); pigeon, Kitchell, Ström, and Zotterman (113, 114, 116, 192, 193, 200, 201); monkey, Gordon, Kitchell, Ström, Zotterman, and Fishman (59, 66, 113, 114, 192, 193, 200, 201). The absence of a distilled "water response" has been recorded as follows: rat by Zotterman and Fishman (61, 192, 193, 198, 200, 201); hamster by Fishman (61); sheep by Baldwin, Bell, Kitchell, Iggo and Leek (9, 28, 96, 113, 114); man by Diamant, Zotterman, Uchida and Mori (120, 122, 123, 193).

With calves, Baldwin, Bell and Kitchell (9, 28, 113) were not able to record the water effect in either the glossopharyngeal nerve or the chorda tympani nerve. Bernard (30) was able to show that a distilled "water response" was consistently present from the posterior receptive field whereas no

response was evident from the anterior receptive field of the calf from both chorda tympani nerve and glossopharyngeal nerve recordings (see page 30 for classification of fields).

Konishi and Niwa (119) using taste nerves from fresh-water fish, found that if distilled water was applied following adaption to salt solutions (having a sufficient concentration to depress receptor activity) a distilled water effect would inevitably occur. This effect would not occur as long as the chemoreceptors were responsive to the salt solutions. A similar effect was observed with non-electrolytes.

Responses to simple and complex sugars

Andersen, Funakoshi, and Zotterman (3, 4) stimulated the tongue receptors of mongrel dogs to equimolar solutions of D-fructose, L-sorbose, D-galactose, D-glucose, D-mannose, sucrose, maltose, and lactose. Dogs were used because a previous electrophysiological study by Andersson, Landgren, Olsson and Zotterman (5) showed that the dog possessed specific sweet fibres in the afferent nerves of the tongue. When stimulating with 0.5M solutions of the monosaccharides, they found fibres which were activated by all the sugars, but also fibres which responded only to D-fructose. The rule obtained when stimulating with monosaccharides and disaccharides was that the stimulating power of D-fructose > sucrose > D-glucose > maltose > D-galactose > lactose. In a

few of their preparations, the disaccharide maltose elicited a massive barrage of impulses on stimulation whereas the other sugars caused a much smaller response. It was tentatively classified as a "maltose" fibre.

Diamant et al. (39; summarized in 202) studied the relative effectiveness of different biological sugars on man by recording the neural activity from the whole chorda tympani nerve. They found that the relative effectiveness (determined by the largest response observed) of different biological sugars was obtained with sucrose, then fructose, mannose, lactose, glucose and maltose, sorbose and arabinose, and finally galactose.

Hagstrom and Pfaffmann (69) and Hardiman (77) looked into the relative taste effectiveness of 5 sugars -- sucrose, glucose, fructose, lactose and maltose -- by recording chorda tympani responses from the rat tongue. They found the order of relative effectiveness (relative to NaCl) to be sucrose > fructose > glucose $\geq$ lactose $\geq$ maltose which corresponded well to psychophysical findings in man. However, this sequence was directly opposite to preference-threshold findings. Pfaffmann (145) observed a chorda tympani relative effectiveness of sucrose > fructose > lactose > glucose for the squirrel monkey.

Effect of gymnemic acid on sugar response Gymnemic acid is a plant extract which selectively modifies or

suppresses electrophysiological and/or behavioral responses to particular gustatory stimuli. Halpern (72) has stated that gymnemic acid and its potassium salt (potassium gymnemate) act primarily on the peripheral portion of the gustatory system. Responses from sweet-tasting compounds are supressed, while inorganic salts and organic acids are unaffected. That gymnemic acid will temporarily eliminate the ability to taste sweet substances has been confirmed from electrophysiological recordings of chorda tympani responses in man by Diamant, Oakley, Ström, Wells, and Zotterman (40; summarized in 193), in the dog by Andersson, Landgren, Olsson, and Zotterman (5); in the hamster by Pfaffmann and Hagstrom (153; summarized in 146, 147).

Gustatory impulses from flavor enhancers

A class of taste modifying compounds known as flavor enhancers or flavor potentiators[1] has been known to the Japanese for years. Foremost amongst these is monosodium glutamate (MSG) which was discovered in 1908 as an extract of the marine plant sea tangle. Recently several extracts

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[1]Gustatory enhancement: the response magnitude to a mixture is larger than the sum of the individual responses to the several components of the mixture when presented separately (72).
Potentiation: a synergistic reaction in which small amounts of a chemical may exaggerate the effect of one or more chemicals taken at the same time.

referred to collectively as 5' -ribonucleotides have appeared. These include sodium 5' -guanylate (5' -GMP), sodium 5' -uridylate (5' -UMP), sodium 5' -ionosinate (5' -IMP), and sodium 5' -cytidylate (5' -CMP).

Several Japanese researchers; including Sato and Akaike (162), and Sato, Yamashita, and Ogawa (165), have studied the flavor enhancing ability of the 5' -ribonucleotides when given with MSG. They noted the following particulars from rat chorda tympani neural data: (1) marked enhancement in response magnitude when 5' -GMP or 5' -IMP was added to L-MSG; (2) small enhancement in response magnitude when 5' -UMP or 5' -CMP was added to L-MSG; (3) no enhancement when 5' -GMP and 5' -IMP were mixed together or when 5' -GMP or 5' -IMP was mixed with 5' -UMP or 5' -CMP; (4) stimulating effectiveness was in the order of L-MSG > D-MSG and 5' -GMP > 5' -IMP > 5' -UMP > 5' -CMP.

Other work by Adachi, Funakoshi, and Kawamura (2) indicated that a mixture of 5' -IMP + 5' -GMP gave little if any enhancement to the response from "salt" fibres in the cat as compared to individual solutions of 5' -IMP and 5' -CMP. Conversely, a mixture of MSG + 5' -IMP, or MSG + 5' -GMP always enhanced the nerve response when used individually. When they added MSG, 5' -IMP, or 5' -GMP to a salt solution, the neural activity of the "salt" fibre increased, whereas

the activity of a "quinine" fibre became depressed when these same chemicals were mixed with quinine HCl.

Amino acids as gustatory stimuli

Amino acids are key structural elements in all living systems. Halpern, Bernard, and Kare (74; summarized in 70) studied electrophysiological data from the whole chorda tympani nerve of albino rats to glycine, alanine (D-, L-, and DL-), DL-valine, DL-methionine, and DL-tryptophan. Response magnitudes were found to be low in comparison to NaCl. Order of effectiveness at concentrations near the solubility limits for amino acids was DL-methionine > DL-tryptophan > DL-alanine > glycine > 0.1M NaCl.

Tateda and Hidaka (175, 179) found that sugars and α -amino acids stimulated the same receptor site. Nejad (136) showed that the response to amino acids in Rana pipiens glossopharyngeal nerves decreases with increasing chain length.

Ethyl alcohol as a gustatory stimulus

The summated response from the whole chorda tympani nerve to ethyl alcohol in the cat, dog, and rat was studied by Hellekant (82; summarized in 79). He found that the threshold concentration was lower in the cat than in the dog and that the strongest solutions were required for the rat. Maximum response was obtained at 5.0M ethyl alcohol in the cat and at 6.5M in the rat.

In a single fibre study from the cat chorda tympani nerve, Hellekant (81; summarized in 79) showed that all fibres which responded to at least one of the conventional test solutions (represented by 0.5M NaCl, 0.5M sucrose, 0.05M acetic acid, and 0.05M quinine HCl) also responded to ethyl alcohol. When alcohol was mixed with the test solutions there was a moderate increase in activity for solutions of NaCl alone as compared to NaCl in ethyl alcohol., and solutions of acetic acid alone as compared to acetic acid and alcohol. There was a moderate increase in activity for sucrose in alcohol as opposed to sucrose alone. The most surprising result was observed between quinine HCl alone and solutions of quinine HCl and alcohol. Alcohol had the effect of completely reducing the quinine response that was evident when quinine alone was used.

Following application of alcohol, gustatory, thermal, and mechanical stimuli to the cat tongue, Hellekant (80; summarized in 79) observed specific single fibres that would not respond to the basic gustatory modalities, yet would respond to ethyl alcohol, to cooling, and to mechanical stimuli. In fact all fibres responding to alcohol also responded to mechanical stimulation as well as to tongue cooling.

The alkaline taste

Several investigators have considered an alkaline taste to be one of the basic taste modalities (17, 132). Liljestrand and Zotterman (128) studied the effects of NaOH on cat taste receptors. Negligible neural responses were recorded from the chorda tympani to solutions of pH = 11 or less; whereas at pH = 12 or more, neural activity was quite pronounced. Results from few fibre preparations showed that "water" fibres, "salt" fibres, and some "quinine" fibres are stimulated by alkaline substances. They concluded that the alkaline taste is the result of a general stimulation of several kinds of taste fibre endings.

Gustatory Electrophysiological Topics of Current Interest

The extensive literature in recent years on the electrophysiology of taste attests to the diversified approach many investigators have taken in their attempt to understand the mechanism underlying the system. With reference to the peripheral gustatory nervous system the following topics are reviewed below: (1) classification of temporal activity; (2) attempts to break the afferent neural code; (3) taste-temperature interaction; (4) regenerated and cross-regenerated taste nerve studies; (5) psychophysical-neural correlations in man; (6) Beidler's fundamental taste equation; (7) electrically

induced taste sensation; (8) efferent control of gustatory receptors; (9) sensitivity enhancement through tongue movement.

Classification of temporal activity

Beidler (19) and Pfaffmann (144) used temporal activity and response magnitude to characterize different fibre types, yet they failed to categorize neural activity into any specific patterns. Fishman (61), however, was able to classify neural activity into five general types of response.[1] These are summarized below using Fishman's terminology.

> The first type has a rapid initial response of one or two seconds duration followed by a rapid decrement to a steady state of activity which shows little evidence of fatigue on constant stimulation for three minutes or longer. The second type is similar to the first type but for the absence of a rapid initial burst and that steady state activity is attained in the first second. Monovalent salts are always found to evoke responses of the first and second types. The third type of response showed a rapid initial burst of 1/10 to 1/5 second duration followed by a short period of inactivity and then a prolonged increase in activity to a maximum with a subsequent slow decline to some low level steady state or to zero activity. The fourth type of response was introduced by a gradual increase in activity from the time of stimulation to a maximum followed by a slow decline to zero activity or some low level steady state. Divalent salts, in general, gave the fourth type of response although $MgCl_2$ and $CaCl_2$ varied between the third and fourth types of response amongst individual fibres. The fifth type

[1] In a later paper, Kimura (109) was able to classify temporal activity into five categories which seem to corroborate those of Fishman.

> was the rarest kind of all responses and was found only in a few fibres that were responsive to sweet substances. It was exemplified by a rhythmic burst-like activity in which impulses are grouped together with a fairly constant interval between groups.

Halpern (70) gave four characteristics of chorda tympani multiunit responses to relatively high concentrations of the amino acids glycine and alanine. These were: (1) a relatively long latency; (2) a slow increase in response magnitude; (3) little if any large spike, high frequency initial burst; (4) little adaption after reaching peak magnitude. These temporal characteristics are very different from those of other stimuli, e.g. NaCl.

Attempts to break the afferent neural code

Interest in peripheral nerve coding seems to have had its origin with Pfaffmann (142). Noting from his own work (142, 144), and the work of Cohen et al. (35) and Fishman (61) that a given taste fibre may be responsive to more than one type of stimulus, he proposed that the relative amounts of activity across several fibres may determine the taste quality; as an example he cited the relative performance of two parallel fibres designated "fibre A" and "fibre B". Regardless of stimulus concentration the frequency of nerve discharge was higher for fibre A over fibre B when NaCl was the stimulus. However, when sucrose was the stimulus the discharge rate of

fibre B was consistently higher than that of fibre A over the entire concentration range tested. Pfaffmann suggests a frequency code based on the ratio of activity in fibres A and B as A/B > 1 = salty and A/B < 1 = sweet. Along a similar line, Sato [cited in (181, p. 1026)] was able to statistically categorize taste fibres into several basic units in which quality discrimination depended on the relative amount of activity in each category.

Probably the boldest approaches to afferent coding are those outlined in a series of papers by Erickson (55, 56), and Erickson et al. (57). Starting with Pfaffmann's concept of across-fibre patterning Erickson isolated many single rat chorda tympani fibres and recorded the number of impulses in the first second of evoked activity to various chemical taste solutions. He then found a product-moment correlation coefficient between chemical stimuli for all single fibres tested. If a high correlation existed, as it did between NH_4Cl and KCl, then it was felt that these patterns were quite similar and therefore quality discrimination would be difficult. Conversely, if a poor correlation was evident, as was found between NaCl and KCl, and between NaCl and NH_4Cl the assumption was that quality discrimination would be quite pronounced. Using shock-avoidance techniques, Erickson was able to support his hypothesis.

Making note of the fact that most afferent neural systems have well defined stimulus dimensions which may then be

related to the quality of sensation, Erickson set out to determine the unknown stimulus dimension and response function for the gustatory system. Since the visual system has a well defined stimulus dimension (wavelength of light) and associated neural response functions (difference absorption spectra for specific visual cones), he forced some visual data to parallel the taste data obtained (activity in first second of evoked response from many single fibres and several chemical stimuli). Then using the taste data he was able to retrace his steps, as based on the a priori knowledge of the visual system parameters and come up with a suitable gustatory neural response function and stimulus dimension. Psychophysical data were shown to conform to neural data.

This model was later tested by Marshall (131) on opossums (Didelphis virginiana) with good agreement. A matrix mathematical model by Schiffman and Falkenberg (166) extended Erickson's model to include interactions among neurons and among stimuli. Doetsch (45) used Erickson's model to compare taste quality information of rat chorda tympani fibres with second order cells in the nucleus tractus solitarius. His main conclusion was that synaptic transmission tends to smooth information content to provide a very stable sensory message to the higher order neurons.

Intensity coding seems to be related to the number of active fibres and their discharge rate (141).

Taste-temperature interaction

Abbott (1) studied the effects of temperature on the taste endings of white rats. He recorded the action potentials in the chorda tympani and lingual nerves to chemical stimuli of NaCl, NaBr, KCl, and HCl at selected concentrations. Each concentration of a particular stimuli was allowed four specific temperatures, which were 15°, 22°, 37°, and 45°C respectively. To abolish the effect of temperature reception, Abbott had to preadapt the tongue to the selected temperature for a period of 30 seconds before the chemical stimulus at the same temperature could be applied. The preadaption solution used was wash water. He found that regardless of concentration, the greatest response to NaCl was obtained at 22°C, and the smallest at 15°C. With NaBr and KCl, responses to 37° and 45°C solutions were greater than those to 15° and 22°C solutions. No significant difference in response magnitude was demonstrated when HCl was varied with temperature. He concluded that no single temperature coefficient exists for taste, and that insofar as salts are concerned the temperature effect must be attributed to the combination of ions rather than the cation and anion specifically.

Beidler (21) reported that there was no change in rat chorda tympani response to 0.5M NaCl at 20°, 25° and 30°C. Since preadaption of the tongue was not part of Beidler's technique, his summated results must be interpreted in that

a temperature change has little influence on the sum of the responses to thermal stimuli and chemical stimuli.

Recently, several Japanese investigators have studied the taste-temperature interactions further on several species of animals. From cats, Nagaki, Yamashita, and Sato (135; summarized in 160) obtained summated chorda tympani responses to various taste concentrations over a temperature range from 5° to 50°C. They found that when 1.0M NaCl solutions were applied to the tongue the corresponding impulses were independent of the temperature change, thus confirming Beidler's results above (21). Moreover, when they subtracted the response to Ringer's solution from that to the taste solution at each temperature they obtained results which were in concert with those of Abbott. There is, however, some disparity between their and Abbott's findings. For instance, they found that the greatest response to NaCl, quinine, HCl, and saccharine was obtained at about 30°C. Yamashita, Yamada, and Sato (189) later confirmed these results using the preadaption method of Abbott.

The effects of temperature on the taste response of the common Japanese frog was investigated by Yamashita (186) who recorded summated neural activity from the glossopharyngeal nerve to various taste solutions over the temperature range 10° to 40°C. Using the method of preadaption he found that a maximum response magnitude to NaCl, $CaCl_2$, distilled water, and 0.1M sucrose occurred at about 20°C. Optimum temperature

for maximum response varied from 20° to 30°C for quinine and acetic acid. The temperature coefficient Q_{10}[1] was found to be a function of concentration, temperature range, response criterion chosen, and even the type of chemical used.

The chorda tympani response of rats to various chemical taste enhancers at temperatures varying from 20° to 45°C was investigated by Sato and Yamashita (164). Specifically, the taste stimuli were NaCl, MSG, 5' -GMP, 5' -IMP, 5' -UMP, 5' -CMP and their mixtures. An increase in temperature caused a decrease in response magnitude for NaCl and MSG, whereas the response to 5' -GMP and 5' -IMP attained a maximum value at about 30°C. Mixtures of MSG with one of the 5' -ribonucleotides acted as if two types of temperature dependent processes were involved one for MSG and the other for the 5' -ribonucleotide. For these mixtures, enhancement in response magnitude started at 10° to 15°C and increased gradually with rising temperature. In this sense, response magnitude was recorded in the following order: 5' -GMP > 5' -IMP > 5' -UMP > 5' -GMP.

Yamashita and Sato (188; summarized in 161) noted the effects of temperature on impulse discharges in the rat chorda tympani nerve to taste stimuli of NaCl, KCl, $CaCl_2$,

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[1] Q_{10} is defined as the change in magnitude of the summated response per 10°C (141).

HCl, quinine, and sucrose at temperatures ranging from 10° to 45°C. Summated records from the preadapted tongue afferent nerves indicated that the response magnitude to all stimuli increased except for small concentrations of sodium salts (0.01 to 0.03M). For 0.01M NaCl the response magnitude was found to decrease with increasing temperature. The fact that small negative Q_{10} values (based on the magnitude of response) occurred with the taste solutions applied, led the investigators to conclude that the reaction between taste receptor and stimuli is of a physical nature rather than an enzymatic one. Nejad (137) has indicated that temperature affects the taste response latency in the rat tongue as well as the response magnitude.

Regenerated and cross-regenerated taste nerve studies

In two recent papers, Robbins (157, 158) compared data relating to normal frogs with intact nerve supply, frogs in which the lingual nerve had been denervated, frogs in which the cut lingual nerve was allowed to regenerate, and frogs in which a cross-anastomosis between the lingual nerve and the cutaneous branch of the seventh nerve (ramus hyoideus) had been performed. As a criterion for the reinnervation of the taste buds he noted the mean taste bud area for each of the 4 types of preparation listed above. At the 90% confidence limit he recorded the following mean taste bud areas: 0.022

square microns, normal nerve; 0.002 square microns, denervated nerve; 0.008 square microns, regenerated nerve; 0.008 square microns, cross-regenerated nerve. This indicated that the effects of denervation were somewhat reversed by either cutaneous or lingual nerve reinnervation, and that taste cell nourishment (trophic role) was not confined exclusively to gustatory nerves.

In the rat, the chorda tympani branch normally innervates taste buds on the anterior two-thirds of the tongue, and the glossopharyngeal the posterior one-third. Moreover, on a relative basis Ringer's solution and NaCl are more responsive to the chorda tympani nerve receptive field, whereas quinine and saccharine are more effective on the glossopharyngeal nerve receptive field. These two features enabled Oakley (139, 140) to conduct an electrophysiological inquiry into the taste effectiveness of normal, regenerated, and cross-regenerated nerves. The regenerative portion was conducted on chorda tympani nerves that had been cut and resutured. By cross-union, the glossopharyngeal nerve was made to innervate the front of the tongue in some rats, and in other cases the chorda tympani nerve to the back of the tongue. After an elapse of 15 post-operative weeks summated responses were taken. Chorda tympani regenerative preparations gave comparable summated responses (on a relative basis) to normal untouched chorda tympani nerve preparations. Cross-regenerative

preparations in which the chorda tympani was made to innervate into the posterior part of the tongue changed the relative magnitude and time course of the summated responses so that they closely resembled those from the normal glossopharyngeal nerve. In a parallel fashion the role is similarly reversed when the glossopharyngeal nerve is made to innervate the anterior part of the tongue. Oakley contends that his results indicate that (1) the nerve ending itself does not function as a taste receptor in direct contact with the applied taste solution, and (2) the taste nerve ending does not determine the chemical specificity of the taste cell without tissue-nerve modification.

Psychophysical-neural correlates in man

Recently, Diamant et al. (40; updated in 31, 32) compared summated chorda tympani nerve responses in man to appropriate psychophysical responses for various taste solutions. Psychophysical tests were based on a ratio scaling method in which the subject had to handle figures and make ratio estimations. The correspondence, at least for sugars, between psychophysical and neural data was quite good. They found that it was useless to compare the nerve responses from one patient with the psychophysical responses from another since individual differences in gustatory sensitivity varied widely. When citric acid was the chemical stimulus,

they were able to describe the relationship between response R and concentration C by a simple power function:

$$R = a\,C^{n} \qquad (1)$$

where

a is an appropriate constant,

n = 0.5 for both psychophysical and neural data.

Using 0.2M NaCl salt solutions Diamant et al. found that psychophysical adaption times of 79, 90, and 120 seconds in 3 patients compared favorably to values of 50 and 79 seconds from the neural recordings of 2 patients.

Beidler's fundamental taste equation

In a very important paper in 1954, Beidler (19; summarized in 16, 18, 77) developed a fundamental equation of taste. This equation is based on the notion that the reaction between taste stimuli and taste receptor is of a physical nature or more specifically: "-the stimulating ions are loosely bound to the taste receptor membrane." This theory has been in conflict with the views of some research groups, but the fact that electrophysiological data can be directly applied to it has given it an aura of awe over the other proposed theories; hence its inclusion in this literature survey.[1]

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[1] In the light of the additional taste data now available, Beidler (11) has attempted to re-examine his basic site theory. A multiple site theory has been proposed by Gander, Griffin, and Fischer (64).

The essence of the theory lies in the assumption that each receptor is a single cell having several receptor sites to which the cell selective molecules are adsorbed. For a given solution concentration a certain number of receptor sites will be filled. As the concentration is varied, the number of receptor sites filled also varies. The highest response occurs when all receptor sites are filled. Accordingly, he arrived at the fundamental taste equation:

$$\frac{C}{R} = \frac{C}{R_m} + \frac{1}{KR_m} \qquad (2)$$

where

C is the concentration of the stimulus,

R is the magnitude of response,

R_m is the magnitude of the maximum response,

K is the equilibrium constant.

Beidler's theory was found to account for the sense of taste of salts very well in hamsters and rats. In the case of non-sodium salts, acids, sugars, and bitters certain complications arose (20, 21). Hardiman (77) has proposed that a multiplicity of K and R_m terms should be added to the basic equation to account for these complications. Nejad (137) has suggested that a secondary taste mechanism must be considered to account for variations in response magnitude with temperature.

Other laboratories have attempted to fit their electrophysiological data to Beidler's equation. Kimura and Beidler

(112) were able to fit changes in taste receptor resting potential to the equation. Fishman has corroborated Beidler's results on single fibres in the rat and hamster (61) and on whole fibre recordings in squirrels and bats (62). Tateda and Hidaka (175, 179) have shown a good fit to sucrose for the rat.

Response magnitude-concentration curves by Diamant et al. (39) on human subjects appear to fit a modified form of Beidler's equation for a variety of taste solutions. Using a subjective response Stone (170) found an excellent fit to L-amino acids for man.

In contrast, some investigators were not able to fit their neural data to Beidler's equation. For instance, Halpern et al. (73) could not fit their integrated response data from the rat to this equation for various amino acids. Tateda and Hidaka (175, 179) found that stimulus-response curves for fructose and glucose did not conform to Beidler's equation. Griffith (67) was unable to interpret responses to distilled water, NaCl, and glucose in terms of Beidler's theory for the frog.

Recently, Sato (161) has pointed out that there is a difference in the excitatory process of taste receptors between mammals and frogs, since a difference in body temperature is evident between the two species. He believes that for the case of mammals, the fundamental taste equation adequately

describes the initiation of nerve impulses as a first process for solutions such as sodium salts, KCl, $CaCl_2$, quinine HCl, and sucrose. For frogs his data seems to conform to a theory supported by Duncan (46) in that enzyme reactions participate in the excitatory mechanism of frog taste receptors.

Electrically induced taste sensation

By stimulating single fungiform papillae in man, chemically as well as electrically, Békésy was able to obtain subjective evidence that the two methods do produce identical taste sensations and that only the four basic taste qualities could exist (25, 26, 27). Nejad (137) had previously given evidence that the integrated neural activity from the rat chorda tympani nerve is very similar whether the tongue is stimulated electrically or chemically.

Ichioka (94) quantified the relationship between electrical stimulus applied and the stimulus interval in terms of the following equation:

$$V = K_1 (1 - e^{-I/K_2}) \qquad (3)$$

where

V is the stimulus intensity in volts,

I is the stimulus interval in milliseconds,

K_1 and K_2 are constants relating to the applied pulse duration and the number of pulses.

Curves similar to those obtained for chemical response-concentration data were observed.

Efferent control of gustatory receptors

Halpern (72) has stated that "neural control of a receptor system requires that physiologically meaningful stimuli, when applied to a receptor locus which is separate from the locus under study, modulate the adequately evoked action potential pattern of the receptor system."

Kimura (110) studied the effects of electrical and chemical stimulation on sympathetic nerves, and the application of epinephrine, atropine, strychnine, acetylcholine, and eserine on the nerve activity of the chorda tympani nerve in the rat. Neural activity was found to be markedly increased whenever the sympathetic nerve was stimulated electrically while simultaneously chemical taste solutions were applied to the tongue. Chemical stimulus (sodium citrate) to the nerve resulted in an increase in spontaneous activity and response to taste solution. Intravenous injections of epinephrine gave similar results to nerve chemical stimulation although increase was not as marked. Injection of eserine and atropine did not appear to have any effect on neural activity. Strychnine and acetylcholine increased the nerve response to taste solutions without increasing the spontaneous discharge.

In Russia, Esakov (52, 53, 54) has studied the efferent control of spontaneous activity and taste activity in frogs

(Rana temporaria, Rana ridibunda, and Rana esculenta). Gastric distention with water increased nerve activity during application of NaCl and water to the tongue, but activity decreased during application of quinine HCl. Application of peptone into the stomach markedly decreased peripheral activity to water, slightly decreased activity to NaCl, but did not alter activity to quinine HCl. Stimulation of the interoceptors of the stomach indicated the spontaneous activity of the tongue chemoreceptors would adapt the tongue for improved perception. He also recorded efferent activity in the sublingual nerve to applications of 5% NaCl solutions.

Halpern and Postles (72; summarized in 76) monitored changes in taste response during gastric distention by balloon and during interoceptor stimulation with protein hydrolysate. Responses to 0.5M NaCl were consistently larger during gastric distention thereby indicating that gastric receptors provide feedback control to the gustatory receptor system.

Sensitivity enhancement through tongue movement

That movement of the tongue surface enhances taste sensitivity was probably first noticed by Appelberg (8). He found that if a blunt glass rod was used to stroke the tongue surface while simultaneously applying the chemical stimulus an enhancement of the integrated response from the glossopharyngeal nerve would occur. He interpreted this to mean

that movement of the tongue surface allows ions to stimulate deep lying receptors not otherwise accessible. Bell and Kitchell (28) later observed that movement of the circumvallate papillae in goats, sheep, and calves would enhance gustatory responses. The most detailed study of the effects of tongue movement on gustatory responses comes from the work of Ishiko and Amatsu (98, 99) on cats with and without tongue movement. No significant alteration of the spontaneous discharge level was noted between stretched and relaxed tongue position. Tongue movement that was not sufficient to activate tactile receptors resulted in marked increases of integrated response activity to taste solutions in both the chorda tympani and glossopharyngeal nerve. The increase in chorda tympani nerve activity was most obvious with quinine and NaCl. They state that the most probable explanation of this effect is one that Beidler advanced (20). Beidler contends that movement interferes with existing concentration gradients near the receptors so that these gradients are redistributed, consequently the change in neural activity.

Experiments on Non-Gustatory Receptors in the Tongue

Neural activity from the somesthetic receptors of the tongue has been studied almost exclusively by Zotterman and his colleagues in Sweden. In general, this work has con-

centrated on thermal receptors alone, although some attempts have been made to quantify activity from fibres responding to both mechanical and thermal stimuli. Most of this work has been confined to analyzing responses from the chorda tympani and lingual nerve of the cat. Two excellent review papers by Zotterman (196, 197) and two publications by Hensel (83, 84) summarize their work.

In this survey the following topics are covered: (1) cold receptors; (2) warm receptors; (3) comparison between cold and warm receptors; (4) thermoreceptors in species other than cat; (5) subsidiary topics on thermoreceptor phenomena; (6) mechanoreceptors; (7) fibres responding to both mechanical and thermal stimulation.

Cold receptors

Zotterman has studied the discharge of cold receptors under conditions of constant temperature and under temperature changes.

Discharges at constant temperatures In conjunction with Hensel, he noted cold receptor impulses in fine strands of lingual nerve to constant cooling of the tongue surface via a water thermode (86, 91). At temperatures below 23°C, for a given constant temperature, a continuous steady barrage of impulses at constant frequencies existed for periods of more than 1 hour. Rewarming the tongue caused an immediate

cessation of impulses. In other work [see Hensel and Zotterman (88), Hensel and Witt (87)] intracutaneous temperature gradients were applied to preparations containing cold nerve fibres which innervated only the upper surface of the tongue. Upon direct cooling of the lower surface of the tongue, no immediate reaction was observed until the cold temperature had penetrated to the upper tongue surface. At this juncture impulses from cold receptors appeared with increasing frequency as more receptors were recruited. Rewarming the lower surface had no effect on cold fibre impulses until the warmth had penetrated to the upper surface whereupon activity ceased. Hence the cold receptor does not depend on slope or direction of temperature gradient but only on the cooling of the receptor layer, i.e. on the absolute temperature. (The impulse frequency was found to rise even if the slope of the gradient decreases or becomes zero.)

A quantitative study which differentiated between the single cold fibre discharge and the temperature of the receptor indicated that over the temperature range from 10° to 41°C a maximum frequency of about 10 impulses per second could be obtained. The site at which the maximum frequency occurred varied. The response usually occurred between 30° and 32°C. As long as the tongue was warmed to temperatures under 22°C the cold impulses continued to appear. Warming to temperatures

above 22°C caused the cold induced inpulses to gradually disappear. This was referred to as the persisting cold sensation (90). Dodt (41) has shown that a secondary smaller discharge maximum occurs in the range 10° to 15°C. No cold receptor activity was noted below 8°C.

Discharges through temperature change Rapid cooling of single cold receptors was found to raise the discharge level to values of about 140 impulses per second, a value more than 10 times greater than that observed under conditions of steady temperature. This frequency of discharge was attributed to the temporal gradient of temperature change set up in the tongue. The initial absolute tongue temperature was found to be important when applying rapid cold stimuli. Different degrees of activity were observed for the same magnitude of cold temperature change or "cold jump" at different values of initial tongue temperature. Within certain ranges of temperature there was an approximately linear relation between initial and final frequency. This relationship was 3:1 for 1°C cold jumps and 6:1 for 2°C cold jumps (197).

Warm receptors

Spikes from specific warm fibres were first recorded by Zotterman in 1936 from the lingual nerve of the cat (199). Much later, Dodt and Zotterman (44) showed that warm fibres react in an analogous way to that of cold fibres. Their main

conclusions are deferred until the next section "Comparison between cold and warm receptors."

Comparison between cold and warm receptors

Hensel (83) has differentiated cold receptors from warm receptors in the following manner. A cold (warm) receptor is one having: (1) frequency rise (fall) on sudden cooling; (2) no response on sudden warming (cooling) if the fibre is silent, or inhibition of a resting discharge; (3) a steady discharge dependent on temperature; (4) no response to weak mechanical stimulation.

Dodt and Zotterman (44) have compared various characteristics of the behavior of warm and cold receptors. Warm receptors are characterized by: (1) steady discharge rate at constant temperatures between 20° and 47°C; (2) maximum frequency of steady discharge at 3.7 impulses per second, within a generalized temperature range of 38° to 43°C; (3) sporadic discharge of impulses in response to constant temperature; (4) "paradoxical" discharge of phasic character to a fall in temperature of more than 8° to 15°C. Cold receptors exhibit: (1) steady discharge rate at constant temperatures from about 10° to 41°C; (2) maximum frequency of steady discharge at 9.8 impulses per second within a generalized temperature range of 25° to 35°C; (3) periodic discharge of impulses in response to constant temperatures; (4) "paradoxical" steady discharge in response to constant temperatures of 45° to 50°C.

Hensel (83) has stated that the steady discharge rate associated with constant temperature application to the surface of the tongue is not a valid means of concluding whether a cold or warm receptor is invoked, since both have a positive temperature coefficient below the maximum and a negative coefficient above the maximum. He states that the discharge frequency will always change in the direction of sudden temperature change. A cold receptor will display a transient increase in activity during cooling and a transient decrease or inhibition during warming; whereas a warm receptor will behave in the opposite direction. Therefore a receptor's dynamic behavior should be used rather than its static behavior for precise definition.

Thermoreceptors in species other than cat

A few studies have concentrated on other species. The presence of cold and warm fibres in the dog has been confirmed (83). Kitchell, Ström, and Zotterman (116) have studied thermal receptors in the chicken and pigeon; Iggo and Leek (96) have concentrated on the sheep.

Kitchell et al. monitored the neural activity associated with steady temperature conditions and sudden temperature drops from lingual nerve and laryngo-lingual nerve preparations. For instance, at a constant temperature of 44°C a steady barrage of 2 to 4 impulses per second was associated with a lingual nerve cold fibre in the chicken. A rapid drop

of 9°C from this constant temperature resulted in an abrupt increase to 29 impulses per second, which gradually declined to a frequency of 8 to 10 impulses per second, before reaching pre-stimulus activity. Similar observations were noted in the laryngo-lingual nerve in chickens. Rapid temperature drops failed to activate the lingual nerve in pigeons; however, some activity was noted in the laryngo-lingual nerve.

Other salient observations in these two species of birds were: (1) no evidence of warm fibre activity; (2) thermal receptors in chickens are active at constant temperatures much higher than those found in mammals and pigeons; (3) the paradoxical cold sensation is not observed electrophysiologically in the chicken.

In the sheep Iggo and Leek found numerous cold fibres in the lingual, chorda tympani, and glossopharyngeal nerves. They noted an increase in the rate of discharge when the tongue cooled suddenly, and a steady discharge at constant temperatures. Only one warm fibre was confirmed.

Subsidiary topics on thermoreceptors phenomena

Three topics of current interest have appeared in the literature in recent years. These include: (1) paradoxical sensations; (2) menthol action on thermoreceptors; (3) factors governing thermal impulses. Each of these are treated briefly below.

Paradoxical sensations Paradoxical sensations are usually relegated to sensations in which warming (cooling) the skin produces a feeling of cold (warmth). At constant temperatures above 45°C, Dodt and Zotterman (43) found that the cold fibres from the cat's tongue start discharging again. This corresponds well with the paradoxical cold sensations in man (83). Below 45°C, paradoxical excitation of cold receptors does not occur. It is still dubious whether a corresponding paradoxical warmth can be found. The paradoxical response found in cooling warm receptors by more than 8°C has been quoted by Dodt and Zotterman to have "more the character of an off-discharge of phasic nature" (197).

Menthol action on thermoreceptors The effect of menthol on cold receptors was studied by Hensel and Zotterman (89). For a given constant temperature it was noted that the effects of menthol increased the steady state activity not normally associated without menthol. Menthol was found to activate neural discharges at those constant warm temperatures at which cold receptors usually do not fire. Sudden heating compensates for the effects of menthol.

Factors governing thermal impulses The thermoreceptor discharge is dependent on the absolute temperature θ, the temporal gradient $d\theta/dt$ of temperature change, and the receptive field area F (83, 197). The factor $d\theta/dt$ is the most important factor, in that the maximum frequency during rapid

temperature changes can exceed the frequency of the steady discharge (which is dependent on θ) by about 10 times.

Zotterman (summarized in 182, 197) states that the conscious cold sensation probably has a higher threshold in the central nervous system than the peripheral threshold for cold fibre discharges. The decline in frequency of cold fibre discharges on either side of an optimal temperature range (usually 25° to 35°C) has suggested to Zotterman that receptor discharge is governed by two processes, one excitatory and one inhibitory. Support for this hypothesis is also based on the transient increase in frequency when temperature is rapidly dropped as well as the post-excitatory depression for temperature changes in the opposite direction.

Hensel (85; summarized in 182) has specifically studied the time factor involved in the excitation of thermal receptors. He found that the impulse frequency of the cold receptor system can be related to the difference between an excitatory and an inhibitory process.

Mechanoreceptors

Mechanoreceptors in the tongue of sheep have been studied by Iggo and Leek (96) by recording neural impulses from the lingual and glossopharyngeal nerves of the animal.

Three categories of receptor types were recognized: (1) rapidly-adapting mechanoreceptors in the mucosa, from which

controlled mechanical stimuli would elicit a brief burst of impulses on contact and upon removal of the stimulating probe; (2) slowly-adapting mechanoreceptors in the mucosa, from which a constant tongue pressure would evoke a steady discharge of impulses; (3) slowly-adapting receptors having a high mechanical threshold, which were excited upon stretching or distorting the tongue.

Fibres responding to both mechanical and thermal stimulation

Hensel and Zotterman (92) have described a number of mechanoreceptors which may be activated by cooling. Recordings from strands from the lingual nerve of the cat showed that there were specific cold fibres having small amplitude impulses (199), large specific mechanoreceptor fibres (12 to 15 microns diameter) having a spike amplitude of almost 8 to 10 times that of the cold fibre impulses, and smaller mechanoreceptors (8 to 10 microns diameter) which also responded to cooling with spike amplitudes of about 3 to 5 times that of cold fibres. The latter fibres respond only to very low temperatures and rapid cooling. As soon as the temperature was constant, the discharge activity ceased in those mechanoreceptors sensitive to temperature. This is in direct contrast to the behavior of cold fibres. Later, Hellekant (80) indicated that pure mechanoreceptors could not be stimulated by ethyl alcohol. Iggo and Leek (96) gave evidence that only the superficial,

slowly-adapting mechanoreceptors in the tongue of the sheep were temperature sensitive. The rapidly-adapting mechanoreceptors were not excited, and deep, slowly-adapting units were generally unaffected by low temperatures.

