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BOWDICH (GASTROPODA: PULMONATA), IN OLFAC-
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THE ROLE OF THE POSTERIOR TENTACLES OF THE GIANT AFRICAN LAND SNAIL, ACHATINA FULICA BOWDICH (GASTROPODA: PULMONATA), IN OLFACTION AND ORIENTATION

A DISSERTATION SUBMITTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY JANUARY 1968

By
Lester Yoshio Ichinose

Committee:
Professor Pieter B. van Weel
Professor Sidney J. Townsley
Associate Professor Fred I. Kamemoto
Associate Professor Howard F. Mower
Assistant Professor Martin D. Rayner
I would like to acknowledge my wife, Nancy, for the confidence and inspiration she has shared with me in this endeavor.
THE ROLE OF THE POSTERIOR TENTACLES OF THE GIANT AFRICAN LAND SNAIL, ACHATINA FULICA BOWDICHI (GASTROPODA: PULMONATA), IN OLFACTION AND ORIENTATION

ABSTRACT

The role of the posterior tentacles of the giant African land snail, Achatina fulica Bowdich, in olfaction and orientation was studied in a three-part investigation; anatomy, behavior and electrophysiology.

Anatomical studies revealed a close homology with other stylommatophore pulmonates. A freely moving tubular retractor muscle attached distally inside the tentacle tip contains the tentacular artery, and the optic and tentacular nerves. The latter nerve connects with the pro-cerebrum proximally and with a ganglion distally. The ganglion sends digitiform branching projections throughout the tip epithelium, where they receive afferent fibers from bipolar cell aggregates in the sub-epithelial region.

A second set of 4-6 afferent nerves serving the ventro-lateral epithelium of the tentacle tip was found to insert between the ventral digitiform projections, and extend to bipolar cell body aggregates in the sub-epithelial region. These "chemotactile" nerves extend centrally along the ventro-lateral wall and undergo several bifurcations at the base of the tentacle, forming a complex nerve net as a result. The left and right peritentacular nerves, which terminate proximally in the
posterior procerebrum, receive the bifurcations from chemotactile nerves serving the left and right ventrolateral epithelium of the tip, respectively. They also receive bifurcations from the same ventral (middle) chemotactile nerve.

The bipolar cell aggregates mentioned above send distal fibers to the tentacle tip epithelium. They extend between the columnar epithelial cells and terminate at the cuticle in these types of nerve endings: 1) Blunt, expanded endings terminating at the cuticle base; 2) bifurcating nerve endings within the cuticle; 3) 5-7 filamentous unbranched endings in the cuticle, apparently emanating from a sub-cuticular conical sheath.

The olfactory ability of eight 1½ year old laboratory raised snails was investigated by noting their behavior in a four-choice biradially symmetrical maze. A vial containing vegetable juice serving as the stimulus was randomly placed in front, rear, left, and right of the snail in known ratios. The remaining vials contained distilled water, and the maze was entirely darkened during a trial. 68% of the total trials demonstrated positive chemotaxis for this group. For the experimental group, the epithelium of the posterior tentacles was removed in 7 members of the normal control group. Positive chemotaxis for the experimental snails occurred in 25% of the total trials.
Two cerebropetal channels serving olfaction were demonstrated in electrophysiological experiments on isolated posterior tentacles. Tentacular nerve activity increased with odor stimulation, but no adaptation was evident up to the time the experiment was terminated 5.5 minutes later. No activity was noted in response to tactile stimuli. The activity of the chemotactile nerves increased when olfactory and tactile stimuli impinged on the tentacle tip; the activity in response to the olfactory stimulus diminished 80-120 seconds after the introduction of the odor.

The ability of a single posterior tentacle to provide directional information in response to olfactory and tactile stimuli was demonstrated electrophysiologically on the chemotactile nerves.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREFACE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>viii</td>
</tr>
<tr>
<td>PART I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>PART II. MATERIALS OF RESEARCH</td>
<td>8</td>
</tr>
<tr>
<td>PART III. GROSS AND MICROSCOPICAL ANATOMY</td>
<td>12</td>
</tr>
<tr>
<td>Observations</td>
<td>14</td>
</tr>
<tr>
<td>External Description</td>
<td>14</td>
</tr>
<tr>
<td>Internal Gross Anatomy</td>
<td>16</td>
</tr>
<tr>
<td>Microscopical Anatomy</td>
<td>20</td>
</tr>
<tr>
<td>Discussion</td>
<td>24</td>
</tr>
<tr>
<td>Conclusion</td>
<td>33</td>
</tr>
<tr>
<td>PART IV. BEHAVIOR</td>
<td>35</td>
</tr>
<tr>
<td>Methods of Research</td>
<td>35</td>
</tr>
<tr>
<td>The Behavior Maze</td>
<td>35</td>
</tr>
<tr>
<td>Placement of the Snail</td>
<td>38</td>
</tr>
<tr>
<td>Selection of Stimulus Position</td>
<td>38</td>
</tr>
<tr>
<td>Trial Operation</td>
<td>39</td>
</tr>
<tr>
<td>Control Trials</td>
<td>39</td>
</tr>
<tr>
<td>Normal and Experimental Groups</td>
<td>39</td>
</tr>
<tr>
<td>Observations</td>
<td>41</td>
</tr>
<tr>
<td>Normal and Experimental Animals</td>
<td>41</td>
</tr>
<tr>
<td>Behavior Control Experiment</td>
<td>43</td>
</tr>
<tr>
<td>Olfaction With a Single Posterior Tentacle</td>
<td>45</td>
</tr>
<tr>
<td>Discussion and Conclusions</td>
<td>45</td>
</tr>
<tr>
<td>PART V. ELECTROPHYSIOLOGY</td>
<td>49</td>
</tr>
<tr>
<td>Methods of Research</td>
<td>49</td>
</tr>
<tr>
<td>Air Purification System</td>
<td>49</td>
</tr>
<tr>
<td>Isolation of the Olfactory Field</td>
<td>50</td>
</tr>
<tr>
<td>Pickup and Recording of Electrophysiological Responses</td>
<td>55</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (Continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial Operation</td>
<td>57</td>
</tr>
<tr>
<td>Observations</td>
<td>57</td>
</tr>
<tr>
<td>Tentacular Nerve Activity</td>
<td>57</td>
</tr>
<tr>
<td>Activity of Chemotactile Nerves</td>
<td>57</td>
</tr>
<tr>
<td>Chemotactile Nerves and</td>
<td>59</td>
</tr>
<tr>
<td>Directional Stimuli</td>
<td>61</td>
</tr>
<tr>
<td>Simultaneity in Nerve Bifurcations</td>
<td>62</td>
</tr>
<tr>
<td>Discussion</td>
<td>66</td>
</tr>
<tr>
<td>Conclusion</td>
<td>71</td>
</tr>
<tr>
<td>PART VI. GENERAL DISCUSSION</td>
<td>73</td>
</tr>
<tr>
<td>PART VII. SUMMARY</td>
<td>78</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>82</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>ISOTONIC SALINE FOR ACHATINA FULICA</td>
<td>13</td>
</tr>
<tr>
<td>II.</td>
<td>NUMERICAL DISTRIBUTION OF THE BEHAVIOR TRIALS OF ACHATINA FULICA</td>
<td>42</td>
</tr>
<tr>
<td>III.</td>
<td>PERCENTAGE DISTRIBUTION OF THE BEHAVIOR TRIALS OF ACHATINA FULICA</td>
<td>42</td>
</tr>
<tr>
<td>IV.</td>
<td>BEHAVIOR CONTROL TRIALS FOR ACHATINA FULICA</td>
<td>44</td>
</tr>
<tr>
<td>V.</td>
<td>BEHAVIOR OF ACHATINA FULICA WITH A SINGLE TENTACLE</td>
<td>44</td>
</tr>
<tr>
<td>Figure</td>
<td>Illustration Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Photograph of <em>Achatina fulica</em></td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Drawing of the distal portion of the posterior tentacle of <em>Achatina fulica</em></td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>Drawing of the posterior tentacle of <em>Achatina fulica</em> showing nerves and ganglia</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>Photograph of the posterior tentacle tip epithelium with associated nerves</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>Cross-section of the ventral epithelium of the posterior tentacle tip</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>Dorsal-ventral sagittal section of the posterior tentacle tip</td>
<td>21</td>
</tr>
<tr>
<td>7</td>
<td>Cross-section of the posterior tentacle tip</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>Drawings of distal nerve endings in the epithelium of the posterior tentacle tip</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>Photograph of distal nerve endings</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>Photograph of distal nerve endings</td>
<td>26</td>
</tr>
<tr>
<td>11</td>
<td>Photograph of distal nerve endings</td>
<td>27</td>
</tr>
<tr>
<td>12</td>
<td>Photograph of distal nerve endings</td>
<td>27</td>
</tr>
<tr>
<td>13</td>
<td>Photograph of distal nerve endings</td>
<td>28</td>
</tr>
<tr>
<td>14</td>
<td>Photograph showing the side view of the behavior maze</td>
<td>36</td>
</tr>
<tr>
<td>15</td>
<td>A. Diagram showing top view of maze&lt;br&gt;B. Diagram showing side view of maze</td>
<td>37</td>
</tr>
<tr>
<td>16</td>
<td>Diagram of the air purification system</td>
<td>51</td>
</tr>
<tr>
<td>17</td>
<td>Photograph of the olfactory chamber for frontal flow</td>
<td>52</td>
</tr>
<tr>
<td>18</td>
<td>Diagram of the olfactory chamber for frontal flow, top view</td>
<td>52</td>
</tr>
</tbody>
</table>
## LIST OF ILLUSTRATIONS (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>PHOTOGRAPH OF THE OLFACTOR Y CHAMBER FOR DIRECTIONAL FLOW</td>
<td>53</td>
</tr>
<tr>
<td>20</td>
<td>DIAGRAM OF ELECTRONIC EQUIPMENT FOR ELECTROPHYSIOLOGY</td>
<td>56</td>
</tr>
<tr>
<td>21</td>
<td>ELECTROPHYSIOLOGICAL ACTIVITY OF THE TENTACULAR NERVE</td>
<td>58</td>
</tr>
<tr>
<td>22</td>
<td>ELECTROPHYSIOLOGICAL ACTIVITY OF THE CHEMOTACTILE NERVES</td>
<td>60</td>
</tr>
<tr>
<td>23</td>
<td>ELECTROPHYSIOLOGICAL ACTIVITY OF LATERAL CHEMOTACTILE NERVES WITH DIRECTIONAL STIMULI</td>
<td>63</td>
</tr>
<tr>
<td>24</td>
<td>HISTOGRAMS SUMMARIZING THE ACTIVITY SHOWN IN FIGURE 23</td>
<td>64</td>
</tr>
<tr>
<td>25</td>
<td>ELECTROPHYSIOLOGICAL ACTIVITY OF LEFT CHEMOTACTILE NERVES WITH DIRECTIONAL STIMULI</td>
<td>65</td>
</tr>
<tr>
<td>26</td>
<td>ELECTROPHYSIOLOGICAL ACTIVITY OF LATERAL CHEMOTACTILE NERVES WITH DIRECTIONAL STIMULI</td>
<td>65</td>
</tr>
</tbody>
</table>
PART I

