An Examination of the Concept of Arousal within the Context of the Sexual Behaviour of the Snail, *Helix aspersa*

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April, 1990

A thesis submitted to the

Faculty of Graduate Studies and Research of

McGill University in partial fulfillment of the

requirements for the degree of Ph.D.

To my parents, with love and gratitude for all their support and encouragement

Abstract

Sexual 'arousal' in Helix aspersa can be divided into 2 components, sexual proclivity (the tendency of a snail to respond to conspecific contact with courtship) and sexual arousal (the intensity with which the snail courts). Sexual proclivity and sexual arousal have different effects on feeding and locomotion and are differentially affected by sexual isolation, daily conspecific contact, and by a courtship pheromone found in the digitiform gland mucus. Therefore sexual arousal and sexual proclivity are probably mediated by 2 separate physiological mechanisms. Behavioural state, or the animal's general level of activity, correlates positively with mating behaviour. However, although a central system controlling behavioural state probably exists, it has no direct effect on either sexual proclivity or sexual arousal. Confusion over the term 'arousal', which impedes neuroethological research in this area, would be decreased by the adoption of the terms used in this thesis.

Résumé

"L'éveil" sexuel chez Helix aspersa peut être divisé en deux composantes: la disposition sexuelle (soit la tendance d'un escargot à faire la cour lors d'un contact avec un congénère), et la stimulation ou l'éveil sexuel proprement dit (c'est-à-dire l'intensité avec laquelle l'escargot fait la cour). Non seulement la disposition et l'éveil sexuels ont-ils des effets distincts sur les comportements d'alimentation et de locomotion, mais ils sont affectés de manière différente par l'isolation sexuelle, le contact quotidien avec les congénères, et par une phéromone reliée au comportement de cour que l'on trouve dans le mucus des glandes multifides. Il est donc fort probable que la disposition sexuelle et l'éveil sexuel soient servis par deux mécanismes physiologiquement distincts. L'état comportemental (le niveau général d'activité de l'animal), est en corrélation positive avec le comportement d'appariement. Cependant, bien qu'il soit possible qu'un système central contrôlant l'état comportemental existe, celui-ci s'avère sans effet, autant sur la disposition sexuelle que sur l'éveil sexuel. La confusion règnante en ce qui concerne le terme "éveil", et qui de fait freine la recherche en neuroéthologie dans ce domaine, se trouverait diminuée si l'on adoptait les termes utilisés dans cette thèse.

Preface

The research presented in this thesis, and the preparation of the thesis itself, are solely the work and responsibility of the candidate. However, in the experiments requiring blind controls, Barbara Tolloczko, Caroline Tidd and Shelley LaBerge assisted with the data collection.

The thesis chapters which have been published, or have been submitted for publication, are listed below:

Chapter 2. Adamo, S.A. and Chase, R. (1988), Canadian Journal of Zoology, 66: 1446-1453.

Chapter 3. Adamo, S.A. and Chase, R. (in press), Journal of Experimental Zoology.

Chapter 4. Adamo, S.A. and Chase, R. (in press), Behavioral and Neural Biology.

Chapter 5. Adamo, S.A. and Chase, R. (in press), Behavioural and Neural Biology.

Chapter 6. Adamo, S.A. and Chase, R. (submitted), Behavioural and Neural Biology.

Chapters 2, 3, 4, 5, and 6 are in the formats required of them by their respective journals.

Chapter 1 gives a general introduction to the topic and outlines the scientific problem addressed in this thesis. The discussion (Chapter 7) summarizes the results of the thesis in the context of the scientific question posed in the introduction.

Contributions to Original Knowledge

Below is a listing of the main contributions to original knowledge contained in this thesis:

1) The thesis gives a clear description of Helix aspersa sexual behaviour and copulation (Chapter 2) which previously had been poorly characterized.

2) It presents the first known function for dart shooting behaviour (Chapter 3). It demonstrates that the digitiform gland mucus, which coats the dart, contains a courtship pheromone.

3) It establishes that the phenomenon of 'arousal' can be divided into 2 distinct components, arousal and proclivity (Chapter 4, Chapter 5) and that this division can be usefully applied to other molluscs (Chapter 7).

4) It demonstrates that the relationship between sexual behaviour and feeding behaviour in Helix can best be described as a time-sharing arrangement (Chapter 5). This is the first time that this complex behavioural interaction has been shown in a mollusc.

5) It clarifies the meaning of the term central 'arousal' (Chapter 6). Chapter 7 shows that much of the controversy in the literature dealing with central 'arousal' is due to a confusion in terminology. Clarifying the term 'arousal' will aid neuroethologists in their study of motivated behaviours.