METHODOLOGY, MATERIALS AND INSTRUMENTATION

General

In the following sections of this chapter the methodology for preparing the animal for neural activity extraction and the way in which the data were subsequently transcribed for analysis are first presented. This is followed by a description of the materials used and the instruments designed to carry out data extraction and its subsequent conditioning.

Methodology

A total of 22 cross-bred pigeons (cross between Homer, King, Carneaux, and domestic) were used in the experiments. Pigeons were chosen because previous studies [Kitchell *et al.* (116)] had indicated the presence of far fewer active fibres than most other species and consequently single fibre data would be easier to extract. For coding purposes, the pigeon should be an ideal specimen since basically only two of the four modalities--sour and salt--activate the chemoreceptor sites and therefore the model should be relatively simple. It is conceded that other solutions such as hydroxides, distilled water, or sweets can activate receptor sites; however, our study indicates the latter two solution types are not consistent from

bird to bird, while the former is probably in a class by itself. Then too, the very limited number of studies on the pigeon warranted a fuller investigation of the electrophysiological properties of chemoreceptors. Pigeons numbered 1 through 12 were used to locate and identify the anatomical position of the peripheral nerves sensitive to chemoreceptor stimulation of the tongue. Pigeons numbered 13 through 22 were used to extract both single fibre and multiple fibre activity from the peripheral nerves identified as subserving chemoreception. A wide variety of chemical stimuli were used. In all cases the solvent was distilled water. Not all stimuli were used with any particular pigeon. Also, electrode types for extracting neural spike data were not consistent from experiment to experiment. This was primarily due to the fact that as experiments and techniques progressed, certain electrode types were found to be superior to others. This aspect is given more coverage on page 125.

Twelve hours before the experiment started, the pigeon was taken off both food and water. It was anaesthetized with injections of anaesthetic agent[1] at a dosage level of 2.5 milliliters per kilogram in the breast region muscles.[2] The bird was

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[1]Equi-Thesin. Jensen-Salsbery Laboratories, Division of Richardson-Merrell Inc., Kansas City, Missouri.

[2]At first, the birds were anaesthetized by way of a cannulated wing vein but this proved to be unsatisfactory as well as time consuming because of the small blood vessel size. It was also very easy to over-anaesthetize the bird by this method.

securely fastened on its back to a retaining board by means of canvas webbing straps and the trachea exposed and excised in preparation for artificial respiration. The feathers in the abdominal region were removed, an abdominal mid-line incision (approximately 3 inches long) was made, and gross separation of fascia, muscle and peritoneum took place to obtain access to the abdominal air sacs which were subsequently ruptured by means of forceps.[1] A self-retaining retractor[2] was used to maintain the abdominal incision open to allow unimpeded flow of respiratory gases.

At this time the tracheal tube from a unidirectional flow respirator was inserted into the trachea to maintain artificial respiration (see page 102 for details). The expired gases were exhausted through the incised abdominal air sacs.

The flow of filtered air mixture from the respirator was held at 2500 cubic centimeters (cc) per minute; carbon dioxide (CO_2) flow was set at 50 cc per minute. This level of hyperventilation together with periodic injections of 0.5 cc anaesthetic agent kept the pigeon in a satisfactory surgical-

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[1]Iris forceps, 1x2 teeth, half-curved. Arista Surgical Co., 67 Lexington Avenue, New York, New York 10010.

[2]Alm retractor, self-retaining, stainless steel. Arista Surgical Co., 67 Lexington Avenue, New York, New York 10010.

anaesthesial plane throughout the preparatory and recording sessions. Since respiratory muscle movement is markedly reduced by hyperventilation, it was necessary to monitor heart beat by means of an auxilliary amplifier in conjunction with an audio monitor to determine the viability of the bird (see page 135 for details).

A thermistor probe was inserted into the vent of the bird and its temperature noted. Temperatures ranged from 31° to 35°C on the 10 birds used in the study. Once the temperature was recorded, it was maintained there throughout the experiment by thermostatically regulating the water heater-circulator (see pages 108 and 109 for details). Knowing the bird's temperature provided a convenient standard for setting the temperature of the chemical taste solutions. This standard was based on the fact that the bird's tongue, which was exposed to the room temperature atmosphere, was approximately 3°C lower than the vent temperature of the bird. It was noted that chemical solutions kept at the vent temperature would encounter about a 3°C drop from the time of placement into the taste burette to the time of application to the tongue surface. Solutions were kept close to the tongue temperature to avoid responses due to thermal fibre activity (116). The vent temperature, solution temperature, and tongue temperature were monitored (specific details on page 126).

Other preparatory procedures involved anchorage of the head to the retaining board by means of a copper spring clip inserted into the external auditory meatus. Sutures, applied through the nasal aperture and the top of the lower beak, allowed access to the buccal cavity when tension was applied. These sutures were fastened to the retaining board by means of hook screws or thumb tacks. A tension suture, placed in the lateral aspect of the anterior third of the tongue, was fastened to the retaining board so that the pharyngeal portion of the tongue was exposed for chemical solution application from a dispensing burette. A small gauze sponge moistened in physiological saline covered the exposed tongue until neural recording took place. Non-toxic modeling clay[1] was used to form a taste solution run-off channel from the tongue area to a drainage receptacle.

The feathers in the lateral head and neck region were clipped. A one inch skin incision was made parallel and 1/4 inch ventral to the external auditory meatus at the level of the ramus of the mandible. The skin was bluntly separated from the superficial masseter muscle and mandible to expose the cornu of the hyoid bone. Approximately 6 stay sutures were placed through the periphery of the incision and fastened to a skin retractor ring to provide a suitable mineral oil pool. (Mineral oil at the bird temperature was used to slow

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[1]Permoplast modeling clay. American Art Clay Co., Indianapolis, Indiana.

down degeneration of neural tissue and to reduce shunting between electrode tips.)

The caudal attachment of the cornu of the hyoid bone was separated; a stay suture was attached, and the cornu retracted cranio-laterally so as to expose the main surgical field.

The mineral oil pool was filled, a dissecting microscope and a high intensity lamp were placed over the surgical field.

The laryngeal artery was located and exposed up to the point where it branched from the external carotid artery. A loose fine thread was passed around the former artery to retract and make accessible the deeply situated glossopharyngeal nerve trunk. Two major nerve branches were found to arise from the glossopharyngeal nerve, both cranial to the junction of the laryngeal and external carotid arteries. The posterior branch, referred to as the pharyngeal branch,[1] had been tested initially for neural response to chemical solutions without any results, and was therefore ignored in subsequent work. The anterior branch, referred to as the lingual branch was severed by means of very fine scissors,[2,3] from the glossopharyngeal nerve trunk for further

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[1]Ghoshal, N. G., Department of Veterinary Anatomy, College of Veterinary Medicine, Iowa State University of Science and Technology, Ames, Iowa. Comments on nerve supply to head of pigeon. Private communication. 1968

[2]Number C410, Castroviejo corneal scissors, angular. Arista Surgical Co., 67 Lexington Avenue, New York, New York 10010.

[3]Number C450, Westcott tenotomy scissors, spring handle. Arista Surgical Co., 67 Lexington Avenue, New York, New York 10010.

nerve preparation and also to prevent recording of central nervous system efferent activity. When the epineural sheath was stripped away from the lingual branch with very fine jewellers' forceps,[1] two separate individual nerves (in the majority of cases) were revealed. The anterior nerve, referred to as the lingual nerve by Kitchell et al. (116), was found to enter the lateral aspect of the cornu whereas the posterior nerve, labelled laryngo-lingual nerve by Kitchell et al. (116), was seen to enter the medial side of the cornu.

When the lingual branch nerve complex was severed from the glossopharyngeal nerve trunk, the immediate appearance of a tremor in the musculature covering the cornu of the hyboid bone was noticed. This provided a useful cue for establishing the identity of the lingual branch nerve complex since other adjacent nerve branches did not provide this method of indication for motor nerve supply to the cornu.

Both the lingual and the laryngo-lingual nerves were placed separately on recording electrodes and their tongue receptive fields tested and defined by means of a blunt glass rod. The dorso-anterior portion of the tongue was found to set up neural impulses in the lingual nerve to touch; the

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[1]Number 4 Dumont jewellers' forceps. S & H Clausin and Co., 41 N. 12th Street, Minneapolis, Minnesota.

posterior and pharyngeal regions activated the laryngo-lingual nerve to touch. Test chemical solutions, applied to the tongue receptive fields by pipette, indicated that neural activity was subserved only by the laryngo-lingual nerve; the lingual nerve remained quiescent. This is in contrast to the observations of Kitchell et al. (116) as they recorded neural activity from both nerves to chemical stimuli; however, it does substantiate the observations of Halpern (71, p. 542) on chickens.

The procedure carried out thus far can be visualized by observing the series of photographs and diagrams now introduced. In Figure 1 a simple schematic diagram is shown in order to indicate how the bird's head was securely fastened and how the skin retractor ring was used to form a suitable mineral oil pool. In addition, the gross anatomical features of the recording field are indicated within the skin retractor ring area. The photographs in Figures 2a and 2b show the pigeon under conditions of actual experimentation. Figure 2a is very similar to Figure 1. In Figure 2b the taste dispensing burette, the electrode positioning assembly, the dissecting microscope, and the recording field illuminating lamp are all shown. Photomicrographs of the nerve and arterial supply within the recording area of a typical pigeon are shown in Figures 3a and 3b. A composite representation of the overall nerve and blood supply in the nerve recording

Figure 1. Simple schematic representation of pigeon preparation showing skin retractor ring set-up used to form a suitable mineral oil pool. Beak and tongue are shown to be secured by means of thumb tacks; the head region by way of an ear bar. Gross anatomical features of recording field are indicated within the mineral oil pool area. The specific nerve recorded from is shown as being draped over an electrode of the bipolar type

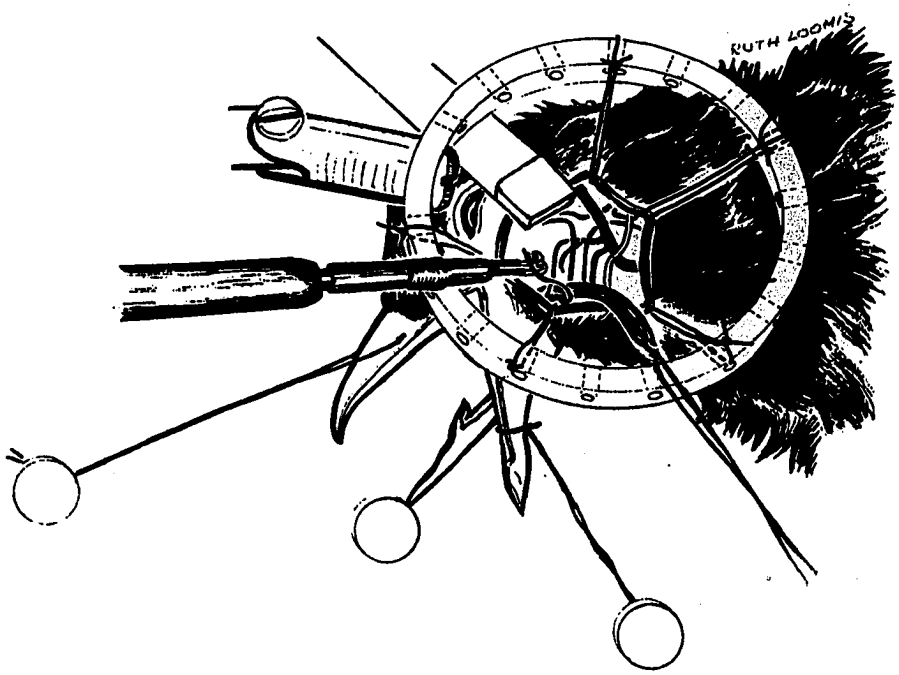

RUTH LOOMIS

Figure 2a. Photograph showing an uncluttered view of experimental pigeon preparation. Note the taste solution drainage channel at the bottom center of the photograph

Figure 2b. As in Figure 2a, except that all items normally used during experimentation are now in their appropriate positions. Note particularly the taste dispensing burette, (middle left side) the electrode positioning assembly (bottom right side) the dissecting microscope (upper center) and the recording field illuminating lamp (upper right side). A small thermistor bead placed on the tongue for monitoring solution temperature is also shown

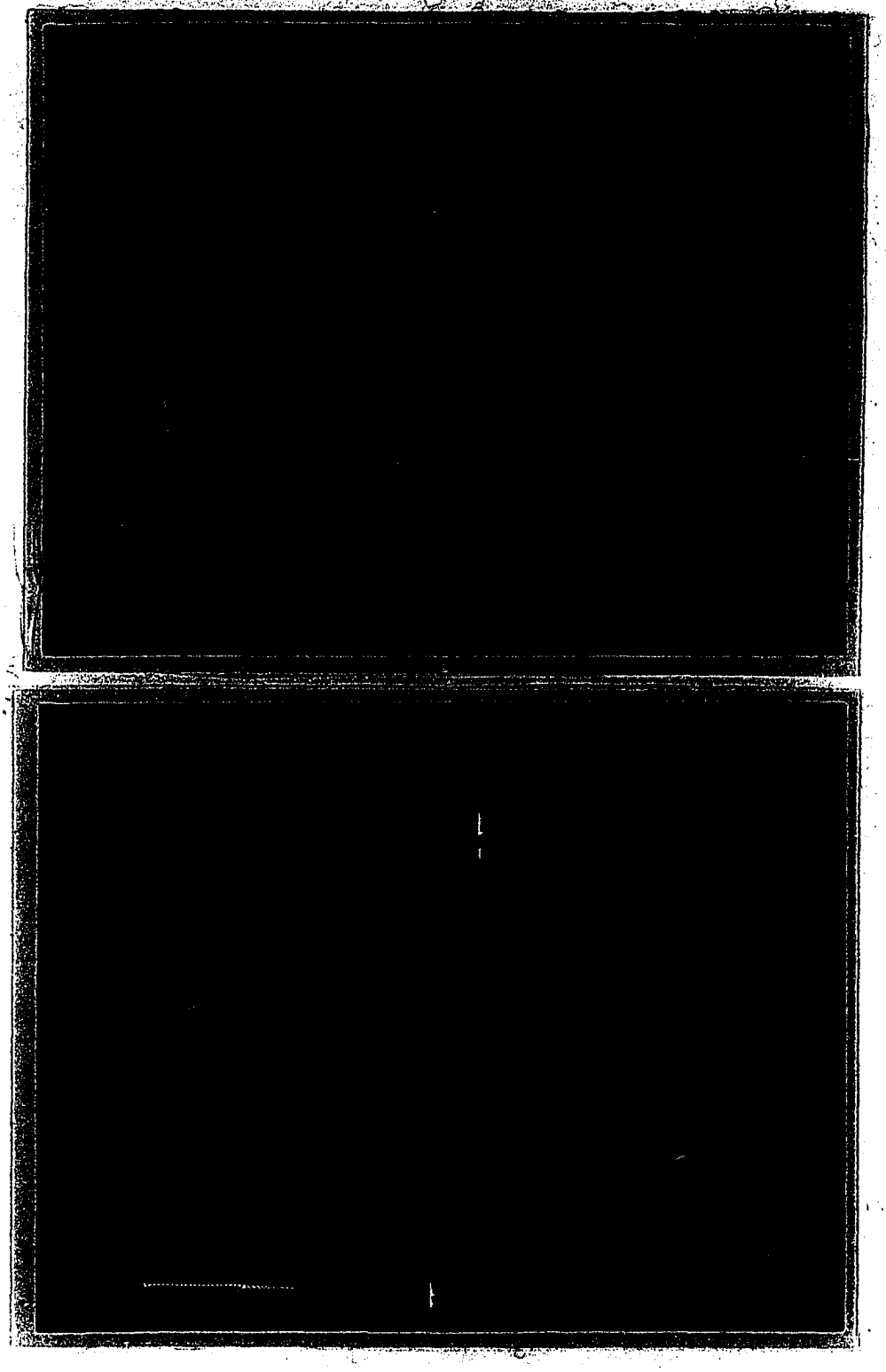


Figure 3a. Photomicrograph of nerve recording area showing:

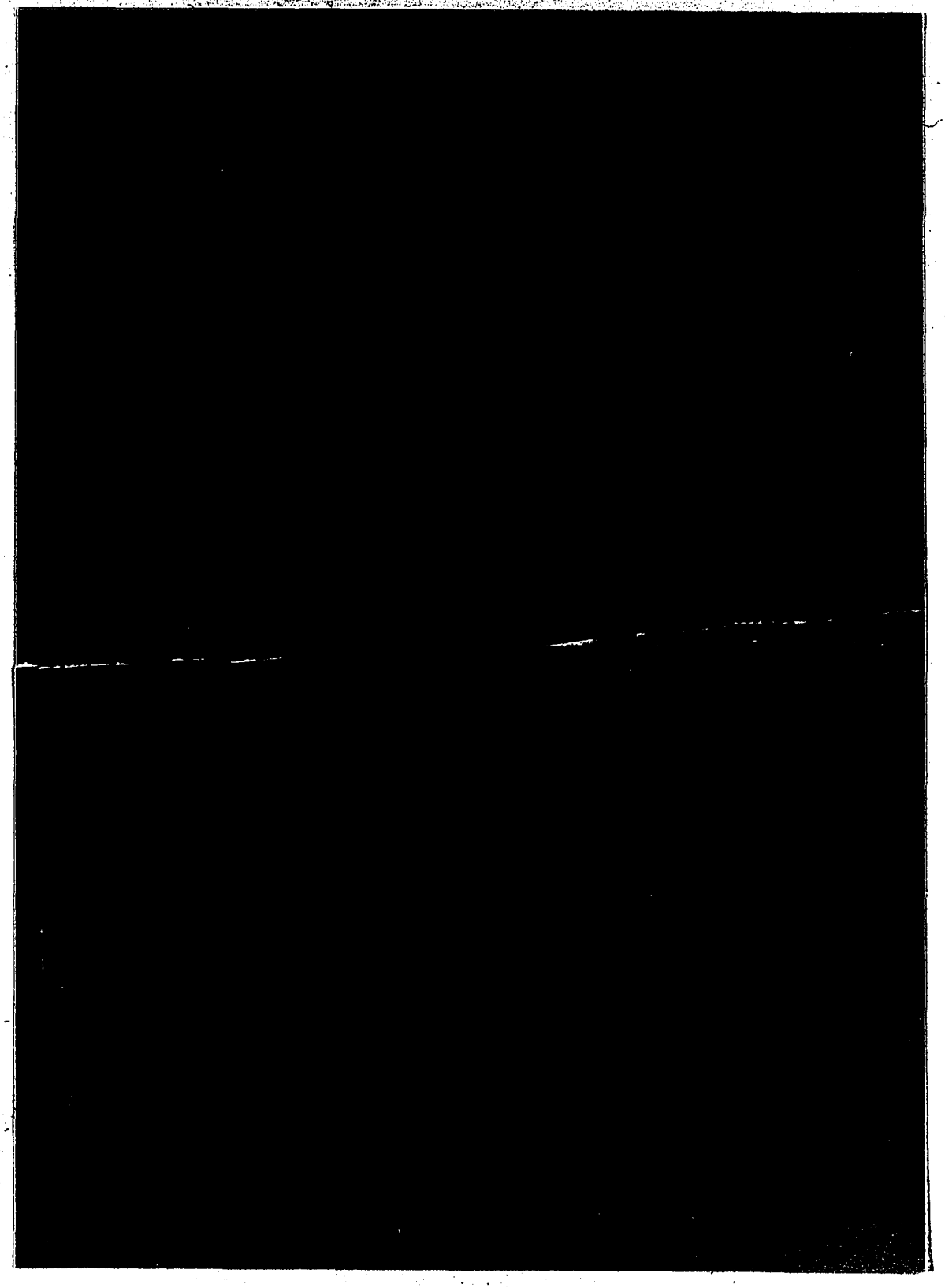
a. external carotid artery
b. laryngeal artery
c. lingual branch of IX nerve containing both lingual nerve and laryngo-lingual nerve (confirmed electrophysiologically)
d. laryngeal and pharyngeal branches of vagus nerve (X)

Magnification: 7X approximately

Figure 3b. Same surgical field as in Figure 3a but laryngeal artery has been retracted to show:

a. glossopharyngeal nerve (IX)
b. lingual branch arising from IX nerve
c. pharyngeal branch of IX nerve
d. retracted cornu of hyoid bone
e. laryngo-lingual nerve circling around cornu to enter at its medial side

Magnification: 7X approximately

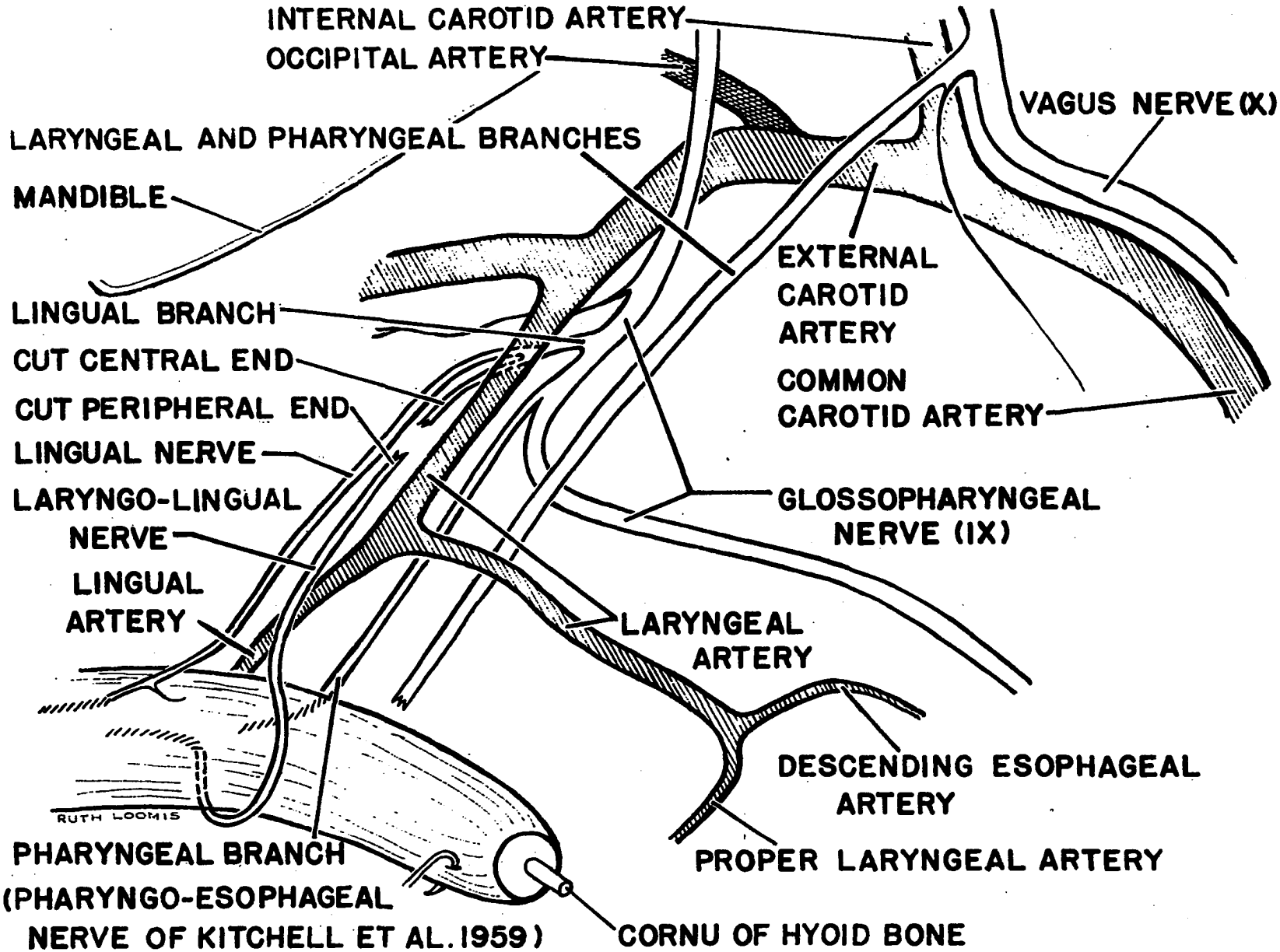


region is shown schematically by way of Figure 4. Although anatomical variations were noted from pigeon to pigeon, the schematic diagram in Figure 4 does represent the most commonly found features as based on 12 separate dissections. The arterial blood supply designations were those used by Kaupp (105); nerve supply notations were taken from Kolda and Komárek (118, p. 235), and Schwarze and Schröder (167, p. 194) as needed.

For recording multiple nerve fibre activity, no additional preparation was necessary; the desheathed nerve was placed over the recording electrode tip and slightly stretched. Electrodes were either bipolar or monopolar (specific details on pages 123 to 126). When using the bipolar electrode type the cut end of the nerve was made to adhere to one of the electrode tips so that monophasic activity would be picked up. With the monopolar electrode type, the nerve was merely draped across the electrode tip and an indifferent electrode, brought into proximity to it, was positioned and imbedded into adjacent muscle tissue where pick-up noise and artifacts (EKG, movement, etc.) were minimized. A ground reference electrode was similarly placed into muscle tissue for maximum cancellation of pick-up noise and EKG artifact to the differential preamplifier system (see page 141 for some details on preamplifier).

Few fibres and single fibres required further preparation. Some of the dissecting instruments used in this phase of

Figure 4. Schematic representation of the IX and X cranial nerves with their accompanying major arteries. Note that laryngeal artery has been stretched in retraction of the cornu of the hyoid bone. Neural recording was confined to the cut peripheral end of the laryngo-lingual nerve


INTERNAL CAROTID ARTERY
OCCIPITAL ARTERY
LARYNGEAL AND PHARYNGEAL BRANCHES
MANDIBLE
VAGUS NERVE (X)
EXTERNAL CAROTID ARTERY
COMMON CAROTID ARTERY
LINGUAL BRANCH
CUT CENTRAL END
CUT PERIPHERAL END
LINGUAL NERVE
LARYNGO-LINGUAL NERVE
LINGUAL ARTERY
GLOSSOPHARYNGEAL NERVE (IX)
LARYNGEAL ARTERY
DESCENDING ESOPHAGEAL ARTERY
PROPER LARYNGEAL ARTERY
RUTH LOOMIS
PHARYNGEAL BRANCH (PHARYNGO-ESOPHAGEAL NERVE OF KITCHELL ET AL. 1959)
CORNU OF HYOID BONE

experimentation are shown in Figure 5. The cut peripheral end was held by means of finely sharpened jewellers' forceps or ultra-micro forceps[1,2] and a pair of micro-dissecting tissue scissors[3] or a sharp cutting knife, fabricated from a sharp chip of a razor blade, were used to nick into the whole nerve bundle until several nerve fibres were thought to be isolated. By means of ultra-micro dissecting needles[4,5,6], in conjunction with the ultra-micro forceps, the nicked part of the nerve bundle was carefully separated from the parent bundle to form a thin strand of approximately 0.5 centimeters in length. This strand was placed over a monopolar electrode; a glass rod or chemical solutions were applied to the tongue,

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[1]Number V37379A, Trident forceps, ultra-micro, straight tip. Aloe Scientific, Health and Sciences Division, Brunswick Corp., 1831 Olive Street, St. Louis, Missouri 63103.

[2]Number V37379B, Trident forceps, ultra-micro, 45° angle tip. Aloe Scientific, Health and Sciences Division, Brunswick Corp., 1831 Olive Street, St. Louis, Missouri 63103.

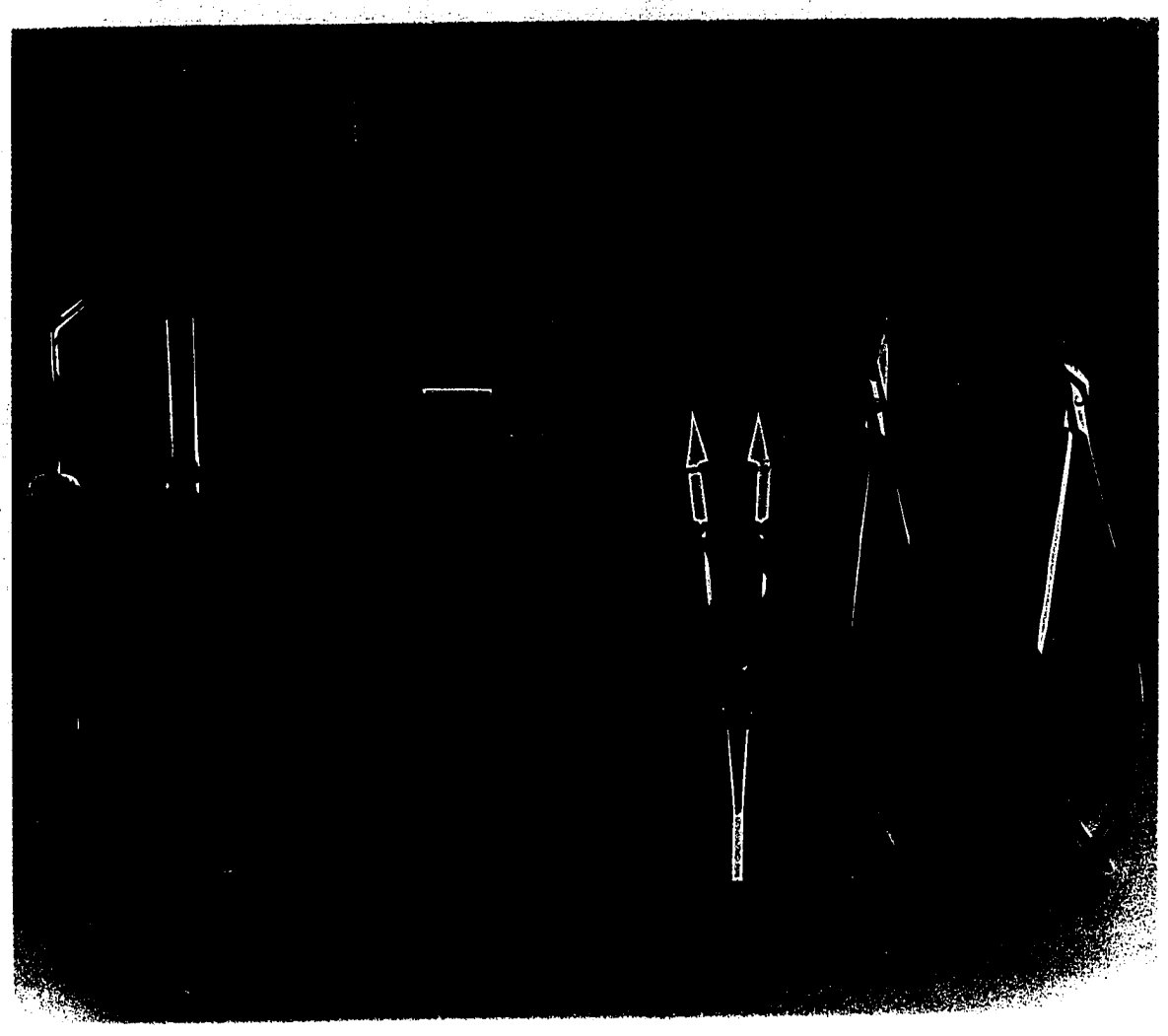
[3]Number V38559CR, Trident scissors, micro-dissecting tissue, clipper type, straight ultra-fine points. Aloe Scientific, Health and Sciences Division, Brunswick Corp., 1831 Olive Street, St. Louis, Missouri 63103.

[4]Number V38053, Trident hook, ultra-micro, 75 micron. Aloe Scientific, Health and Sciences Division, Brunswick Corp., 1831 Olive Street, St. Louis, Missouri 63103.

[5]Number V38056, Trident needle, ultra-micro. Aloe Scientific, Health and Sciences Division, Brunswick Corp., 1831 Olive Street, St. Louis, Missouri 63103.

[6]Number V38057, Trident chuck handle, micro-tool. Aloe Scientific, Health and Sciences Division, Brunswick Corp., 1831 Olive Street, St. Louis, Missouri 63103.

Figure 5. Surgical instruments used in nerve dissection showing from left to right Trident 45° angle tip ultra-micro forceps, Trident straight tip ultra-micro forceps, Dumont jewellers' forceps, Trident ultra-micro needle in chuck handle, Trident ultra-micro hook, Trident micro-dissecting tissue scissors, Westcott tenotomy scissors, and Castroveijo corneal scissors



and the neural activity monitored on a cathode ray oscilloscope. If a single constant height action potential or at the most 2 or 3 separate constant height action potentials were noted, no further nerve preparation was deemed necessary and the series of chemical solutions could be applied to the tongue and neural activity recorded on magnetic tape. If the above condition was not satisfied, further pruning of the fine strand was required. When the few fibre or single fibre strand deteriorated, the process was repeated and a new strand was isolated from the parent nerve. To slow down nerve deterioration, mainly through blood serum seepage into the mineral oil pool, the mineral oil was periodically swabbed out with cotton gauze and replenished with fresh solution.

Chemical solutions were placed on the tongue by means of a special taste burette (see Figure 2b and page 122) except in the case of one experiment (see Table 5). Capacity of the taste burette was set at 10cc and a flow rate to the tongue was measured at 1cc per second. This represented one stimulus. Small 8 inch pipettes were used to transfer the pre-heated chemical solutions from special tanks (see pages 130 to 132 for details) to the dispensing burette. After each application of chemical stimulus, both the burette and tongue were flushed with solutions of physiological saline and/or tap water to return neural activity to the resting state. An additional flush of saturated $NaHCO_3$ (buffer solution) again

followed by tap water was used when acid represented the chemical stimulus as it was noted that acid had a depressant effect on receptor site activity. Between each stimulus a 3 minute time lapse was observed. A simple mercury switch, attached to the burette stopcock, was activated when the system was open to flow. The time delay between activation of stopcock mercury switch to the instant when chemical solution first touched tongue surface was of the order of 0.8 seconds.

The neural activity from the laryngo-lingual nerve was fed from the recording electrode to a high impedance probe and then to a high gain ac preamplifier (details on page 138). From the preamplifier output four parallel paths were possible: (1) directly to a magnetic tape recording system; (2) through a discriminator and integrator network and then to the tape recorder; (3) directly to an audio monitor; (4) directly to a visual monitor--a cathode ray oscilloscope. Paths (1), (3) and (4) were usually used. Gain of the ac preamplifier was set at 50,000; low frequency cut-off was set at 30 hertz; high frequency cut-off was set at 10 kilohertz.

A time mark generator was designed so that a suitable time scale could be related to the neural activity recorded (details on page 145). To conserve a magnetic tape recording channel it was decided to superimpose the event signal from the taste dispensing burette onto the time mark signal. A simple base line shift of the time mark signal provided a

suitable event marker. Period of the time signal was 100 milliseconds, with resolution to less than 30 milliseconds.

A thermistor bead (see Figure 2b) in conjunction with a driver amplifier could be used to record temperatures from the tongue onto the magnetic tape recording system if required.

Provisions were made to record up to four distinct signals on the tape recorder. All signals were recorded and reproduced from the recorder at 7 1/2 inches per second using the FM mode feature available.

For the single fibre data analysis, the neural signal and time/event signals were extracted from the tape recorder by passing both signals simultaneously to a cathode ray oscilloscope where they were photographed on paper film with a Kymograph® camera. The neural signals were first channeled to a band-pass filter set at cut-off frequencies of 120 hertz and 2 kilohertz respectively. This removed the power mains 60 hertz interference plus high frequency components with only negligible loss to individual action potential wave shape. Camera speed was set at either 25 millimeters per second or 50 millimeters per second for single fibre analysis.

After developing the paper film, selected strips suitable for single fibre data analysis were studied. The criterion used to identify a single fibre response was to scan the record and search for uniformity of the all-or-none height of

the action potentials. Records in which "doubling-up" of impulses took place were discarded (145, p. 22). When 2 or 3 fibres were involved, a series of hypothetical windows were drawn along the paper record. Within each window only one single fibre could fall. The width of each window was predetermined by the noise level of the record, as the impulses tended to follow small noise fluctuations. Details of this gating procedure can be found in Bureš et al. (33, pp. 264-265). Only those records in which the amplitudes of the action potentials were well above the noise level were given any consideration. For a given single fibre the responses to several solutions were extracted from the records and histograms made by counting the number of impulses in each 0.25 second interval as a function of time. Counting started with the first impulse noted after solution had touched the tongue surface. Further statistical work on single fibre and few fibre activity was confined to using Spearman's rank correlation and product-moment correlation techniques on 4 specific single units, each activated by 2M NaCl, 2M NH_4Cl, 2M KCl, 0.5M HCl, 0.5M acetic acid, and 0.5M oxalic acid. Details of this are given in the section, RESULTS AND DISCUSSION.

Extraction of multiple fibre activity from the magnetic tape recording system was carried out by passing the neural data through a band-pass filter, then into a discriminator

and integrator circuit, and finally to a paper pen recorder. The integrator part of the circuit had a time constant set at 2.0 seconds throughout the reproduction session. From the integrated data displayed by the paper pen recorder, the time from initial nerve activity to peak nerve activity was extracted and the peak response to each test solution was compared to the peak response of 2M NaCl. Further details of this phase are given in the section, RESULTS AND DISCUSSION.