INTRODUCTION

After Cuvier (1806) produced the first documented observation of olfaction in stylommatophore pulmonates, subsequent research on olfaction in this group has pursued the problem from two aspects; behavior and anatomy. However, although anatomical descriptions of the posterior tentacle and behavior experiments have dominated investigations since 1851, no unequivocal evidence correlating olfactory ability, anatomy, and function in the whole animal is available. The application of electrophysiological techniques in the field of gastropod chemoreception has been difficult due to the non-rigid tentacle support for the olfactory epithelium, the presence of a mucus layer, and the lack of definite results. Therefore, the application of electrophysiological techniques combined with anatomical and controlled behavior studies may substantiate much of the information that has been accumulated concerning the olfactory ability of stylommatophore pulmonates. *Achatina fulica* Bowdich was selected as the subject because of its large size, abundance, and hardiness.

The only reliable information available on the olfactory behavior of stylommatophores is by Schulz (1938), Kieckebusch (1953) and Kittel (1956). All previous
workers have reported either single observations or have used an inadequate sample of animals; also, any measures that may have been taken to control sensory stimuli from other sources are seldom mentioned. The most comprehensive observations and reviews on the anatomy of the stylommatophore posterior tentacle are by Hanström (1925, 1928) for gross and microscopical anatomy of Helix pomatia L., Schulz (1938) for the microscopical anatomy of the terminal nerve endings in Helix, and Demal (1955) in a comprehensive review. Since Retzius (1892) was the first to use the silver impregnation technique of Golgi on the posterior tentacle of gastropods, descriptions of its microscopical anatomy before 1892 have limited value. With these qualifying comments in mind, a more comprehensive review of the literature will be undertaken.

The first experimental investigation on the sense of olfaction in stylommatophores is attributed to Moquin-Tandon (1851), who observed that Limax maximus L. always crept to an apple, even with changes in the relative position of the snail to it. He concluded that the posterior tentacles were the principal seat of olfaction as no positive chemotactic behavior was observed when the tentacles were amputated. Stahl (1889) observed that Limax crept to a pile of mushrooms when air was blown over it toward the snail, and Adams (1898) noticed that it oriented to this food at a distance of over two meters.
However, Yung (1904) reported that Helix pomatia "smells" lettuce from a distance of 5-6 meters, but noticed no difference in behavior when both posterior or all four tentacles were removed. For this reason, he postulated that the tentacles did not have an olfactory function. Schulz (1938) disagreed, since he observed a clear lessening of olfactory ability when the tentacles of Helix were amputated. Also, when he removed a single posterior tentacle or both tentacles on the same side, Helix was still able to orient to a lettuce stimulus. He tried to control extraneous visual stimuli by performing all of his experiments under red light. Kieckebusch (1953) observed that Helix pomatia crept from a distance of 10-20 centimeters to a dandelion homogenate, but he conceded the possibility of a humidity gradient guiding the animal to the stimulus. In an attempt to control humidity and to increase the sampling significance, Kittel (1956) tested the olfactory ability of 200 pulmonates in their environment; they were Limax cinero-niger Wolf, Arion empiricorum Fér. and Limax maximus. On the basis of his experiments, he concluded that the posterior tentacles were the principal seats of olfaction, and therefore held greater significance for the survival of the animal than the anterior tentacles. Ghose (1962a) stated that Achatina fulica was able to detect food at a great distance; however, his statement was entirely unsupported by
experimental evidence.

The definition and use of the term "olfaction" has been a point of confusion for some workers. Hanström (1926) found that the posterior tentacle of *Helix pomatia* was stimulated by both olfactory and gustatory stimuli, but noted that the afferent nerve fibers all terminated in the single tentacular ganglion. Although he conceded that Helix was able to "smell", he concluded that it was done through a non-specialized common chemical sense. Hodgson (1953) reinforced this statement by showing that the aquatic beetle, *Laccophilus*, was able to sense liquid and gaseous chemicals through the same antennal receptors. He concluded that a physical distinction between olfaction and gustation is meaningless at the cellular level (Hodgson 1955). However, Schulz (1938) provided an acceptable distinction between olfaction and gustation by defining them operationally: Distance chemoreception was considered "olfaction"; contact chemoreception was "gustation".

The only description of the gross anatomy of the posterior tentacle of *Achatina fulica* with its proximal connections to the cerebral ganglion is by Ghose (1962a, 1962b). Unfortunately, his description of the "olfactory organ" has limited value in comparative studies with other snails because of its brevity and superficial treatment (1962a). There is a description of a "club-shaped
ganglion", more commonly termed the distal, tentacular, or olfactory ganglion in literature dealing with the neuroanatomy of other stylommatophore pulmonates. In this respect, Hanström (1925, 1928) pointed out that all the terrestrial stylommatophore pulmonates investigated for their tentacular neuroanatomy appear to have the same basic structure.

Samassa (1894) described the posterior tentacle of *Helix pomatia* as an invaginated tube with an inner retractor muscle extending throughout its length and inserting at the tip. This retractor muscle was described as a hollow tube containing the tentacular artery and the optic and tentacular nerves, with the nerves terminating in the procerebrum of the cerebral ganglion. He also noted that the distal extension of the tentacular nerve originated from a pyriform distal ganglion that sent six "finger-like" projections into the ventral epithelium.

The microscopical anatomy of the procerebrum, tentacular nerve and distal ganglion of *Helix pomatia* was described by Hanström (1925). He noted the presence of motor fibers as well as afferent and efferent internuncial interneurons in the tentacular nerve. Also, the distal tentacular ganglion was described as a dense, fine neuropile resembling the fine consistency of the procerebrum. He postulated that the unique presence of the distal ganglion in stylommatophore pulmonates is indicative of a
greater dependence on exteroceptive stimuli, particularly olfaction (Hanström 1928).

Before the use of silver impregnation, Boll (1869) described two types of terminal receptors; nucleated sub-epithelial cells sending distal extensions between fringed epithelial cells, and fringed epithelial cells with centripetal nerves. Fleming (1872) verified the presence of cells with filamentous extensions, sending nervous projections centrally. Retzius (1892), who was the first to use the silver impregnation technique of Golgi, described many bipolar cells whose distal extensions terminated in a knob, and central fibers that inserted in the tentacular ganglion. In Helix, Samassa (1894) noted the presence of unipolar as well as bipolar cells that send branches centrally. Hanström (1925), like his predecessors, described the presence of bipolar cells below the digitiform extensions of the tentacular ganglion. The distal extensions of these cells were sometimes found to form an arborization from which branches formed a rich network of fine sub-cuticular fibers. He also noted that the central extensions terminated in the superior portion of the tentacular ganglion. Schulz (1938) described different types of sub-epithelial sensory cells in Helix, as well as their distal nerve terminals. He assigned a special sensory function to each type of nerve ending; this was based on a statistical correlation of
relative areal abundance and behavioral responses to olfactory, gustatory, tactile and visual stimuli.
PART II

MATERIALS OF RESEARCH

The availability of the giant African land snail, *Achatina fulica*, posed no problem because of its almost ubiquitous distribution and abundance on the island of Oahu. Specimens for anatomical and electrophysiological studies were obtained from a single area in Pauoa Valley on the island of Oahu due to its accessibility, the relatively young age distribution of the population and the comparatively healthy physical appearance of the snails. The specimens collected were approximately one to two years of age. No individuals with skin lesions, skin discolorations or deformed tentacles were used.

One of the problems in selecting animals for behavior experiments is that of obtaining biologically uniform specimens in terms of health, age and environmental experience. It has been pointed out by Mead (1956, 1961) that the apparent health of *Achatina* found in the field is quite variable. He states that the giant African snail found in the Hawaiian Islands is afflicted with a microbial disease that reaches epidemic proportions, and which may very well be a factor in the drastic population reductions found in certain areas on the island of Oahu. In addition, the age of an individual snail taken from the field cannot be determined accurately because most of
their growth is attained in a little more than a year. Relative age differences can be determined for individuals from the same area on the basis of erosion of the periostracum, thickness of the shell and the angle of the columella, but the observer has to reconstruct these standards for each separate area (Mead, personal communication).

Therefore, in order to circumvent the difficulties involved in obtaining biological uniformity, the animals used in the behavior experiments were hatched and raised in the laboratory.