The following statement is added in accordance with the regulations of the Faculty of Graduate Studies and Research of McGill University:

> The candidate has the option, subject to the approval of the department, of including as part of the thesis the text, or duplicated published text (see below), of an original paper, or papers. In this case the thesis must still conform to all other requirements explained in Guidelines Concerning Thesis Preparation. Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient detail (e.g. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported. The thesis should be more than a mere collection of manuscripts published or to be published. It must include a general abstract, a full introduction, and literature review and a final overall conclusion. Connecting texts which provide logical bridges between different manuscripts are usually desirable in the interests of cohesion.
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> It is acceptable for thesis to include as chapters authentic copies of papers already published, provided these are duplicated clearly on regulation thesis stationery and bound as an integral part of the thesis. Photographs or other materials which do not duplicate well must be included in their original form. In such instances, connecting texts are mandatory and supplementary explanatory material is almost always necessary.
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> The inclusion of manuscripts co-authored by the candidate and others is acceptable but the candidate is required to make an explicit statement on who contributed to such work and to what extent, and supervisors must attest to the accuracy of the claims, e.g. before the Oral Committee. Since the task of the Examiners is made more difficult in these cases, it is in the candidate's interest to make the responsibilities of authors perfectly clear. Candidates following this option must inform the department before it submits the thesis for review.

Acknowledgements

Although it is established etiquette to thank one's supervisor for his time and expertise, my thanks to Dr. Ronald Chase are sincerely warm and heart-felt. He has always been supportive of me and my research. His keen ability to analyze, and his own integrity and imagination as a scientist, has provided me with an invaluable learning experience. I would also like to thank the members of my supervisory committee, Dr. Valerie Pasztor, Dr. Gerry Pollack, and Dr. Henry Reiswig for their encouragement and constructive criticisms.

I would like to thank Robert Lamarche and Guy L'Heureux for their advice and expert technical assistance with the photographic materials and with the laser printer. I would also like to thank Matthias Schmidt for his clear and literate translations of Meisenheimer (1912) and Syzmanski (1913).

I thank the Natural Sciences and Engineering Research Council of Canada for their financial support.

I would also like to thank my colleagues in the Chase Lab., especially Holly Bradshaw and Barbara Tolloczko, for their constant encouragement and for their gifts of both time and expertise.

Finally I would like to thank my relatives and friends, especially my parents and Jim Belanger, who were always willing to listen.

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Chapter 1. Thesis Introduction

Introductory remarks on the snail, Helix aspersa

Helix aspersa is a terrestrial snail found in many temperate parts of the world (Herzberg and Herzberg, 1962). Its distribution is restricted by its need for high humidity and moderate temperatures. Possibly due to its sensitivity to dry air, H. aspersa tends to be most active at night.

Snails are capable of aestivating when conditions become adverse. A snail will withdraw into its shell and seal the opening with a membrane to retard moisture loss. By aestivating when conditions are poor, snails can survive for several years, reproducing annually (Potts, 1975; Bailey, 1981).

H. aspersa is a general herbivore, grazing on a wide variety of plants (Ingram, 1946). In California its successful foraging abilities have led it to become a major agricultural pest (Ingram, 1946). Under laboratory conditions, snails will also feed on dead conspecifics (personal observations), and snails probably feed on dead animal tissue in the field as well.

Like all molluscs, Helix relies on a hydrostatic skeleton for the support of its body parts against gravity (Dale, 1973). The snail has a semi-open circulatory system with arteries leading blood away from

the heart. Blood returns to the heart via hemocoels, which are large, open, blood sinuses (Dale, 1973). During emergence from the shell, increases in blood pressure are needed to 'inflate' the body cavity before any active movement can occur. Blood pressure can be increased by constricting arteries to shunt blood flow, and, as is done during dart shooting, by partially contracting the body wall (Dale, 1973; Chung, 1986). Increases in heart rate, as well as in the amplitude of its contraction, may be involved as well (Lehman and Greenberg, 1987).

Coordinating the activity of the circulatory system with the rest of the snail, are the central and peripheral nervous systems. Molluscs have extensive peripheral nerve nets which have their own cell somata (Kandel, 1979). General coordination, however, is thought to be executed by the central nervous system. The central nervous system consists of a cerebral ganglion, which sits dorsal to the crop, as well as a series of 7 fused ganglia which are located immediately ventral to the crop. As in other molluscs, the central nervous system is amenable to neurophysiological analysis because it has relatively large cells, many of which are uniquely identifiable and have already been characterized (Pentreath et. al., 1982). This has made Helix, and other molluscs, notably Aplysia, popular as model systems

for the exploration of the biological basis of complex behaviours (Chase, 1986; Kupfermann, 1974).

Some of the most complicated behaviours performed by the snail are those involved in reproduction.