Materials and Instrumentation

General

The overall system can be broken down into 5 basic subsystems for purposes of description. Each subsystem and its relationship to the other subsystems is outlined in the block diagram of Figure 6. Specifically, the 5 subsystems have been classified as:

(1) the animal respirator subsystem;

(2) the animal preparation and signal extraction subsystem;

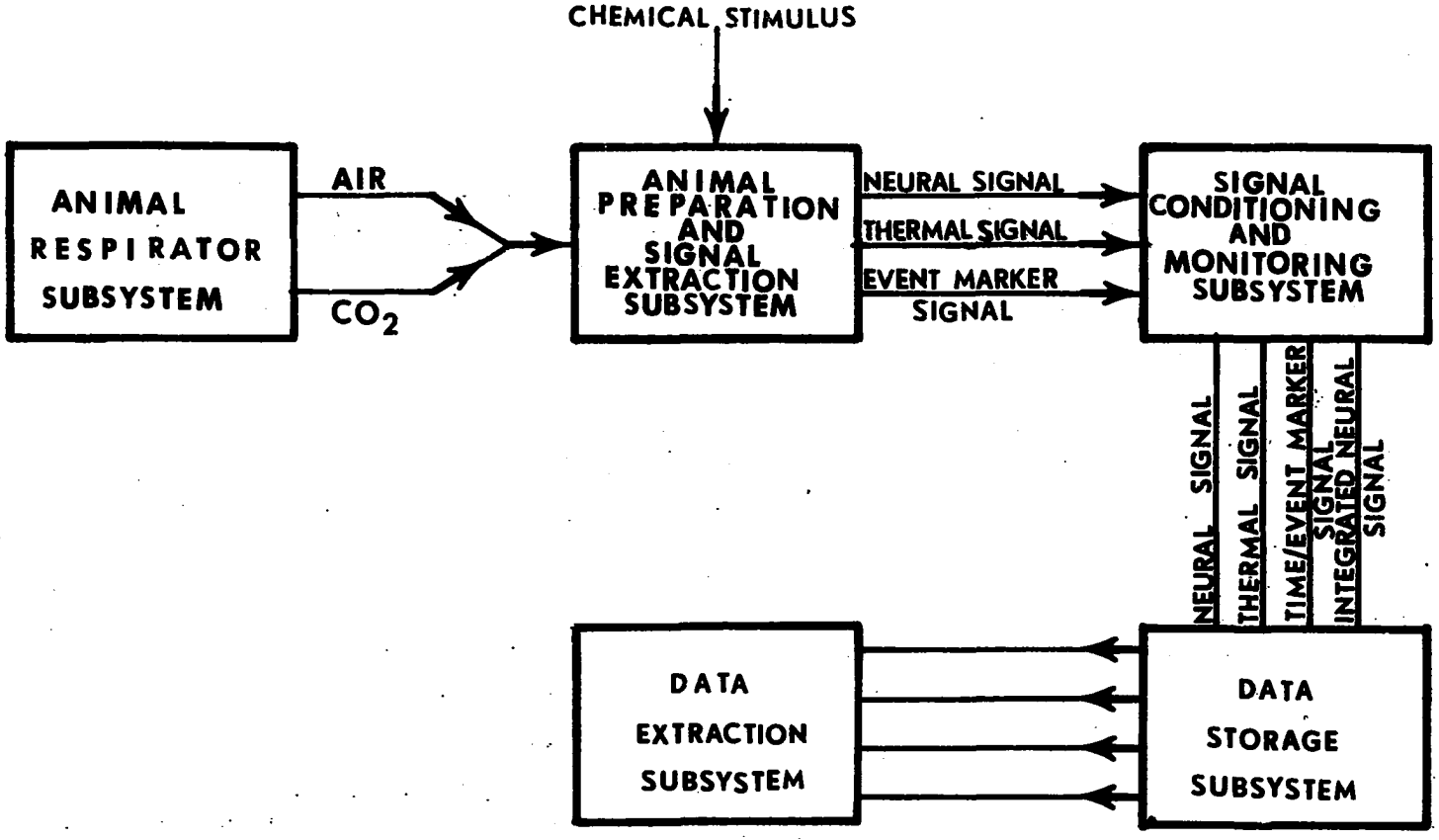
(3) the signal conditioning and monitoring subsystem;

(4) the data storage subsystem;

(5) the data extraction subsystem.

Each subsystem will now be covered in some detail.

Figure 6. Block diagram illustrating interrelationship between major subsystems


CHEMICAL STIMULUS
ANIMAL RESPIRATOR SUBSYSTEM
AIR
CO_2
ANIMAL PREPARATION AND SIGNAL EXTRACTION SUBSYSTEM
NEURAL SIGNAL
THERMAL SIGNAL
EVENT MARKER SIGNAL
SIGNAL CONDITIONING AND MONITORING SUBSYSTEM
NEURAL SIGNAL
THERMAL SIGNAL
TIME/EVENT MARKER SIGNAL
INTEGRATED NEURAL SIGNAL
DATA EXTRACTION SUBSYSTEM
DATA STORAGE SUBSYSTEM

The animal respirator subsystem

Since neural recordings are very susceptible to movement artifact (particularly respiratory muscle movement in our case), it was necessary to anaesthetize the animal very deeply so that these artifacts became minimal. Unless one is extremely careful in the type and dosage level of anaesthetic agent administered, however, death through depression of respiratory brain centres is a quitc common sequela when operating on birds (116, p. 134). This factor, above all, led to the fabrication of a suitable respirator so that deep levels of anaesthesia could be tolerated while maintaining adequate respiratory ventilation.

The design chosen employed a unidirectional flow of gas (air and carbon dioxide) as outlined by Burger and Lorenz (34) to which was added a gas heater and humidifier as described by Fedde and Burger (58), so that bird body temperature could be maintained during hyperventilation under deep levels of anaesthesia.

Although the method used was essentially that of the above two experimental groups, the final respirator design differed somewhat and therefore merits some description.

The air--water mixing chamber The main component of the respirator subsystem was the air--water mixing chamber. It was composed of a 12 inch long lucite cylindrical sleeve having 1/8 inch wall thickness and 2 1/2 inches inside

diameter (ID), plus a 4 inch long lucite collar (sealed onto the upper portion of the cylindrical sleeve) having 1/4 inch wall thickness and 3 1/2 inches ID. A series of very small orifices were drilled into the cylindrical sleeve in order that heated water could enter in the form of a spray. The tiny orifices, located 2 inches from the top of the cylindrical sleeve, covered a band of approximately 2 inches in length around the periphery and were placed so that the water spray was directed into the geometric center of the system where it could mix with incoming room temperature air. All ends of the cylindrical sleeve--collar complex were enclosed with circular lucite plate of 1/4 inch thickness. All interfaces were sealed with epoxy resin and hardener[1] so that the system was water tight. The air--water mixing chamber is shown in cross-section in Figure 7.

Heated water from a motor driven circulator was made to enter the cylindrical collar in two diametrically opposite positions by means of brass tubing connectors. A total of 4 connectors were used; 2 in each position lay in the same vertical plane and were about 3 inches apart. Tygon® tubing interconnections between the 4 tubing connectors were such that water pressure was effectively equalized around the

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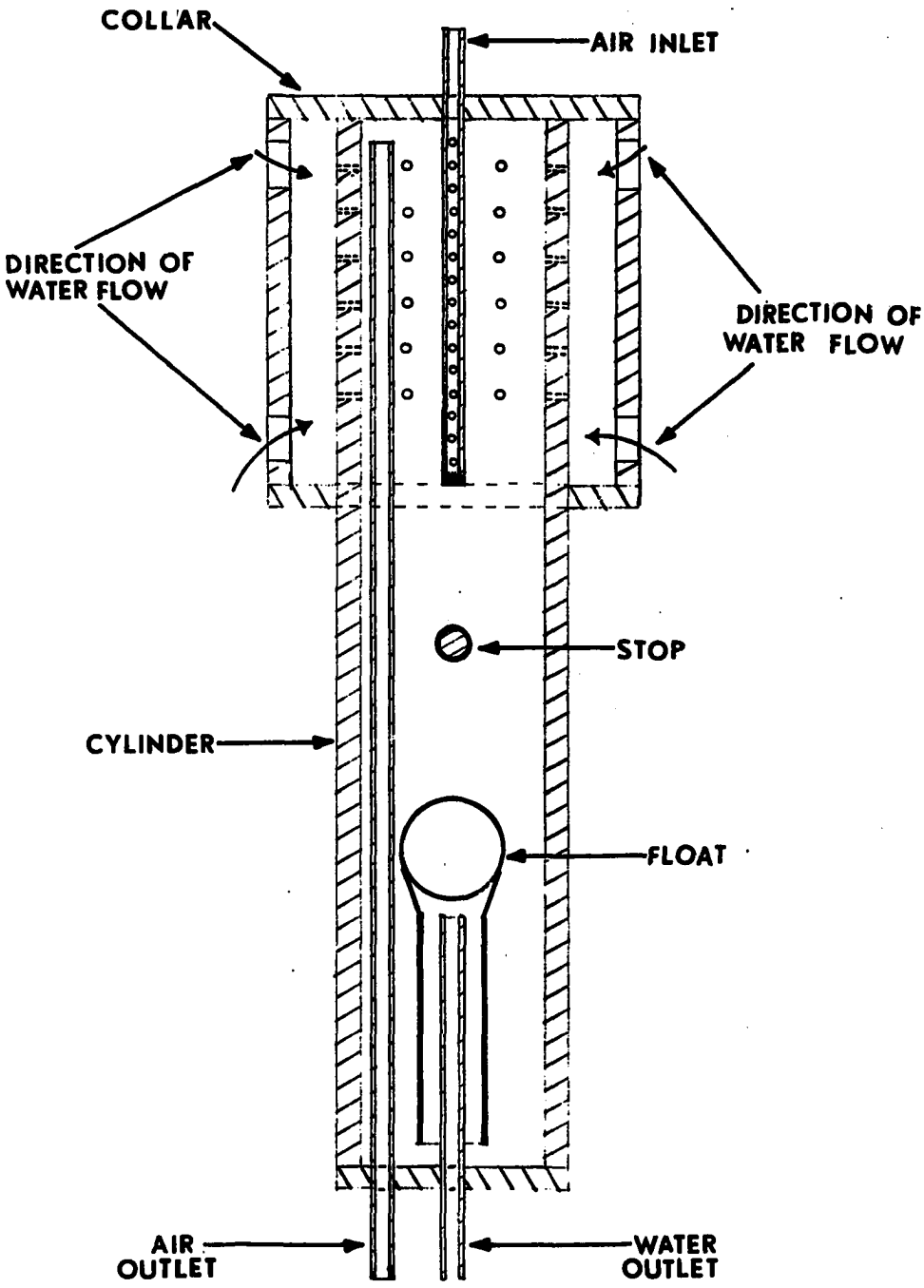
[1]Epoxy Dura plastic. The Woodhill Chemical Corporation, Cleveland 28, Ohio.

Figure 7. Schematic diagram illustrating the air--water mixing chamber of the animal respirator subsystem


COLLAR
AIR INLET
DIRECTION OF WATER FLOW
DIRECTION OF WATER FLOW
STOP
CYLINDER
FLOAT
AIR OUTLET
WATER OUTLET

cylindrical collar, thereby creating a uniform spray. To avoid congestion of the drilled pores in the cylindrical sleeve, distilled water was used during the experimental procedures. The cylindrical sleeve-collar complex represented the gas heater and humidifier portion of Fedde and Burger's apparatus.

Air entered the top of the cylindrical sleeve at 14.7 pounds per square inch (psi) through a 1/4 inch ID plexiglass tubing. Fine holes were drilled around the perimeter of this tubing through which the air was made to flow by sealing the normal exit end of the tubing. The tubing was centered in the midst of the water spray.

The lucite plate covering the bottom of the mixing chamber had two pieces of plexiglass tubing exiting from it. One of these was to return water to its container, and the other to provide an exit for the moistened and heated air mixture.

To exit the returning water a piece of 3/8 inch ID plexiglass tubing was cut so that at least a 3 inch length projected into and through the bottom of the mixing chamber. Around this projection was placed a 3/4 inch ID plexiglass tubing, 2 inches in length with a standard ping-pong ball secured at one end to act as a float. A stop within the mixing chamber gave assurance that the float system would enclose the 3 inch plexiglass projection at all times. The plexiglass

tubing projecting outward from the mixing chamber was fitted with a length of 5/8 inch ID black latex tubing in order to return water to its container. Water was forced from the mixing chamber by way of gravity and cylinder air pressure. With the aid of a hose clamp the returning water flow could be regulated.

The purpose of the float system was two-fold.[1] Firstly, to assure that the humid air only enters into the bird preparation, and secondly, to maintain the same volume of air in the bird constant.

As stated above, moistened air was made to exit through a length of plexiglass tubing (5/8 inch ID). The tubing was of sufficient length so that moistened air from within the mixing chamber entered it well above the water spray line, thus preventing excess water from possible entry into the bird preparation. Air leaving the mixing chamber entered a special configuration[2] of glass tubing where it was mixed with carbon dioxide having a 10 psi manifold pressure. Carbon dioxide is soluble in water, forming carbonic acid, and therefore had to be replaced in the air mixture. This was

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[1]Nightingale, T., Dept. of Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas. Comments on unidirectional respirator. Private communication. 1967.

[2]Ibid., Sample provided by T. Nightingale.

accomplished by by-passing the carbon dioxide from the air--water mixing chamber. The temperature of the humid air and carbon dioxide mixture leaving the special configuration of glass tubing was monitored by a standard thermometer. All air exits from the lucite mixing chamber and the special configuration of glass tubing were enclosed in insulation rubber hosing[1] and then wrapped in masking tape to prevent vapor condensation from going into the bird. An 18 inch piece of latex tubing[2] joined the special configuration of glass tubing to a glass water trap. From the water trap an additional 12 inch piece of latex tubing joined to a T-connector. One end of the T-connector was fastened to a short piece of 3/16 inch ID infant suction catheter tubing[3] which was used as a tracheal tube for the bird. The remaining end of the T-connector was connected by way of a valve to an aspirator pump.

The heated water supply system A water container was formed by insulating a Pyrex® glass jar[4] of 12 inch height and 12 inch ID with a 1 inch thick fibreglass layer. The

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[1]Armstrong Armaflex 1/2 inch ID x 3/8 inch.

[2]Amber latex tubing. Aloe Medical Division of Brunswick, St. Louis 3, Missouri.

[3]Number V5-S-1022 sterile suction catheter, 10 French. Becton Dickinson and Co., Rutherford, New Jersey.

[4]Pyrex laboratory glassware. Corning Glass Works, Corning, New York.

insulated unit was then placed inside a 1/4 inch thick rectangular plywood box of external dimensions 15 inches x 15 inches x 13 inches. Expanded polyethylene foam chips were used to fill all empty corners so that water heat loss would be reduced. A 1 1/2 inch thick masonite cover, having a portion of its underside centrally indented and cut out so as to fit snuggly over the Pyrex® glass jar, completed the water container.

A commercially available heater--circulator[1] was fitted through the water container cover. This attachment was capable of thermostatically regulating and circulating the water to the remotely located air--water mixing chamber.

The gas mixture supply The air supply necessary to hyperventilate the bird was drawn from the laboratory air pump system. This system provides an air supply at 80 psi; consequently, a pressure regulator and reducer valve[2] were needed to reduce the pressure to the one atmosphere (14.7 psi) required. The air was first filtered[3] and then led by way of

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[1]Thermomix II, 115 volts, 60 herz, 750 watts. B. Braun, Melsungen, Germany. Distributed in U.S.A. by Bronwill Scientific Division, Will Corporation, 277 North Goodman St., P. O. Box 277, Rochester, New York 14601.

[2]Type 289H-42. Fisher Governor Company, Marshalltown, Iowa.

[3]Pollutex air filter. The Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128.

1/4 inch polyethylene tubing through a safety relief valve unit[1] set at 20 psi. The safety valve was then connected by means of a short piece of 1/4 inch outside diameter (OD) copper tubing to a fine metering valve[2], which in turn was connected by more of the same copper tubing to a regulated gas flow meter tube[3] capable of providing air up to 5800 cc per minute at 70°F and 14.7 psi. Tygon® tubing was used to complete the loop to the air--water mixing chamber.

The 100% carbon dioxide supply needed was drawn from a gas cylinder by way of a two-stage gas regulator[4] to a fine metering valve assembly[5] before passing out through the

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[1]Made up of (a) safety relief valve, Catalog Number 400-6R-4M-10; (b) female T-connector, Catalog Number 400-334-TTF; (c) 1/4 inch Swagelok inlet and outlet connectors. Nuclear Products Company, 15635 Saranac Road, Cleveland, Ohio 44110.

[2]Made up of (a) fine metering valve, Catalog Number SS-4M; (b) 1/4 inch Swagelok inlet and outlet connectors. Nuclear Product Company; 15635 Saranac Road, Cleveland, Ohio 44110.

[3]Rotometer, Type 4-15-2 thru-taper tube with glass and stainless steel floats. Ace Glass Incorporated; Vineland, New Jersey 08360.

[4]Two-stage regulator with two 2 1/2 inch diameter gauges, inlet pressure gauge reads to 3000 psi, delivery pressure gauge reads to 100 psi. The Matheson Company; Joliet, Illinois.

[5]Made up of (a) fine metering valve, Catalog Number SS-2S; (b) 1/8 inch Swagelok inlet and outlet connectors; (c) Swagelok reducing union, 1/4 inch to 1/8 inch, Catalog Number 400-6-2. Nuclear Products Company, 15635 Saranac Road, Cleveland, Ohio 44110.

special configuration of glass tubing. Polyethylene tubing was used to link the regulator to the metering valve assembly. Short pieces of 1/8 inch OD and 1/4 inch OD copper tubing were used for interconnections within the metering valve assembly. The desired flow could be adjusted with the metering valve assembly and read out on a gas flow meter tube[1] (capable of providing air up to 440 cc per minute at 70°F and 14.7 psi) before entering the special configuration of glass tubing by way of Tygon® tubing.

The assembled apparatus of the animal respirator system as used during actual experimentation is shown by way of a labelled photograph (Figure 8).

Feasibility test A test on three White Leghorn roosters of 6 months age was carried out to determine the ability of the respirator for hyperventilation. The test consisted in measuring the physiological parameters of pH, P_{O_2}, and P_{CO_2} before ventilation, under several levels of hyperventilation, and under conditions of rapidly incremented doses of anaesthesia. Concomitant with these measurements the vent temperature of the bird and time of blood extraction were recorded. Blood samples drawn from

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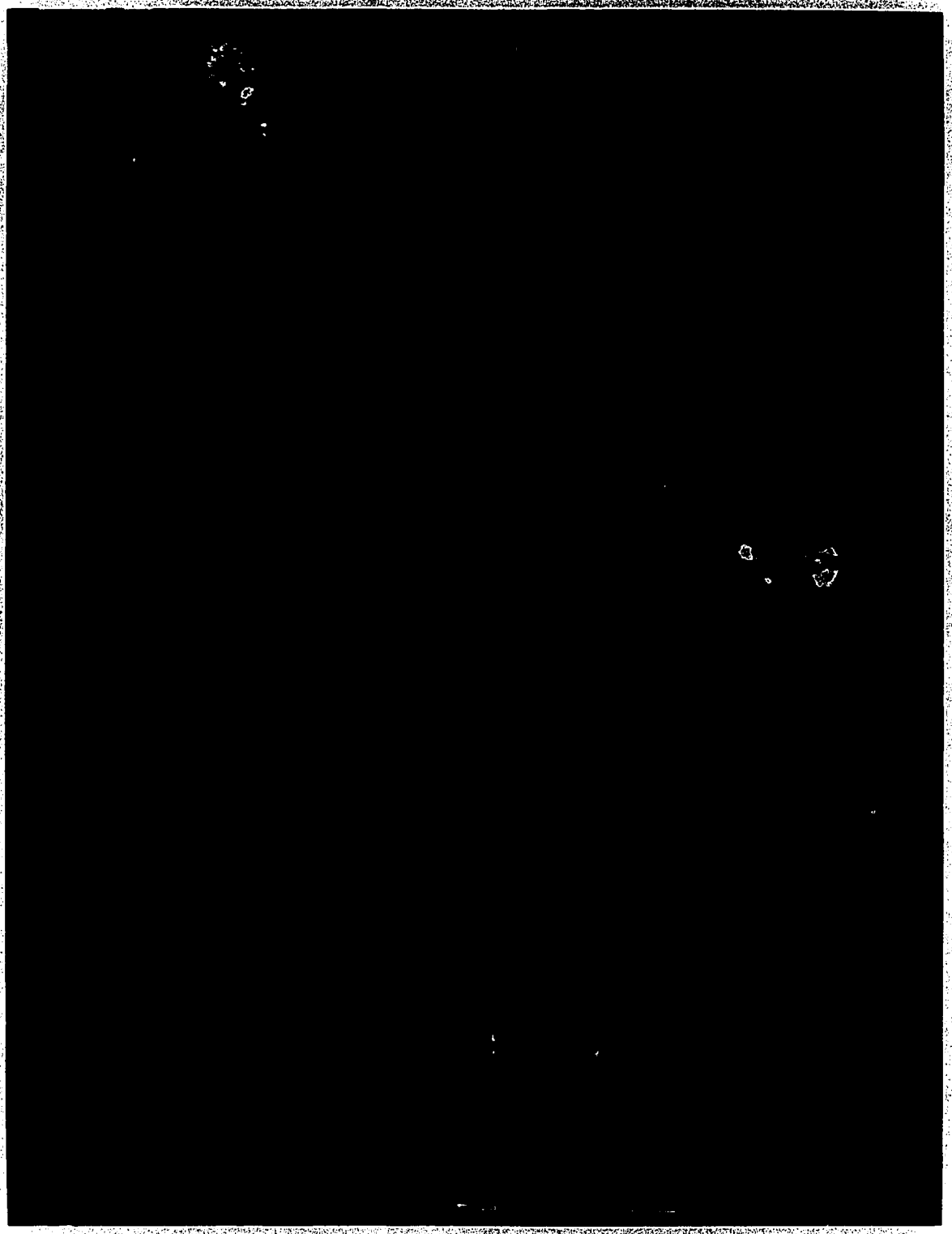
[1]Rotometer, Type 1A-15-1 thru-taper tube with glass and stainless steel foats. Ace Glass Incorporated, Vineland, New Jersey 08360.

Figure 8. Animal respirator subsystem showing:

a. air--water mixing chamber
b. insulated water container
c. motor driven heater--circulator
d. water traps
e. tracheal tube
f. laboratory air supply
g. CO_2 fine metering valve assembly
h. air supply fine metering valve assembly



a convenient wing vein, were assumed to be representative of an average blood sample and therefore should represent the blood supply to the tongue. Normal hyperventilation for the rooster was taken as a flow of 4000 cc per minute, which was made up of 3840 cc per minute of air and 160 cc per minute of carbon dioxide[1]. Values of pH, P_{O_2}, and P_{CO_2}, as measured on a commercial physiological gas analyzer[2] with multiple cuvette[3] arrangement are included in Tables 1, 2, and 3 for the 3 preparations considered.

In general, values of pH, P_{O_2}, and P_{CO_2} under normal conditions, i.e. before respiratory ventilation, agreed fairly closely with values quoted by Sturkie (171, pp. 180-183). Under conditions of hyperventilation the venous P_{CO_2} value decreases somewhat and the venous P_{O_2} correspondingly increases as might be expected. Anaesthesia tends to decrease pH values slightly from the norm in the two preparations administered. The fact that abnormal changes in pH, P_{O_2}, and P_{CO_2} do not occur as the levels of ventilation and anaesthesia are changed

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[1]Nightingale, op. cit.

[2]Model 160 Physiological Gas Analyzer. Beckman Instrument Inc., Palo Alto, California.

[3]Multiple cuvette. Beckman Instrument Inc., Palo Alto, California.

Table 1. pH, P_{O_2} and P_{CO_2} values for Rooster number 1

Conditions	Time (minutes)	Vent temperature (°C)	pH	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)
Before ventilation	0	39.4	7.02	35.0	48.5
Hyperventilation at 3840 cc/min. air; 160 cc/min. CO_2	49	39.8	7.24	51.0	44.0
	84	40.2	6.90	63.0	32.0

Table 2. pH, P_{O_2} and P_{CO_2} values for Rooster number 2

Conditions	Time (minutes)	Vent temperature (°C)	pH	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)
Before ventilation	0	38.4	7.16	45.8	34.4
Hyperventilation at 3840 cc/min. air; 160 cc/min. CO_2	23	39.4	7.16	47.5	34.0
	47	39.4	7.15	64.0	34.3
	72	39.2	7.15	64.0	34.4
Hyperventilation changed at time = 99 minutes to 5000 cc/min. air; 360 cc/min. CO_2	111	39.4	7.11	64.0	33.4
	132	39.4	7.12	61.0	31.8
Hyperventilation level unchanged; 2.5 cc anaesthesia administered at time = 148 minutes	176	39.3	7.10	51.0	32.2

Table 3. pH, P_{O_2} and P_{CO_2} values for Rooster number 3

Conditions	Time (minutes	Vent temperature (°C)	pH	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)
Before ventilation	0	39.9	7.18	56.0	40.0
Hyperventilation at 3840 cc/min. air; 160 cc/min. CO_2	17	39.4	7.16	64.0	40.0
	41[a]	39.7	7.15	58.0	42.0
	62	39.9	7.14	63.0	39.0
	84	40.0	7.12	61.0	39.5
Hyperventilation changed at time = 84 minutes to 5000 cc/min. air; 360 cc/min. CO_2	109	39.7	7.11	59.0	36.0
Hyperventilation level unchanged; 2.5 cc anaesthesia administered at time = 115 minutes	141	38.8	7.08	52.0	37.0

[a]Hyperventilation was accidentally interrupted just prior to this measurement.

is sufficient evidence that an adequately rich blood supply reaches the tongue region and therefore nerve degeneration or tissue necrosis should not occur due to a deficient blood supply.

The animal preparation and signal extraction subsystem

Basically, the subsystem was made up of those components necessary to prepare the animal for neural extraction together

with ancilliaries required for proper application of the chemical stimuli.

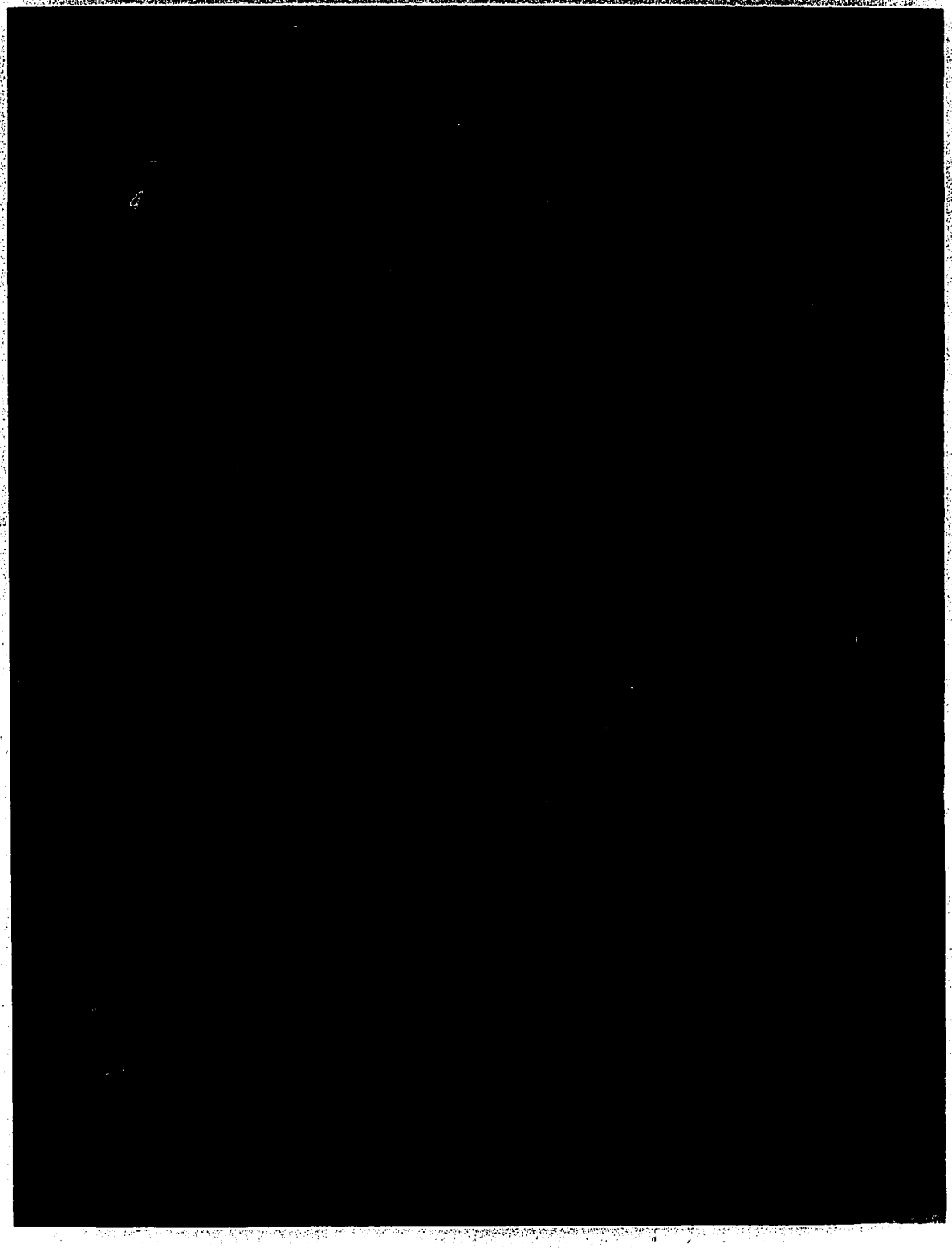
Components for neural extraction A sturdy supporting table, on which was mounted a shielded bronze screened cage, represents the basic building block of the subsystem. Directly within the screened cage, a special animal preparation platform was built on which a movable animal retaining board was positioned and secured by means of a bar clamp. These items can be seen in Figure 9 together with other assemblage.

To reduce extraneous mechanical motion from being transmitted, through floor vibrations, to the animal preparation (where it would contaminate the low level neural voltages to be extracted), it was necessary to construct a sufficiently rigid supporting table. This was accomplished by reinforcing a heavy wooden table with 2 inch x 4 inch plank braces diagonally crossed between the table legs. This adequately compensated for vibratory motions.

To remove electomagnetic interference, particularly interference at 60 hertz, a shielded bronze screened cage of dimension 42 inches long x 32 inches wide x 36 inches high was fabricated. An aluminum channel allowed the window to be opened and closed in a vertical direction. Window dimensions were 42 inches x 32 inches. An 8 hole copper feed-through strip provided access to all electrical cables. The cage and

Figure 9. Animal preparation and signal extraction subsystem showing:

a. binocular dissecting microscope
b. micromanipulator for positioning of electrodes
c. taste dispensing burette
e. thermistor probe selector switching unit
f. high intensity lamp
g. skin retractor ring
h. high impedance field effect transitor (FET) probe
i. shielded screened cage
j. animal preparation platform
k. animal retaining board
l. EKG monitor amplifier



all electrical equipment were securely grounded to the water mains at one single point to avoid extraneous ground loops.

The special animal preparation platform was positioned solidly inside the screened cage (so that no vibrations could be transferred to the electrode pick-up apparatus during preparation and recording from the animal.) The platform consisted of a 5/8 inch thick piece of plywood (18 inches wide x 30 inches long) from which a small portion (6 inches wide x 12 inches long) was removed from one corner and replaced by a piece of 1/4 inch thick cold rolled steel plate. A 1 1/4 inch wide strip of 1/8 inch thick angle iron was fastened around the perimeter of the platform. Numerous holes of 1/2 inch diameter were drilled through the metal plate and angle iron so that metal binding posts could be mounted at various positions for greater flexibility. The binding posts were used for instrument and equipment attachment. Overall thickness of the platform was approximately 5 inches.

The animal retaining board was made from 1 1/2 inch lumber (12 inches wide x 18 inches long) and had a sharply bevelled block (6 inches wide x 9 inches long) fastened to it at one end. This block was used to position the bird's head for neural recording. Bevelling was done so that taste solutions applied to the tongue would run off to be collected in a drainage receptacle. To secure the bird in a supine

position, 1/2 inch wide canvas webbing belts were fastened onto the retaining board. This included 2 belts for the legs, 4 belts for the wings and 1 belt for the neck region. A 2 1/2 inch long spring clip ear bar, for securing the head to the block and 2 hook screws for fastening tension sutures from the beak completed the retaining board.

The binding post--metal plate combination provided attachment for the following items:

(1) A dissecting microscope[1] from which the base plate was removed and replaced with a 3 inch threaded stud for mounting.

(2) An electrode holder assembly made from a right hand micromanipulator[2] (having tilt movement) from which the horizontal plate and fine vertical control dovetail slide mechanism had been removed. A vertical brass plate with attached binding post bracket was then added in replacement. A polystyrene mounting block (see Figure 11b) for retaining the electrodes in place, was then fastened

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[1]American Optical stereoscopic microscope, Series 23, Objectives: 1X, 3X, 6X. American Optical Company, Scientific Instrument Division, Buffalo, New York 14215.

[2]Prior, England. Serial number 38387. Distributed in U.S.A. as Catalog Number 930/T by Eric Sobotka, 110 Finn Court, Farmingdale, N. Y.

to the extremity of the tilt mechanism.

(3) A 15 cc taste dispensing burette formed by removing the upper portion of a 60 cc separatory funnel[1]. The lower stem was bent at a 60° angle in the direction away from the stopcock ratchet. A small piece of Pyrex® glass tubing was then fastened to the stem end. This tubing was bent so that taste solutions could run onto the tongue area when positioned properly. A simple mercury switch, fastened to the remote end of the stopcock, acted as an event marker whenever the stopcock was open to flow.

(4) A rack for holding up to four electrodes. Electrodes were either bipolar or monopolar in design and were constructed in two different ways:

(a) All bipolar electrodes were constructed by soldering either Number 20 gauge (0.036 inch wire for few fibre recording) silver wire to copper electrical cables. The active electrode end was forced into small diameter glass tubing of about 3 to 4 inches in length. The electrode--glass tubing assemblage was then

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[1]Squibb's pear shaped separatory funnel. Kimble Products, Owens--Illinois, Toledo, Ohio 43601.

placed inside a 1/4 inch ID piece of plexiglass tubing of equivalent length and wrapped with electrical tape for protection.

Electrical cables were either braided 2 wire conductors or else made up of 2 wire coaxial cable[1]. To reduce extraneous electromagnetic pick-up, cables were fabricated as short as possible (12 inches at maximum). In Figure 11b a bipolar electrode is shown. Note that electrodes are hook shaped for good recording geometry so that exposed nerve could be draped onto the electrodes and stretched slightly. Separation of each electrode wire pair was of the order of 1/4 centimeter.

(b) Monopolar electrodes were designed using either treated silver wire or treated platinum--iridium alloy wire[2]. A common shaft for both electrode types was made by soldering an

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[1]Preparatory work was done with coaxial type in which silver bipolar electrode was held together with acrolite fast cure dental plastic. Samples provided by T. Fletcher, Dept. of Anatomy, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota.

[2]Samples provided by J. C. Sinclair, Buena Vista College, Storm Lake, Iowa.

insulated copper wire to the female end of a needle adapter[1]; the entire assembly then placed into a 4 inch length of 3/8 inch ID plexiglass tubing.

The platinum--iridium alloy wire was first electropolished in a solution of sodium cyanide (NaCN) and NaOH until the tip had a diameter in the order of microns. A molten glass bead was then formed and the wire pulled through it until only the extreme etched tip was left untouched. Because of surface tension, a very thin coating of glass insulation could be made to adhere to the etched wire. The length of the electrode tip left unexposed to insulation determined the impedance of the electrode. Further details can be found in Guld (68). The best suited electrodes in this class had an impedance in the order of 100 kilohms. The etched wire was forced into the shaft of a Number 26 gauge hypodermic needle until only a 1/2 inch of electrode was left exposed.

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[1]Luer slip type needle adaptor, Catalog Number 9414. Arthur H. Thomas Co., Philadelphia 5, Pennsylvania.

Monopolar silver electrodes were fabricated from Number 30 gauge wire. The shaft from a Number 22 hypodermic needle was removed and a 1/2 inch length of silver wire soldered to its hub. The electrode tip was etched in a solution of silver nitrate ($AgNO_3$) to form a very fine point. The impedance of this type of electrode was measured and found to be 2500 ohms or less. The platinum--iridium electrode, because of its high impedance, tended to accentuate the neural activity in fibres lying close to the electrode surface. The activity in those fibres furthest away from the electrode surface became buried in the noise of the system. The silver monopolar electrode did not seem to differentiate between close-and far-lying fibres; as a consequence all neural activity appeared to be of the same height above the noise level. Effectively then, even though the impedance level was considerably higher with the platinum--iridium electrode and consequently the noise level was higher, a superior signal-to-noise ratio was evident than with the low impedance silver type.

(c) A simple indifferent electrode and a ground lead made from thick gauge silver wire were

fabricated in a similar manner to that used for the bipolar type. In Figure 11b some of the electrodes used during experimentation are shown.

(5) A thermistor probe selector switching unit which enabled up to 5 individual temperatures to be monitored or recorded. Specifically, thermistor probes were used to monitor the temperature of the taste solution bath[1], the bird's vent[2], and the solution contacting the bird's tongue[3].

(6) Two types of high intensity light sources to illuminate the surgical field. They were:

(a) A glare free, white light lamp[4] consisting of metal shade, 11 inch flexible gooseneck, and transformer. The flexible gooseneck was removed from the transformer and reconnected by way of a 6 foot length of 2 wire copper

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[1]YSI tubular thermistor probe, Catalog Number 403. Yellow Springs Instrument Co., Inc., Yellow Spring, Ohio 45387.

[2]YSI general purpose thermistor probe, Catalog Number 401. Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio 45387.

[3]YSI small surface temperature probe, Catalog Number 427. Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio 45387.

[4]Tensor Hi-Intensity lamp, Student Model 7200, 120 volts, 60 hertz, 25 amperes. Tensor Corporation, Brooklyn, New York 11207

conductor. This enabled the transformer to remain outside the shielded cage. The lamp and gooseneck were fastened to a metal binding post on the animal preparation board.