A single clutch of 175 eggs taken from Pauoa Valley on May 12, 1965, was hatched in three days in the laboratory. The snails were maintained in covered glass petri dishes with damp filter paper bases until they attained a shell length of approximately 8 millimeters, thereafter they were transferred to glass terraria measuring 0.65 cubic feet in volume. For logistic reasons most of these animals were removed after 8 months, and the remaining snails were then kept in terraria measuring 1.2 cubic feet in volume. From the time of hatching, the animals were fed almost exclusively a diet of lettuce, oatmeal and chalk; occasionally, cabbage and vegetable juice were given, but the former was not as acceptable as lettuce. The snails appeared to obtain sufficient moisture from the food, obviating the need for frequent watering.
The general health of the animals appeared to be very
good. No deaths occurred except for accidental reasons,
and pathological lesions prevalent in field snails were
non-existent in the laboratory raised animals.

Ideally, the stimulating substance to be utilized in
the behavior and electrophysiology experiments should be
selected according to two criteria: 1) The stimulant em­
ployed in the behavior trials should also be suitable for
use in the electrophysiology experiments; 2) the stimulant
should be uniform in quality and intensity with each
trial. The first criterion can be satisfied by the use of
odors attractive to the whole animal, since a behavioral
reaction to repellent substances may be due to excessive
stimulation of the epithelium; in other words, a reaction
to "pain" sensation. The second criterion can be satis­
fied by the use of a pure chemical as the stimulating
substance, since its physical properties would be known,
and thus could be controlled precisely with appropriate
dilution methods.

Unfortunately, no pure chemical was found that had
attractant properties, and although homogenates of green
vegetables (lettuce, cabbage) did have this property, the
intensity and quality of the odor deteriorated rapidly
with time. However, a satisfactory attractant was found
in a commercial brand of vegetable juice, V-8 (Campbell
Soup Company). It was used exclusively in all the
behavioral and electrophysiological experiments. Fresh amounts were used with each trial to insure uniform odor quality and intensity.

Special techniques used in the investigation will be discussed in detail in the appropriate parts of this dissertation.
PART III

GROSS AND MICROSCOPICAL ANATOMY

The gross anatomy studies made use of two basic preparations; the posterior tentacle extirpated at its base without the cerebral ganglion, and the posterior tentacle excised with its cerebral ganglion connectives intact.

The former preparation was easily obtained from an extended non-anesthetized snail by simultaneously excising the tentacle at its base with a razor while holding it between the thumb and index finger of the other hand. When the tentacle was not held securely for 5-10 seconds after excision, it inverted itself, very much like a glove turned inside-out. A longitudinal cut made along the dorsal midline of the tentacle wall from the base to the wall-tip junction revealed all the pertinent structures intact and in their true relationships.

The latter preparation was obtained after immobilizing the animal by injecting 0.05 milliliters of 0.5% succinylcholine chloride in isotonic saline (Table I, page 13) into the haemocoele through the ventral surface of the foot. The animal was then decapitated while maintaining the extension of the tentacle by suction. All nerves terminating in the cerebral ganglion not associated with the tentacle were excised; the tentacle with
TABLE I. ISOTONIC SALINE FOR ACHATINA FULICA (van Weel, personal communication)

<table>
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<th>Chemical</th>
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</tr>
</thead>
<tbody>
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<td>NaCl</td>
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</tr>
<tr>
<td>KCl</td>
<td>0.005</td>
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<tr>
<td>CaCl₂</td>
<td>0.001</td>
</tr>
</tbody>
</table>

its ganglion connectives was now able to be removed from the animal intact.

The internal gross anatomy of the tentacle tip was determined by pulling all structures connected to the tip in an anterior direction, thus causing it to invert. The nerves in all the dissected preparations were supra-vitally stained with a 1:1000 dilution of methylene blue in isotonic saline.

The difficulty in obtaining a successful silver impregnation technique selective for molluscan nervous tissue was discussed by Demal (1955). After reporting the disappointing results of many familiar nerve stains (Cajal, Golgi, Bielchowsky, Bodain), he recommended the ammoniated silver carbonate technique by Van Campenhout (1953) because it produced the best impregnation. This method was therefore used in this investigation.

The excised tentacles obtained by the method described above were fixed in Bouin's Picro-formol fixative for three days. The tissues were then embedded in paraffin, sectioned at a thickness of eight microns and
mounted on slides coated with gelatin, according to the procedure of Masson (Edwards 1950). After the silver staining, most of the tissues were counterstained with a 1% aqueous solution of Ponceau Red and a 0.2% aqueous solution of Fast Green.

Photomicrographs were taken with a 35 mm camera, using Kodacolor-X and Panatomic-X film.

Observations

The giant African land snail (Figure 1) is a stylommatophore pulmonate with two pairs of tentacles: The anterior pair is situated antero-ventrally on the head, slightly dorsal to and flanking the buccal cavity; the posterior tentacles are situated at the top of the head and bear the eyes in the distal region of the tip.

The following descriptions will be confined to the posterior tentacles only.

External Description

In the extended state, the tentacle attains a length of over two centimeters in the fully grown snail. Its cylindrical form tapers from the base to the tip where it expands into a terminal bud with a smooth and glossy surface that contrasts strongly with that of the rest of the tentacle. The color of the wall varies from tan to dark brown, whereas the terminal bud is always found with a yellowish pigmentation (Figure 4).
Figure 1. The giant African land snail, *Achatina fulica* Boeltch. Posterior tentacles (PT); anterior tentacles (AT). x0.8
Viewed from the front, the tip has a convex surface which is divided by a shallow groove into two fields; the dorsal one is annular and slightly narrower, and the ventral field is circular and larger. The eye is situated slightly ventral to the bottom of the groove (Figure 2).

Internal Gross Anatomy

The tentacle is essentially a tube within a tube, a hollow dermomuscular cylinder (Figure 2) within which runs the tubular retractor muscle that inserts at the tip. This inner cylinder houses the tentacular artery, and the optic and tentacular nerves. The tentacular nerve terminates distally in a large pyriform olfactory ganglion that ramifies into branching extensions serving the sensory epithelium.

The course of these nerves and the artery through the tentacle retractor muscle is quite sinuous, with the optic and tentacular nerves inserting into the procerebrum of the cerebral ganglion.

A second set of (4-6) suspected sensory nerves (Figures 3 and 4, N₁-N₅) extends through the length of the tentacle between the inner ventral tentacular wall and the retractor muscle. They insert distally on the digitate ventral projections of the olfactory ganglion that lie along the inner ventral wall of the external circular portion of the tentacular tip (Figure 3). These relatively
Figure 2. Diagrammatic representation of the distal portion of the posterior tentacle of *Achatina fulica*. Dorso-lateral view. Sensory epithelial region (SE).
Figure 3. Diagrammatic dorsal view of the ventral wall of the everted right posterior tentacle of *Achatina fulica*, demonstrating its principal neural elements and cerebral ganglion connectives. The distal tip has been turned inside-out with the olfactory ganglion and its associated nerves pulled forward. Note the finger-like extensions of the olfactory ganglion with branching. Tentacular nerve (TN); afferent chemotactile nerves (N₁–N₅); connecting nerves to the cerebral ganglion (C₁, C₂).
Figure 4. Outer view of the ventral portion of the tentacle tip with its associated nerves. The muscular wall connecting the tip with the base has been dissected away. Note the yellow pigmentation of the epithelium. Tentacular artery (TA); tentacular nerve (TN); ventral sensory epithelium (VSE); chemotactile nerves (N1-N5) extending from the VSE to the tentacular base. Supra-vitally stained with 1:1000 methylene blue solution in isotonic saline. x12.5
broad insertions extend proximally as thin discrete nerves that often form collateral branches between adjacent bundles only. These nerves undergo one to two bifurcations at the base with branches from adjacent bundles coalescing (Figure 3) to form a complex neural network held firmly by connective tissue. The anastomosing branches from the three left nerves (Figure 3; N₅, N₄, N₃) combine to form a single connective to the procerebral portion of the cerebral ganglion (Figure 3, C₂); a second connective, C₁, is formed by anastomosing branches from the three nerves from the right (N₁, N₂, N₃).

Microscopical Anatomy

The olfactory ganglion and its branches appear to have a very homogeneous and fine consistency (Figure 7). The ventral branches originating from the anterior portion of the olfactory ganglion (Figure 2) receive nerve fibers from bipolar cell aggregates (Figure 5, CB) situated in the sub-epithelial region of the tentacle tip.

The distal fibers of the chemotactile nerves appear to course between the ventral digitiform branches of the ganglion (Figure 6) and lead to bipolar cell aggregates situated in the sub-epithelial region of the tip. The chemotactile nerves are in very close association with the olfactory ganglion branches, "wrapping" around them in many instances (Figure 7).
Figure 5. Cross-section of the ventral epithelium of the posterior tentacle tip of Achatina fulica. Ventral epithelium (VE); olfactory ganglion branches (B); chemotactile nerves (N); cell body aggregates (CB). Van Campenhout nerve stain. x156

Figure 6. Dorso-ventral sagittal section of the posterior tentacle tip of Achatina fulica. Note the chemotactile nerves extending between the branches of the olfactory ganglion. The abbreviations used in the above figure apply. x53
Figure 7. Cross section of the posterior tentacle tip of Achatina fulica. Optic nerve (O); chemotactile nerves (N); olfactory ganglion (G); branches of the olfactory ganglion (B). x59
The bipolar cell aggregates of both the olfactory ganglion branches and the chemotactile nerves appear to send distal processes to the epithelium (Figure 5, P). The sensory epithelium consists of an outer cuticular mucus layer approximately 4-6 microns thick and a lower layer of simple columnar epithelium with no apparent basement membrane (Figures 9-13). Nerve fibers from the bipolar cell body aggregates were observed to course between the epithelial cells and terminate in nerve endings that exhibited a high degree of polymorphism. The most predominant morphological types (Figure 8) could be classified into two groups according to two defining characteristics: 1) The existence of terminal extensions within the cuticle; b) the degree of penetration into the cuticle.