H. aspersa reproductive biology

H. aspersa is a simultaneously reciprocal hermaphrodite. Each snail has both male and female genital organs and an ovotestis which makes both sperm and eggs. It cannot self-fertilize; it must have access to foreign sperm for successful reproduction (Tompa, 1984).

The following paragraphs give a brief summary of Helix aspersa reproductive physiology. Fig. 1 in Chapter 2 gives a schematic outline of its reproductive organs.

Snails package their sperm in spermatophores prior to copulation. During copulation, each snail everts its penis and pushes it into its partner's genital atrium and bursa tract. The penis has a bulbous hook at one end to maintain the connection during spermatophore transfer. The spermatophore enters the bursa tract diverticulum and slowly dissolves, releasing and activating the sperm (personal observations; Lind, 1973).

The sperm swim down the tail of the spermatophore, back into the genital atrium, and then up the

spermoviduct. Sperm are stored in a fertilization pouch at the end of the spermoviduct (Lind, 1973).

Ovulation does not necessarily follow copulation and the 2 activities appear to be independently controlled. During ovulation, ova mature in the ovotestis and then pass through the hermaphroditic duct which conducts them to the fertilization pouch. Eggs are fertilized and then coated with a nutrient material made by the albumen gland. The eggs pass through the spermoviduct and are extruded out the genital atrium (Tompa, 1984). Snails require an average of 9 hours to complete egglaying (Herzberg and Herzberg, 1962).

Snails tend to be slow at completing all reproductive behaviours; copulation requires about 7 hours and courtship usually requires more than 30 min (Chapter 2). Because sexual behaviour requires a substantial time investment, the behaviour is likely to be ecologically costly (Peake, 1978). Nonetheless, sexual behaviour has evolved yet another feature, dart shooting, which adds an additional cost to reproduction.

In one of the most violent precopulatory behaviours known in the animal world, snails impale their partners with a 1 cm calcareous dart. The dart is propelled by the eversion of the muscular dart sac (Meisenheimer, 1912), and is coated by a mucus secreted by the digitiform glands (Chung, 1986).

Malacologists have speculated on the purpose of dart shooting for over a century (Tryon, 1882), but an actual function has never been demonstrated. This is partly due to the nature of the previous studies on H. aspersa courtship, which have tended to be anecdotal (i.e. Tryon, 1882; Taylor, 1900; Herzberg and Herzberg, 1962). One paper (Giusti and Lepri, 1980) does give a more quantitative description of the behaviour, but it is somewhat cursory, and does not discuss such phenomena as genital eversion stages, spermatophore transfer or the order and duration of the various courtship behaviours. Moreover, these previous studies often contradict one another, especially in their descriptions of dart shooting (Tryon, 1882; Goddard, 1962; Herzberg and Herzberg, 1962).

The questions of when and how dart shooting occurs during courtship have been largely resolved in a related species, Helix pomatia (Jeppesen, 1976; Lind, 1976). Unfortunately, although H. aspersa and H. pomatia are very similar in their ecology and internal anatomy, their mating behaviours are substantially different (Table 6, Chapter 2). Therefore, the sexual behaviour of H. aspersa must be described separately; it cannot be assumed that the characteristics of courtship and dart shooting will be identical to that found in H. pomatia.

In neither species is the function of dart shooting nor the physiological control of courtship known. One difficulty in studying the courtship behaviour of either species, is that, unlike other, simpler, behaviours, it cannot be reliably elicited by the same external stimulus.

The problem of behavioural variability

"Under the most rigorously controlled conditions of pressure, temperature, volume and humidity the organism will do as it damn well pleases." (p. 26, Weiss et al., 1982). Thus states the somewhat pessimistic Harvard Law of Animal Behaviour. It summarizes the common observation that any animal, from mollusc to mammal, will vary in its behavioural responsiveness to a stimulus, even under constant conditions. Some behaviours, such as feeding and sex, are notorious for the inability of the appropriate external stimuli to reliably induce them. Behaviours like sex and feeding are often called 'motivated behaviours' to distinguish them from the more simply controlled reflexive behaviours.

Initially, variability in an animal's behaviour, even for that observed in invertebrates, was ascribed to the workings of the animal's conscious intellect (i.e. Romanes, see Boakes, 1984). Later workers such as Loeb

and Watson advocated the view that animals were mere 'mindless machines' (see Kendler, 1987). This left the problem of behavioural variability in need of a mechanistic solution. In response, Craig, Lorenz, and Tolman developed concepts involving 'intervening variables' in an attempt to explain behavioural variability (see Kendler, 1987). An intervening variable 'explained' variations in behavioural responsiveness by inferring the existence of internal processes which resulted in the variability of the behaviour. For example, food deprivation increased the intervening variable called hunger, and this increased the animal's responsiveness towards food stimuli. Intervening variables tended to be specific for a particular motivated behaviour, such as feeding.