(b) A cold light lamp consisting of a regulated power supply and a commercially available stereoilluminator[1]. The lamp power supply, shown schematically in Figure 10, is a series regulated, full wave rectifier bridge configuration. Two large capacitors C_1 and C_2 are connected in π-configuration for filtering to dc. The high effective capacitance of the circuit gives a ripple factor of 1.5% and provides an exponential rise in output voltage so that the lamp is brought to full voltage very slowly in order to prolong bulb life.

In the voltage feedback regulator part of the circuit, a sample of the output voltage is extracted from the bridge resistor R_2 and compared by Q_4 with the reference Zener diode voltage. The common-emitter comparison stage Q_4 acts as a dc amplifier to raise the level of

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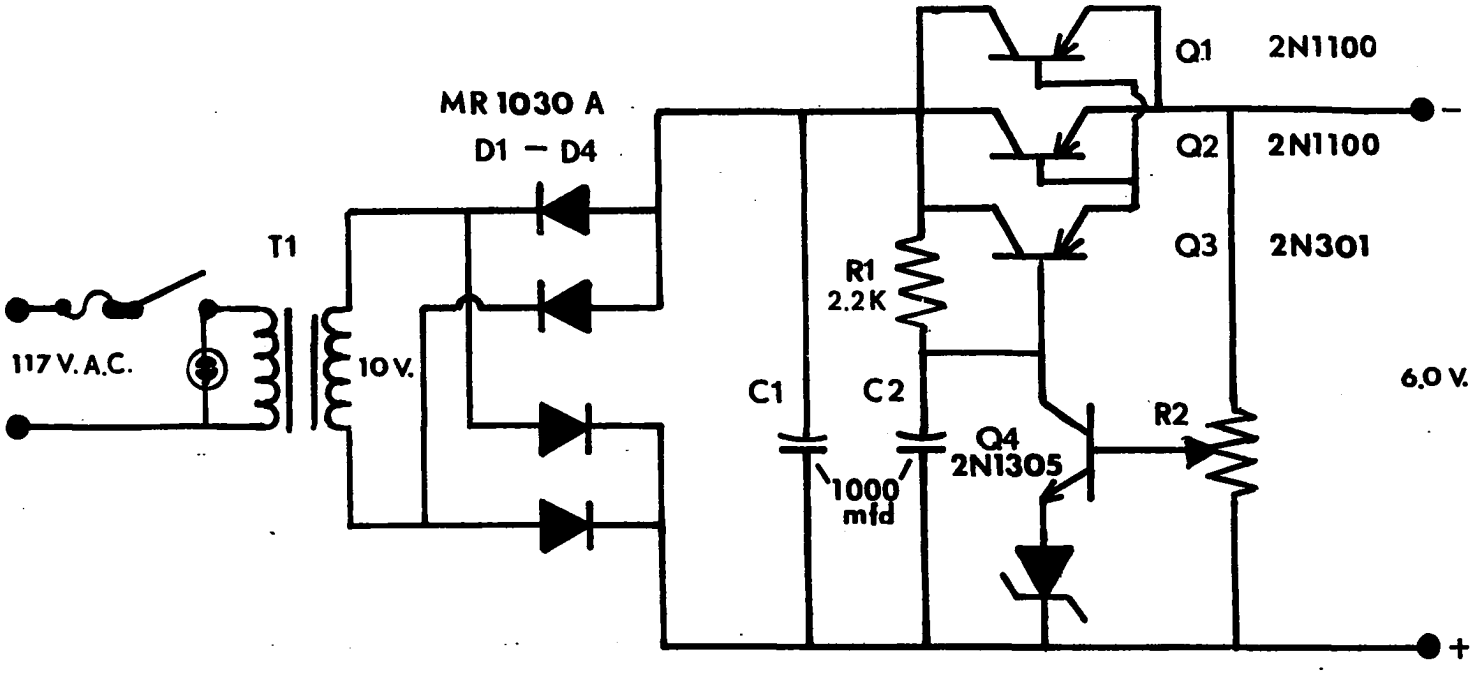
[1]Model 76150 stereoilluminator. Frank E. Fryer., 60 E. Main Street, Carpentersville, Illinois 60110.

Figure 10. Circuit diagram of regulated power supply used with illuminating lamp


MR 1030 A
D1 – D4
T1
117 V. A.C.
10 V.
C1
C2
1000 mfd
R1
2.2K
Q1 2N1100
Q2 2N1100
Q3 2N301
Q4 2N1305
R2
6.0 V.
–
+

the collector current in order to drive the control element. The control element is made up of 2 parallel transistor stages Q_1 and Q_2, compound connected in Darlington configuration with Q_3. The Darlington connection was utilized to provide sufficient current gain to the required lamp load. The parallel configuration of Q_1 and Q_2 acts as a heat sink while also increasing the load current through power amplification. Details on voltage regulators of this type can be found in Walston and Miller (180, pp. 145-167).

(7) A 2 inch OD lucite skin retractor ring of 1/4 inch height having numerous 1/16 inch diameter holes center drilled around the periphery (see Figure 1). Stay sutures from the incised outer skin covering were stretched, threaded through these holes and tied to form a mineral oil pool. The skin retractor ring was glued to an adjustable fibreglass epoxy rod which was anchored to a binding post. By means of the adjustable rod the optimum position of the retractor ring over the nerve region was realized.

Equipment for preparation of chemical stimuli Sapid solutions were prepared and then warmed in two specially designed plexiglass rectangular containers.

The larger container had internal dimensions 20 inches long x 20 inches wide x 15 inches high using 1/2 inch thick material. Solutions were kept at the required temperature by continuously flowing water from the mains through an orifice centrally located near the bottom of the container. Excess water was returned to the sink by way of two exit orifices centrally located at the top of the tank. Entrance and exit orifices were at opposite ends of the tank. Hot and cold water could be adjusted to within 1°C of the required temperature. A total of 6 solution flasks[1] (either 500 milliliter or 2 liter capacity) could be used at any one time with this tank. The neck of each flask, to which some insulation putty[2] had been applied, was firmly secured by a circular hose clamp. A metal arm was connected from the hose clamp to a steel rod. The steel rod was placed inside a copper sleeve which was fastened to the water container. As the chemical taste solution level was changed, the steel rod would slide up and down within the copper sleeve (due to flask buoyancy), until an equilibrium position was attained. Four wheels and a handle completed the unit.

The smaller container had internal dimensions 19 inches long x 12 inches wide x 7 inches high using 1/4 inch thick

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[1]Pyrex Erlenmyer flasks. Corning Glass Works, Corning, New York.

[2]Scotchfil electrical insulation putty, 1 1/2 inches wide. Minnesota Mining and Manufacturing Co., St. Paul 6, Minnesota.

material. It was fabricated in a similar manner to the larger container. Solutions were heated by fastening an adjustable automatic aquarium heater[1] to a bracket on the container. Up to 12 Erlenmyer® flasks of 500 milliliter capacity could be warmed at any one time. Provisions were made so that air from the laboratory air supply could be bubbled through the water container to prevent thermal gradients from forming. Finally, a bracket was made to hold a YSI, Number 403, tubular thermistor probe so that the water bath temperature could be closely compared and regulated to the temperature of the bird's body.

A commercially available temperature controlled water bath[2] was used to warm 5 Erlenmyer® flasks each of 250 milliliter capacity. Tap water, distilled water, physiological saline, mineral oil, and 2M NaCl were always warmed in this bath. These solutions were used for bird preparation and for testing degree of neural activity. A circular bakelite cover kept the flasks in place. The three items described above are shown in Figure 11a.

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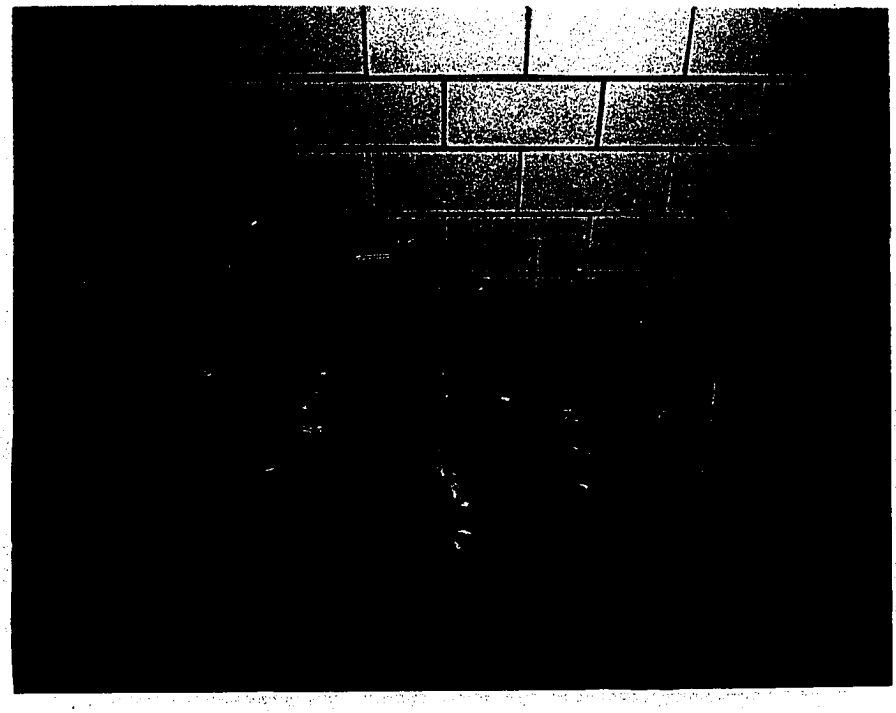
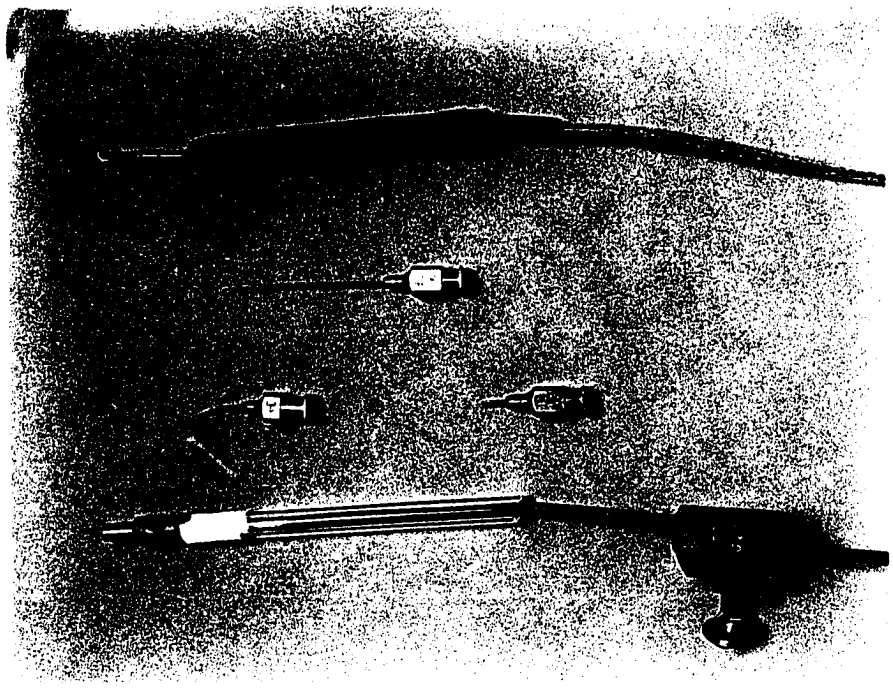
[1]Dial-A-Matic, Model 33, 100 watts, 1% accuracy. 1964 Aquarium Incorporated, Maywood, New Jersey.

[2]Technicon constant water bath, Serial Number 0815. The Technicon Corp., Ainsley, New York 10502.

Figure 11a. Water baths for warming chemical solutions showing from left to right the large taste solution container, the small taste solution container, and the commercially purchased water bath

Figure 11b. Electrode ancilliaries showing from top to bottom the silver wire bipolar electrode assembly, the platinum--iridium wire monopolar electrode, two samples of silver wire monopolar electrodes, and common electrode shaft (left) with polystyrene mounting block (right)





Electrocardiogram (EKG) monitor amplifier Under conditions of hyperventilation it was extremely difficult to determine the state of viability of the bird since muscular activity was barely noticeable. Once the nerve region was exposed, however, the pulsations in the adjacent arterial blood supply furnished a sufficient monitor of cardiac activity. Often, in the time interval between administration of anaesthesia and securement of the bird's trachea onto the unidirectional respirator, cardiac activity would stop. By means of an EKG amplifier an advance warning of any impending cessation of cardiac activity would be given during this crucial period. The bird could often be rejuvenated by injection of a suitable heart stimulant.[1]

An EKG amplifier as designed is shown in Figure 12. Three integrated circuits have been used; the first two provide a dual amplifier input, and the remaining one the output stage of a standard differential amplifier. A differential input configuration was chosen with each amplifier stage noninverting to give a very high input impedance. The cross-coupling resistor R_{12} reduced common mode gain between amplifiers to unity while still amplifying the differential signal up to

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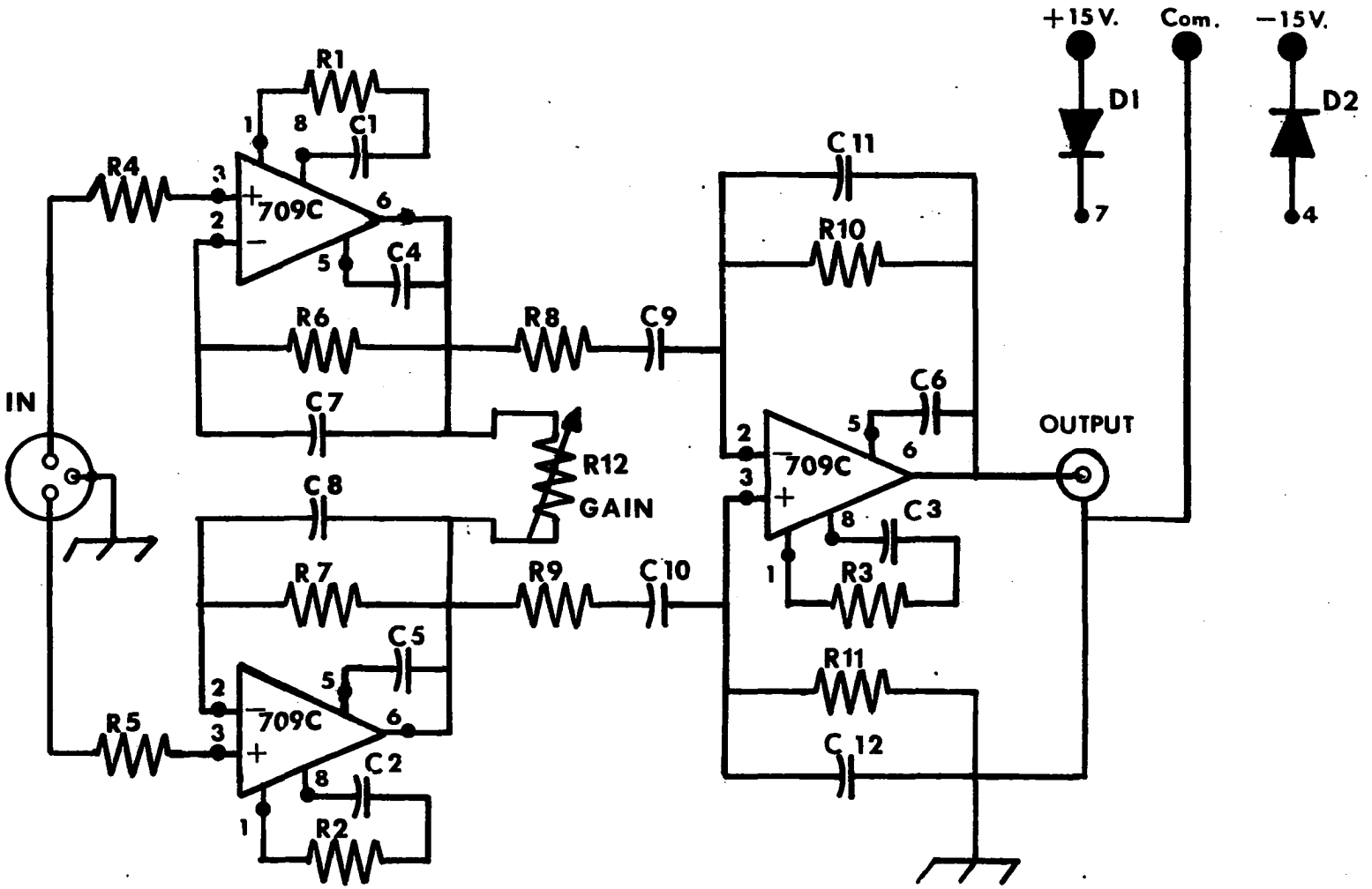
[1]Dopram (doxapram hydrochloride). A. H. Robins Co. Inc., 1407 Cummings Drive, Richmond, Virginia 23220.

Figure 12. Circuit diagram of electrocardiogram (EKG) amplifier used during experimentation



R1, R2, R3	= 1.5K	R10, R11	= 1.0 M	C7, C8	= 3 nfd	R12	= 50K
R4 R5, R8, R9	= 8 K	C1, C2, C3	= 5.1 nfd	C9, C10	= 1.0 mfd		
R6, R7	= 301K	C4, C5, C6	= 200pfd	C11, C12	= 1 nfd		

40 times. Common mode voltages were further reduced in the output stage. Two 15 volt batteries were used to supply power. Further details on this type of amplifier can be found in Giles (65, pp. 141-142).

The packaged EKG amplifier was placed in the shielded animal preparation cage to further cut down on pick-up. It was connected into one channel of an audio monitor. Three pin electrodes fastened to the bird's breast were used to monitor the EKG activity.

The signal conditioning and monitoring subsystem

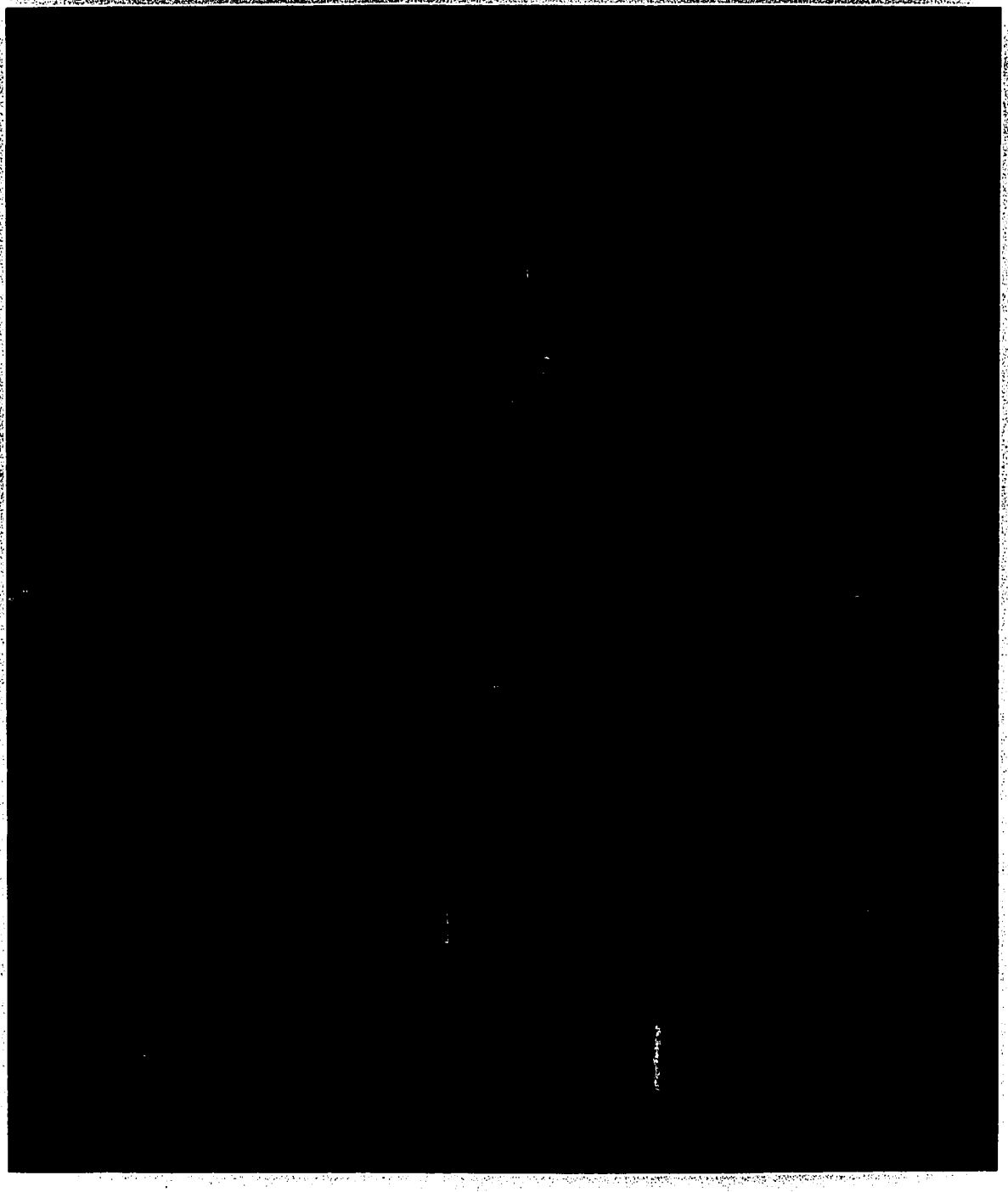
Once the nerve had been exposed and prepared, it was necessary to extract and condition the neural signals of interest. Signal conditioning consisted of (1) amplifying and integrating desired neural impulses; (2) correlating neural activity to a suitable time mark signal; (3) signifying release of sapid solution from dispensing burette by means of an event marker; (4) indicating tongue solution temperature changes by way of an electrical signal. In Figure 13, the rack mounted assembly for signal conditioning and monitoring is shown. Each of the major components is described below.

Neural signal processing Neural signals were first passed to a high impedance probe[1] which utilized field effect

[1]Grass Model HIP511 B FET probe. Grass Medical Instruments, Quincy, Massachusetts.

Figure 13. Signal conditioning and monitoring subsystem showing:

a. audio monitor
b. YSI Tele-thermometer and driver amplifier
c. discriminator and integrator package
d. patch panel
e. ac preamplifier for amplifying neural activity
f. ac preamplifier power supply
g. time/event generator and power supply
h. power supply for high intensity lamp
i. speaker for audio monitor



transitors (FET) for its active components. This feature enabled a 50,000 to 1 common mode rejection ratio at 60 hertz to be realized when used in conjunction with a high performance ac preamplifier.[1] Input impedance to the probe was 10^{11} ohms. Several means for examining the amplifier neural signals were accessible. Two direct modes were available for monitoring the neural activity picked up by the electrodes; one visual by virtue of a cathode ray oscilloscope[2], and the other aural by way of an audio monitor.[3] An indirect mode of monitoring involved feeding the neural signals into a discriminator and integrator circuit, the output of which was then displayed by a paper recorder.[4] Up to 4 different signals could be individually monitored with the audio monitor by simply switching channels. Both neural activity and EKG activity (see page 135) were monitored with the audio monitor during experimentation.

Since many of the instruments used in neural signal conditioning and monitoring were designed and fabricated in our laboratory, a brief description of the more important circuits is carried out below. These include: (1) discriminator and

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[1]Grass Model P511 DR ac preamplifier. Grass Medical Instruments, Quincy, Massachusetts.

[2]Tektronix Type 502 dual-beam oscilloscope. Tektronix Incorporated, Box 500, Beaverton, Oregon 97005.

[3]Grass Model AM5 BC precision solid-state audio monitor. Grass Medical Instruments, Quincy, Massachusetts.

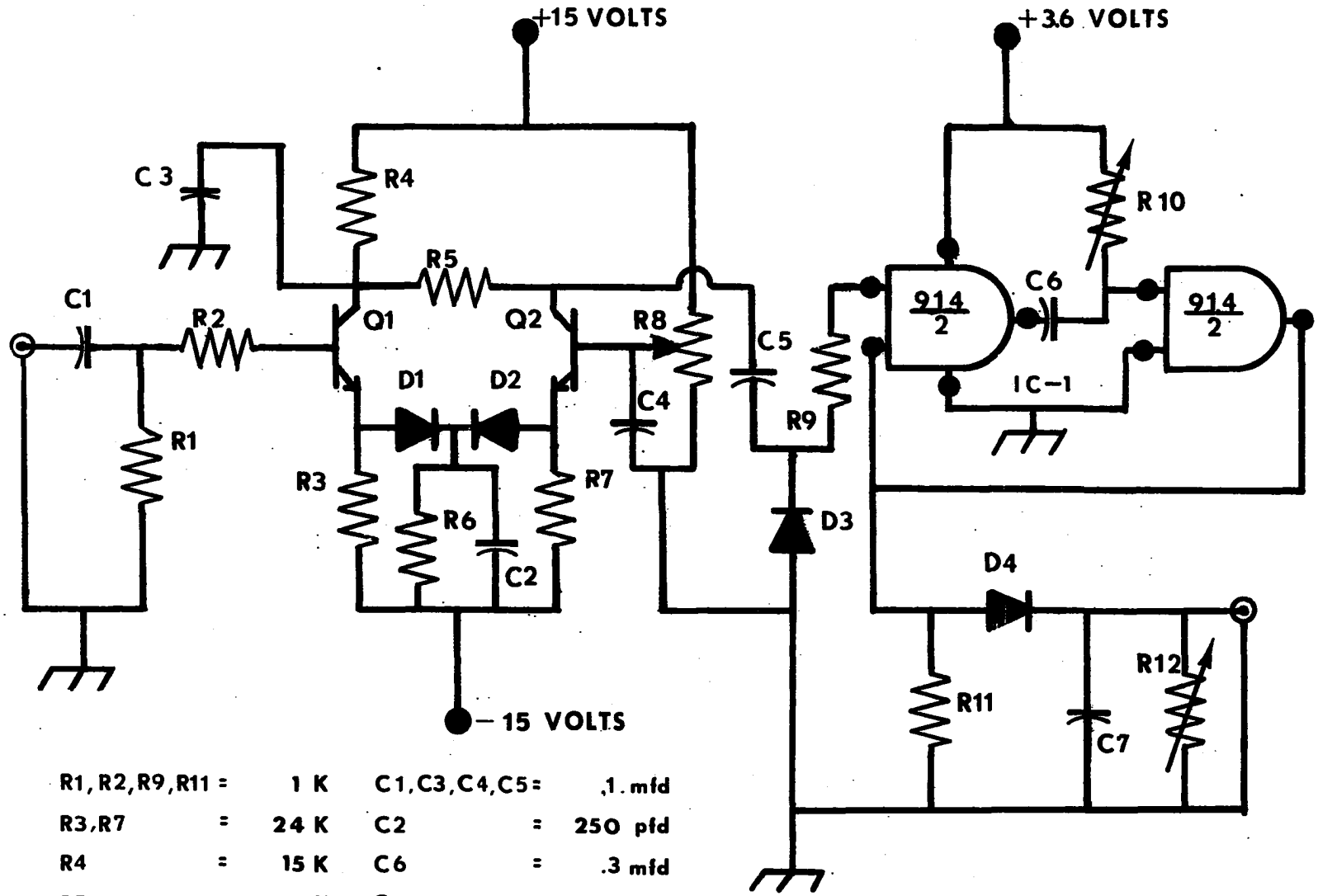
[4]Brush Mark 220 recorder, Model Number 15-6327-50. Brush Instrument Division, 37th and Perkins, Cleveland, Ohio 44114.

integrator circuit; (2) time/event marker circuit; (3) YSI driver amplifier circuit; (4) patch panel; (5) power supplies.

Discriminator and integrator The discriminator and integrator circuit, shown in Figure 14, was used as a frequency rate meter for the neural activity from whole nerve bundles. Although the instrument was sensitive enough to measure discharge rates in single fibre preparations, it was not used as such in the experiments.

The discriminator itself was divided into two parts--a level detector and a monostable multivibrator. The level detector--a differential amplifier--operated in an amplitude difference mode. Input neural signals entered the base of transistor Q_1 and were compared with a preset reference voltage applied to the base of transistor Q_2. Any neural signal larger than the reference voltage at the collector stage of Q_2 was used to trigger a monostable multivibrator (one-shot) to provide an output pulse of uniform amplitude and constant width. The reference voltage at R_8 could be adjusted to allow varying levels of activity to trigger the one-shot. In practice, however, the reference voltage was set to pass only neural signals above the intrinsic neural noise level. Consequently, the reference level was adjusted and set for noise activity happening prior to tongue stimulation.

Figure 14. Circuit diagram of neural activity discriminator and integrator



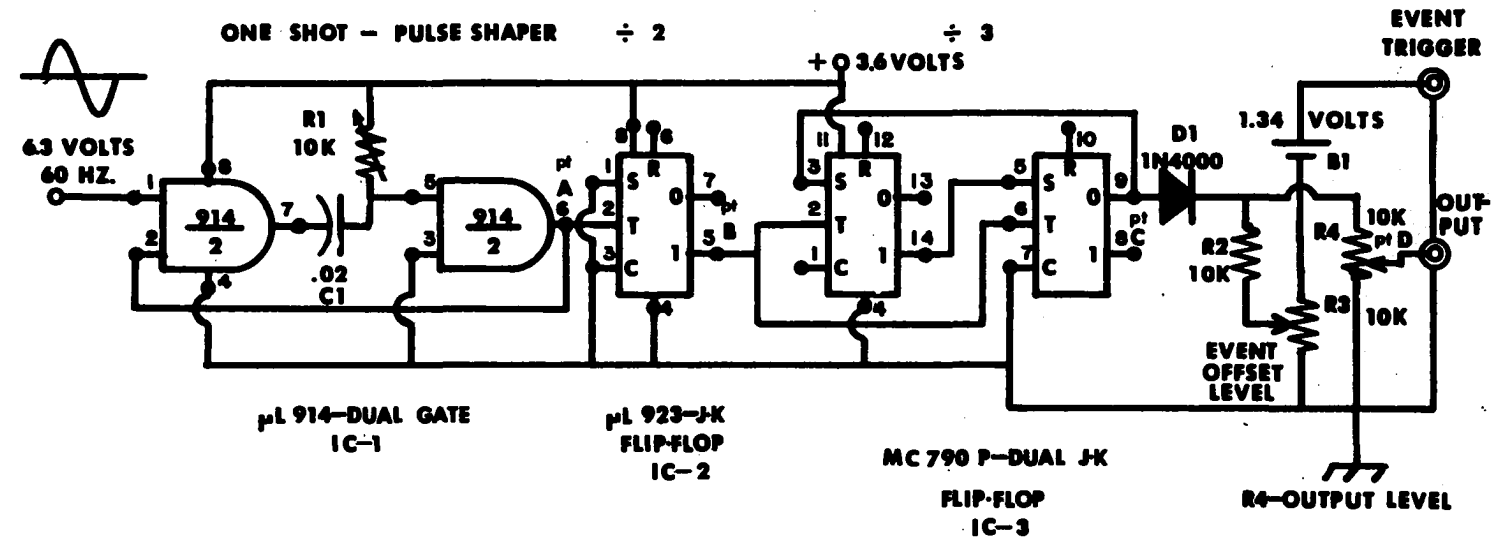
R1, R2, R9, R11	= 1 K	C1, C3, C4, C5	= .1 mfd		
R3, R7	= 24 K	C2	= 250 pfd		
R4	= 15 K	C6	= .3 mfd		
R5	= 2 K	C7	= 100 mfd		
R6	= 8 K	Q1 Q2	= 2N3904	IC–1 = µL 914	Dual Gate
R8, R10, R12	= 50 K	D1, D2, D3, D4	= 1N56		

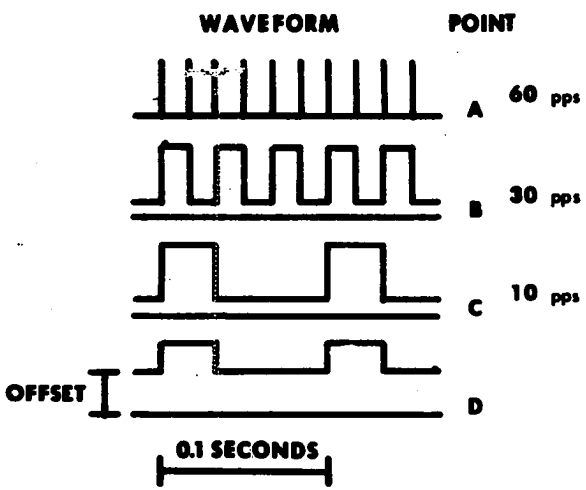
The output of the one-shot was passed through an isolating diode D_4 to an RC integrator. The time constant of the integrator was adjustable.

The above design differs from most integrators used in gustatory studies in that the entire activity is integrated in other designs, whereas in the present design all activity above a preset level is uniformly weighted and then integrated. A related design seems to have been developed by Kenshalo (107, p. 404) for use on thermal receptor units.

Time/event marker A time signal was needed to provide a suitable measurement scale to interpret the neural impulses as a time series. The three-stages IC-1, IC-2 and IC-3, shown in Figure 15 represent a time generator design using logic elements. The system envisaged frequency-divides and shapes a 6.3 volt, 60 hertz signal into a pulse train having a 0.1 second period. Stage IC-1, representing a two-input dual gate connected as a monostable multivibrator, generates a very narrow rectangular pulse train at 60 pulses per second (pps) (point A waveform in Figure 15). The flip flop circuit in stage IC-2 effectively divides the pulse train by two to provide another pulse train of 30 pps (point B waveform in Figure 15). Note that the flip flop was triggered on the negative slope of the waveform at point A. The two flip flop circuits in stage IC-3 were interconnected for further division; the resulting waveform is shown as a 10 pps train

Figure 15. Circuit diagram of time/event marker. Waveforms appearing at specific points (pt) in the circuit are illustrated for clarity





IC-1 FμL91429-Dual Two-Input Gate,
Fairchild Semiconductor

IC-2 FμL92329-JK FlipFlop,
Fairchild Semiconductor

IC-3 MC790P-Dual JK FlipFlop,
Motorola Semiconductor Products Inc.

at point C. Waveform representation at point C in Figure 15 does not show the correct phase relationship to the waveform at point B. The exact phase relationship in addition to the interaction of states between IC-1, IC-2, and IC-3 is shown by the truth tabulation Table 4. Further details on timing circuitry and one-shot configurations are given in Millman and Taub (134).

Since frequency division was the mode used to provide a suitable time scale, it was important that constancy of input frequency to the time generator was maintained. A check on this indicated that the frequency was normally held at maximum limits of 60 ± 0.02 hertz for long term conditions. A time correction factor was applied to the system periodically to keep it within these limits.[1] Such a frequency deviation would cause at most a 7 millisecond change in waveform at point C in Figure 15, which was considerably less than the resolution between impulses in the nerve train. Normally, transients provide a voltage spike on the power line for a maximum of 0.1 hertz. The probability of having longer transients was extremely small. In any case, changes in the integrity of the time signal would be readily noted and compensations could be made accordingly.

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[1]Peterson, H., Iowa Electric Light and Power Co., Boone, Iowa. Comments on line frequency stability. Private communication. 1968.

Table 4. Truth table of states in time generator circuit

IC-1 output pulse number	IC-2 output state	IC-3 output state	
		First flip flop	Second flip flop
1	1	0	0
2	0	0	0
3	1	1	0
4	0	1	0
5	1	1	1
6[a]	0	1	1
7	1	0	0

[a]The sequence of states is repetitive with pulse number 6, 12, 18, etc.

The terminal stage in Figure 15 represents an event marker, which was activated whenever the contacts of a mercury switch were short circuited. The mercury switch, mounted on the taste burette stopcock, became short circuited whenever the stopcock was open to chemical solution flow. An auxiliary push button switch, connected in series with the mercury switch, provided a means of triggering an independent event. Activation of either switch caused a reference voltage to appear at the output of the clamping diode D_1. This raised the level for which D_1 could conduct and consequently the output voltage indicated a base line shift to signify an event had taken place (point D waveform in Figure 15).

Temperature recording Tongue temperature changes were monitored with a YSI, Model 427 thermistor bead, having a time constant of 0.5 seconds. Output from the probe was

fed to a thermistor temperature indicator[1] and then to a driver amplifier (Figure 16) before storage on magnetic tape. The circuit in Figure 16 used an operational amplifier as the basic component. The OFFSET control, in conjunction with the GAIN control, allowed very small temperature changes to be amplified. Ambient temperatures could be set to give a null voltage output from the driver amplifier. In this way both increases and decreases about the ambient temperature could be recorded. The maximum output of the driver amplifier was adjusted to conform to an IRIG standard of 3 volts peak-to-peak for the magnetic tape input.

Patch panel A patch panel, illustrated schematically in Figure 17, was built as a focal point for selecting and shunting all signals obtained. All source signals (neural, integrated, time/event, and thermal) were first passed to the patch panel before further transmission; all signals to be played back from the magnetic tape unit were sent to the patch panel before further processing. Source signals could either be monitored or passed to the magnetic tape recorder from the patch panel.

Power supplies Three separate power supplies were used for the subsystem. One, a commercially available

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[1]YSI Thermistemp Tele-thermometer, Model 43, Range: 0 to 50°C. Yellow Springs Instrument Co., Yellow Springs, Ohio 45387.