The nerve endings with terminal extensions within the cuticle (Figure 8, D-F) send 1-7 processes into that layer; these were predominantly found in the anterior and lateral regions of the tentacular tip. Rarely, several were observed to terminate below the cuticle, but this may have been an artifact of sectioning. There appeared to be two types of extensions into the cuticle from the nerve fibers; some extending singly into the cuticle (Figure 8, D) or bifurcating into two or possibly three non-filamentous processes (Figure 8, F), others having 5-7 relatively long unbranched filaments emanating from a
non-nervous conical sheath (Figure 8, E). Whether this latter type is the result of branching of a nerve fiber within the sheath or simply due to the funnelling of a group of approaching nerve fibers to the surface will not be speculated upon. The conical sheath was not impregnated with silver (Figure 11, E) and was probably formed by the neighboring cells.

The nerve endings that did not form filamentous extensions within the cuticle terminated in blunt bulbous expansions, most of which seemed to extend into the cuticle up to 0.5 microns above the base (Figure 8; A, B). Both types A and B were found to occur over the entire surface of the tentacle tip epithelium, but the former was far more numerous than the latter, which appeared to be in greater abundance in the ventral portion of the tip. Type C, which appeared to terminate in a slight swelling immediately below the cuticle, seemed to predominate in the epithelial region surrounding the cornea of the eye (Figure 9, C).

Discussion

The gross internal anatomy of the posterior tentacle of Achatina fulica does not differ from that described in great detail for Helix pomatia by Samassa (1894), with the exception of the chemotactile nerves. The tentacular nerve with its distal ganglion is apparently a common
Figure 8. Diagrammatic representation of sensory nerve endings observed in histological sections of the distal tip of the posterior tentacle of Achatina fulica. Cuticle (C); cell body (CB).
Figure 9. Histological section of the posterior tentacle tip demonstrating nerve endings. This represents the lateral surface of the tip. The letters denoting the nerve endings refer to the diagrammatic types in Figure 8. Type C (C); type D (D); cuticle (Cu). Van Campenhout nerve stain, with Ponceau Red and Fast Green. x1548

Figure 10. The comments and abbreviations in the above figure apply. This section represents the proximal ventral surface of the tentacle tip. x1328
Figure 11. Histological section of the tentacle tip epithelium. The abbreviations used in Figure 9 apply. Type B (B); type E (E); cuticle (Cu). Van Campenhout nerve stain, with Ponceau Red and Fast Green. x1328

Figure 12. Histological section of the tentacle tip epithelium. The abbreviations used in the above figure apply. x2153
Figure 13. Histological section of the posterior tentacle tip epithelium demonstrating nerve endings. The abbreviations used in Figure 9 apply. Type F (F); cuticle (Cu). Van Campenhout nerve stain, with Ponceau Red and Fast Green. x1328
characteristic of stylommatophore pulmonates which are thought to represent the highest evolutionary order of that group (Demaal 1955). As Hanström pointed out (1925, 1928), most prosobranchs have mixed nerves serving the tentacle with no distal ganglion present and no procerebrum in evidence, while the basommatophore pulmonates do possess a procerebrum but still lack a distal ganglion. Therefore, a reasonable insight into the fine internal nervous structure of the tentacular nerve-olfactory ganglion complex of Achatina can be obtained by considering its close homologues (and presumably, analogues) in Helix pomatia.

The excellent work by Hanström (1925) on Helix described the distal ganglion of the posterior tentacle as receiving primary neuronal fibers into its distal portion from the epithelium and synapsing with distal processes from two sources: 1) Cells from the proximal segment of the ganglion; 2) cells from the distal portion of the ganglion. He presumed that the former were related to efferent controls over the sensory input by higher levels, and that the latter were afferent. The tentacular nerve was thought to contain motor nerve fibers as well as centrifugal and centripetal internuncial neurons associated with the specialized input.

The set of chemotactile nerves (Part V) that has been described in the posterior tentacle of Achatina fulica has
not been mentioned in any of the literature dealing with the anatomy of the stylommatophore pulmonate tentacle. The fact that this has been overlooked is not surprising considering the heavy innervation of the tentacular ventro-lateral wall and the early lack of adequate electronic equipment. In contrast to the afferent fibers of the tentacular nerve, these chemotactile nerves appear to be bundles of primary neurons, because internuncials were not observed in the tip epithelium.

In *Achatina fulica*, Ghose (1962b) has named the C\textsubscript{1} and C\textsubscript{2} cerebral ganglion connectives (Figure 3) the superior and inferior frontal nerves, respectively. The nerves homologous to C\textsubscript{1} and C\textsubscript{2} in the posterior tentacle of *Helix pomatia*, however, were more aptly termed the external and internal peritentacular nerves, respectively (Hanström 1925). These latter nerves were described to innervate the lateral and medial walls at the base of the posterior tentacle, which is also the case for Achatina. However, the bifurcating network of the chemotactile nerves in their proximal extension is so well enmeshed in connective tissue that their formation into these discrete cerebral ganglion connectives is well masked. Therefore, these neural connectives are probably mixed nerves with efferent fibers terminating throughout the proximal half of the tentacle wall and afferent fibers inserting into the procerebrum.
On the basis of the anatomical evidence, there appear to be two cerebropetal channels for sensory reception, the olfactory ganglion-tentacular nerve and the chemotactile nerves. As only primary neurons appear to serve the sensory receptors of the posterior tentacle in the prosobranchs and basommatophore pulmonates, it may be postulated that this system persists in the more highly developed stylommatophore pulmonates as the chemotactile nerves, superimposed on the peripherally integrating olfactory ganglion-tentacular nerve complex. It is also noteworthy that the tentacular nerve terminates in the anterior portion of the procerebrum while the ganglionic connectives (Figure 4; C₁, C₂) insert in its posterior portion in a region close to the mesocerebrum, a phylogenetically older portion of the cerebral ganglion.

Schulz (1938) assigned functional properties to morphologically different nerve endings in the tip of the posterior tentacle of Helix by correlating the predominant sensibility in a given region of the body surface with the most common type of receptor in that area. The predominant chemical sensibility of an area was determined by timing the onset of an appropriate behavioral response when the open end of a micropipette (0.5 mm inner diameter) containing an attractant or repellent solution was placed against the area in question.

According to his classification, small knob-like
endings (Figure 8; A, C) are photic, spreading unbranched filamentous endings, olfactory, and branching filamentous types, tactile. The morphology of some endings observed in the tentacle tip of Achatina (Figure 8) were slightly different; extensively branching filamentous types were not seen, and most of the unbranched filamentous endings were of the type \( \text{variety} \) which he designated as a primitive cell serving the common chemical sense. He did not report the presence of any of the large knob-like endings (Figure 8, B) found in the tentacle of Achatina.

Although it is tempting to assign sensibility characteristics to the nerve endings shown in Figure 8 on the basis of the classification by Schulz (1938), acceptable correlations between structure and function of these terminal receptor types can only be established by electrophysiological means.

The presence of a pigmented olfactory epithelium (Figure 4) characteristic of the tentacle tip is also found in the olfactory mucosa of many vertebrate groups, and is widely assumed or postulated to function in olfaction (Gesteland et al 1963, Heyninix 1919, Moncrieff 1951). This idea gained support from the assumption that albino animals have a poorly developed sense of smell, for they appear to lack such pigmented epithelium (Ogle 1870, Moncrieff 1951). However, experimental data on this topic is fragmentary and widely conflicting (Ottoson 1963).
There are several reports associating the function of the visual and olfactory system on the basis of their pigments. This idea gained support when Milas et al. (1939) claimed the presence of vitamin A and carotenoids in the olfactory mucosa of the steer, but later histological and biochemical experiments failed to confirm this (Gerebtzoff and Shkapenko 1951, Philippot and Gerebtzoff 1958). Wright (1954) postulated that the pigment may serve as an energy acceptor and that excitation in the receptor occurs as a result of the "de-excitation" of the pigment molecules. The quality of the odor would be determined by the vibration frequency of the odor molecules, and the threshold of excitation by the closeness of coupling between pigment and odor molecules. This theory also failed to gain confirmation in later experiments (Ottoson 1958, Jackson 1960).

**Conclusion**

In addition to the tentacular nerve-distal olfactory ganglion complex serving the tip epithelium of the posterior tentacle, approximately 5 longitudinal chemotactile nerves extend from the digitiform processes of the olfactory ganglion to the tentacle base where they branch and coalesce into two nerves (Figure 3; C₁, C₂) terminating in the posterior portion of the procerebrum. Distally, the chemotactile nerves course between and
around the digitiform processes of the olfactory ganglion, and both receive fibers from bipolar cell body aggregates.

The terminal nerve endings are highly polymorphic variations of two basic types: Filamentous or non-filamentous extensions into the cuticle; club-shaped with no such extensions. No attempt was made to correlate structure and function of these receptor types.
PART IV

BEHAVIOR

Methods of Research

The Behavior Maze

The ability of *Achatina fulica* to respond to an odor was determined by placing the animal in a maze situation of four possible choices. The biradially symmetrical maze (Figures 14 and 15) was constructed of % and ½ inch plexiglas with the exception of the base, cover and central ports, which were of glass. The external surface of the maze was painted black or covered with black paper with the exception of observation areas bordering the opening at the top; these were entirely covered by four black flaps hinged to the cover (Figure 15, W). With the cover bolted over the top opening, the inside area was completely darkened when the flaps were kept down over the observation areas.

The air outlet on the cover was connected to a suction pump which drew room air (21.5-22.5°C) through an activated charcoal filter and into a mixing chamber containing water; these served to purify and humidify the air. The filtered air was then distributed to the four central ports and eventually to the four stimulus chambers through their respective side ports. Air drawn from these
Figure 14. Side view of the behavior maze. Cover with air outlet (C); side port (SP); central port (CP); air inlet with activated charcoal (AC); mixing chamber (MC); flaps for observation areas (W). x0.12
Figure 15. Diagrammatic representations of the behavior maze.

A. Top view. The central circle is a 5 inch diameter hole in the top. The air coming through the central port enters the stimulus chamber through the side ports. Vials (V); central port (CP); side port (SP); stimulus chamber (SC); behavior areas (A,B,C,D).