Figure 16. Circuit diagram of driver amplifier used to amplify temperature changes metered by means of a YSI Tele-thermometer for subsequent storage on magnetic tape

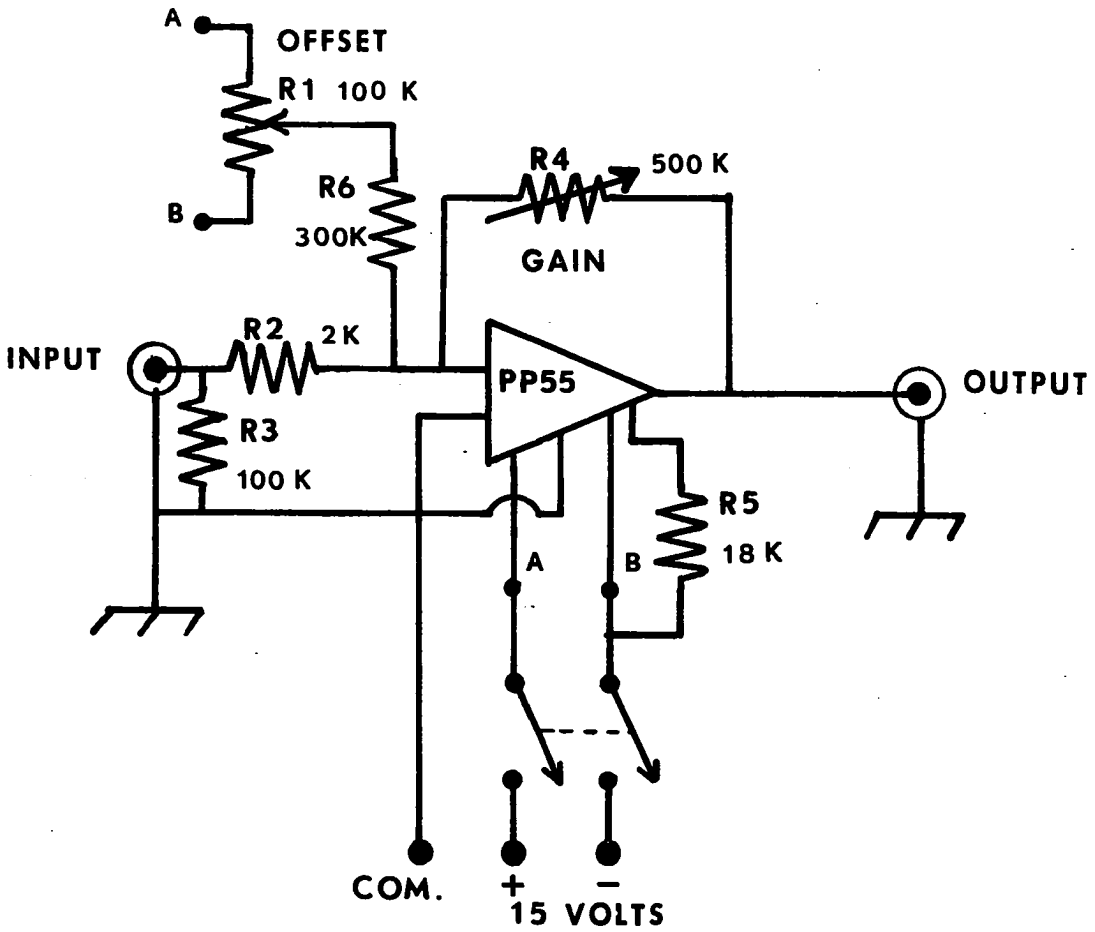
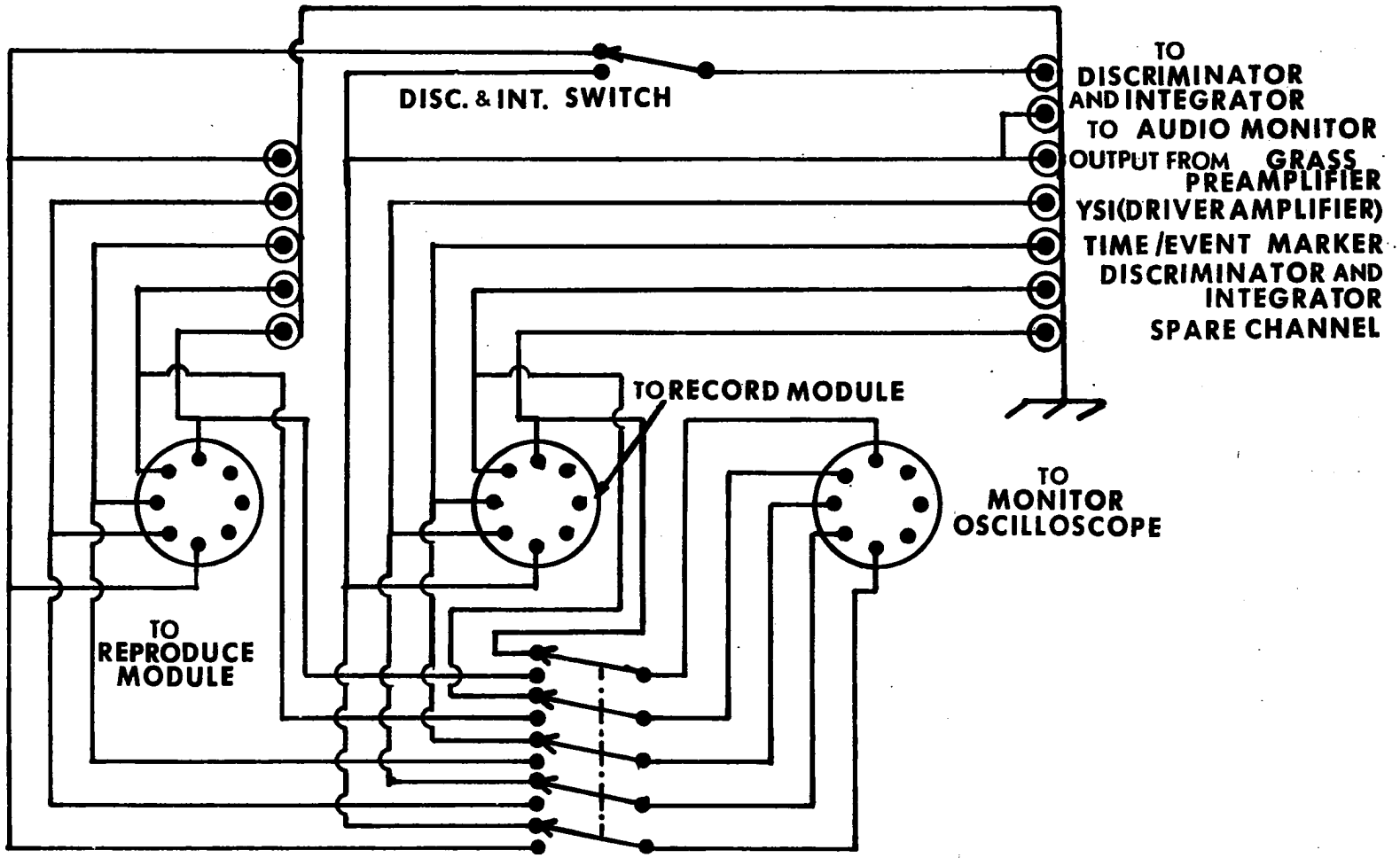

A
OFFSET
R1 100 K
B
R6
300K
R4
500 K
GAIN
INPUT
R2
2 K
PP55
OUTPUT
R3
100 K
R5
18 K
A
B
COM.
+
15 VOLTS
−

Figure 17. Schematic diagram of patch panel used to select or shunt signals to various locations during experimentation


DISC. & INT. SWITCH
TO
DISCRIMINATOR
AND INTEGRATOR
TO AUDIO MONITOR
OUTPUT FROM GRASS
PREAMPLIFIER
YSI(DRIVER AMPLIFIER)
TIME/EVENT MARKER
DISCRIMINATOR AND
INTEGRATOR
SPARE CHANNEL
TO RECORD MODULE
TO
MONITOR
OSCILLOSCOPE
TO
REPRODUCE
MODULE

unit[1] was purchased to supply power to the ac preamplifier used to amplify the gustatory neural signals. It had the capability of supplying power to 4 individual ac preamplifiers if the need arose.

A 3.6 volt power supply, shown in Figure 18, was designed to handle both the time/event marker and part of the discriminator and integrator circuit complex. It featured a full wave rectifier bridge circuit, a π-configuration circuit for smoothing and filtering, and a series emitter follower combined with a reference voltage (Zener diode) for regulation. A ripple factor of 0.2% was measured. A ± 15 volt dual power supply, shown schematically in Figure 19, was used in conjunction with the YSI driver amplifier, and the remaining circuitry of the discriminator and integrator. The design is similar to Figure 18 and will not be elaborated upon any further. Ripple factor was 0.1%.

The data storage subsystem

Once the various signals had been satisfactorily conditioned they were passed to a magnetic tape recording system[2] for future retrieval. Provisions were allowed for storing

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[1]Grass Model 106-B regulated power supply. Grass Medical Instruments, Quincy, Massachusetts.

[2]DAS-100 magnetic tape recording system. Ampex Corporation, Redwood City, California.

Figure 18. Circuit diagram of power supply used in conjunction with the time/event marker and a portion of the discriminator and integrator circuit complex

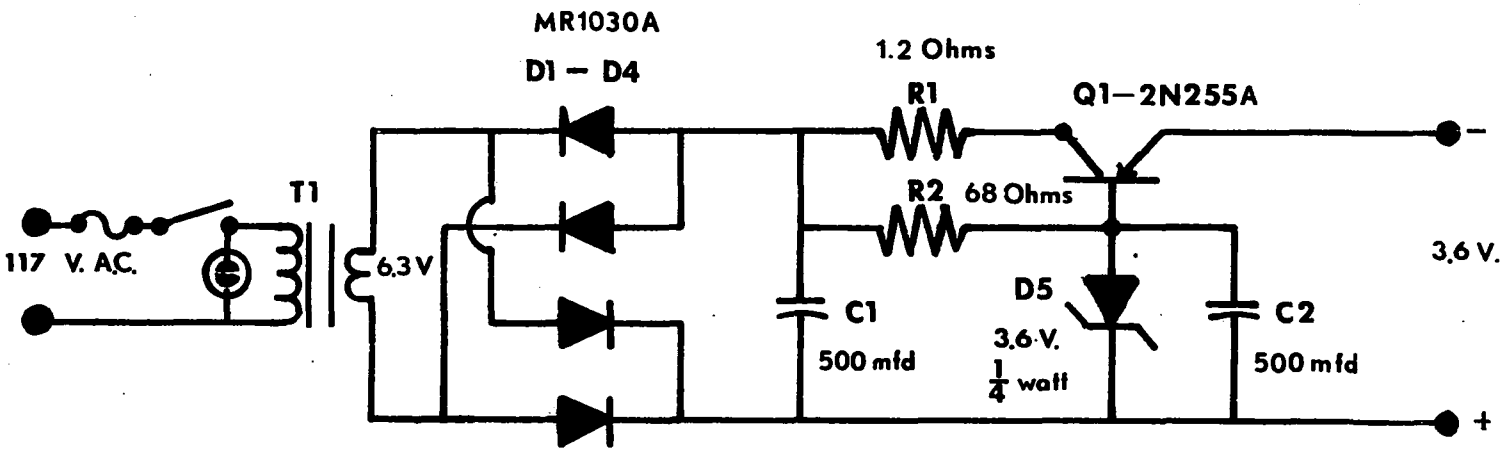
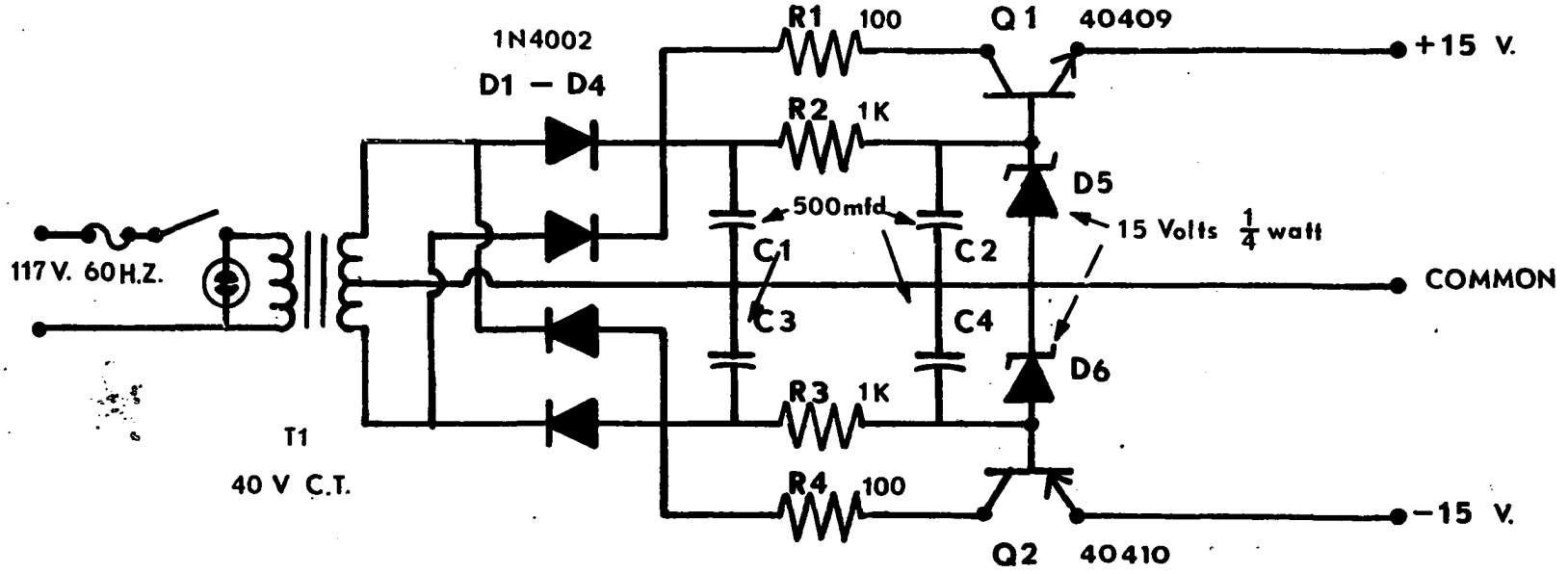

MR1030A
D1 – D4
1.2 Ohms
R1
Q1–2N255A
T1
R2 68 Ohms
117 V. A.C.
6.3 V
3.6 V.
D5
C1
C2
3.6 V.
1/4 watt
500 mfd
500 mfd
–
+

Figure 19. Circuit diagram of power supply used in conjunction with the YSI driver amplifier and part of the discriminator and integrator circuits


1N4002
D1 – D4
R1 100
Q1 40409
+15 V.
R2 1K
D5
500mfd
15 Volts ¼ watt
C1
C2
117 V. 60 H.Z.
COMMON
C3
C4
D6
R3 1K
T1
40 V C.T.
R4 100
–15 V.
Q2 40410

up to four electrical signals at any one time on the tape recording system. These were: (1) neural signals from ac preamplifier; (2) thermal signals from tongue of animal preparation by way of YSI Tele-thermometer; (3) event marker signal superimposed on a running time signal when activated; (4) integrated neural signals.

The DAS-100 magnetic tape recording system, shown in Figure 20a, consisted of a tape transport mechanism[1], a time code generator[2], a record/reproduce channel selector[3], an audio system, and a monitoring cathode ray oscilloscope system[4], all integrally interconnected to make up a composite portable unit. Five frequency modulated (FM) record/reproduce channels and one direct record/reproduce channel were available in the system. The time code generator signal was reserved for the direct channel. Tape speeds available in the record mode of operation were 60, 30, 15, 7 1/2, 3 3/4,

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[1]Ampex Model FR-1300 recorder/reproducer. Ampex Corporation, Redwood City, California.

[2]Milgo Model 4049-1A time code generator. Milgo Electronics Corporation, Redwood City, California.

[3]Catalog Number 1800269-01. Ampex Corporation, Redwood City, California.

[4]Cathode ray oscilloscope system comprised of (a) Tektronix Type RM 561A oscilloscope; (b) Tektronix Type 2B67 time base plug-in unit; (c) Tektronix Type 3A74 four-trace amplifier plug-in unit. Tektronix Incorporated, Box 500, Beaverton, Oregon 97005.

and 1 7/8 inches per second. Plug-in filters enabled conversion to various reproduce mode operating speeds. Available were filters for 1 7/8 inches per second (bandwidth--dc to 625 hertz), 3 3/4 inches per second (bandwidth--dc to 1250 hertz), and 7 1/2 inches per second (bandwidth--dc to 2500 hertz). Maximum input voltage to the FM record modules was set at 3 volts peak-to-peak. One-half inch magnetic tape[1] was used.

The data extraction subsystem

Two methods were employed in extracting data from the magnetic tape storage system. The first method consisted in passing neural data through a band-pass filter to the pulse discriminator and integrator before displaying it on a 2-channel paper tape analog recorder.[2] The second recorder channel was used to print out the corresponding time/event signal.

The second method of extracting the neural data consisted in transmitting the information from the tape channel through a band-pass filter[3], and then onto the face of a dual-

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[1]Ampex Corporation, Redwood City, California.

[2]Brush Mark 220 recorder, Model Number 15-6327-50. Brush Instruments Division, 37th and Perkins, Cleveland, Ohio 44114.

[3]Ultra-low frequency band-pass filter, Model 330-A. Krohn-Hite Instrument Co., 580 Massachusetts Avenue, Cambridge, Massachusetts 02139.

beam oscilloscope[1] for subsequent photography by means of a Kymograph® recording camera.[2] The entire set-up was made portable and is shown in Figure 20b as it was used in operation. The band-pass filter had a frequency response set from 0.02 to 2000 hertz. Two dual-trace amplifier plug-in units[3] in the chopped mode of operation enabled up to four simultaneous tracings to be displayed on the oscilloscope face. A light-tight connecting hood,[4] with viewing window was mounted between the camera lens and oscilloscope bezel. Shielded cables were used for interconnection between the oscilloscope, tape recording unit, camera, and filter by way of the patch panel. The shield was connected to a common ground (water mains) so as to prevent ground loops from occurring.

The recording camera was capable of providing up to 12 fixed film speeds ranging from 0.25 to 1000 millimeters per second. Eight f-stops ranged from f/2 to f/22. Although both 35 millimeter film or paper may be used with the camera only paper (unperforated)[5] was used. Standard magazines

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[1]Tektronix Type RM 565 dual-beam oscilloscope. Tektronix Incorporated, Box 560, Beaverton, Oregon 97005.

[2]Grass Model C4 oscilloscope recording camera. Grass Medical Instruments, Quincy, Massachusetts.

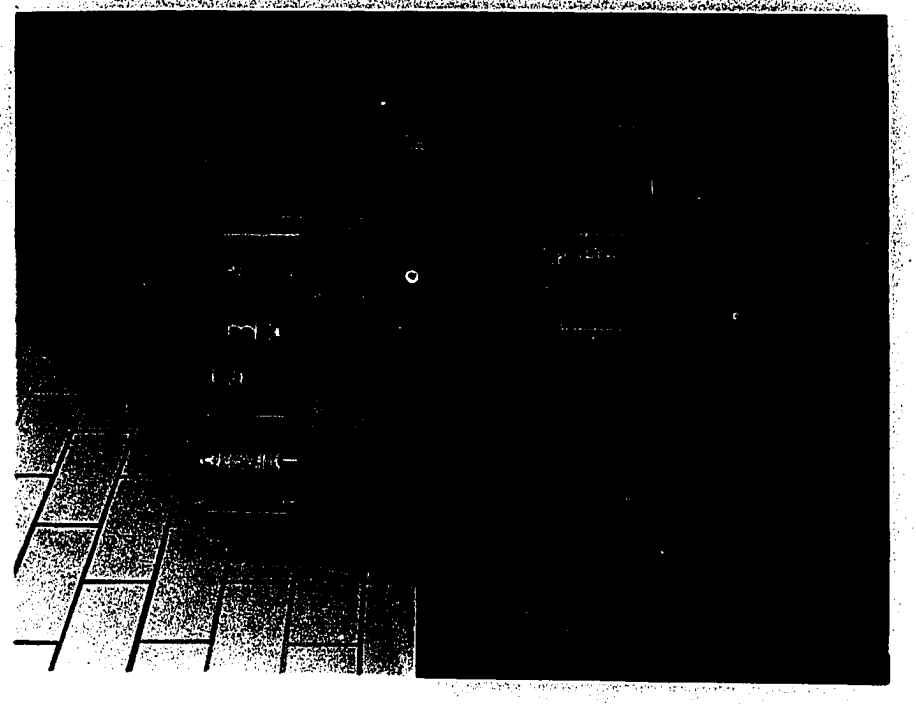
[3]Tektronix Type 3A72 dual-trace amplifier plug-in unit. Tektronix Incorporated, Box 500, Beaverton, Oregon 97005.

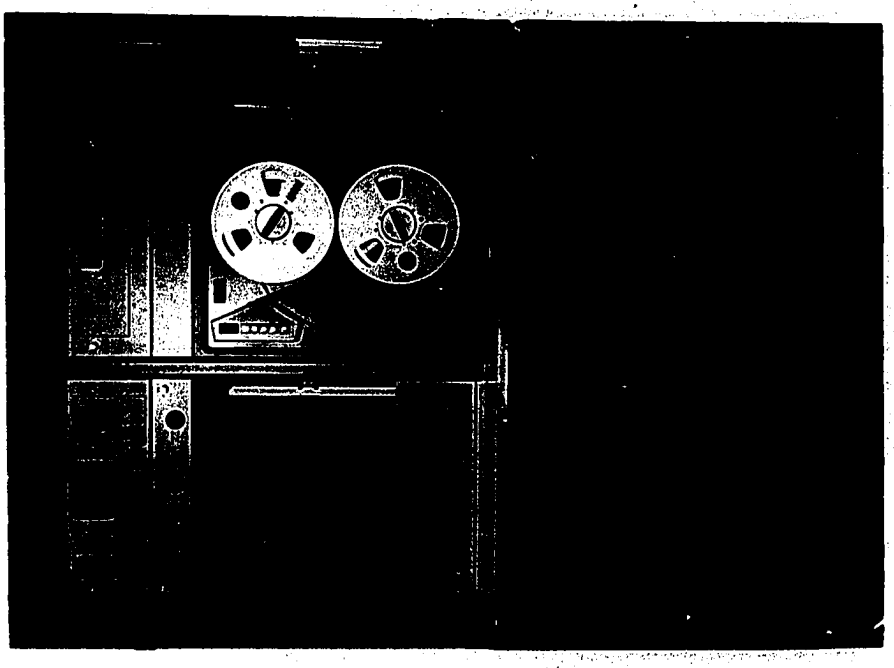
[4]Grass Medical Instruments, Quincy, Massachusetts.

[5]Kodak Linograph Paper Kind 1732. Supplied by Grass Medical Instruments, Quincy, Massachusetts.

Figure 20a. DAS-100 magnetic tape recording system as used for data storage and retrieval

Figure 20b. Data extraction subsystem showing from top to bottom the bandpass filter, the Kymograph® recording camera (left), the recording cathode ray oscilloscope and associated plug-in units (right), and the film developing outfit plus other photographic supplies





took rolls having a maximum of 200 feet capacity. A footage counter indicated the recorded material exposed.

Since the moving paper in the camera provides a time sweep, it was necessary to disconnect the horizontal traces from the cathode ray oscilloscope. This was simply done by switching both time bases of the recording oscilloscope to the EXTERNAL mode. A circular piece of black construction paper having a vertical slit 1/8 inch wide in its center was gummed onto the scope face to minimize light glare. The vertical signal traces were then aligned in the center of the scope face to project through the vertical slit and into the camera lens. A special short persistance cathode ray tube phospher (P 11) was used so that oscilloscope tracing afterglow would not overexpose the paper film. Paper film was developed and processed by means of a commercially available developing outfit.[1] The unit was designed to handle up to 200 feet of 35 millimeter paper film. It consisted of a light tight stainless steel tank, a motor drive unit, and two 35 millimeter reels. A simple crank mechanism allowed loading of paper film onto either reel. Commercially available

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[1]Smith automatic developing outfit, Model F-214. Philadelphia Air Transport Co., Norristown, Pennsylvania.

developer[1], stop bath[2], and fixer[3] were used in processing. Permanent prints were terminally washed in a fixative solution.[4]

The overall system set-up

The photograph, shown in Figure 21, indicates how each subsystem was integrated to make up the overall system set-up used during each experimental run. The information flow diagram, shown in Figure 22, summarizes the major pathways of each recorded signal in the overall system set-up.

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[1]Kodak Linograph developer, single powder, Eastman Kodak Stores, Inc., 2205 Ingersol, Des Moines, Iowa.

[2]Kodak indicator stop bath. Eastman Kodak Stores, Inc., 2205 Ingersol, Des Moines, Iowa.

[3]Kodak rapid fixer with hardener, liquid. Eastman Kodak Stores, Inc., 2205 Ingersol, Des Moines, Iowa.

[4]Pakosol. Pako Corporation, 6300 Olson Memorial Highway, Minneapolis 40, Minnesota.

Figure 21. Overall laboratory set-up illustrating:

a. large taste solution container
b. small taste solution container
c. animal respirator subsystem
d. animal preparation and signal extraction subsystem
e. magnetic tape recording system
f. monitor cathode ray oscilloscope
g. signal conditioning and monitoring subsystem

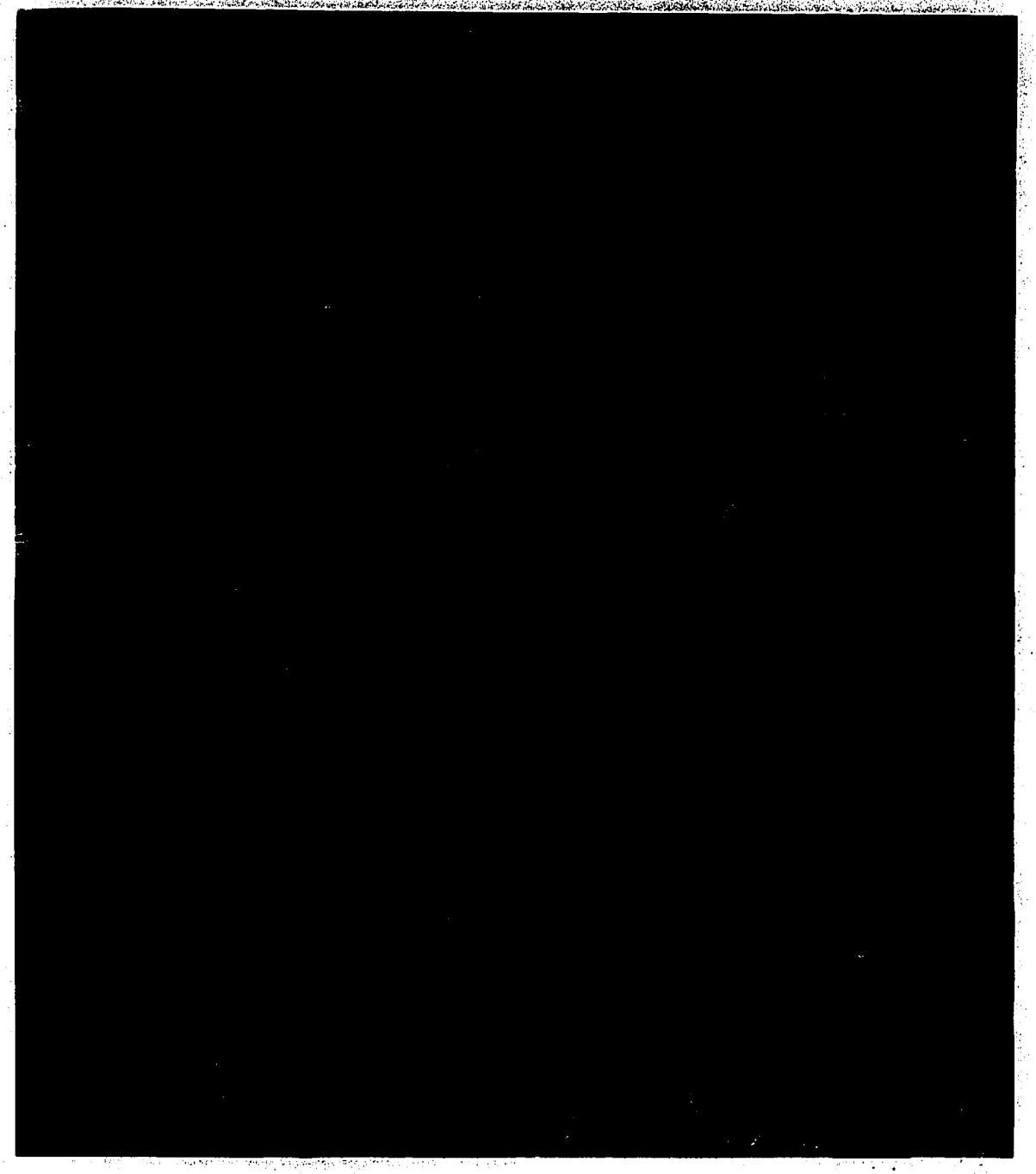
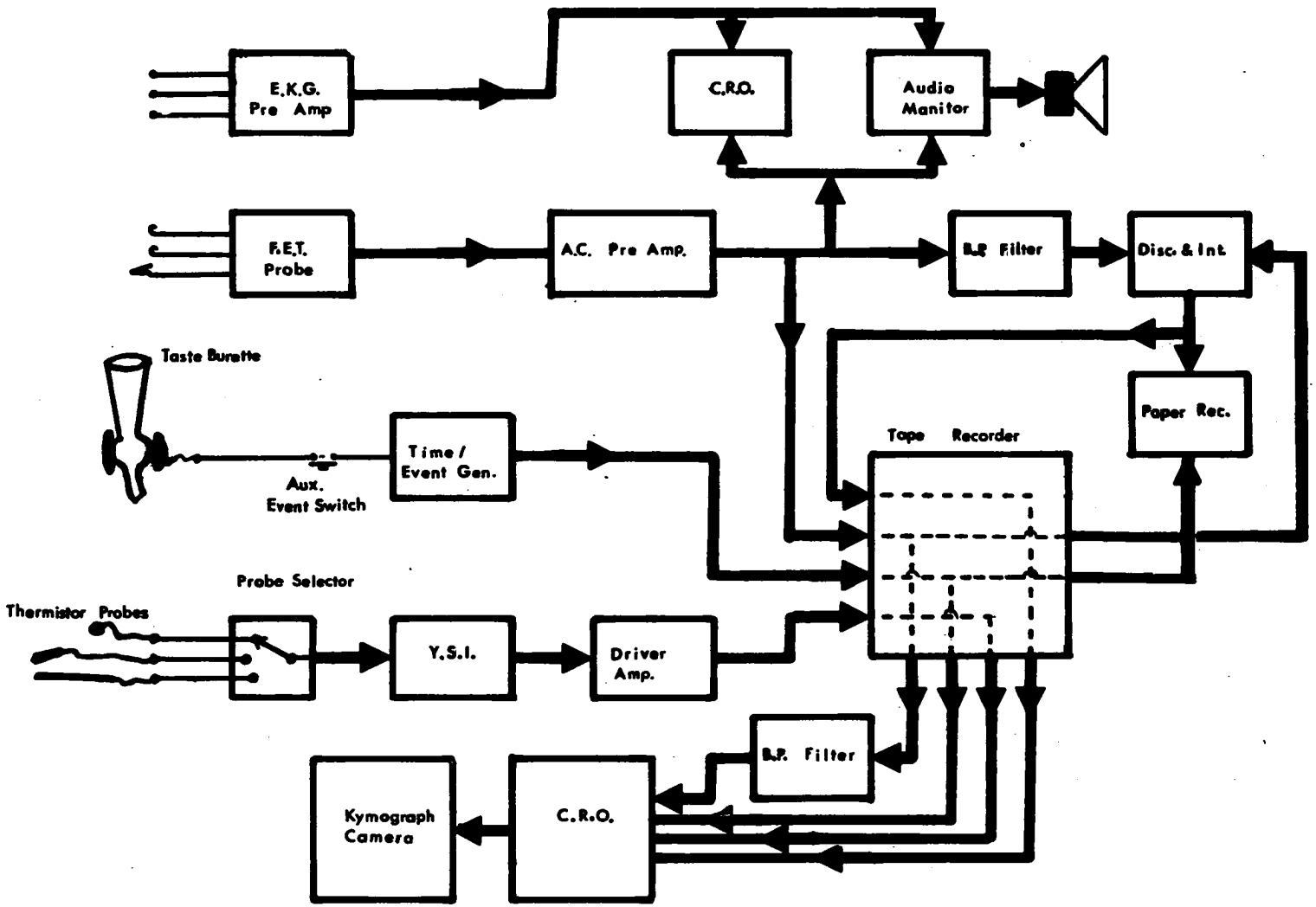


Figure 22. Information flow diagram of each signal source recorded in the overall system set-up


E.K.G.
Pre Amp
C.R.O.
Audio
Manitor
F.E.T.
Probe
A.C. Pre Amp.
B.P Filter
Disc. & Int.
Taste Burette
Time /
Event Gen.
Aux.
Event Switch
Paper Rec.
Tape Recorder
Probe Selector
Thermistor Probes
Y.S.I.
Driver
Amp.
B.P. Filter
Kymograph
Camera
C.R.O.

RESULTS AND DISCUSSION

Some General Observations

The date of experimentation, the breakdown of chemical stimuli applied, the electrode type used in recording neural information, the mode of fibre isolated, i.e. whether multiple fibre or few fibre preparations from which single fibre data could be obtained, and the specific chemicals which induced responses for each pigeon are shown in Table 5.

Sweet stimuli, such as the monosaccharide dextrose, the disaccharide sucrose, and glycerol gave no notable neural responses, although one preparation was found to respond to a 15% ethylene glycol solution. This was in marked contrast to the findings of Kitchell et al. (116) in both the pigeon and the chicken and Halpern (71) in the chicken. From both species Kitchell's group consistently recorded responses to both ethylene glycol and glycerol but not to sucrose (dextrose was not tried). Halpern recorded responses to all these sweet tasting solutions. Kitchell[1] has indicated that a long delay time takes place before a response is initiated with

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[1]Kitchell, R. L., Dean, College of Veterinary Medicine, Iowa State University of Science and Technology, Ames, Iowa. Comments on neural response to ethylene glycol. Private communication. 1968.

Table 5. Resumé of date of experiment, stimuli applied, electrode used, fibre type isolated, and responding stimuli for each pigeon

Pigeon no.	Date of experiment	Chemical stimuli applied to tongue	Type of electrode used	Mode of fibre isolated	Definitive response to	Remarks
1-12	Spring 1968	-	-	-	-	used for dissection purposes to locate peripheral nerves of interest (see Figure 4)
13	April 26, 1968	0.5M, 1M, 2M, 5M NaCl; 2M NH_4Cl	silver monopolar	few fibre	all chemicals tested	
14	May 7, 1968	0.5M, 1M, 2M, 5M NaCl; 2M NH_4Cl; 2M $CaCl_2$; saturated $NaHCO_3$; 2M LiCl; 2M Na citrate; 2M NaI; 2M $FeCl_3$; 2M Na acetate; 2M $MgCl_2$; 2M $SrCl_2$; 2M KCl; 2M Na_2CO_3; 15% ethylene glycol; distilled water	silver bipolar	multiple fibre	0.5M, 1M, 2M, 5M NaCl; 2M $CaCl_2$; 2M $FeCl_3$; 2M Na citrate	Na acetate = CH_3COONa; Na citrate = $Na_3C_6H_5O_7$ ethylene glycol = 1,2-ethanediol

Table 5. (Continued)

Pigeon no.	Date of experiment	Chemical stimuli applied to tongue	Type of electrode used	Mode of fibre isolated	Definitive response to	Remarks
15	May 10, 1968	as for Pigeon no. 14 plus saturated $CaCO_3$; 15% glycerol; 0.06% saccharine; 20mM quinine hydrochloride; 2M NaBr; 2M $BaCl_2$; 0.1M, 0.2M, 0.5M, 1M acetic acid	platinum--iridium monopolar	multiple fibre	0.5M, 1M, 2M, 5M NaCl; distilled water; 2M $FeCl_3$; 2M Na citrate; 2M KCl; 2M Na_2CO_3; saturated $NaHCO_3$; 2M NaBr; 0.06% saccharine; 2M $CaCl_2$; 2M NaI; 2M NH_4Cl	saccharine = $C_7H_5NO_3S$; glycerol = $HOCH_2CHOHCH_2OH$; quinine hydrochloride = $C_{20}H_{24}N_2O_2 HCl$; acetic acid = CH_3COOH
16	May 19, 1968	as for Pigeon no. 15	platinum--iridium monopolar	multiple fibre	2M, 5M NaCl; 2M $FeCl_3$; 2M NaBr; 2M Na acetate; 2M Na_2CO_3; 2M $MgCl_2$; 2M $SrCl_2$; saturated $NaHCO_3$; 2M Na citrate; 2M NH_4Cl; 2M LiCl; 2M KCl; 2M $CaCl_2$; 0.1M, 0.2M, 0.5M, 1 M acetic acid	

Table 5. (Continued)

Pigeon no.	Date of experiment	Chemical stimuli applied to tongue	Type of electrode used	Mode of fibre isolated	Definitive response to	Remarks
17	May 21, 1968	as for Pigeon no. 15	platinum--iridium monopolar	multiple fibre	0.5M, 1M, 2M, 5M NaCl; 2M Na_2CO_3; 2M Na acetate; saturated $NaHCO_3$; 2M NH_4Cl; 2M Na citrate; 2M $FeCl_3$; 2M KCl; 2M NaBr; 2M LiCl; 2M $SrCl_2$; 2M NaI; 2M $CaCl_2$; 2M $BaCl_2$; 15% ethylene glycol; distilled water	8 inch pipette used to irrigate tongue. Tongue irrigation by burette on all other pigeon preparations

Table 5. (Continued)

Pigeon no	Date of experiment	Chemical stimuli applied to tongue	Type of electrode used	Mode of fibre isolated	Definitive response to	Remarks
18	May 29, 1968	0.5M, 1M, 2M, 5M NaCl; saturated $NaHCO_3$; 2M LiCl; 2M NaI; 2M $FeCl_3$; 2M Na acetate; 2M KCl; 2M Na_2CO_3; distilled water; 2M NaBr; 0.1M, 0.2M, 0.5M, 1M acetic acid; 2M $SrCl_2$; 2M NH_4Cl; 2M Na citrate; 15% sucrose; 15% dextrose; 0.5M NaOH; 0.5M boric acid; 0.5M HCl; 0.5M oxalic acid; 0.5M citric acid; 0.5M molybdic acid; 0.5M picric acid; 20mM sucrose octa-acetate	platinum--iridium monopolar	few fibre	2M, 5M NaCl; saturated $NaHCO_3$; 0.5M NaOH; 0.5M molybdic acid; 2M NaI; 0.1M, 0.2M, 0.5M, IM acetic acid; 0.5M oxalic acid; 2M KCl; 2M Na citrate; 2M NH_4Cl; 2M Na_2CO_3; 0.5M HCl	sucrose octa-acetate = $C_{28}H_{38}O_{19}$; dextrose = $C_6H_{12}O_6$; sucrose = $C_{12}H_{22}O_{11}$; boric acid = H_3BO_3; citric acid = $H_3C_6H_5O_7$; picric acid = $C_6H_2OH(NO_2)_3$; oxalic acid = $H_2C_2O_4$; molybdic acid = H_2MoO_4. High noise level due to deteriorating nerve masked possible response to HCl, $FeCl_3$, picric acid, and citric acid

Table 5. (Continued)

Pigeon no.	Date of experiment	Chemical stimuli applied to tongue	Type of electrode used	Mode of fibre isolated	Definitive response to	Remarks
19	May 31, 1968	as for Pigeon no. 18	platinum--iridium monopolar	multiple and few fibre	0.5M, 1M, 2M, 5M NaCl; 0.5M HCl; 0.5M citric acid; 0.5M oxalic acid; 0.5M NaOH; 2M NaBr; 2M KCl; 2M NH_4Cl; 2M Na citrate; saturated $NaHCO_3$; 2M NaI; 2M $SrCl_2$; 2M Na acetate; 2M Na_2CO_3; 2M LiCl; 0.1M, 0.2M 0.5M, 1M acetic acid; 2M $FeCl_3$	
20	June 4, 1968	as for Pigeon no. 18	platinum--iridium monopolar	multiple and few fibre	0.5M, 1M, 2M, 5M NaCl; 0.5M oxalic acid; 2M NaI; 0.5M NaOH; 2M Na_2CO_3; 2M Na acetate; 2M $FeCl_3$; 2M KCl; 0.5M citric acid; 2M NH_4Cl; distilled water; 0.5M HCl; saturated NaHCO3; 0.5M picric acid	

Table 5. (Continued)

Pigeon no.	Date of experiment	Chemical stimuli applied to tongue	Type of electrode used	Mode of fibre isolated	Definitive response to	Remarks
21	June 7, 1968	0.5M, 1M, 2M, 5M NaCl; 2M Na_2CO_3; 2M NH_4Cl; saturated $NaHCO_3$; 2M KCl; 0.1M, 0.2M, 0.5M, 1M acetic acid; 0.5M oxalic acid; 0.5M HCl; 0.5M citric acid; 0.5M NaOH	platinum--iridium mono-polar	multiple and few fibre	0.5M, 1M, 2M, 5M NaCl; saturated $NaHCO_3$; 2M Na_2CO_3; 2M NH_4Cl; 2M KCl; 0.5M oxalic acid; 0.5M citric acid; 0.5M HCl; 0.5M NaOH; 0.1M, 0.2M, 0.5M, 1M acetic acid	
22	June 13, 1968	as for Pigeon no. 21 plus distilled water; 2M Na acetate; 2M $FeCl_3$	platinum--iridium mono-polar	multiple and few fibre	1M, 2M, 5M NaCl; saturated $NaHCO_3$; 2M Na_2CO_3; 2M NH_4Cl; 2M KCl; 0.5M oxalic acid; 0.5M citric acid; 0.5M NaOH; 0.5M HCl; 2M Na acetate; 0.1M, 0.2M, 0.5M, 1M acetic acid	

ethylene glycol. This could possibly account for the absence of activity in some of our preparations since this writer's recording intervals were terminated 10 seconds after release of each solution.