B. Side view of a cross-section through the central ports by areas A and C. The symbols in the above diagram apply. The air outlet cover is bolted over the 5 inch diameter hole. Cover with the exhaust tube (CO); flap for observation area (W); activated charcoal (AC); mixing chamber (MC).
four chambers into the square area passed over each of the four vials in the process.

The average velocity of the odor flow from the vials to the exhaust port in the cover was determined indirectly by passing air containing hydrochloric acid vapor over a vial of concentrated ammonium hydroxide. The resulting product, ammonium chloride, served as an adequate marker because it was visible as a white mist. The average velocity was calculated to be approximately 12 centimeters per second, a value that was maintained for all the trials.

**Placement of the snail**

The animal was always placed in the maze facing the vial at area D (See Figure 15A). Its head region was positioned immediately below the exhaust port of the cover, and the antero-posterior axis of the foot was placed on an imaginary line connecting the vials of B and D. With respect to the snail, vial D is straight ahead, vial A is 90° to its left, vial C is 90° to its right, and vial B is 180° to its rear.

**Selection of Stimulus Position**

A behavior experiment consisted of ten trials run consecutively using the same snail. The proportion of these ten trials in which the stimulus vial was placed at areas A, B, C and D was 3:2:3:2 respectively. The order
in which the olfactory stimulus was placed in these four areas over the course of an experiment was determined by random selection. The other three vials contained distilled water.

**Trial Operation**

Before the snail was placed in the maze, the glass base was thoroughly moistened with water. The flaps were kept over the observation areas throughout most of the trial, but were raised momentarily after five minutes to allow the observer to note the progress of the animal. The trial was terminated when the snail was found in any of the areas designated A, B, C or D. Also, a trial was considered positive when the snail was found in the area containing the stimulus. The glass base was thoroughly cleaned with absolute alcohol after each trial in order to remove the slime trail. Fresh stimulus was used with each run.

**Control Trials**

Sham trials were performed whereby all four vials contained distilled water, but one was designated as the "stimulus". This vial was randomly moved to different areas as described, and a trial was considered to be positive if the snail was found in its area.

**Normal and Experimental Groups**
The normal group was composed of eight snails with all of their tentacles intact; seven of these were later operated on to form the experimental group.

The operation consisted of the removal of the epithelium covering the tip of both posterior tentacles (Figure 2). This was accomplished by first immobilizing the animal with 0.05 milliliters of 0.5% succinylcholine chloride in isotonic saline injected into the haemocoele through the ventral surface of the foot. The snails relaxed within two minutes and became quite flaccid, but the posterior tentacles still maintained an ability to invert in response to a tactile stimulus. In order to keep the tentacle extended, the tip was placed within the orifice of an eye-dropper with its bulb compressed. When the bulb was released, the orifice slid to the base of the tentacle causing the rest of the tentacle to extend within the lowered air pressure of the eye-dropper. The tips of a fine pair of forceps were applied to the tentacle base next to the orifice of the eye-dropper, then the dropper was slowly pulled away from the base with the forceps following the orifice until the constriction below the tentacle tip was reached. At this point, the tentacle was held gently with the forceps while the dropper was removed. The epithelium was quickly dissected away with a microdissection scalpel.

The anesthetized animals revived after 4-5 hours with
no mortality or apparent behavioral abnormalities.

**Observations**

The behavior of eight normal snails were observed from the middle of September 1966 to November 1966, after which seven of these animals were operated on to form the experimental group. The trials of this latter group began in the middle of December 1966 and terminated in April 1967.

Regeneration of the tentacle tip epithelium was noted for all the members of the experimental group by February 21, 1967. However, the regenerated region was malformed with irregular bulbous surfaces, and in many cases, multiple eyes. The epithelium was pale and leucodermic with no apparent yellow pigmentation. This latter condition persisted when the behavior experiments were terminated in April 1967.

**Normal and Experimental Animals**

The results of the behavior trials are summarized in Tables II and III. The numerical and percentage data refer to the total number or percentage of trials in which the stimulus was placed in a given area.

The duration of each trial was approximately 15 minutes, and therefore, it was not always possible to complete the theoretical ratio of 3:2:3:2 stimulus placements in areas A, B, C and D for a series of experiments. This
TABLE II. NUMERICAL DISTRIBUTION OF THE BEHAVIOR
TRIALS OF ACHATINA FULICA

<table>
<thead>
<tr>
<th>Position* of the Stimulus</th>
<th>Normal Snails</th>
<th>Experimental Snails</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Trials</td>
<td>Number of Positive Responses</td>
</tr>
<tr>
<td>Area A</td>
<td>106</td>
<td>72</td>
</tr>
<tr>
<td>Area B</td>
<td>77</td>
<td>40</td>
</tr>
<tr>
<td>Area C</td>
<td>96</td>
<td>71</td>
</tr>
<tr>
<td>Area D</td>
<td>75</td>
<td>58</td>
</tr>
<tr>
<td>All Areas</td>
<td>354</td>
<td>241</td>
</tr>
</tbody>
</table>

*See Figure 15A

TABLE III. PERCENTAGE DISTRIBUTION OF THE BEHAVIOR
TRIALS OF ACHATINA FULICA

<table>
<thead>
<tr>
<th>Position* of the Stimulus</th>
<th>Percentage of Total Trials</th>
<th>Percentage of Positive Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Snails</td>
<td>Experimental Snails</td>
</tr>
<tr>
<td>Area A</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Area B</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Area C</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>Area D</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>All Areas</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*See Figure 15A
difficulty is reflected in the first column of Table III. However, it is clear that the percentage of total trials for experimental snails in column two is comparable.

Although the percentage of positive chemotaxes observed for all the trials regardless of stimulus position was 68% for normal snails as opposed to 25% for experimental snails, it is useful to consider their responses according to the stimulus area. In reference to the behavior maze (Figure 15A), the amount of overt behavior necessary for positive chemotaxis is undoubtedly related to the angle of turn the snail would have to execute in order to face the stimulus source. In other words, the probability that positive chemotaxis would occur for a snail oriented towards area D would decrease as the stimulus is placed at D, A and C, and B, respectively. These qualitative probabilities are manifested in the percentage of positive responses for both normal and experimental groups in Table III. The percentage distribution for the experimental group is very symmetrical, with no positive chemotaxis observed with the stimulus placed in area B.

Behavior Control Experiment

Experiments with two members of the normal group of snails were followed immediately by sham trials, in which the vegetable juice stimulus was replaced by distilled
### TABLE IV. BEHAVIOR CONTROL TRIALS FOR ACHATINA FULICA WITH AND WITHOUT THE OLFACTORY STIMULUS

<table>
<thead>
<tr>
<th>Snail</th>
<th>With Stimulus</th>
<th>No Stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Trials</td>
<td>Number of Positive Responses</td>
</tr>
<tr>
<td>No. 37</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>No. 17</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

### TABLE V. BEHAVIOR OF ACHATINA FULICA WITH A SINGLE TENTACLE IN RESPONSE TO AN OLFACTORY STIMULUS

<table>
<thead>
<tr>
<th>Area* of Stimulus</th>
<th>Number of Trials</th>
<th>Number of Positive Responses</th>
<th>Percentage of Positive Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>5</td>
<td>71</td>
</tr>
<tr>
<td>All Areas</td>
<td>35</td>
<td>18</td>
<td>51</td>
</tr>
</tbody>
</table>

*See Figure 15A
water. The results indicate that the operation of the trials was not affecting the behavior of the snail (Table IV).

Olfaction With a Single Posterior Tentacle

The left posterior and both anterior tentacles were removed from one individual member of the normal group of snails on April 1967. The snail exhibited positive chemotaxis in 51% of the total trials (Table V), but it appeared to have a tendency to move in the direction of its intact tentacle. As a member of the normal group with all the tentacles intact, it responded positively to the olfactory stimulus in 56% of 25 trials.

Discussion and Conclusions

Although previous behavior experiments on stylommatophore pulmonates have been convincing in demonstrating the existence of an olfactory sense separate from a gustatory one for this group, their results are still questionable for several reasons. Most of the previous descriptions were single observations (Moquin-Tandon 1851, Stahl 1889, Adams 1898, Kieckebusch 1953) and lack statistical veracity. Although Kittel (1956) utilized over 200 snails and Schulz (1938) ran 16 trials for each experimental group, they failed to control some of the variables. Kittel made the majority of his observations in the field during the day, thus allowing possible visual responses;
also, no allowance was made for possible anemotactic behavior. Positive anemotaxis was demonstrated for *Achatina fulica* by Chamberlin (1952) who stated that these movements may not necessarily be chemokinetic since their upwind course was not straight, as would be expected if the movements were in response to chemical stimulation (Fraenkel and Gunn 1940).

Schulz (1938), in his experiments with Helix, tried to remove all visual stimuli by illuminating the room with red light, but there is no evidence that Helix does not perceive this wave length. Also, the lettuce stimulus may have created a small but sharp humidity gradient, for it was positioned only 4 centimeters in front of and above the head of the animal. It is well known that microclimatic factors may have a strong effect on the behavior of animals.

The behavior experiments performed on Achatina were designed to control all the variables discussed here. A sufficiently large number of trials for each group was performed using snails of the same age and learning experience; the reliability of the results was enhanced by the described method of controlled randomization in the placement of the stimulus. The humidity of the air entering each of the four areas in the behavior area was the same, because the three vials containing water and the fourth, the stimulant, had identical surface areas.
subjected to the same rate of air flow. This was further controlled by the use of a common mixing chamber for all the air entering the maze.

The possibility of positive anemotaxis affecting the behavior of Achatina was controlled by maintaining the air flow velocities constant for the four areas, thus allowing for the expected qualitative probabilities of response discussed previously. Finally, the possibility of a visual response to moving objects outside the box was eliminated by running the trials in a darkened and enclosed maze.