Two bitter stimuli, quinine hydrochloride, and sucrose octa-acetate gave no responses. This conforms to the observations of Kitchell et al. (116) for bitter substances in the pigeon. The fact that both stimuli evoked responses in the chicken clearly indicates a species difference for bitter solutions (71, 116).

Of the 7 acids tested, responses were obtained to HCl (5 pigeons responded from a total of 5 pigeons tested--5/5), oxalic acid (5/5), acetic acid (5/8), citric acid (4/5), picric acid (1/3), and molybdic acid (1/3). Only boric acid (0/3) failed to activate the chemoreceptor sites. Acetic acid responses have been noted by both Kitchell et al. (116) and Duncan (49) from the pigeon. Both citric acid and picric acid have taste components other than the sour taste in man (132, p. 247). One might expect that of the two anthropomorphic taste components of bitter and sour in picric acid, the sour component would be more effective in stimulating the receptor sites in the pigeon since completely negative results were obtained with bitter taste solutions. Similarly of the two components of sour and sweet in citric acid the receptor sites are in all probability stimulated by the sour component rather

than the sweet component in view of the poor results obtained with sweet stimuli.

The depressant action of acids on themselves and other solutions is well documented (71, 116). It was possible to neutralize this effect by application of $NaHCO_3$ solution after each acid irrigation to return the receptor sites to normal physiological conditions.

Several specific salt series were used to determine the effectiveness of both cations and anions on chemoreceptor sites. Of the monovalent chloride cation series tested, good responses were obtained to NaCl (10/10), KCl (8/8), and NH_4Cl (9/10) from most pigeons; only LiCl (2/7) did not respond well. In contrast, only $CaCl_2$(4/4) gave meaningful data in the divalent chloride cation class; whereas $BaCl_2$ (1/3), $SrCl_2$ (3/7), and $MgCl_2$ (2/4) responded in only 50% or less of the pigeons tested. With the trivalent chloride cation salt $FeCl_3$ (7/8), good responses were obtained from most pigeons but a different receptor site may have been stimulated, as $FeCl_3$ is noted for its astringency. For the sodium monovalent anion series, good pigeon responses were noted to all substances tested; i.e. NaCl (10/10), NaI (5/7), NaBr (4/6) $NaHCO_3$ (8/9), and Na acetate (5/8). Positive electrophysiological responses were recorded to the sodium divalent anion salt Na_2CO_3 (8/9), and the sodium trivalent anion salt Na citrate (6/7) from most pigeons. Acid salts such as Na

acetate and Na citrate are noted as having a sour taste component (132, p. 539) because of hydrogen ions available to the receptor sites; consequently, this feature should not be overlooked in the interpretation of the corresponding neural activity.

Solutions tested which cannot be specifically categorized into the above 4 modalities include NaOH, $CaCO_3$, saccharine, and distilled water. Excellent responses were obtained to NaOH (5/5), but this may be due to irritation to the tongue rather than specific chemoreception. With some preparations, the application of NaOH caused the pigeon to go into a convulsive state as if vomiting, even though the pigeon was in a deep anaesthetic plane. Levi (127, p. 375, p. 463) has reported a vomit reflex to impalatable food in the pigeon. It could be that NaOH causes such a conditioned reflex reaction.

Limestone pellets are readily accepted by the pigeon for egg shell structure. Solutions of $CaCO_3$, a basic limestone constituent, were applied to the pigeon tongue to test for chemoreceptor neural activity. In all instances, $CaCO_3$ solutions (0/3) failed to evoke any responses. Since limestone pellets are picked up with the tip of the bird's beak and projected backward into the esophagus with very little tongue contact, it is not surprising that chemoreceptor

stimulation is absent. However, some mechanoreceptors may be stimulated in transit.

A distilled water response (3/8) was recorded from less than 50% of the pigeons tested. This does not agree with the electrophysiological investigations of Kitchell et al. (116) or the behavioral studies of Duncan (49). A saccharine response (1/3) was rarely found. Kitchell et al. (116) recorded a saccharine response from 50% of their preparations.

The fact that in many instances some pigeons respond well electrophysiologically to a given chemical stimuli whereas others do not, raises the question of how sensitive is a given species to a particular chemical stimulus. There is ample evidence from behavioral studies that individual variations do occur within a species. Duncan (49) noted great variations between domestic pigeons to all of the basic taste modalities. Kare and Ficken (101, pp. 292-293) have given evidence of individual differences in pigs and dogs to saccharine. In cross-bred chickens they found significantly different thresholds for different individuals to several concentrations of NH_4Cl, $CaCl_2$, and $FeCl_3$. However, all birds eventually responded once the concentration reached a high enough level. An individual variation was also noted between chlorides. A similar observation was later reported by Kare (100) on Japanese quail. That genetic differences can account for individual variations has been confirmed by Williamson [cited

by Kare and Ficken (101, p. 293)] who selectively bred birds having low thresholds among themselves. After three generations he concluded that $FeCl_3$ threshold differences are genetic in origin. That response variations might also be seasonal can be ruled out in our studies as experimentation was confined to the spring of the year (see Table 5). Cross-breeding between domestic and other strains of pigeon could account for response variations as has been indicated recently by Kare and Maller (104). They felt that domestic fowl become insensitive to chemical stimuli which are related to caloric intake, whereas wild fowl make use of this feature to regulate intake according to need.

Even electrophysiological response results have supported the presence of an individual variation in the bird. Halpern (71) had to group his experimental chickens into two groups; those that responded well to chemical solutions at 24°C and those that did not. Our electrophysiological results indicate that individual variations are very prominent with the divalent chloride cation salt class and some of the sour stimuli.

Much data is now available from behavioral studies on the pigeon. This has mainly come from the contributions of Englemann (51), Kare (100), and Duncan (47, 48, 50). The entries in Table 6 represent a comparison of electrophysiological and behavioral responses to those chemicals documented.

Table 6. Comparison between electrophysiological and behavioral responses

Solution	Electrophysiological response	Behavioral response[a] Observation	According to
Acetic acid	+	Rejection	Duncan, Englemann
HCl	+	Rejection	Duncan, Englemann
Citric acid	+	Indifferent at pH=3.65	Duncan
NaOH	+	Rejection	Duncan
Sucrose	-	Indifferent[b]	Duncan, Englemann, Kare
Dextrose	-	Indifferent	Duncan, Kare
Glycerine	-	Rejection	Englemann
Saccharine	+[c]	Rejection	Englemann, Kare
Quinine HCl	-	Rejection Indifferent	Duncan Englemann
NaCl	+	Preference and then rejection above 0.15M Rejection	Duncan Englemann
KCl	+	Preference and then rejection above 0.35M	Duncan

[a]Relative to acceptance of water.

[b]15% sucrose solutions are slightly preferred [Duncan (47)].

[c]After Kitchell *et al.* (116).

Table 6. (Continued)

Solution	Electro-physiological response	Behavioral response[a]	
		Observation	According to
NH_4Cl	+	Preference and then rejection above 0.25M	Duncan
$CaCl_2$	+	Preference and then rejection above 0.37M	Duncan
$MgCl_2$	+	Rejection	Englemann

This table updates and adds to the information listed by Kitchell et al. (116).

In general, there seems to be a good comparison between behavioral response and those of electrophysiological origin; nevertheless, there are some striking exceptions. Sucrose, for instance, showed a statistically significant behavioral perference at 15% solution, yet it was not possible to record any neural responses at this concentration. Although Duncan quoted a marked rejection to quinine HCl, our results tend to support Englemann's observations. The neural recordings of Kitchell et al. for glycerine tended to support Englemann's conclusions, whereas our findings are in direct contrast.

Duncan compared behavioral responses of the pigeon with those of several species of mammals. He found good agreement

for salt (48) and sour (50) taste solutions, but no correlation for bitter and sweet substances.

Multiple Fibre Activity

Integrated neural response analysis

The interpretation of multiple fibre activity was exclusively confined to the results obtained from the output of the discriminator and integrator. Typical patterns of neural activity from multiple nerve fibres are shown in Figure 23 to 7 chemical solutions tested. Although some variations were noted; in general, multiple fibre patterns to a specific chemical stimuli were reproducible from pigeon to pigeon if neural activity could be evoked. In Figure 24, a spectrum of typical integrated records is shown for 16 distinct chemical solutions. Integrated records such as this were used in all subsequent analysis of the multiple fibre activity.

In Tables 7 through 26 are tabulated the response time, the peaking time, and the peak response magnitude to both 2M NaCl and the chemical solution being analyzed, for the responding pigeons listed. The time to respond, for a given bird, includes the physiological latency, and the lag time from activation of burette stopcock to contact of solution to tongue surface (0.8 seconds). Differences in response time

Figure 23. Typical patterns of multiple fibre neural activity to 7 chemical solutions applied. Upper trace in each record represents the untouched neural activity recorded from multiple nerve fibres to each chemical solution quoted. Lower trace is both a time signal and an event marker. The sharp shift in amplitude of the time signal base line indicates that burette stopcock has been activated to initiate solution flow (event marker). Impulses on the upper records corresponding to time of activation of event marker are usually artifacts. Time signal = 0.1 second per period

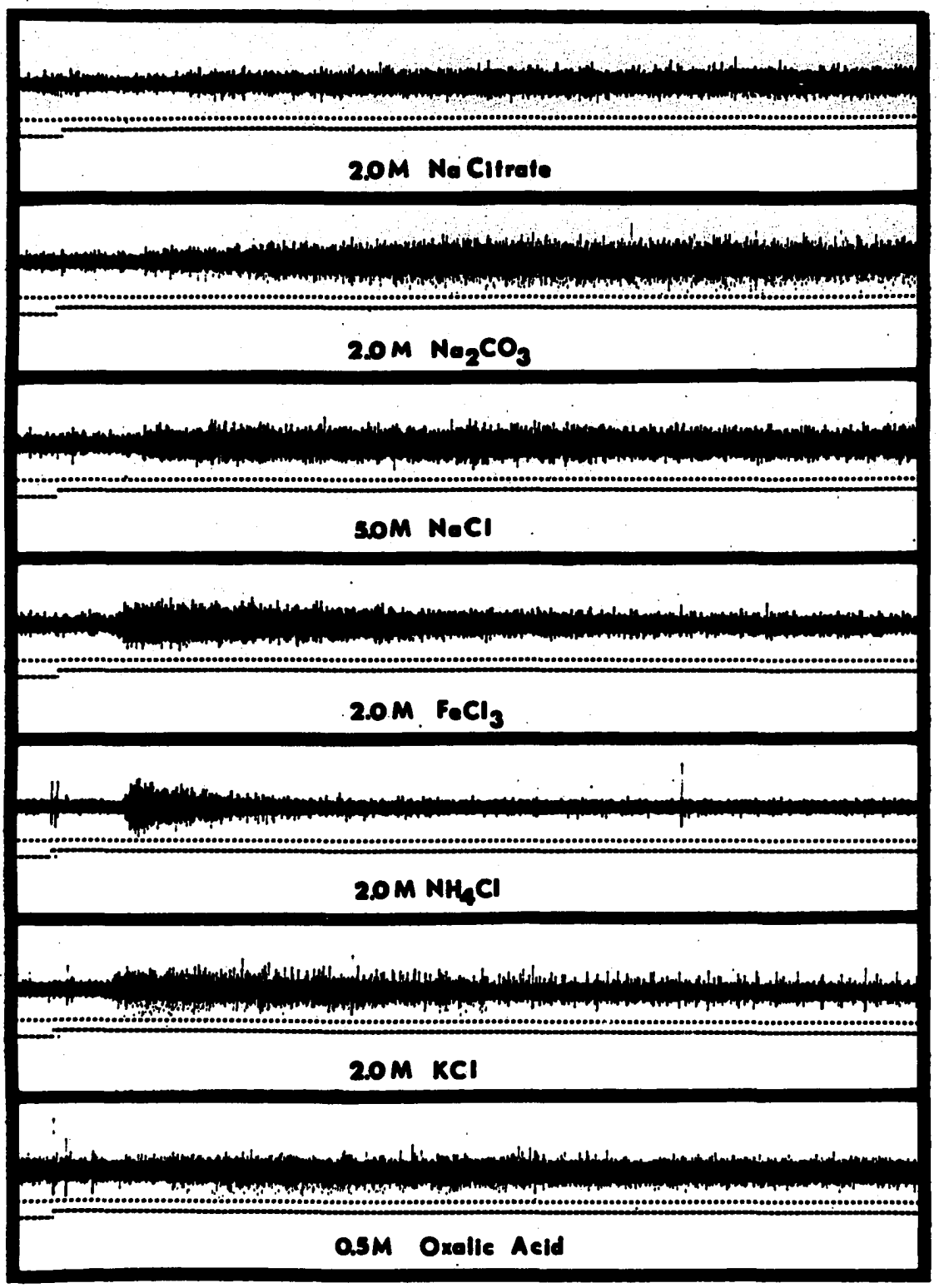
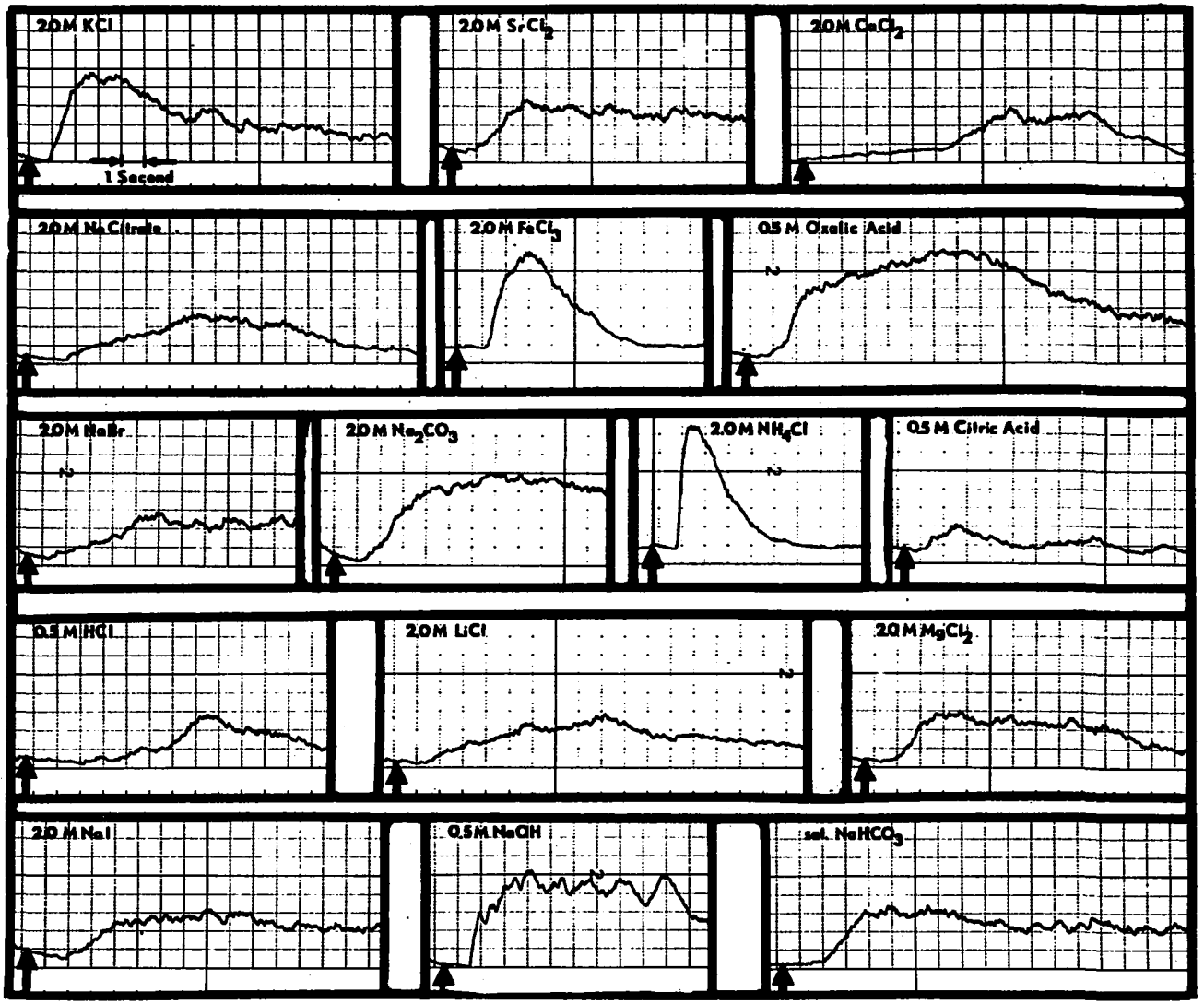

2.0 M Na Citrate
2.0 M Na_2CO_3
5.0 M NaCl
2.0 M $FeCl_3$
2.0 M NH_4Cl
2.0 M KCl
0.5 M Oxalic Acid

Figure 24. Typical integrated records from multiple nerve fibres to 16 different chemical solutions. Arrow indicates activation of burette stockcock. Amplitude of each integrated record is arbitrary. Time = 1 second per vertical division


2.0 M KCl
1 Second
2.0 M $SrCl_2$
2.0 M $CaCl_2$
2.0 M Na_3 Citrate
2.0 M $FeCl_3$
0.5 M Oxalic Acid
2.0 M NaBr
2.0 M Na_2CO_3
2.0 M NH_4Cl
0.5 M Citric Acid
0.5 M HCl
2.0 M LiCl
2.0 M $MgCl_2$
2.0 M NaI
0.5 M NaOH
sat. $NaHCO_3$

between pigeons for the same chemical solution may arise through individual variations plus the fact that it is very difficult to preserve the same relative position between burette stem tip and tongue surface from pigeon to pigeon. Both response and peaking times were referred back to the time of initiation of the burette stopcock. Because of the uncontrollable variations in the response time data, it was very difficult to place any quantitative judgement on them. It was, however, possible to quantitate the rise time over several pigeons for most of the spectrum of taste solutions used. This was simply done by taking the difference between the sum of the peak times and the sum of the response times and averaging this by the number of pigeons. Symbolically, the average rise time can be written as:

$$\overline{\Sigma T_R - \Sigma T_L} = (\Sigma T_R - \Sigma T_L)/N \qquad (4)$$

where

ΣT_R = summation of all pigeon response times T_R to the designated solution,

ΣT_L = summation of all pigeon peaking times T_L to the designated solution,

N = number of pigeons in sample responding to taste solution.

Average response times are included in the tabulations referred to above. Since only about 10 seconds of neural activity were recorded, it was not possible to justifiably quantitate any fall times.

A second parameter capable of being quantified is the peak response magnitude R of the integrated neural activity. The standard method has been to define a new parameter--the average relative effectiveness measure--which compares the peak magnitude R of each chemical solution to the peak magnitude R_{NaCl} of a standard base solution (2M NaCl in this study). Average relative effectiveness values are also listed in each table.

It should be pointed out that all missing values in Tables 7 through 26 are due either to lack of available information or else to data of questionable nature. Also good quantitative data of multiple unit activity for HCl, $MgCl_2$, $SrCl_2$, and $BaCl_2$ were quite limited but have been included for completeness. (Note that in Table 15 only the HCl response data to one pigeon have been recorded even though five out of five pigeons tested were found to respond to this taste solution.) In some instances artifacts prevented a satisfactory quantification of the data; in other instances only single fibre data were extracted. This discrepancy applied to other solutions tested also. Whenever a sequence of chemicals was repeated on the same nerve preparation, the new recordings are represented in the tables by following the pigeon number with a dash and then the repetition number; e.g. 20-2. Each seriation of neural recording was accepted only if the electrode remained in the same fixed position during any given

Table 7. Integrated response data to 0.5M NaOH

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
19	1.8	6.0	8.0	2.7
20-1	1.2	3.6	5.0	3.0
20-2	2.0	5.0	3.5	3.0
21	2.6	3.8	5.1	2.5
Total(Σ)	7.6	18.4	21.6	11.2

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 2.70$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 1.93$

Table 8. Integrated response data to 2M Na_2CO_3

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
15-1	7.8	13.6	5.2	4.7
15-2	5.2	12.0	6.0	4.0
16	1.2	4.0	4.0	2.3
17	-	-	4.7	4.1
19	1.5	5.1	6.2	2.7
20-1	2.0	6.6	4.9	3.0
20-2	2.0	6.8	5.0	3.0
21	1.6	6.0	3.4	2.5
22	0.9	3.1	7.3	3.4
Total(Σ)	22.2	57.2	46.7	29.7

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 4.38$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 1.57$

Table 9. Integrated response data to 2M NH_4Cl

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
15-1	1.0	1.4	7.4	4.7
15-2	1.1	1.5	4.0	4.0
16	0.7	1.3	5.3	2.3
17	-	-	3.4	4.1
19	1.2	2.4	5.9	2.7
21	1.1	2.3	5.2	2.5
22	1.1	1.4	3.3	3.4
Total(Σ)	6.2	10.3	34.2	23.7

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 0.68$

Average relative effectiveness - $\Sigma R/\Sigma R_{NaCl} = 1.44$

Table 10. Integrated response data to 2M $FeCl_3$

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
14	1.0	1.5	10.2	6.5
15-1	1.3	2.6	6.4	4.7
16	2.0	2.6	3.0	2.3
17	-	-	2.9	4.1
20	3.6	8.4	4.7	3.0
Total(Σ)	7.9	15.1	27.2	20.6

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 1.80$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 1.32$

Table 11. Integrated response data to 0.5M oxalic acid

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
19-1	1.5	7.0	6.0	2.7
19-2	3.2	9.4	2.2	2.7
20	6.9	7.8	2.7	3.0
21	4.8	5.3	1.8	2.5
Total(Σ)	16.4	29.5	12.7	10.9

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 3.28$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 1.16$

Table 12. Integrated response data to saturated $NaHCO_3$

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
15-1	6.3	11.2	4.2	4.7
16-1	2.2	4.4	3.3	2.3
16-2	1.6	4.3	5.1	2.3
17	-	-	4.6	4.1
19	4.0	6.3	2.9	2.7
21	4.8	6.6	2.0	2.5
22	-	-	2.5	3.4
Total(Σ)	18.9	32.8	24.6	22.0

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 2.78$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 1.12$

Table 13. Integrated response data to 2M Na citrate

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
14	2.2	15.2	8.7	6.5
15-1	3.1	4.8	4.0	4.7
15-2	3.2	8.2	4.1	4.0
16	3.0	7.2	2.5	2.3
17	-	-	2.1	4.1
19	7.0	12.2	3.7	2.7
Total(Σ)	16.3	47.6	25.1	24.9

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 5.82$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 1.01$

Table 14. Integrated response data to 2M NaCl

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
14	1.8	5.0	6.5	6.5
15-1	1.7	6.0	4.7	4.7
15-2	3.6	6.8	4.0	4.0
16	1.7	2.4	2.3	2.3
17	-	-	4.1	4.1
19	1.4	2.4	2.7	2.7
20	2.8	7.5	3.0	3.0
21	1.2	4.2	2.5	2.5
22	4.0	6.2	3.4	3.4
Total(Σ)	18.2	40.5	33.2	33.2

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 2.54$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 1.00$

Table 15. Integrated response data to 0.5M HCl

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
19-1	5.2	7.6	2.7	2.7
19-2	10.0	11.4	2.7	2.7
Total(Σ)	15.2	19.0	5.4	5.4

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 1.90$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 1.00$

Table 16. Integrated response data to 2M KCl

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
15-1	1.5	2.2	2.2	4.7
15-2	3.3	5.0	2.4	4.0
16	1.0	2.1	4.8	2.3
17	-	-	2.0	4.1
19	1.4	2.8	4.3	2.7
20	2.4	4.6	3.0	3.0
21	1.3	1.8	2.5	2.5
22	1.1	2.6	4.7	3.4
Total(Σ)	12.0	22.1	25.9	26.7

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 1.44$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 0.97$

Table 17. Integrated response data to 2M Na acetate

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
16	1.1	3.8	4.5	2.3
17	--	-	3.6	4.1
19	5.6	7.0	2.2	2.7
20	10.4	12.2	2.3	3.0
22	-	-	1.7	3.4
Total(Σ)	17.1	23.0	14.3	15.5

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 1.97$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 0.92$

Table 18. Integrated response data to 0.5M acetic acid

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude 2M NaCl--R_{NaCl} (arbitrary units)
16	4.2	9.7	2.2	2.3
19	4.1	5.4	3.1	2.7
21	1.9	3.0	3.1	2.5
22	5.4	7.0	1.6	3.4
Total(Σ)	15.6	25.1	10.0	10.9

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 2.38$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 0.92$

Table 19. Integrated response data to 2M NaBr

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
15-1	2.9	4.7	3.8	4.7
16	5.0	9.0	2.1	2.3
17	-	-	3.5	4.1
19	2.0	5.0	2.6	2.7
Total(Σ)	9.9	18.7	12.0	13.8

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 2.93$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 0.87$

Table 20. Integrated response data to 2M LiCl

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
16	2.6	9.0	2.7	2.3
17	-	-	2.4	4.1
Total(Σ)	2.6	9.0	5.1	6.4

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 6.40$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 0.80$

Table 21. Integrated response data to 2M $SrCl_2$

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
16	1.6	4.2	3.0	2.3
17	-	-	1.0	4.1
19	1.3	3.1	3.1	2.7
Total(Σ)	2.9	7.3	7.1	9.1

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 2.20$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 0.78$

Table 22. Integrated response data to 2M $MgCl_2$

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
16	1.8	3.2	2.8	2.3
17	-	-	2.2	4.1
Total(Σ)	1.8	3.2	5.0	6.4

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 1.40$

Average relative effectiveness = $\Sigma R/\Sigma R_{NaCl} = 0.78$

Table 23. Integrated response data to 2M NaI

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
15-1	3.6	6.3	2.3	4.7
15-2	15.2	18.0	2.2	4.0
17	-	-	4.1	4.1
19	2.3	4.2	2.6	2.7
20	4.2	7.6	2.9	3.0
Total(Σ)	25.3	36.1	14.1	18.5

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 2.70$

Average relative effectiveness = $\Sigma R / \Sigma R_{NaCl} = 0.76$

Table 24. Integrated response data to 0.5M citric acid

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude 2M NaCl--R_{NaCl} (arbitrary units)
19	4.3	5.6	2.4	2.7
20	1.2	2.2	2.0	3.0
21	4.8	5.3	1.8	2.5
Total(Σ)	10.3	13.1	6.2	8.2

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 0.93$

Average relative effectiveness = $\Sigma R / \Sigma R_{NaCl} = 0.76$

Table 25. Integrated response data to 2M $CaCl_2$

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl-- R_{NaCl} (arbitrary units)
14	3.2	6.8	2.1	6.5
15-2	6.6	9.0	2.8	4.0
16	8.2	15.2	2.3	2.3
17	-	-	4.3	4.1
Total(Σ)	18.0	31.0	11.5	16.9

Average rise time = $\overline{\Sigma T_R - \Sigma T_L} = 4.25$

Average relative effectiveness = $\Sigma R / \Sigma R_{NaCl} = 0.68$

Table 26. Integrated response data to 2M $BaCl_2$

Pigeon no.	Time to respond--T_L (seconds)	Time to peak--T_R (seconds)	Peak response magnitude--R (arbitrary units)	Peak response magnitude to 2M NaCl--R_{NaCl} (arbitrary units)
17	-	-	2.3	4.1

Average relative effectiveness = $\Sigma R / \Sigma R_{NaCl} = 0.56$

Table 27. Summary of integrated neural responses

Chemical solution	Average rise time (seconds)	Average relative effectiveness measure	Sample size
0.5M NaOH	2.70	1.93	4
2M Na_2CO_3	4.38	1.57	9
2M NH_4Cl	0.68	1.44	9
2M $FeCl_3$	1.80	1.32	5
0.5M oxalic acid	3.28	1.16	4
Sat. $NaHCO_3$	2.78	1.12	7
2M Na citrate	5.82	1.01	6
2M NaCl	2.54	1.00	9
0.5M HCl	1.90	1.00	2
2M KCl	1.44	0.97	8
2M Na acetate	1.97	0.92	5
0.5M acetic acid	2.38	0.92	4
2M NaBr	2.93	0.87	4
2M LiCl	6.40	0.80	2
2M $SrCl_2$	2.20	0.78	3
2M $MgCl_2$	1.40	0.78	2
2M NaI	2.70	0.76	5
0.5M citric acid	0.93	0.76	3
2M $CaCl_2$	4.25	0.68	4
2M $BaCl_2$	-	0.56	1

run. Between repetitions a new electrode position was allowed.

The major results are summarized in Table 27. Note that the relative effectiveness of NaOH is considerably greater

than that from all other solutions. Other response magnitudes greater than the base NaCl response are Na_2CO_3, NH_4Cl, $FeCl_3$, oxalic acid, $NaHCO_3$ and Na citrate. Note that although $SrCl_2$ and $MgCl_2$ have equivalent relative effectiveness measures, their average rise times are noticeably different. This together with the fall time may provide an important clue to understanding the information processing mechanism of the gustatory system.

Other features can be extracted from Table 27. For instance, the relative effectiveness of the monovalent chloride cation series can be arranged in the following order: $NH_4^+ >> Na^+ \geq K^+ >> Li^+$ if a 5% or smaller variation can be tolerated. It is interesting to note that this is the accepted arrangement with regard to degree of saltiness (146, p. 517) for monovalent chlorides in the human being. Duncan (48, p. 130) has determined the stimulating efficiency of these cations as follows: $Na^+ > NH_4^+ > K^+$ for the pigeon on a preference-aversion basis. This seems to indicate that behavioral responses are at odds with electrophysiological responses; the only common feature being that K^+ is the least effective cation in both cases. Nor does it agree with electrophysiological results on carnivores, yet there are similarities to rodents and frogs (146, p. 517) as indicated by $NH_4^+ > K^+ > Na^+ > Li^+$.

Beidler *et al.* (22) and Fishman (62) have compared the high sodium response of the rodent to the low sodium response

of the carnivore by means of an Na/K ratio. It is interesting to note that the Na/K = 1.03 value for the pigeon does not correspond to either of these classes; rather it compares most favorably with a value of Na/K = 0.97 for the insectivorous bat in Tamar's work [as quoted by Fishman (62, Figure 1)]. In contrast to both carnivores and rodents, K^+ and Na^+ are equally effective stimulants to the pigeon receptor sites.

From the divalent chloride cation salts the following seriation was determined: $Sr^{++} = Mg^{++} > Ca^{++} > Ba^{++}$. The relative effectiveness of any member of the divalent cation series tested seems to be of considerably smaller magnitude than any particular monovalent cation. It should be borne in mind that the sample size for divalent cations is rather limited due primarily to the inability of many pigeon preparations to respond to these salts. Typically, electrophysiological results indicate: $Ca^{++} > Sr^{++} > Mg^{++}$ for both the carnivore and the rodent, while behavioral studies for degree of saltiness in man (146, p. 517) indicate: $Ca^{++} > Mg^{++}$. In contrast to the small measures noted with the divalent cation salts, the trivalent cation salt $FeCl_3$ gave a very large response. The large molar concentration of $FeCl_3$ used, however, may act as an irritant and not true lingual receptor stimulation. Halpern (71, p. 542) has recorded large responses to $FeCl_3$ solutions lower than 0.001M in the chicken.

He suggests that taste in the chicken may be the mechanism responsible for triggering rejection of a given substance.

The sodium monovalent anion series indicated the following sequence of effectiveness: $HCO_3^- \geq Cl^-$ > acetate anion > Br^- > I^-. In particular since $Cl^- > Br^- > I^-$, one would expect smaller response magnitudes with a monovalent or divalent Br^- or I^- series than the comparison Cl^- series as it appears as though both the cation and the anion have a stimulating effect. The above order agrees exactly with the degree of saltiness of sodium halides in the human being; however, this order is exactly reversed in invertebrates (146, p. 517). The sodium divalent anion salt Na_2CO_3 is alkaline. It has been suggested by Moncrieff (132, p. 269) that these substances do not activate taste receptor sites but are a modality of the common chemical sense and as such can cause irritation to the tongue as well as to other parts of the body. NaOH could act in a similar manner.

Concerning the acid series, the following sequence was observed: oxalic acid $\geq$ HCl > acetic acid > citric acid. Of two of these acids tested, Duncan (50, p. 78) quoted a rejection response sequence of stimulating efficiency as HCl > acetic acid at equal normality in the pigeon. This confirms the observation on man in regard to degree of sourness (17).

Testing Beidler's fundamental taste equation

A second study on the integrated data was confined to testing Beidler's fundamental taste equation (see Equation 2, p. 59) on the pigeon to 2 specific chemical stimuli. Since the chemoreceptor mechanism of the pigeon is essentially a two modality system--salt and sour--solutions of NaCl and acetic acid, each at four specific concentrations were chosen as representative. Since Kitchell et al. (116, pp. 139-141) have indicated that tongue receptors are sensitive to NaCl solutions only at concentrations greater than 0.2M, a range from 0.5M to 2M was chosen to test Beidler's equation.

In Table 28, the salt series data collected for each pigeon are shown when normalized for response magnitude to the 2M NaCl concentration. The subscript n indicates that that value has been normalized. As shown in Figure 25, the curves for each pigeon studied are quite similar in shape. The slight differences have mainly been attributed to individual pigeon variations. As such, it seemed advisable to determine an ideal response to fit Beidler's taste equation; even though any one of the 4 curves could itself be fitted quite closely to the taste equation. The method chosen was to take an average value of C/R_n and fit a linear regression curve to the data for each value of concentration chosen. In Table 29, the salient values are shown. Accordingly, the regression taste equation now takes the form:

Table 28. Response-concentration data for NaCl solutions

Pigeon no.	Concentration --C	Response--R (arbitrary units)	Normalized response--R_n	C/R_n
14	0.5M	2.9	0.45	1.11
	1.0M	4.8	0.74	1.35
	2.0M	6.5	1.00	2.00
	5.0M	6.8	1.05	4.76
15-2	0.5M	-	-	-
	1.0M	2.2	0.55	1.82
	2.0M	4.0	1.00	2.00
	5.0M	4.2	1.05	4.76
19	0.5M	0.7	0.26	1.92
	1.0M	1.6	0.59	1.70
	2.0M	2.7	1.00	2.00
	5.0M	3.3	1.22	4.10
21	0.5M	1.5	0.60	0.83
	1.0M	2.0	0.80	1.25
	2.0M	2.5	1.00	2.00
	5.0M	3.0	1.20	4.16

$$\frac{\hat{C}}{R_n} - \frac{\overline{Y}}{Y} = \frac{1}{R_{mn}} (C - \overline{C}) \qquad (5)$$

where

$\frac{\hat{C}}{R_n}$ = linear regression form of C/R_n,

C = concentration in moles per liter of NaCl solution used,

Figure 25. Response-concentration curves for 4 pigeons responding to 4 concentrations of NaCl. Typical integrated records for one pigeon (Pigeon number 21) to each concentration of NaCl tested are shown at the top of the diagram. Large arrow indicates activation of burette stopcock to release sapid solution to tongue surface. Each vertical division on the integrated records indicates 1 second of time. Amplitude of integrated records is arbitrary

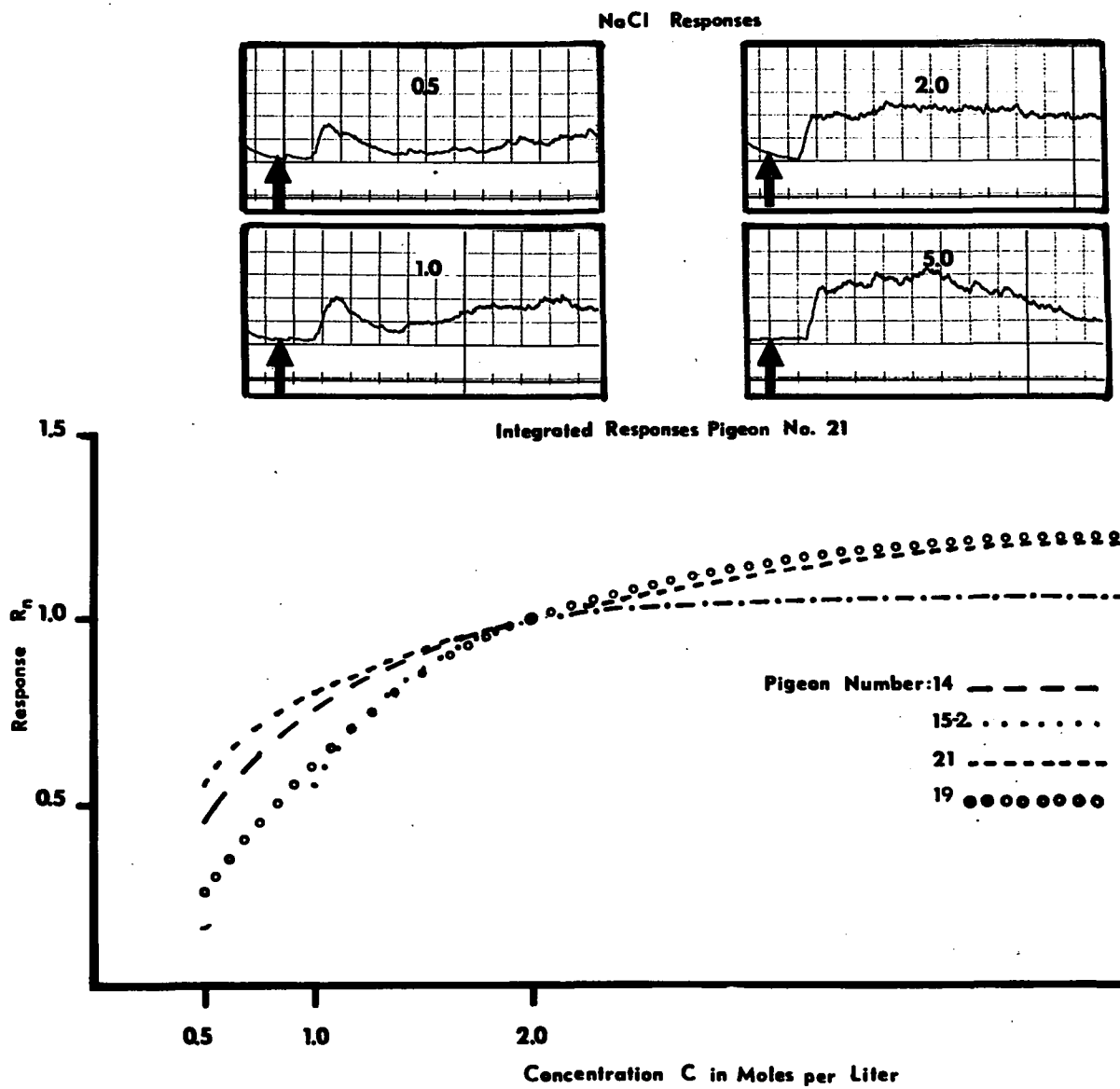

NaCl Responses
0.5
2.0
1.0
5.0
Integrated Responses Pigeon No. 21
1.5
1.0
0.5
Response R_n
Pigeon Number:14
15-2
21
19
0.5
1.0
2.0
5.0
Concentration C in Moles per Liter

Table 29. Least squares fit to Beidler's taste equation for NaCl

Concentration --C (moles/liter)	C/R_n Pigeon number 14	15-2	19	21	Sum	Mean Y	Deviation from overall mean c	y	Squares c^2	Products cy
0.5	1.11	-	1.92	0.83	3.86	1.288	-1.625	-1.028	2.64	1.67
1.0	1.35	1.82	1.70	1.25	6.12	1.531	-1.125	-0.785	1.27	0.88
2.0	2.00	2.00	2.00	2.00	8.00	2.000	-0.125	-0.316	0.02	0.04
5.0	4.76	4.76	4.10	4.16	17.78	4.445	+2.875	+2.129	8.27	6.14
Sum 8.5						9.259	0	0	12.20	8.73

Overall mean $\overline{C} = 2.125$ $\overline{Y} = 2.316$

$\overline{C}$ = overall mean value of C,

Y = mean value of C/R_n over 4 pigeons,

$\overline{Y}$ = overall mean value of Y over all 4 concentrations of NaCl tried,

$$\frac{1}{R_{mn}} = \frac{\Sigma cy}{\Sigma c^2} = \frac{8.73}{12.20} = 0.72 \text{ or } R_{mn} = 1.40,$$

$c = C - \overline{C}$,

$y = Y - \overline{Y}$.

Rearranging Equation 5 and putting in specific values one obtains:

$$\frac{\hat{C}}{R_n} = 0.72\ C + 0.786 \qquad (5a)$$

where

$$\frac{1}{KR_{mn}} \text{ (of Equation 2)} = 0.786 \text{ or } K = 0.91.$$

Further details on linear regression techniques can be found in Snedecor (169, pp. 122-159).

Two interesting features are immediately evident. First the R_m value is far larger than the one found by Beidler for the rat to NaCl (21, p. 136). Since larger concentrations of salt are needed to stimulate the pigeon receptor sites, this seems very reasonable. However, a large R_m also indicates that high concentrations must be used before all receptor sites are to be filled if Beidler's theory is correct. High concentrations are usually harmful to the receptor sites, hence they probably remain in an unfilled state in the pigeon under normal gustatory conditions.

The second obvious fact is that the equilibrium constant K is extremely small. This tends to support the concept that taste reactions are physical rather than enzymatic in nature.

Note that in order for a reaction to be spontaneous, the free energy must be negative. This implies that the equilibrium constant K must be greater than 1 as exemplified by the formula:

$$\Delta F = -RT \ln K \qquad (6)$$

where

ΔF = change in free energy,

R = gas constant,

T = absolute temperature.

In many of our preparations there was quite a time lapse (of the order of several seconds) before neural activity was evoked upon tongue stimulation. If the equilibrium constant K is of the order of 1 or slightly less, as appears to be the case with our results, then values of ΔF will be extremely small and positive. Under these conditions, for spontaneity of chemical reaction on the receptor sites to be realized, the predominant controlling factor would be the absolute temperature T of the solution--tongue interface. As such, one would expect a decreasing temperature T to drive Equation 6 towards spontaneity. The several seconds time lapse noted before neural activity was evoked could be

sufficient to cause a cooling of the solution--tongue interface and thereby provide a plausable explanation for this phenomena.

A complete sequence of multiple fibre responses to all concentrations of acetic acid tested was very difficult to obtain. In fact, only one good preparation (Pigeon number 19) was found that would respond successfully to all 4 concentrations. The response-concentration curve, together with the integrated responses obtained is shown in Figure 26. Beidler's equation was tested and found not to fit.

Few Fibre Activity

Characteristics

Few fibre preparations offer the opportunity to study the effects of stimulus intensity in recruiting different fibres for neural activity. Also several single fibre neural patterns to different chemicals at the same intensity can be observed. In Pigeon number 18 three active fibres were isolated that responded in various degrees to the chemical stimuli applied (see Figure 27--histograms corresponding to neural activity in these 3 fibres). For instance, in the histograms in Figure 27, only one fibre was activated by saturated $NaHCO_3$, whereas the same fibre in addition to two others were activated by the salts 2M KCl and 2M NH_4Cl. Both salt

Figure 26. Response-concentration curve and corresponding integrated records for Pigeon number 19 to 4 concentrations of acetic acid. Large arrow indicates activation of burette stopcock to release sapid solution to tongue surface. Each vertical division in the integrated records indicates 1 second of activity. Amplitude of integrated records is arbitrary

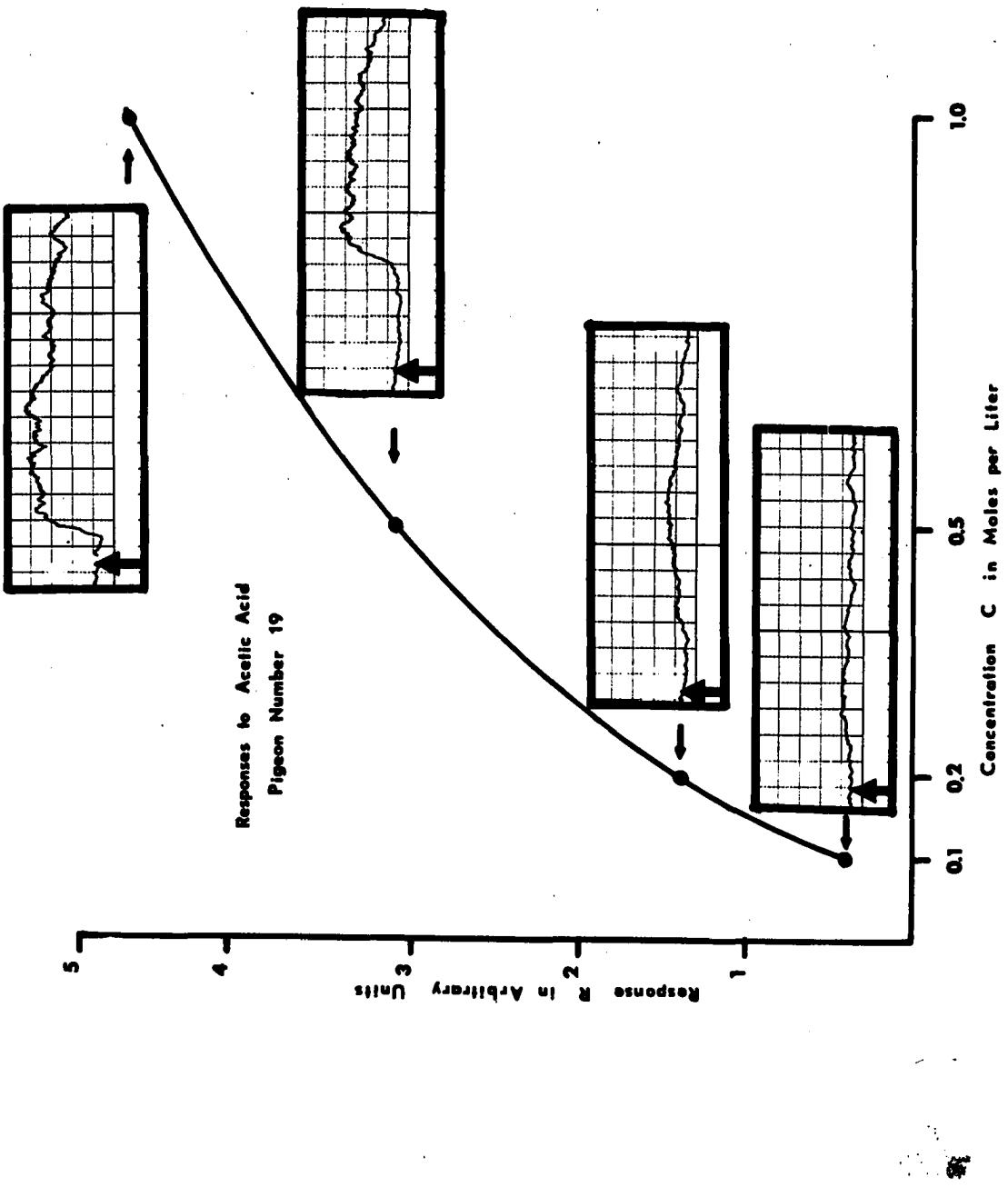
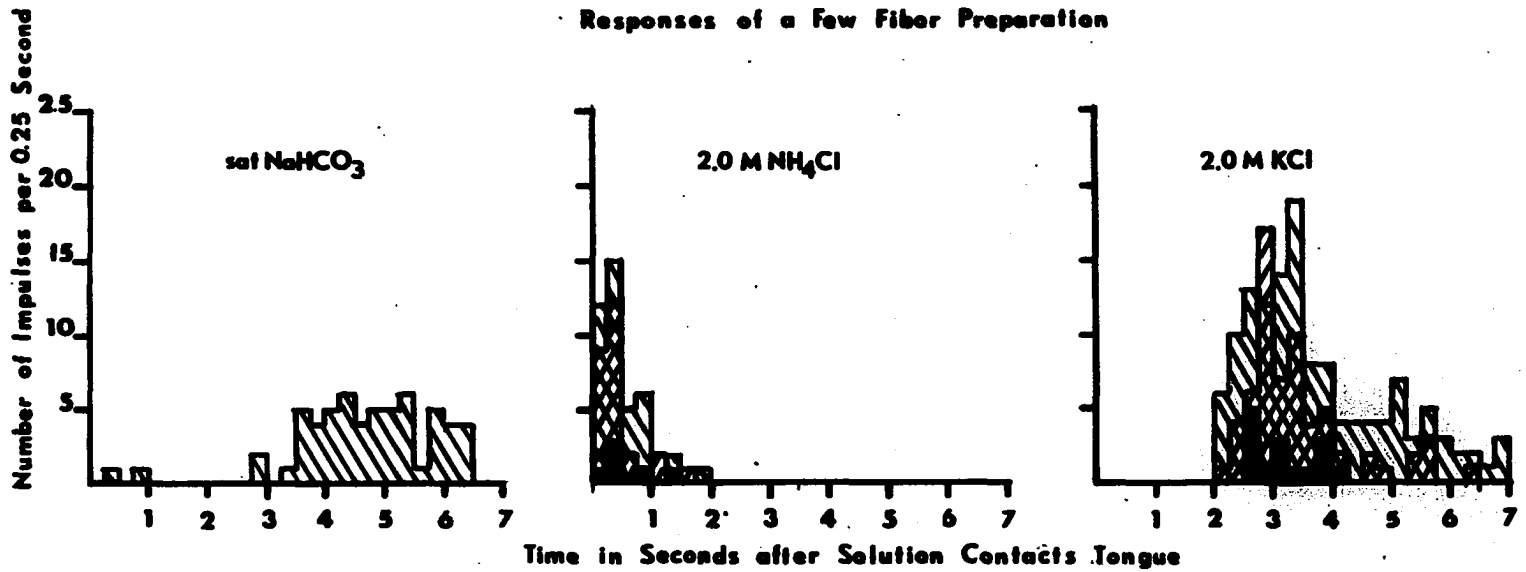

Responses to Acetic Acid
Pigeon Number 19
Response R in Arbitrary Units
1
2
3
4
5
0.1
0.2
0.5
1.0
Concentration C in Moles per Liter

Figure 27. Histograms of the number of impulses per 0.25 second interval. Impulse counts start after solution has made contact with tongue surface. Profiles are from the same few fibre preparation to 3 different solutions


Responses of a Few Fiber Preparation
Number of Impulses per 0.25 Second
25
20
15
10
5
sat NaHCO3
2.0 M NH4Cl
2.0 M KCl
1 2 3 4 5 6 7
Time in Seconds after Solution Contacts Tongue

responses indicate that the three fibres involved are characterized by a rapid rise and then followed by a decrement with essentially only one neural burst peak. Furthermore, the fibres sensitive to NH_4Cl stop responding far earlier than those to KCl. In contrast the single fibre responding to saturated $NaHCO_3$ is characterized by a delay in activity until several seconds have elapsed and then a broader spectrum of activity than the indicated salts. Some few fibre response profiles showed a great tendency for one fibre to be active whenever the other fibre was quiescent and vice versa. Other preparations of few fibre activity gave profile patterns having an almost complete temporal overlap of the neural activity from two or more fibres. Na citrate patterns appeared to belong to this class. By varying the time interval between individual patterns for different solutions, a neural code for taste quality could conceivably be fashioned. However, a word of caution must be introduced with regard to this as a possible coding mechanism, since single fibre preparations have shown some variation in response pattern through repeated stimulation of the same fibre with the same chemical stimulus (see HCl response in Figure 30). Nevertheless, if the variation is consistent from fibre to fibre within a whole nerve bundle for repeated stimuli, quality coding might still be achieved.

Stimulus intensity coding

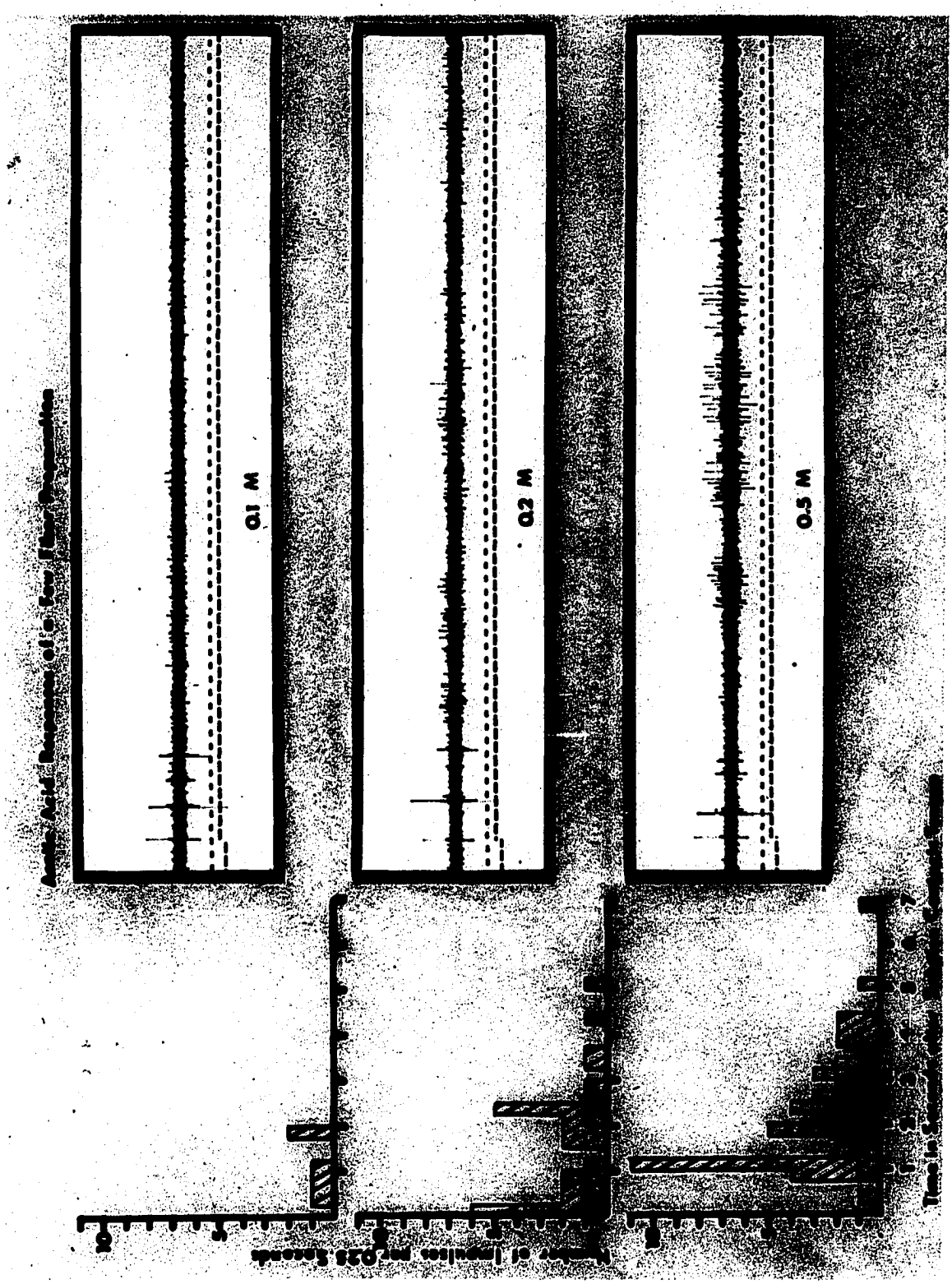
That stimulus intensity coding is related to the number of active fibres and their corresponding discharge rate (141) seems to be confirmed by the acetic acid sequence shown in Figure 28. At the 0.1M concentration level, only a single fibre is activated. Then at 0.2M concentration, the degree of activity in the initial single fibre is increased and a second fibre has been recruited. Finally, at 0.5M concentration the activity continues to increase in both these fibres and a third fibre is now recruited. It may be conjectured that as the stimulus continues to increase, other fibres will be activated while those fibres which were initially discharging now become saturated and continued to discharge at some upper frequency rate. This upper frequency need not be the same in all fibres. Finally, all fibres will be recruited and discharging at their saturation frequency. Accordingly, this should correspond to the maximum response magnitude R_m in Beidler's taste equation (Equation 2).

Single Fibre Activity

Characteristics

Single fibre activity was extracted from several pigeons. The photographic records obtained were scanned and various histograms plotted of the degree of impulse activity as a

Figure 28. Response profile histograms and untouched neural records to 3 concentrations of acetic acid from a few fibre preparation. Neural records are indicated by the upper trace in each block, at the right side of the diagram. Lower trace in each block represents the combined time signal and event marker. Event marker is indicated as a sharp shift in the base line of the time signal. This indicates when burette stopcock has been activated to initiate chemical solution flow. Large impulses in the neural records starting when event marker was activated until 0.8 seconds have elapsed are artifacts. Time from activation of burette stopcock to when solution touches tongue surface is approximately 0.8 seconds. Time signal = 0.1 second per period. Each of the histograms on the left of the diagram represents the degree of neural activity extracted from the neural record to its immediate right

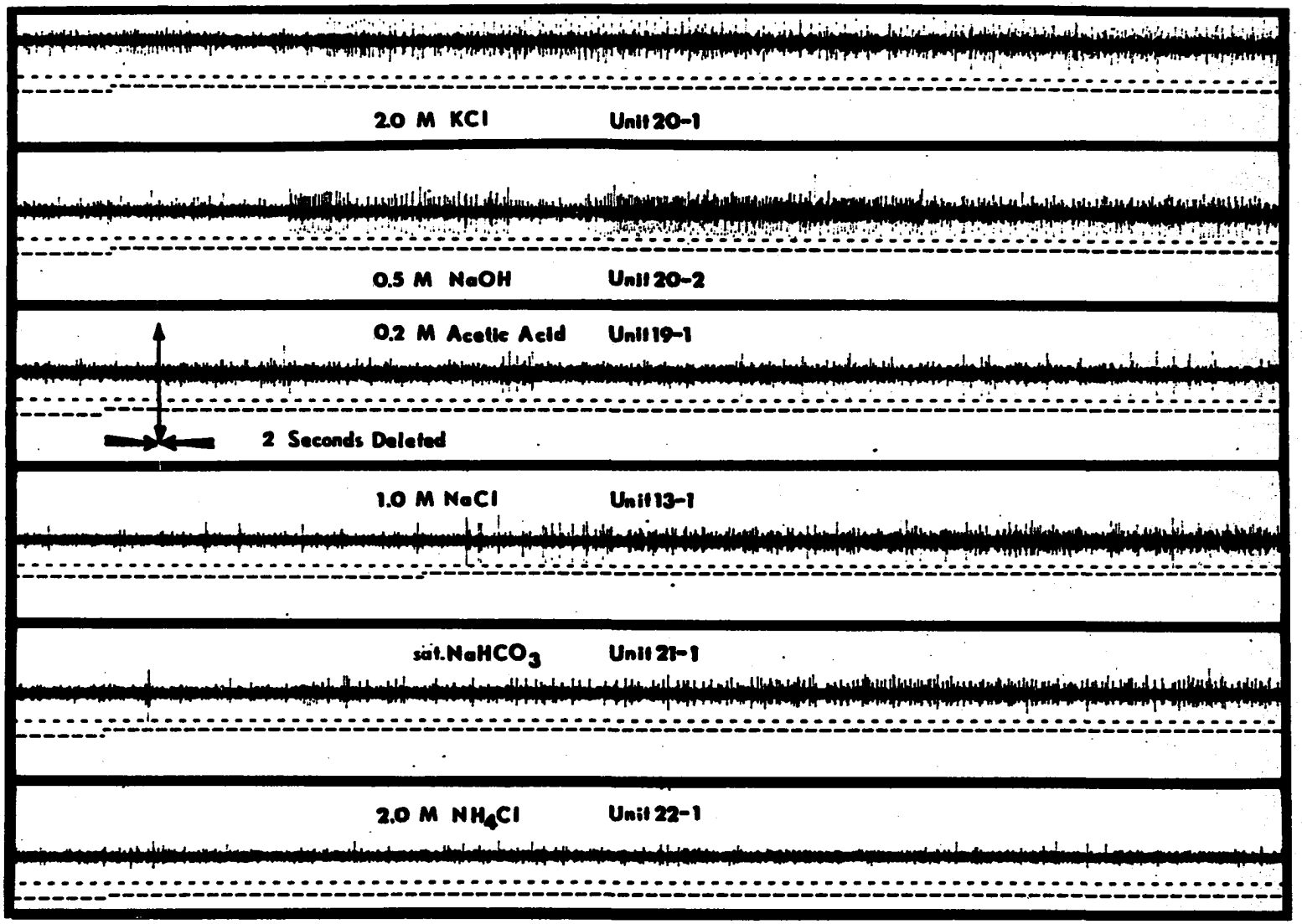

0.1 M
0.2 M
0.5 M

function of time after solution had touched the tongue surface. The data indicated that a very prominent feature of most fibres was the presence of a burst-like type of activity in the neural message. The burst packets were found to vary from fibre to fibre, and from solution to solution with regard to intensity of burst activity, duration of burst activity, and even to time delays between individual bursts. It was not possible to classify fibre types or the specific neural message on the basis of burst activity since the sample size was far too small.

In general, it was noted that the majority of individual fibres isolated did respond to a wide mixture of chemicals tested. In particular, a multiple fibre response to a salt or acid solution practically insured that a given single fibre would be activated by the same acid and salt. This was not the case for NaOH, $NaHCO_3$, and Na_2CO_3 as several specific fibres were observed that responded to any combination of these three solutions and not to acids or salts.

In Figure 29 typical neural recordings from which single fibre impulse data was extracted are shown for different chemical solution stimulation. The nomenclature used in identifying units for single fibre impulse activity is quite straightforward; for instance "Unit 20-2" stands for the second unit isolated and tested in Pigeon number 20.

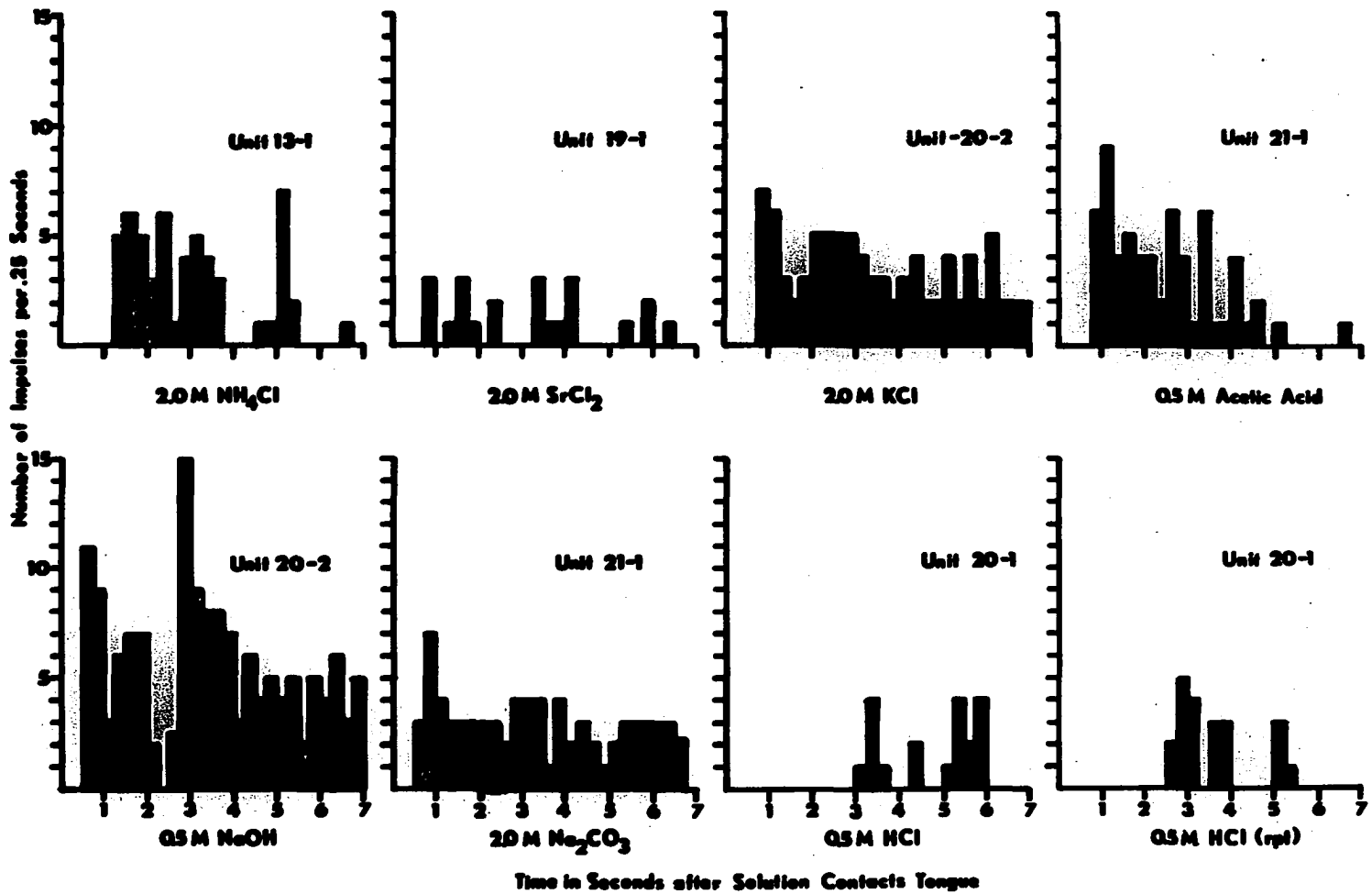
Figure 29. Typical neural recordings to 6 chemical solutions from which single fibre data could be extracted. Upper trace in each block represents neural data. All records, except that for 0.2M acetic acid are untouched. A 2 second strip was removed from the acetic acid response as there was no evidence that neural activity was being evoked during this time interval. Lower trace in each block designates a combined time signal and event marker. Whenever burette stopcock is activated (event marker), a sharp shift in the time signal base line is realized. Time signal = 0.1 second per period


2.0 M KCl
Unit 20-1
0.5 M NaOH
Unit 20-2
0.2 M Acetic Acid
Unit 19-1
2 Seconds Deleted
1.0 M NaCl
Unit 13-1
sat. $NaHCO_3$
Unit 21-1
2.0 M NH_4Cl
Unit 22-1

The KCl response in Unit 20-1 tends to be more regular and less burst-like than many observed. Pfaffmann (144, p. 436) has recorded a similar observation in the rabbit to both KCl and HCl. Although it was not possible to record such a pattern to the latter solution this feature was observed in specific fibres responding to NaOH, $NaHCO_3$, and Na_2CO_3. Because of the irritant nature of NaOH in particular, a regular pattern of neural activity might be interpreted by the central nervous system as a defense mechanism. Because of individual variations between pigeons such solutions as KCl might be offensive or even irritant in some preparations. The low concentrations used by Pfaffmann seem to overrule irritancy in his work, however, concentration of 2M as used in our studies could account for this.

In Figure 30 several typical response profiles are shown for single fibre activity. By using as a measure the total number of impulses per 0.25 second interval it was possible to optimally display the burst-like type of activity noted. The last two profiles show the neural activity to two repetitions of 0.5M HCl from the same fibre (Unit 20-1). The irregularity of impulses displayed by HCl was evident with other solutions in single fibre studies and has been mentioned before in connection with the few fibre profiles analyzed (see page 218). Sato and Kusano (163, p. 80), and Fishman (61, pp. 320-323) have also made reference to random

Figure 30. Typical single fibre response profile histograms from several units to various chemical solutions. Ordinate represents the number of impulses per 0.25 second interval after solution has contacted tongue surface


Number of Impulses per .25 Seconds
Unit 13-1
Unit 19-1
Unit 20-2
Unit 21-1
2.0 M NH_4Cl
2.0 M $SrCl_2$
2.0 M KCl
0.5 M Acetic Acid
Unit 20-2
Unit 21-1
Unit 20-1
Unit 20-1
0.5 M NaOH
2.0 M Na_2CO_3
0.5 M HCl
0.5 M HCl (rpt)
Time in Seconds after Solution Contacts Tongue

variations in impulse patterns. It has been observed that over a certain time period the average number of impulses between repetitions is approximately equivalent for a given solution despite the irregularity encountered. Fishman has indicated that over a long time period the steady-state discharge levels are equal and reproducible between repetitions. Of the five classifications given by Fishman (61, p. 322) in specifying type of single fibre response in the rat and the hamster, only the type identified as displaying a rythmic burst-like pattern seems to apply to our results with the pigeon. If one looks at the profiles in Figure 27 and Figure 30, one can delineate either a single major burst cluster, or two major burst clusters, or even three major burst clusters of activity in the time span covered.

Quality Coding in Chemoreception

General

One of the primary objectives of this research was to obtain some understanding of how neural signals are encoded into appropriate messages of chemical modality and intensity. Intensity coding (Figure 28) is accomplished by both spatial (fibre recruitment) and temporal (impulse frequency variation) phenomena. Quality coding, as indicated by many researchers, is not as readily visualized. Several possible clues for

interpretation have already appeared in the analysis presented. In one instance, it was possible to quantify the relative response magnitude and rise time for different stimuli from the integrated data (see Table 27), which together with fall times (not capable of being quantified in this study) could provide an important insight into a possible coding mechanism in the temporal sense.

The fact that there is a great variation in rise time and/or relative response magnitude indicates that the total neural activity over many fibres for one stimulus may have a different average frequency rate as a function of time from another chemical stimulus, i.e. the gross temporal patterns may be different between stimuli. Because of the wide chemical spectrum used in our studies such factors as variable pH and intensity values must not be overlooked as possible mechanisms responsible for generating different neural patterns.

The approach used in this part of the study was two-fold; firstly, to test for temporal neural pattern variations to different chemical stimuli; secondly, to analyse across-fibre or spatial patterns to different stimuli in order to see whether one or both pattern types are involved in the gustatory afferent coding mechanism of the pigeon. Halpern (70) has indicated that temporal patterns may be important in gustatory afferent coding; Pfaffmann (142) and Erickson (56)

support the across-fibre pattern theory as a mode for coding. In both our approaches statistical correlation techniques were employed as tests for pattern similarities or dissimilarities.

Temporal pattern quality coding

The single fibre data from 4 units to 3 salts (2M NaCl, 2M NH_4Cl, and 2M KCl) and 3 acids (0.5M acetic acid, 0.5M oxalic acid, and 0.5M HCl) were analysed for pattern variation within each salt, (6 combinations) between salts (48 combinations), within each acid (6 combinations), between acids (48 combinations), and between acids and salts (144 combinations). In order to present the burst-like activity observed, the number of impulses in each 0.25 second interval was recorded for a total time of 7 seconds after the first impulse appeared in each fibre. The data are shown in Table 30. Both product-moment and Spearman's rank correlation techniques were used as a measure of the degree of similarity between patterns. Snedecor (169, pp. 160-193) provides further details on the different types of correlation that can be employed.