The tips of the posterior tentacles are apparently the principal sites of olfaction in *Achatina fulica* since the olfactory behavior of the experimental group was random despite the presence of intact anterior tentacles. However, Achatina was still able to exhibit positive chemotaxis in 51% of the trials with only one tentacle remaining (Table V), as compared with 56% with all four tentacles intact. Although only a single animal was used in this experiment, its orientation could only have been influenced by the position of the olfactory stimulus since the other variables were controlled. In addition, Schulz (1938) observed a similar behavior in Helix when it possessed only a single tentacle. Therefore, the total sensory input of directional information concerning the source of air-borne stimuli may not be due to the
differential responses of each of the posterior tentacles, but may be the result of additive responses of both tentacles, each serving as an independent discriminating unit.

It may be concluded that *Achatina fulica* has a definite olfactory ability with its peripheral receptors located principally on the tips of the posterior tentacles. In the reception of the olfactory stimulus, a single posterior tentacle can provide information for orientation and chemotaxis.
An electrophysiological approach to the study of olfaction requires, first, a source of deodorized air that would serve as a vehicle for the stimulus as well as a control; second, a means of isolating the suspected olfactory area from the rest of the preparation; finally, a means of picking up and recording the electrophysiological responses.

**Air Purification System**

Contamination of air by extraneous odors was prevented by a gas purification system (Figure 16) similar to those used by Pfaffman et al. (1958), Mozell (1958), Walsh (1956) and Ueki and Domino (1961). Compressed air was passed at a steady rate of flow through concentrated sulfuric acid, distilled water, 20% sodium hydroxide, anhydrous calcium chloride, activated charcoal and silica gel. The dehumidified and deodorized air was introduced into one of two vials, then sent to the olfactory chamber (Figures 17 and 19) containing the tentacle preparation.

Each of the vials contained 2 milliliters of distilled water. The cover of one vial was fitted with a 3 inch 18 gauge needle extending below the level of the
water. This was convenient for introducing the stimulus with a 5 cc syringe without interrupting the flow rate by switching to the other vial.

The flow rate through the olfactometer was regulated by controlling the head of hydrostatic pressure presented at the tube opening in the air pressure regulator (Figure 16, PR). This was accomplished by varying the depth of the tube, with the level of the air-water interphase always kept at the opening of the tube with no air bubbling out. In order to insure constant pressure, evaporation was minimized by placing a thin layer of mineral oil over the surface of the water.

The flow rate of air through the system was determined by blocking the outflow in the olfactory chambers; this caused the air to escape through the tube opening in the pressure regulator. The average volume of each bubble was estimated to be 0.33 cc. Thus the average flow rate was easily calculated over a given period of time by counting the number of escaping bubbles.

**Isolation of the Olfactory Field**

It was necessary to isolate the tentacle in a chamber where air flow could be introduced while the remaining portion of the tentacle was immersed in isotonic saline or mineral oil. This was accomplished by piercing a piece of rubber dam with a dissecting needle, stretching it to
Figure 16. Schematic diagram of the air purification system used in electrophysiological studies of the posterior tentacle of Achatina fulica. Air pressure regulator (PR); concentrated sulfuric acid (SA); distilled water (W); 20% sodium hydroxide (SH); anhydrous calcium chloride (CC); activated charcoal (AC); silica gel (SG); syringe (S); vial containing distilled water (V).
Figure 17. Chamber for olfactory studies of the posterior tentacle of *Achatina fulica*. The tentacle tip held by a rubber dam is placed in the chamber, and the remaining portion of the tentacle is pinned to the silicon rubber base. The tray and the chamber support is then bolted together. The arrows indicate the inflow and outflow of filtered air through the chamber. Plexiglas chamber support (CS); silicon rubber base (SR); dissecting tray (DT). x0.9

Figure 18. Diagrammatic top view of the chamber in figure 17. The rubber dam holding the preparation is bolted between the tray and the chamber support, with the outer rim of the chamber holding the dam tight against the tray. The abbreviations used in the above figure apply. Rubber dam (R); preparation (P); chamber (C); nut (N); bolt (B). x1
Figure 19. Olfactory chamber for studies on the effect of directional air flow on the tentacle tip of Achatina fulica. The rubber dam holding the tentacle is bolted between the dissecting tray and the directional flow chamber with the tip extending into the lumen of the glass tubing. The arrows indicate the filtered air flow through the chamber. Orifice through one wall of the glass tubing (A); plexiglas support for the tubing (B); glass tubing chamber (C); silicon rubber base (SR); dissecting tray (DT). x0.9
enlarge the hole, and placing the extirpated tentacle so only the tip would protrude from the other side (Figure 18). The rubber dam, besides holding the tentacle, served as an air-tight partition between the dissecting tray and the olfactory chamber.

Two chambers were constructed in order to provide a selection of different areas of the tentacle tip to be stimulated by the impinging air flow. The first (Figures 17 and 18) provided direct frontal air flow on the tip, which was necessary for most of the experiments. The second (Figure 19) provided air flow at right angles to the long axis of the tip, thus enabling selective stimulation of the left or right sides of the convex epithelial surface. The first chamber was a glass cylinder with a volume of 4.7 cc, and it was provided with inflow and outflow ports; the second was a glass tube with an inner diameter of 0.5 centimeters. Both were supported by blocks of plexiglas with anchored bolts and nuts to provide a means of attachment to their respective trays.

The dissecting tray and support for both chambers were then bolted together with the rubber dam forming an effective leak-proof seal in between. The tentacle was pinned to the silicon rubber base with its dorsal side up, and dissected in the manner discussed in Part III (See page 12). The preparation was immersed in isotonic saline and supra-vitally stained with methylene blue solution
while dissecting.

**Pickup and Recording of Electrophysiological Responses**

The olfactory chamber and tray containing the dissected preparation immersed in mineral oil were transferred to an insulated cage (Figure 20) where the nerves to be investigated were supported by two 50 micron stainless steel monopolar electrodes. A common reference electrode was placed in the muscular wall of the tentacle. The responses were amplified by two single-ended Tektronix 122 preamplifiers, and recorded with a Roberts 990 tape recorder at 3½ inches per second. One channel was monitored with a Tektronix RM504 single beam oscilloscope while the other was marked with a Grass S5 stimulator. At the beginning of each experiment, the stimulator was used to mark both channels to provide a common starting point for both recordings. The low and high frequency response filters of both preamplifiers were set at 0.2 and 250 cycles per second, respectively.

Tapes were subsequently played back monophonically at 3½ inches per second, each channel being displayed consecutively on the oscilloscope and recorded on moving photographic paper (Kodak Linagraph 1732) using a Grass C4-K oscilloscope recording camera.

The frequency of spike response was used as a measure of sensory activity.
Figure 20. Diagrammatic representation of the electrophysiological equipment. The cage containing the preparation is shielded with copper screening.
Trial Operation

All trials were started with the introduction of deodorized air into the chamber, originating from the vial containing the 18 gauge needle. The stimulus was then introduced by slowly injecting 5 cc of the vegetable juice into the vial without changing the flow rate of the filtered air. The stimulus marker was used with each change, either in air content or flow rate and direction.

Observations

The following observations are those of the electrophysiological activity manifested by the tentacular and chemotactile nerves (Figure 3; TN, N₁-N₅) in response to olfactory and tactile stimuli. The olfactory chamber (Figures 17 and 18) used in the first experiments (Figures 21 and 22) provided a reasonably uniform air flow over the entire surface of the epithelial tip, while in the next three experiments (Figures 23, 25 and 26) the directional air flow olfactory chamber (Figure 19) was utilized. The direction of flow in relation to the long axis of the tentacle tip is indicated in the records by arrows.

Tentacular Nerve Activity

The deodorized air control, flowing at a rate of 0.64 cc/second, elicited little or no response as a tactile stimulus (Figure 21). However, the olfactory stimulus appeared to produce neural activity 20–30 seconds after it
Figure 21. Compound action potentials recorded from the tentacular nerve of the posterior tentacle of Achatina fulica in response to flowing deodorized air (control) and the olfactory stimulus added to the flowing deodorized air (stimulus). The tentacular nerve was not severed proximally, and the pickup electrode recorded from the nerve midway between the procerebrum and the olfactory ganglion. The control and stimulus air flow rates were 0.64 cc/sec.
was injected into the vial. This latent period was in part due to the time it took for the odor to travel from the vial to the chamber.

Theoretically, deodorized air flowing at the rate of 0.64 cc per second could replace the chamber air in 7 seconds. One would therefore expect a decrease in activity after this period. However, the increasing activity was not diminished when the chamber was flushed with deodorized air for 60 seconds. In addition, the amplitude and frequency of the activity increased steadily after the introduction of the stimulus until the termination of the experiment 5.5 minutes later. Therefore, there was little or no decrease of cerebropetal activity evident in the tentacular nerve for this time period.

Activity of Chemotactile Nerves

Tactile responses to the control flow of deodorized air were apparent for all the chemotactile nerves (Figure 22, N₁-N₅), but this activity decreased markedly with the cessation of air flow (Figure 22; N₃/b, N₄, N₅). When the stimulus was injected into the control air flow, both the mean firing rate and the amplitude of responses were greater after a delay of 10-18 seconds, than those observed for the control (Figure 22; N₁, N₂, N₃/c).

Although it was not possible to discriminate between the tactile and olfactory responses on the basis of
Figure 22. Compound action potentials recorded from the chemotactile nerves of the posterior tentacle of Achatina fulica when the tip was stimulated by flowing deodorized air (control) and the vegetable juice odor (stimulus) borne by the deodorized air flow. The records N\textsubscript{1a}, N\textsubscript{1b} and N\textsubscript{1c} represent a single recording and should be read in alphabetical sequence. N\textsubscript{2a} and N\textsubscript{2b} represent simultaneous recordings from the right and left proximal bifurcations of the same chemotactile nerve, respectively. The tentacle was severed between the cerebral ganglion and the tentacle base. The control and stimulus air flow rates were constant at 0.64 cc/sec.
relative mean firing rates or amplitudinal differences, it was expected that the tactile responses would cease when the air flow was stopped entirely. This indeed proved to be the case (Figure 22; \( N_3/b, N_4, N_5 \)). The olfactory stimulus, on the other hand, remained in the chamber when the air flow was stopped. Therefore, any neural activity that remained in the ensuing period was assumed to be a result of olfactory activity only. Such residual activity was found in all the chemotactile nerves observed (Figure 22).

After the stimulus was introduced into the chamber, the adaptation times observed from these nerves ranged from 80-116 seconds (Figure 22; \( N_1, N_2, N_4, N_5 \)). After adaptation, the level of activity was much reduced, but remained greater than that observed with motionless deodorized chamber air.

**Chemotactile Nerves and Directional Stimuli**

The responses of the two lateral chemotactile nerves to directional deodorized air flow (Figure 23) are summarized by histograms (Figure 24) which reflect their relative activities in consecutive 20 second time periods. It should be mentioned that coincident spikes occurring in the photographic records could only be counted as single responses in the histograms.

The tactile stimulus was presumably greatest on the
side of the tentacle tip on which the air flow impinged. This is graphically illustrated in the histograms where the chemotactile nerve serving the area directly impinged upon by the air flow exhibited a greater activity than the nerve terminating in the opposite side of the tentacle tip. A 180° change in the direction of air flow showed a decrease in the activity of the former nerve mentioned above, and an increase in the latter. On the other hand, responses from two chemotactile nerves serving the left side of the tip were greatest when the stimulus originated from the left (Figure 25); conversely, their mean firing rates and spike amplitudes decreased when the direction of flow was reversed 180°.

Records demonstrating a significant response to directional olfactory stimuli were obtained in which the left chemotactile nerve showed persistent activity after the olfactory stimulus flow was turned off (Figure 26; N_5). Adaptation took place 120 seconds after the introduction of the stimulus into the chamber.

**Simultaneity in Nerve Bifurcations**

Simultaneous activities of proximal left and right bifurcations of a chemotactile nerve were recorded (Figure 22; N_1/a, N_1/b). Visual inspection of the two records indicates that chemotactile nerve bifurcations carry the same information, undoubtedly a result of axonal branching
Figure 23. Compound action potentials recorded from the right and left lateral chemotactile nerves of the posterior tentacle in response to deodorized air flowing at right angles to the tip. The tentacle was severed between the cerebral ganglion and the base of the tentacle. The flow rate was constant at 0.64 cc/second. Recording from the left nerve ($N_c$); recording from the right nerve ($N_l$).
AIR FLOW  OFF  AIR FLOW

8 CM

1 MINUTE  A

AIR FLOW  OFF  AIR FLOW

8 CM

1 MINUTE  B
Figure 24. Histograms A and B summarize the activities exhibited in Figure 23A and B, respectively. The comments in Figure 23 apply.
Figure 25. Compound action potentials recorded from the left chemotactile nerves of the posterior tentacle in response to deodorized air impinging on the left and right epithelial surface of the tentacle tip. The flow rate was constant at 0.64 cc/second. Recordings from the left nerves (N5, N4).

Figure 26. Compound action potentials recorded from the left and right lateral chemotactile nerves of the posterior tentacle in response to deodorized (air flow) and odorized (stimulus) air impinging on the left epithelial surface of the tentacle tip. The flow rate was constant at 0.64 cc/second. Recording from the left nerve (N5); recording from the right nerve (N1).
from the main stem distal to the bifurcation.

Discussion

Although the gross nature of the electrophysiology experiments precludes any detailed discussion of possible sensory receptor mechanisms or coding, the observations do indicate the quality and quantity of cerebropetal activity conducted through the principal sensory inputs.

These results verify the existence of two separate cerebropetal sensory systems serving the tip of the posterior tentacle, as tentatively concluded on the basis of anatomical evidence (Part III). In addition to the fact that the chemotactile nerves have never been reported in the literature, the assumption by previous workers that the tentacular nerve-distal ganglion complex serves an olfactory function in stylommatophore pulmonates was based entirely on correlations of anatomy with behavior.

Although both systems conduct responses to olfactory stimuli, the terminal receptors of the chemotactile nerves also serve a tactile function while those of the olfactory (distal) ganglion do not. Unfortunately, the question of whether a single nerve ending can serve as both mechano- and chemoreceptors is beyond the scope of the evidence presented in this dissertation. However, Ueki and Domino (1961) have postulated the existence of this type of dual reception in dogs and monkeys, and have proposed that the
mechanoreceptor may facilitate chemical reception of odors. Recently, their criteria for "air purity" have been questioned (Moulton and Tucker 1964) on the basis of difficulties other workers have had in removing all active contaminants from the air stream (Adrian 1942, 1951; Ottoson 1963, Tucker 1963).

The mechanoreceptors of the chemotactile nerves apparently respond to continuous deformation (Figure 22, \(N_2\)) and are phasic in their adaptation to extinction. The adaptation of the olfactory receptors of this nerve group, however, seems to be independent of the continued presence or abrupt absence of tactile stimuli (Figure 22; \(N_1, N_2\)). The slow adapting responses recorded from the tentacular nerve may not necessarily be a property of the nerve endings, since peripheral integration probably takes place in the olfactory ganglion. Integration in first order interneurons has been reported in the visual system of crayfish (Wiersma and Yamaguchi 1966), where excitation of the animal causes the fibers to fire at a higher rate than that observed in the quiescent animal.

An olfactory system somewhat comparable to the peripherally integrating olfactory ganglion of Achatina may be the olfactory bulb of vertebrates, where central control over the sensory input takes place (reviewed by Wenzel and Sieck 1966). Both systems probably do not control the sensitivity of the terminal receptors by sending
efferents to the sensory area, which is the case for the vestibular system of vertebrates (Rasmussen and Gacek 1960). This was demonstrated by Adrian (1950) who postulated that olfactory adaptation is due to the inhibition of olfactory signals by the intrinsic activity in the bulb, since he found no signs of failure of the rabbit olfactory receptors with repeated stimulations. Therefore, the olfactory ganglion in stylommatophore pulmonates may play a role in sharpening olfactory sensations or inhibiting prolonged activity so that information may be processed more rapidly. These inhibiting efferents may carry information originating from the pedal and visceral ganglia, and the degree of inhibition might well be a function of the state of the animal.

The observation that a single posterior tentacle can function as an independent directional sensing and transmitting device in behavior (Part IV) may be explained by the anatomical and electrophysiological nature of the chemotactile nerves with their connections to the cerebral ganglion. Rapoport (1955) considered this problem in an analogous sensory system, the retina, where $10^8$ photoreceptors are connected to $10^6$ ganglion cells; he applied the principles of information theory to explain these "bottleneck" systems and the presence of redundancies of information in the optic nerve.

Concerning the posterior tentacle of Achatina, two
conclusions are pertinent: 1) There apparently exists a great reduction in the number of channels carrying information from the olfactory and tactile receptors to the cerebral ganglion (Figure 3), essentially a "bottleneck" situation in cerebropetal convergence; 2) there is a large amount of redundancy in information which seems to increase as the "bottleneck" narrows from the terminal receptors, to the chemotactile nerves, and finally to the peritentacular nerves (Figure 3; C₁, C₂). This is demonstrated in Figure 22 (N₁/a, N₁/b) where 100% redundancy occurs in the bifurcations of a chemotactile nerve. It is also apparent that when two bifurcated nerves carrying information sets coalesce, a certain amount of information would probably be lost due to their refractory periods. Therefore, all distal and proximal neural convergences lead to gains in redundancy at the cost of a loss in information.

Edwards (1964) considered the importance of redundancy in such a system. If a nerve net (such as the one described at the base of the tentacle) becomes partially damaged, the loss in information content will be minimized by the presence of other nerves carrying all or part of the same information. A second advantage lies in the reduction of "noise" or error manifested in the distal portions of the system, primarily due to the binary or "yes-no" nature of the sensory receptors.
Therefore, stimulation of areas of the lateral and ventral epithelium of the Achatina posterior tentacle tip must be sensed in the ipsilateral cerebral ganglion in an overlapping fashion. If the activity of five chemotactile nerves in response to a tactile stimulus directed onto the left surface of the tentacle tip were observed on a multi-channel recorder (hypothetically displaying their activities on five cathode-ray tubes simultaneously), there would be a uniform decrease in the responses observed from nerves N₅ to N₁ (Figure 3). Although this was not technically possible, the results (Figure 23-25) indicate that this would probably be the case.

On the other hand, the addition of a supra-threshold olfactory stimulus to the air flow would be expected to sharpen the gradient considerably, particularly for stimulating periods shorter than the adaptation time of the olfactory receptors. The extreme case is represented in Figure 26 where the flow did not have sufficient force to maintain tactile activity in the right lateral sensory epithelium (served by N₁), while the left (served by N₅) maintained constant activity for a period beyond the cessation of air flow. This difference in activity between the left and right lateral chemotactile nerves (N₅, N₁) may be due to the air flow configuration around the tentacle tip. Air flow directed on the left lateral epithelium would probably be more direct on that surface,
and somewhat laminar on the right side. Therefore, the proportion of odorant molecules dissolved in the cuticular mucus layer would probably be greater on the left side of the tip than on the right.

The tentacular nerve-olfactory ganglion system appears to have no capacity to conduct directional information in a single tentacle. Although no other evidence was obtained in addition to the electrophysiological results (Figure 21), the observation by Hanstrom (1925) that the distal ganglion resembles the fine neuropile consistency of the cerebral ganglion indicates that the former is probably an extension of the latter, and that it functions primarily as a means of controlling the sensory input by the central nervous system. As it was mentioned earlier, this evolutionary innovation in the stylommatophore pulmonates is probably due to a greater dependence on exteroceptive stimuli (Hanstrom 1928).