With both techniques a high correlation coefficient was determined, thereby indicating a direct dependence between patterns. Changing the time interval to 1 second did not show any improvement. (With rank correlation techniques, it was found that the small interval of 0.25 seconds introduced

Table 30. Single fibre impulse counts for 4 units responding to 6 treatments as a function of time

Time (seconds)	Treatment: 2M NaCl					2M NH_4Cl					2 M KCl				
	Unit 18-1	Unit 19-1	Unit 21-1	Unit 22-1	Sum	Unit 18-1	Unit 19-1	Unit 21-1	Unit 22-1	Sum	Unit 18-1	Unit 19-1	Unit 21-1	Unit 22-1	Sum
0.25	1	1	10	2	14	12	1	7	1	21	6	2	3	1	12
0.50	0	0	9	2	11	15	1	11	0	27	10	2	3	1	16
0.75	0	0	4	2	6	5	4	5	3	17	13	2	7	3	25
1.00	1	1	3	3	8	6	10	5	2	23	12	2	3	3	20
1.25	0	2	3	2	7	0	9	11	0	20	14	2	2	0	18
1.50	0	0	1	0	1	2	6	4	0	12	19	5	3	1	28
1.75	1	0	1	4	6	0	8	5	0	13	8	4	2	0	14
2.00	0	0	5	1	6	1	8	1	2	12	8	6	1	2	17
2.25	1	0	4	2	7	0	10	1	1	12	4	7	0	1	12
2.50	0	1	7	1	9	0	5	0	4	9	4	9	1	0	14
2.75	1	0	5	2	8	0	9	3	3	15	4	4	1	1	10
3.00	0	0	3	3	6	0	2	2	7	11	4	2	0	5	11
3.25	0	1	7	3	11	0	4	5	3	12	7	1	0	0	8
3.50	0	0	1	2	3	0	6	1	1	8	3	2	0	1	6
3.75	0	1	2	1	4	0	3	1	3	7	5	3	0	1	9
4.00	0	1	7	1	9	0	5	2	0	7	3	3	0	0	6
4.25	0	1	1	0	2	0	3	1	0	4	2	2	1	1	6
4.50	0	0	6	0	6	0	3	1	2	6	2	2	1	4	9
4.75	0	0	5	2	7	0	3	0	0	3	1	2	0	0	3
5.00	0	0	7	1	8	0	2	3	0	5	3	3	0	1	7
5.25	0	1	3	0	4	0	2	1	0	3	2	5	0	1	8
5.50	0	0	3	1	4	0	0	1	1	2	1	2	0	1	4
5.75	1	0	1	2	4	0	2	2	1	5	1	1	1	1	4
6.00	0	0	0	0	0	0	4	0	0	4	2	1	0	1	4
6.25	0	0	1	1	2	0	2	1	0	3	0	0	0	1	1
6.50	0	0	2	2	4	0	0	1	0	1	4	1	0	2	7
6.75	0	1	6	0	7	0	0	2	0	2	0	1	0	1	2
7.00	0	1	5	1	7	0	0	1	0	1	0	1	0	0	1

Table 30. (Continued)

Time (seconds)	Treatment: 0.5 M acetic acid					0.5 M oxalic acid					0.5 M HCl				
	Unit 18-1	Unit 19-1	Unit 21-1	Unit 22-1	Sum	Unit 18-1	Unit 19-1	Unit 21-1	Unit 22-1	Sum	Unit 18-1	Unit 19-1	Unit 21-1	Unit 22-1	Sum
0.25	1	1	6	1	9	1	3	1	1	6	5	1	1	1	8
0.50	0	7	9	0	16	1	1	0	0	2	1	0	0	0	1
0.75	4	4	4	0	12	1	6	2	0	9	0	1	1	3	5
1.00	11	7	5	1	24	0	11	7	1	19	2	0	1	3	6
1.25	1	8	4	0	13	4	8	4	0	16	2	1	0	1	4
1.50	2	4	4	0	10	3	9	8	0	20	1	0	1	2	4
1.75	5	3	2	0	10	4	5	2	1	12	2	1	0	1	4
2.00	3	2	6	2	13	3	9	4	0	16	4	1	0	3	8
2.25	1	4	4	1	10	6	6	1	0	13	2	0	0	1	3
2.50	2	6	1	1	10	5	4	3	0	12	2	1	0	3	6
2.75	2	7	6	0	15	5	6	5	2	18	0	1	0	4	5
3.00	1	3	1	2	7	3	6	2	2	13	1	1	0	4	6
3.25	1	5	1	2	9	5	7	1	0	13	2	0	0	3	5
3.50	1	3	4	0	8	5	7	1	0	13	0	2	0	3	5
3.75	2	5	1	1	9	8	10	1	1	20	1	1	11	1	14
4.00	2	2	2	2	8	3	7	0	0	10	2	0	2	1	5
4.25	2	0	0	3	5	5	5	0	0	10	1	0	2	0	3
4.50	0	3	1	3	7	1	6	0	0	7	0	4	0	3	7
4.75	0	5	0	5	10	2	4	0	0	6	1	2	0	3	6
5.00	1	4	0	4	9	3	11	0	0	14	0	7	0	0	7
5.25	0	2	0	2	4	3	3	0	0	6	0	4	0	1	5
5.50	0	3	0	2	5	3	4	0	0	7	0	2	0	0	2
5.75	0	2	0	4	6	1	6	0	0	7	0	5	0	1	6
6.00	0	5	1	2	8	2	1	0	0	3	2	1	0	0	3
6.25	0	0	0	2	2	3	8	0	0	11	2	3	0	1	6
6.50	0	2	0	2	4	3	8	0	0	11	0	2	0	2	4
6.75	1	0	0	1	2	0	6	0	0	6	0	2	0	0	2
7.00	0	2	0	1	3	0	5	0	0	5	0	2	0	1	3

too many zero entries and consequently it was not always possible to rank the entries as to importance.) The sample studied indicates that the single fibre data convey similar time patterns irrespective of the stimulus presented.

By lumping the data together for each treatment in Table 30, it was now possible to determine rank correlations between gross patterns of response (lumped data is represented by each "Sum" column in Table 30.) Only 15 possibilities now occur since the activity within each treatment represents the gross activity from 4 fibres. In Table 31 the rank correlation coefficients among the 6 test solutions applied is indicated together with the probability level of occurrence. Specifically for $p < 0.05$ the level of significance is equal to 0.367; for $p < 0.01$ the level of significance is 0.470. Each probability level p expresses the chance that a correlation coefficient quoted would occur if it were actually zero. Correlations less than the levels of significance quoted have a probability level $p > 0.05$ and are designated as N.S. (not significant). The procedure was to rank the times, according to the total impulse count in 4 fibres, within each treatment and to correlate these ranks. A time which ranks high, i.e. it has a large number of impulse counts on one treatment is also likely to rank high on another treatment.

It is quite obvious that many of the entries in Table 31 have correlations greater than 0.30, consequently if a level

Table 31. Rank correlation coefficients among 6 treatments for 4 nerve fibres over 28 time intervals[a]

Treatment	2M NH_4Cl	2M KCl	0.5M acetic acid	0.5M oxalic acid	0.5M HCl
2M NaCl	+0.31 N.S.	+0.06 N.S.	+0.11 N.S.	+0.14 N.S.	-0.18 N.S.
2M NH_4Cl		+0.84 p<0.01	+0.70 p<0.01	+0.35 N.S.	+0.17 N.S.
2M KCl			+0.70 p<0.01	+0.45 p<0.05	+0.16 N.S.
0.5M acetic acid				+0.33 N.S.	+0.17 N.S.
0.5M oxalic acid					+0.30 N.S.

[a]Upper entry indicates correlation coefficient; lower entry indicates level of significance. Complete positive correlation = +1; complete negative correlation = -1; no correlation between response patterns = 0.

of significance of $p < 0.10$ were chosen instead of the commonly used levels of $p < 0.01$ and $p < 0.05$ there would be some evidence for similarity in NaCl - NH_4Cl patterns, NH_4Cl - oxalic acid patterns, acetic acid - oxalic acid patterns, and oxalic acid - HCl patterns in addition to those shown in the table. Because of this little value is placed on the summated 4 fibre temporal activity as a possible coding scheme.

At this stage it was felt that the 4 fibres chosen might conceivably, because of their multisensitive nature to the 6 stimuli presented to them, belong to the same fibre type class and consequently would behave in a similar manner whether analysed from a single fibre viewpoint or a 4 fibre viewpoint. To offset this possibility the data from one pigeon (Pigeon number 19) having good multiple fibre response properties to the 6 treatments above was analysed in terms of rank correlation. The discriminator ciruit was set at a point just above the intrinsic neural noise level and a very narrow uniform pulse was fed into the recording cathode ray oscilloscope--Kymograph® camera combination for each action potential detected. The uniform pulses were counted on paper film in 0.25 second intervals for a total time period of 10 seconds.[1]

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[1]The raw data had been submitted to the Statistical Laboratory, Iowa State University of Science and Technology, Ames, Iowa, for analysis. Rather than analyze a 7 second time strip of evoked (footnote continued on the next page)

The data shown in Table 32, except for action potentials buried in the neural noise level, are a true representation of multiple fibre activity. Because of individual pigeon variations and the fact that some multiple fibre activity buried in the noise level was not extracted, there are discrepancies between entries in Tables 30 and 32. For instance, some treatments in Table 30 (4 fibre data) show greater activity than comparable entries in Table 32 (multiple fibre data), yet one would intuitively expect more activity from the multiple fibre nerve complex. In Table 33, the rank correlation coefficients and the probability levels of occurrence are shown. Since the time intervals have been extended up to 10 seconds of activity, the level of significance for $p < 0.01$ is now equal to 0.393; for $p < 0.05$ it is now 0.304.

Because the values in Table 33 come from a different sample than those in Table 31 plus the fact that the 4 fibres are only a sample from the entire population of nerve fibres, it is not surprising that there are discrepancies between comparable correlation coefficient entires in the two tables.

(footnote continued from the previous page) activity as had been done with the single fibre data in Table 30, they used a 10 second time period to determine rank correlations for the multiple fibre activity. The difference between the above time periods should not alter the interpretation of the end results since pattern similarities or dissimilarities are mainly confined to the first 5 seconds of evoked activity.

Table 32. Multiple fibre impulse counts for Pigeon number 19 to 6 treatments as a function of time

Time (seconds)	2M NaCl	2M NH_4Cl	2M KCl	0.5M acetic acid	0.5M oxalic acid	0.5M HCl
0.25	4	4	3	0	2	1
0.50	2	5	3	0	3	2
0.75	4	0	2	2	2	2
1.00	2	9	4	1	3	3
1.25	8	19	11	2	0	1
1.50	9	28	10	4	0	0
1.75	4	43	12	0	1	1
2.00	10	43	19	2	4	1
2.25	15	45	19	1	2	1
2.50	8	45	22	0	2	4
2.75	5	45	26	2	2	1
3.00	4	24	17	4	2	3
3.25	5	33	23	1	2	4
3.50	6	34	22	2	2	0
3.75	6	23	21	4	4	2
4.00	7	23	16	6	3	4
4.25	8	17	14	11	3	2
4.50	9	13	10	16	2	5
4.75	6	16	11	11	2	5
5.00	4	11	8	12	7	7
5.25	4	11	8	11	3	2
5.50	6	11	14	7	7	5
5.75	6	10	11	3	5	7
6.00	4	7	14	8	3	6
6.25	7	10	11	6	6	9
6.50	6	5	10	7	5	8
6.75	3	7	8	10	4	16
7.00	8	4	6	11	2	13
7.25	5	10	6	3	2	9
7.50	7	8	5	9	6	16
7.75	6	3	4	5	3	11
8.00	7	2	6	8	1	13

Table 32. (Continued)

Time (seconds)	2M NaCl	2M NH_4Cl	2M KCl	0.5M acetic acid	0.5M oxalic acid	0.5M HCl
8.25	9	5	5	8	7	9
8.50	6	10	3	4	5	4
8.75	12	3	4	5	3	6
9.00	15	6	8	6	3	4
9.25	8	6	3	5	4	4
9.50	8	5	4	8	5	6
9.75	7	6	4	5	2	4
10.00	8	8	9	7	0	7

In Table 33, it is apparent that the patterns between the 3 salts are substantially different. However, if a $p < 0.10$ is used, the level of significance falls to 0.258 and many of the patterns agree with each other reasonably well. Nevertheless, it does appear that multiple fibre patterns do vary somewhat in the temporal sense. However, because the multiple fibre level of activity must be reached before any significant differences do occur in the temporal patterns, little value is placed on this scheme as a possible coding mechanism. The above multiple fibre results should be considered preliminary as only one pigeon was analysed over the 6 treatments administered; future studies should include data from several pigeons and specific behavioral experiments to verify the values quoted in Table 33.

Table 33. Rank correlation coefficients among treatments for multiple nerve fibre activity in Pigeon number 19 over 40 time increments[a]

Treatment	2M NH_4Cl	2M KCl	0.5M acetic acid	0.5M oxalic acid	0.5M HCl
2M NaCl	-0.045 N.S.	-0.002 N.S.	+0.468 p<0.01	+0.301 N.S.	+0.355 p<0.05
2M NH_4Cl		+0.255 N.S.	-0.203 N.S.	-0.302 N.S.	-0.253 N.S.
2M KCl			-0.118 N.S.	-0.234 N.S.	-0.238 N.S.
0.5M acetic acid				+0.218 N.S.	+0.748 p<0.01
0.5M oxalic acid					+0.222 N.S.

[a]See comments for Table 31.

Spatial pattern quality coding

The across-fibre pattern theory as developed by Pfaffmann (142), extended by Erickson (56) and more recently supported by Sato [cited by Wayner and Oomura (181)] has received a great deal of attention in academic circles because of the parallelism as a coding technique to other sensory systems such as the eye and ear. It was decided to test the 4 fibre data in Table 30 for 6 treatments considered, from an across-fibre or spatial pattern viewpoint using the product-moment correlation technique used by Erickson. It should be pointed

out that correlations only indicate similarities and dissimilarities in spatial patterns and do not necessarily indicate a mechanism for taste quality encoding. However, if behavioral data can be made to support these patterns, i.e. similar patterns are represented by stimuli that taste similar and dissimilar patterns refer to significantly different tastes, then a possible coding mechanism can be envisaged. Since specific controlled behavioral experiments were not conducted in order to support our data, only a comparative study of neural across-fibre patterns can be attempted. Furthermore, if Erickson's model is a true representation of how taste quality is encoded, then regardless of the species chosen one should be able to determine behavioral patterns from the spatial neural messages. This approach will be used here. In addition to Erickson's data on the rat, there are now available results by Marshall (131) on the opossum.

The data from Table 30 was rearranged as shown in Table 34. The entries represent the total number of impulse counts in the first two seconds of evoked activity. Because of variability in impulse count over very short intervals from one stimulus repetition to another (see page218) the longer time interval or integrating time of two seconds was chosen, as it had been determined that the total impulse count becomes time invariant as the interval is extended (see Figure 30 as

Table 34. Single fibre impulse count in first 2 seconds of evoked activity after application of designated treatment

	Treatment					
Unit	2M NaCl	2M NH_4Cl	2M KCl	0.5M acetic acid	0.5M oxalic acid	0.5M HCl
18-1	3	41	90	37	17	17
19-1	4	51	25	36	52	5
21-1	36	49	24	40	28	4
22-1	16	8	11	4	3	14

an example). Figure 31 has been added to give a visual display of the across-fibre patterns for the 6 treatments used.

Each pair of stimuli was tested for similarity using the product-moment correlation coefficient, i.e.

$$r = \frac{\Sigma xy}{(\Sigma x^2 \Sigma y^2)^{1/2}} \qquad (7)$$

where

r = product-moment correlation coefficient,

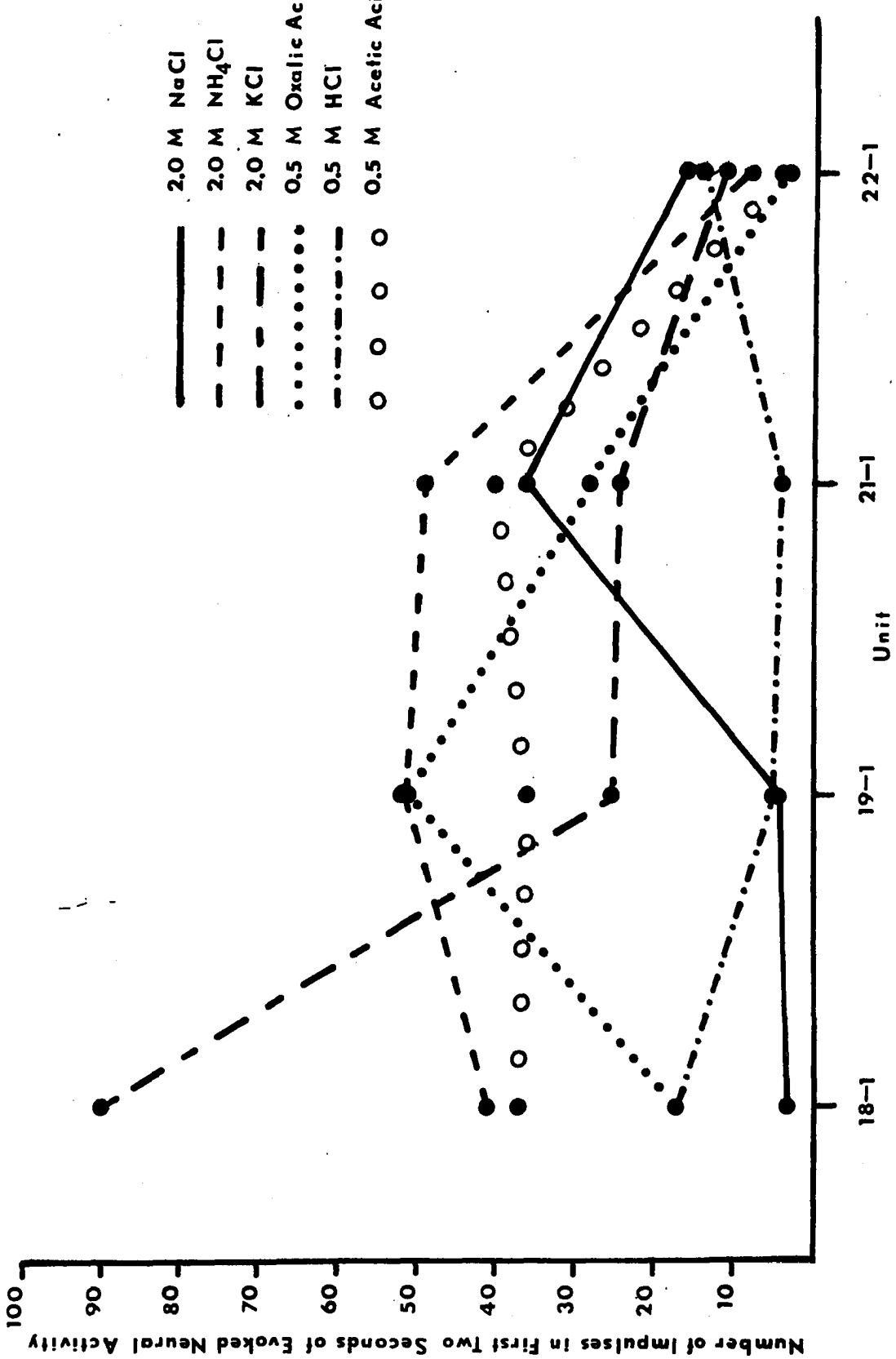
$x = X - \overline{X}$,

X = impulse count in 2 seconds of evoked activity of each unit for first treatment of pair chosen,

$\overline{X}$ = mean impulse count for treatment corresponding to all units in X,

$y = Y - \overline{Y}$,

Figure 31. Across-fibre patterns for 4 units to 6 treatments. Ordinate represents the total impulse count in 2 seconds of evoked activity. Abscissa designates the unit used. Abscissa scale and arrangement of units is arbitrary


Number of Impulses in First Two Seconds of Evoked Neural Activity
100
90
80
70
60
50
40
30
20
10
18–1
19–1
21–1
22–1
Unit
2.0 M NaCl
2.0 M NH_4Cl
2.0 M KCl
0.5 M Oxalic Acid
0.5 M HCl
0.5 M Acetic Acid

Y = impulse count in 2 seconds of evoked activity corresponding to same unit as in X for second treatment of pair chosen,

$\overline{Y}$ = mean impulse count for treatment corresponding to all units in Y.

The entries in Tables 35 through 49 indicate the step by step process in determining product-moment correlation coefficients for each pair of stimuli quoted.

The product-moment correlation coefficients found between each pair of stimuli are summarized in Table 50. Note that the level of significance for $p < 0.01$ is 0.990; for $p < 0.05$ it is 0.950 for the 3 degrees of freedom (or 4 observations) chosen. In each table entry, the upper number indicates the correlation coefficient while the lower figure indicates the probability level of occurrence. The abbreviation N.S. indicates that entry is not significant, i.e. a poor correlation was determined between corresponding pair of stimuli.

Compared to the temporal patterns analyzed where similarities tend to predominate, the spatial patterns indicate marked dissimilarities which leads one to believe that afferent coding, at least at the peripheral level, may be based on the relative amount of neural activity across many fibres as purported by both Pfaffmann (142), and Erickson (56). In only one case are there any similarities in the amounts of neural activity across the 4 fibres chosen; that being between NH_4Cl and acetic acid. If Erickson's model

Table 35. Product-moment correlation analysis between 2M NaCl (X) and 2M NH_4Cl (Y) spatial patterns

	X	Y	x	y	x^2	xy	y^2
	3	41	-11.75	+ 3.75	138.00	- 44.10	14.02
	4	51	-10.75	+13.75	115.50	-147.90	189.00
	36	49	+21.25	+11.75	451.00	+250.00	138.00
	16	8	+ 1.25	-29.25	1.56	- 36.60	855.00
Sum	59	149	0	0	706.06	+ 21.40	1196.02
Mean	14.75	37.25					

r = +21.4/917 = +0.023

Table 36. Product-moment correlation analysis between 2M NaCl (X) and 2M KCl (Y) spatial patterns

	X	Y	x	y	x^2	xy	y^2
	3	90	-11.75	+52.50	138.00	-618.00	2760
	4	25	-10.75	-12.50	115.50	+134.50	156
	36	24	+21.25	-13.50	451.00	-287.00	182
	16	11	+ 1.25	-26.50	1.56	- 33.18	701
Sum	59	150	0	0	706.06	-803.68	3799
Mean	14.75	37.50					

r = - 803.68/1644 = -0.488

Table 37. Product-moment correlation analysis between 2M NaCl (X) and 0.5M acetic acid (Y) spatial patterns

	X	Y	x	y	x^2	xy	y^2
	3	37	-11.75	+ 7.75	138.00	- 91.30	60.00
	4	36	-10.75	+ 6.75	115.50	- 72.70	45.40
	36	40	+21.25	+10.75	451.00	+229.00	115.80
	16	4	+ 1.25	-25.25	1.56	- 3.16	638.00
Sum	59	117	0	0	706.06	+ 61.84	859.30
Mean	14.75	29.25					

r = +61.84/788 = +0.079

Table 38. Product-moment correlation analysis between 2M NaCl (X) and 0.5M oxalic acid (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
3	17	-11.75	- 8.00	138.00	+ 94.30	64.00
4	52	-10.75	+27.00	115.50	-290.00	730.00
36	28	+21.25	+ 3.00	451.00	+ 63.80	9.00
16	3	+ 1.25	-22.00	1.56	- 27.50	485.00
Sum 59	100	0	0	706.06	-159.40	1288.00
Mean 14.75	25					

$r = -159.4/954 = -0.167$

Table 39. Product-moment correlation analysis between 2M NaCl (X) and 0.5M HCl (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
3	17	-11.75	+ 7.00	138.00	- 82.40	49.00
4	5	-10.75	- 5.00	115.50	+ 53.80	25.00
36	4	+21.25	- 6.00	451.00	-127.70	36.00
16	14	+ 1.25	+ 4.00	1.56	+ 5.00	16.00
Sum 59	40	0	0	706.06	-151.30	126.00
Mean 14.75	10					

$r = -151.30/299 = -0.507$

Table 40. Product-moment correlation analysis between 2M NH_4Cl (X) and 2M KCl (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
41	90	+ 3.75	+52.50	14.02	- 44.10	2760.00
51	25	+13.75	-12.50	189.00	-147.90	156.00
49	24	+11.75	-13.50	138.00	+158.90	182.00
8	11	-29.25	-26.50	855.00	- 36.60	701.00
Sum 149	150	0	0	1196.02	- 69.70	3799.00
Mean 37.25	37.50					

$r = -69.70/2130 = -0.033$

Table 41. Product-moment correlation analysis between 2M NH_4Cl (X) and 0.5M acetic acid (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
41	37	+ 3.75	+ 7.75	14.02	+ 29.05	60.00
51	36	+13.75	+ 6.75	189.00	+ 92.80	45.50
49	40	+11.75	+10.75	138.00	+126.20	115.80
8	4	-29.25	-25.25	855.00	+738.00	638.00
Sum 149	117	0	0	1196.02	+986.05	859.30
Mean 37.25	29.25					

$r = +986.05/1025 = +0.962$

Table 42. Product-moment correlation analysis between 2M NH_4Cl (X) and 0.5M oxalic acid (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
41	17	+ 3.75	- 8.00	14.02	- 30.00	64.00
51	52	+13.75	+27.00	189.00	+371.00	730.00
49	28	+11.75	+ 3.00	138.00	+ 35.30	9.00
8	3	-29.25	-22.00	855.00	+644.00	485.00
Sum 149	100	0	0	1196.02	+1020.30	1288.00
Mean 37.25	25					

$r = +1020.30/1240 = +0.822$

Table 43. Product-moment correlation analysis between 2M NH_4Cl (X) and 0.5M HCl (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
41	17	+ 3.75	+ 7.00	14.02	+ 26.21	49.00
51	5	+13.75	+ 5.00	189.00	+ 68.70	25.00
49	4	+11.75	- 6.00	138.00	- 70.50	36.00
8	14	-29.25	+ 4.00	855.00	-117.10	16.00
Sum 149	40	0	0	1196.02	- 92.69	126.00
Mean 37.25	10					

$r = -92.69/388 = -0.238$

Table 44. Product-moment correlation analysis between 2M KCl (X) and 0.5M acetic acid (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
90	37	+52.50	+ 7.75	2760.00	+407.00	60.00
25	36	-12.50	+ 6.75	156.00	- 84.60	45.50
24	40	-13.50	+10.75	182.00	-145.10	115.80
11	4	-26.50	-25.25	701.00	+668.00	638.00
Sum 150	117	0	0	3799.00	+845.30	859.30
Mean 37.50	29.25					

$r = +845.3/1805 = +0.468$

Table 45. Product-moment correlation analysis between 2M KCl (X) and 0.5M oxalic acid (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
90	17	+52.50	- 8.00	2760.00	-420.00	64.00
25	52	-12.50	+27.00	156.00	-338.00	730.00
24	28	-13.50	+ 3.00	182.00	- 40.50	9.00
11	3	-26.50	-22.00	701.00	+583.00	485.00
Sum 150	100	0	0	3799.00	-215.50	1288.00
Mean 37.50	25					

$r = -215.5/2210 = -0.091$

Table 46. Product-moment correlation analysis between 2M KCl (X) and 0.5M HCl (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
90	17	+52.50	+ 7.00	2760.00	+368.00	49.00
25	5	-12.50	- 5.00	156.00	+ 62.50	25.00
24	4	-13.50	- 6.00	182.00	+ 81.00	36.00
11	14	-26.50	+ 4.00	701.00	-160.00	16.00
Sum 150	40	0	0	3799.00	+351.50	126.00
Mean 37.50	10					

$r = +351.5/691 = +0.507$

Table 47. Product-moment correlation analysis between 0.5M acetic acid (X) and 0.5M oxalic acid (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
37	17	+ 7.75	- 8.00	60.00	- 62.00	64.00
36	52	+ 6.75	+27.00	45.50	+182.10	730.00
40	28	+10.75	+ 3.00	115.80	+ 32.30	9.00
4	3	-25.25	-22.00	638.00	+556.00	485.00
Sum 117	100	0	0	859.30	+708.40	1288.00
Mean 29.25	25					

$r = +708.4/1050 = +0.674$

Table 48. Product-moment correlation analysis between 0.5M acetic acid (X) and 0.5M HCl (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
37	17	+ 7.75	+ 7.00	60.00	+ 54.20	49.00
36	5	+ 6.75	- 5.00	45.50	- 33.80	25.00
40	4	+10.75	- 6.00	115.80	- 64.60	36.00
4	14	-25.25	+ 4.00	638.30	-101.00	16.00
Sum 117	40	0	0	859.30	-145.20	126.00
Mean 29.25	10					

$r = -145.2/1082 = -0.140$

Table 49. Product-moment correlation analysis between 0.5M oxalic acid (X) and 0.5M HCl (Y) spatial patterns

X	Y	x	y	x^2	xy	y^2
17	17	- 8.00	+ 7.00	64.00	- 56.00	49.00
52	5	+27.00	- 5.00	730.00	-135.00	25.00
28	4	+ 3.00	- 6.00	9.00	- 18.00	36.00
3	14	-22.00	+ 4.00	485.00	- 88.00	16.00
Sum 100	40	0	0	1288.00	-177.00	126.00
Mean 25	10					

$r = -177.0/403 = -0.440$

Table 50. Summary of product-moment correlation coefficients between pairs of treatments for spatial patterns

Treatment	2M NH_4Cl	2M KCl	0.5M acetic acid	0.5M oxalic acid	0.5M HCl
2M NaCl	+0.023 N.S.	-0.488 N.S.	+0.079 N.S.	-0.167 N.S.	-0.507 N.S.
2M NH_4Cl		-0.033 N.S.	+0.962 $p<0.05$	+0.822 N.S.	-0.238 N.S.
2M KCl			+0.468 N.S.	-0.091 N.S.	+0.507 N.S.
0.5M acetic acid				+0.674 N.S.	-0.140 N.S.
0.5M oxalic acid					-0.440 N.S.

is correct, then there should be similar taste qualities between NH_4Cl and acetic acid.

It might be argued that 4 fibres are not a truely representative sample of the pigeon taste afferent fibre system. While this is true to some extent, and future work will expand on this, it is also known from observing multiple fibre activity records that very few fibres are activated in the pigeon as compared to the dog or cat (115, 116). In the latter species it is impossible to quantify multiple fibre activity, as we did in Table 32 for the pigeon, since too many fibres are involved. The data in Table 32 indicate that

possibly 10 fibres (to a first approximation) are activated by each of the 6 treatments used. Accordingly, a 4 fibre sample is not too meager a choice.

With the rat in Erickson's model, in which the criterion of impulse counts was based on the first second of evoked activity across 13 fibres, the following observations were noted: (1) high correlation between NH_4Cl - KCl pairs, NH_4Cl - HCl pairs, and KCl - HCl pairs; (2) poor correlation between NaCl - NH_4Cl pairs. Marshall, using the Virginia opossum considered both 1 second and 2 second time interval impulse counts, both starting when activity was initiated. Both his 1 second and 2 second counts indicate: (1) high correlation between KCl - NH_4Cl pairs, KCl - HCl pairs, and NH_4Cl - HCl pairs; (2) poor correlation between NaCl - NH_4Cl pairs and NaCl - HCl pairs across 31 fibres. However, with the 1 second count a good correlation was noted for the NaCl - KCl pair, whereas a poor correlation was recorded with a 2 second count. Other than this, good agreement occurs for the other pairs whether 1 second or 2 second counts are used.

Since both Erickson and Marshall supported their neural results with behavioral experiments one should be able to determine species differences solely on the basis of these spatial patterns. For instance, whereas NH_4Cl - KCl pairs, NH_4Cl - HCl pairs and KCl - HCl pairs indicate a high correlation for both the rat and the opossum, very poor correlation is demonstrated by the pigeon. This could indicate that these

solutions taste differently to the opossum and the rat than to the pigeon. With other pairs quoted above there was good agreement between the pigeon, rat, and opossum.

Thus it appears that quality coding, at least on the basis of pattern similarity, is realized in the form of neural activity across many fibres and that activity in the temporal sense has little meaning other than alerting higher centers of the presence of the stimuli. Whether the burst-like feature in single fibres acts as a fine control to the coding mechanism or whether it reflects an inherent peculiarity of the receptor mechanism remains unanswered.

SUMMARY AND CONCLUSIONS

Electrophysiological studies of lingual chemoreception have mainly concentrated on mammals and frogs. Few studies have been concerned with Aves, primarily because until a few years ago it was felt that the gustatory mechanism of the avian species was very rudimentary and that taste buds did not exist. This attitude was shattered when Lindenmaier and Kare (130) isolated 24 taste buds in the chicken, and Moore and Elliott (133) reported an average of 37 taste buds in the pigeon. Electrophysiological investigations in peripheral nerves soon followed; the work of Kitchell et al. (116) in pigeons and chickens and Halpern (71) in chickens provided valuable insight into the gustatory system of Aves. The behavioral work of Englemann (51), Kare (100), and Duncan (47, 48, 49, 50) on various avian species has aided immeasurably in the interpretation of the electrophysiological data collected. However, as Kitchell et al. (116, p. 147) have pointed out, electrophysiological studies merely indicate receptor sensitivity to chemical solutions, whereas behavioral studies reveal how the central nervous system reacts to the given sensory input, consequently discretion must be used in correlating these two distinct and separate kinds of information.

With this background in mind it was decided to embark on a research program, using the pigeon as the species for study, to extend the overall range of solutions tested into specific categories of sweet (both natural and artificial sugars), bitter, sour (both mineral and organic acids), salt (to include a monovalent chloride cation series, a divalent chloride cation series, a sodium monovalent anion series, plus a trivalent chloride cation salt, a sodium divalent anion salt and a sodium trivalent anion salt), and NaOH, $CaCO_3$, and $NaHCO_3$. Single fibre, few fibre, and multiple fibre data were collected from 10 pigeons.

The average rise time and relative effectiveness as based on integrated response data from multiple fibre activity were found for each solution. Beidler's fundamental taste equation was tested using integrated activity from multiple fibres to four concentrations of both NaCl and acetic acid.

Few fibre preparations were isolated and tested to various solutions in order to observe the interaction of several single fibre temporal patterns. By changing the concentration of a specific chemical stimulus, an indication of taste intensity coding could be observed.

Several few fibre preparations, from which single fibre data could be extracted, were dissected free from the parent nerve bundle and the unit activity observed and analysed, by

means of histograms, the object being to visualize the temporal variations for different stimuli.

Since afferent fibres in the pigeon seem to convey only two taste modalities--salt and sour--to higher nerve centers with any degree of reliability it was conjectured that a simple coding scheme should be inherent to differentiate between these two modalities. With this in mind both temporal patterns and spatial patterns (across-fibre patterns) of activity were studied with the hope of providing a clue to the underlying coding mechanism.

Even though the sample sizes used in this study were sometimes small, the following conclusions, listed below, can be inferred from the results on the pigeon.

1. There are large individual pigeon variations to the taste solutions tested.
2. Chemoreception is conveyed only by one nerve of the lingual branch; i.e., the laryngo-lingual nerve which activates the posterior and pharyngeal regions of the tongue. This is supported by studies on chickens by Halpern (71), but refuted by Kitchell et al. (116) from observations in both pigeons and chickens. The latter group localized chemoreception to both the anterior and posterior portions of the tongue; the posterior receptive field was found innervated by a second nerve--the lingual nerve.

3. No responses were observed to bitter solutions; whereas questionable results were recorded to sweets. For instance sweet tasting substances such as sucrose, dextrose, and glycerine did not evoke any responses, whereas only one preparation responded to ethylene glycol and saccharine.
4. Sour taste solutions, both mineral and organic acids were found to be effective as stimulating agents in most cases. Only picric acid, molybdic acid, and boric acid failed to evoke activity in the majority of preparations.
5. Most salt solutions were effective stimuli. Exceptions to this were the entire divalent chloride cation class where evoked activity was found in less than 50% of the preparations studied.
6. Good responses were obtained to NaOH; no responses to $CaCO_3$, and only a few responses to distilled water. In contrast, distilled water responses were consistently found by Kitchell et al. (116) in both pigeons and chickens. Halpern (71) had to categorize his findings from chickens into two groups--those that gave large responses to distilled water and those that did not.

7. In most instances there was good correlation between electrophysiological results and behavioral studies, although some exceptions were noted.
8. Relative effectiveness of the monovalent chloride cation series was: $NH_4^+ >> Na^+ \geq K^+ >> Li^+$ which agrees with the arrangement for degree of saltiness in human beings. Behaviorally, Duncan (48) quotes: $Na^+ > NH_4^+ > K^+$ for the pigeon.
9. A value of Na/K = 1.03, indicates that Na^+ and K^+ are equally effective as stimulating agents. Contrast this with rodents where Na^+ responses are superior, and carnivores where K^+ responses are higher (22, 62).
10. Relative effectiveness of the divalent cation series was: $Sr^{++} = Mg^{++} > Ca^{++} > Ba^{++}$.
11. The relative effectiveness measure for each divalent chloride cation was far smaller than that found for each monovalent chloride cation tested.
12. Relative effectiveness of the halogen series was: $Cl^- > Br^- > I^-$ which compares with the degree of saltiness in human beings.
13. Both anions and cations take part in receptor stimulation.
14. Relative effectiveness of the acid series was: oxalic acid $\geq$ HCl > acetic acid > citric acid.

Duncan (50) has confirmed a behavioral stimulating efficiency of HCl > acetic acid in pigeons which compares with the degree of sourness in man (17).

15. Integrated data to various concentrations of NaCl indicate that Beidler's fundamental taste equation can be fitted and that the mechanism of taste reaction is physical rather than enzymatic. Acetic acid responses did not fit the fundamental taste equation.

16. Taste intensity coding seems to be mediated by an increase in single fibre nerve activity as the concentration is increased. More fibres also are recruited for concentration increases.

17. Repeated stimulation with the same concentration taste solution indicates that there is a temporal variation in afferent activity for any specific nerve fibre. However, over a relatively long time interval (e.g. 2 seconds) the impulse count between repetitions becomes equivalent.

18. Regular features of nerve activity in single fibres were the presence of burst-like patterns. Patterns could be visually categorized as having one major burst cluster, two major burst clusters, and even three major burst clusters. Nevertheless, it was

not possible to classify fibre type or neural message on the basis of this activity.

19. Temporal patterns in single, few, and multiple fibres were analysed for 6 treatments using both rank and product-moment correlations to test for pattern similarities. With single and few fibre data, patterns tended to be very similar. With multiple fibre activity there were indications that differences between patterns of NH_4Cl, NaCl, and KCl do occur, however all acid responses showed great similarity in pattern features. Because it was necessary to go to the multiple fibre level before dissimilarity in pattern feature is obvious, it is felt that coding in the temporal sense is negligible.

20. Testing the across-fibre pattern theory of Pfaffmann (142) and Erickson (56) indicates that a coding mechanism, at least at the peripheral level, could be realized with the pigeon. Product-moment correlations indicate that pattern similarities are confined to only one case; specifically, high correlations have been found only between the NH_4Cl - acetic acid pairs. According to the model this should indicate similar taste qualities between NH_4Cl and acetic acid. Specific controlled behavioral studies were not conducted to support the model.

Future studies should include exactly controlled behavioral studies to substantiate or refute the across-fibre pattern theory in the pigeon. A larger sample than the one considered herein should be used to test the validity of the theory. To reduce individual variability a specific pigeon breed rather than the cross-breed chosen could be used. Because of temporal variations upon repeated stimuli it might be desirable to localize the preferred single fibre time of firing by way of a post-stimulus histogram for each treatment. Microelectrode techniques could be utilized to record from a single nerve fibre axon, since with the present method there is always a chance that activity from more than one fibre is being recorded.

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