**Conclusion**

The posterior tentacle of *Achatina fulica* has two separate sensory channels converging on the ipsilateral cerebral ganglion from the tip epithelium. The terminal receptors of the tentacular nerve-olfactory ganglion system do not respond to a tactile stimulus, but appear to exhibit prolonged activity in response to an olfactory stimulus. The chemotactile nerves, on the other hand,
conduct responses from both mechanoreceptors and olfactory receptors; the former type respond to continuous deformation and is phasic in its adaptation, while the latter appears to be intermediate in their ability to adapt.

Due to the "bottleneck" pattern of the chemotactile nerves, the ventro-lateral epithelium of the posterior tentacle has overlapping sensory representation in the ipsilateral cerebral ganglion. The ipsilateral cerebral ganglion receives sensory information through two channels, the left and right peritentacular nerves (Figure 3; C₂, C₁). The left nerve conducts sensory information from the left ventro-lateral epithelium, and the right nerve from the right ventro-lateral epithelium. Their sensory representations of the ventral epithelium overlap to some extent.

The tentacular nerve-olfactory ganglion system appears to have no capacity to conduct directional information in a single tentacle.
PART VI

GENERAL DISCUSSION

Adrian (1928) pointed out that there is a reciprocal relationship between the perception of stimulus intensity and stimulus change, and that these in turn are related to the rate at which the particular receptors adapt. Although adequate information is lacking concerning the ability with which the receptors of the olfactory ganglion of the snail can perceive different intensities of the stimulus, its relatively long (Figure 22) activity recorded from the tentacular nerve indicates that it would not be able to perceive fairly rapid temporal changes in the stimulus. The receptors of the chemotactile nerves, on the other hand, possess relatively short adaptation times, the mechanoreceptors being phasic in this respect. The receptors of this latter system are apparently very sensitive to sudden stimulus changes, and air-borne odors would provide additional information. Therefore, on the basis of the activities observed in the tentacular and chemotactile nerves, the receptors of the posterior tentacle tip appear to fall into three basic categories: 1) Slow adapting and sensitive to odor intensity differences; 2) intermediate adapting and relatively insensitive to odor concentrations above threshold; 3) fast adapting to changes in mechanical deformation. These categorical
types are widely found in different animals (Marler and Hamilton 1966) where specialization in either direction is achieved only by sacrificing the efficiency of one function.

The spatial properties of olfactory and tactile stimuli in the environment of Achatina should be considered in order to gain some understanding concerning the respective roles of the sensory systems served by the tentacular and chemotactile nerves. The spatial properties of the stimulus are defined by essentially two factors: 1) The presence or absence of air movement; 2) the distance of the snail from the stimulus source. In the case of no air movement, the snail would be guided to the source of the stimulus by "sampling" the odor concentration at different points along the diffusion gradient. The slope of the three-dimensional stimulus gradient would increase as the animal approaches the source. Therefore, the intensity of the stimulus and the ability of the animal to discriminate a gradient would decrease nonlinearly with increasing distance. Since the maximal distance between the tentacle tips of a full-grown Achatina averages a little less than 3 centimeters, it is very unlikely that it can sense an odor gradient through either its chemotactile nerve patterns or bilateral tentacular nerve-olfactory ganglion systems, unless it is very close to the stimulus source. Conversely, its perceptive
ability at greater distances may increase with the presence of air movement, as indicated by the electrophysiology results in Figure 26. This was shown to be true for the silkworm moth, *Bombyx mori* (L.): The male can be attracted to the female at a distance of ¾ mile by flying upwind (Jacobson and Beroza 1963), but it has to be within 1 meter to find the female in still air (Schwinck 1954, Wilson and Bossert 1963).

If the long adaptation time recorded for the tentacular nerve is a property of the olfactory receptors it serves or of internuncial integration in the olfactory ganglion, then it can be postulated that its activity probably produces a higher level of random motile behavior in Achatina; this is probably modulated by the central nervous system through cerebrofugal efferents controlling sensory flow at the first synaptic level in the olfactory ganglion. The sensory receptors with the chemotactile nerve pattern, on the other hand, provide two types of information: 1) Direction of air movement; 2) source of an olfactory stimulant. Therefore, each tentacle would provide identical or similar sets of directional information that are "summed" in the central nervous system.

Although Achatina has sensitive olfactory and directional senses, their importance to this snail as a successful species is very doubtful. Observations made in the laboratory and in the field indicate that individuals
with amputated posterior tentacles are equally active at night as the normal animals. Mead (1961) pointed out that Achatina is rarely limited in its environment by food, due to its omnivorous appetite and ability to aestivate for long periods of time. He also concludes that the great success Achatina has shown is due primarily to its hermaphroditic nature, great fecundity, hardiness, and convenient human dispersal vectors. However, the ability to locate food determines the success of the individual only, not that of the species. But chemoreception can be a density-dependent factor in a newly introduced population in terms of aggregation, homing ability, and the selection of a new environment.

Unfortunately, no controlled studies on the aggregating behavior of terrestrial molluscs exist. Aggregates of individuals were frequently noticed in the field, but this was not unexpected in a non-random environment. Chamberlin (1952) observed the same phenomenon and concluded that such aggregates were probably formed as a result of individuals responding to the same environmental stimuli. Therefore, no conclusion can be made concerning the aggregating ability of Achatina and its dependence on olfaction.

The homing ability of gastropods, particularly *Helix pomatia*, has been discussed by Edelstam and Palmer (1950). Experiments with Helix demonstrated positive homing
behavior with an angular error of less than 30° for distances under 40 meters. They attributed this capacity to the olfactory ability and memory of Helix. In another investigation, Hatai and Kato (1943) marked 61 Achatina individuals in a prepared shelter and recorded their re-occurrences six times in the next three weeks. The presence of a marked snail in the shelter during a check was considered to be an indication of its return to the shelter. In this manner, they recorded 70% homing individuals during the three week period. Unfortunately, there was no proof of any individual leaving the shelter after the previous check.

Therefore, although olfactory and directional abilities do exist in *Achatina fulica*, their importance in aggregation, homing ability, and environment selection have not been definitely established.
PART VII

SUMMARY

1. The posterior tentacle contains an inner tubular retractor muscle that connects with the outer wall only at the tip. It contains the tentacular artery, and the optic and tentacular nerves within its lumen.

2. The tentacular nerve connects proximally with the anterior portion of the procerebrum; distally, it expands into a pyriform ganglion which sends digitiform extensions throughout the tentacle tip epithelium. These extensions receive proximal fibers from bipolar cell aggregates situated in the sub-epithelial region.

3. 4-6 chemotactile nerves insert between the digitiform extensions in the ventro-lateral tip epithelium and lead to bipolar cell aggregates in the sub-epithelial region. These nerves extend proximally between the retractor muscle and the ventro-lateral wall of the tentacle to form a complex nerve net at its base. The net is formed by the proximal anastomoses of the branches of adjacent chemotactile nerves; these form the left and right peritentacular connectives to the posterior portion of the procerebrum.

4. The distal fibers of the bipolar cell aggregates extend between the epithelial cells to terminate at the base of the cuticle, or within it. The nerve endings
are of three basic types: 1) Blunt, expanded endings at the base of the cuticle; 2) bifurcating endings within the cuticle; 3) filamentous non-branching endings, some (5-7) emanating from a sub-cuticular conical sheath.

5. Normal laboratory-raised snails demonstrated positive chemotaxis in response to an olfactory stimulus in 68% of 354 trials when in a darkened biradially symmetrical maze with four possible choices.

6. Laboratory-raised snails with their posterior tentacle epithelium removed demonstrated positive chemotaxis in 25% of 294 trials under the same conditions.

7. In a control experiment in which the olfactory stimulus was replaced with distilled water, a normal laboratory-raised snail demonstrated no significant taxis to the stimulus control vial.

8. A laboratory-raised snail with one posterior and both anterior tentacles removed demonstrated positive chemotaxis in response to an olfactory stimulus in 51% of 35 trials. However, it seemed to have a tendency to move in the direction of its intact tentacle.

9. The electrophysiological activity recorded from the tentacular nerve increased when the posterior tentacle tip was subjected to an olfactory stimulus for a short time period; this activity increase continued until the termination of the experiment, 5.5 minutes after
10. No increase in electrophysiological activity was recorded from the tentacular nerve when the posterior tentacle tip was stimulated with a tactile stimulus.

11. Electrophysiological activity recorded from the chemotactile nerves increased when the posterior tentacle tip was subjected to an olfactory stimulus. The duration of the olfactory response ranged from 80 to 120 seconds. The simultaneous presence or absence of a tactile stimulus appeared to make no difference in the magnitude or duration of the olfactory response.

12. An increase in electrophysiological activity recorded from the chemotactile nerves was immediate when the tentacle tip was subjected to a tactile stimulus. This level of activity was maintained for the duration of the stimulating period, after which an immediate return to the resting level was observed.

13. Synchronous electrophysiological responses were recorded from two proximal branches of a chemotactile nerve at the base of the posterior tentacle when the tip was stimulated with olfactory and tactile stimuli. It was concluded that the proximal bifurcations of each chemotactile nerve probably conduct the same information as the main stem.

14. Tactile stimuli directed on the left surface of the
posterior tentacle tip produced a proportionately greater electrophysiological activity recorded from the left chemotactile nerve than from the right. Similarly, stimuli directed on the right lateral surface of the tip produced a greater response in the right chemotactile nerve than in the left.

15. Increased electrophysiological activity was recorded from two left chemotactile nerves in response to tactile stimuli directed on the left epithelial surface on the tentacle tip; the activity of both nerves was diminished when tactile stimuli were directed on the right epithelial surface.

16. An olfactory stimulus directed on the left lateral surface of the posterior tentacle tip produced electrophysiological activity lasting 120 seconds when recorded from the left chemotactile nerve, while no activity in response to this stimulus was recorded from the right chemotactile nerve.
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