Adding to the behaviour-specific concept of intervening variables, Hull proposed that animals also possessed an internal process called 'drive', which determined the intensity of a behavioural response. Changes in the level of 'drive' resulted in fluctuations in the intensity of a behaviour. Unlike an intervening variable, Hull's general 'drive' was not specific for any particular behaviour, but had an effect on all motivated behaviours (Kendler, 1987).

Later, Hebb developed a concept of general 'arousal', an idea which was akin to Hull's theory of general

'drive' in that it too was non-specific and, when activated, resulted in increased responsiveness in all motivated behaviours. Hebb (1955) proposed that general 'arousal', which increased both the animal's general level of activity as well as its responsiveness to environmental stimuli, was mediated, at least in mammals, by a brain structure called the reticular formation. In this way, Hebb, unlike earlier workers, gave his motivational hypothesis a plausible physiological mechanism (Kendler, 1987).

At the same time, other workers were postulating the existence of a bewildering variety of 'drives' specific for a particular behaviour (Hinde, 1970). The definitions and the concepts behind the terms 'drive', 'arousal', and 'motivation' became (and, to a large extent, still are) confused. The concepts lacked well defined boundaries partly because the terms were never formally proposed, but had continued to change over the years as various experimenters tried to come to grips with the problem of behavioural variability (Kupfermann, 1974). Without well defined terms and basic concepts, the study of motivation floundered. Bolles (1975), reviewing the field of motivation in the psychological literature, found examples of theories of behavioural variability that invoked the concept of 'general drive', some that had only behaviour-specific 'drives', and some

which used neither general 'drive' nor behaviour-specific 'drives'. Halliday (1983) rather despairingly concluded that "...there are no unifying principles or widely accepted theories in the study of motivation. There is probably less consensus about the nature of motivational mechanisms than there is in any other area of ethology." (p. 130).

Controversy over specific and non-specific 'drives'

Despite the vagueness of most motivational terms, it was clear that a rigourous and testable distinction could be made between general and behaviour-specific 'drive' theories (Hinde, 1970). After extensive study, it was found that different measures of general 'arousal', such as activity level, responsiveness to stimuli, and brain activity, did not always correlate (Hinde, 1970). This suggested that these phenomena were not all positively modulated by the same underlying mechanism as was predicted by the general 'drive' or 'arousal' theory. This led to the collapse of Hull's theory of general 'drive'. After reviewing the evidence, McFarland and Sibly (1972) concluded that general motivational constructs such as 'arousal' or 'drive' accounted for an insignificant part of behavioural variability and that behaviours were controlled largely by mechanisms specific

to each motivated behaviour. After this, the concepts of general 'drive' or 'arousal' no longer played a prominent part in either psychological or ethological behavioural theories (Toates, 1986). Work was concentrated on motivational processes specific for a particular behaviour, such as feeding.

From this work arose a number of behaviour-specific motivational theories of animal behaviour (i.e. see Gallistel, 1980; Toates, 1986). Most postulate the existence of neural centres which are responsible for the control of specific motivated behaviours. Behavioural variability is partly explained in these theories by the way in which different behaviours interact with each other (McCleery, 1983).

Neurophysiologists, however, continued to work on the reticular formation, which is still viewed as exerting a general effect on an animal's behavioural responsiveness (Trulson and Jacobs, 1979). Work on circadian rhythms also stresses, and even assumes, that some mechanism of general 'arousal' exists (Zucker, 1983). As animals move from sleep to wakefulness, activity increases as does the responsiveness to all behavioural stimuli (Halliday, 1983). This work suggested that general motivational mechanisms make an important contribution to behavioural variability, contrary to the conclusion reached by McFarland and Sibly (1972).

Despite the lack of agreement over many issues in motivation, most workers believe a real understanding of the phenomenon will occur only once the physiological substrates mediating variability are understood. Unfortunately, studying the complex problem of behavioural variability in any animal is a daunting challenge, and technically impossible in most. Even the seemingly simple question: 'do general, as opposed to specific, motivational processes significantly influence behaviour?' has proven to be exceedingly difficult to answer in the standard animal of psychological research, the white rat. Technical problems, including the lack of identifiable neurons, have made a neurophysiological explanation of motivation slow in developing. Moreover, in higher animals, a question such as this is made even more complex by the ability of the animal to modulate its behaviour using higher order cognitive processes. In higher animals, any stimulus carries with it some 'affective' and 'learned' components which may differ for each animal and which can alter the animal's response (Epstein, 1982). To try to separate these factors, some physiologists, for example those working on drinking motivation, differentiate between primary drinking, which is due to tissue deficits, and secondary drinking, which is due to other 'causes' such as social factors (see

McFarland and Sibly, 1972). This technique has met with limited success.

A more manageable motivational model - the Mollusc

Molluscs, like mammals, show behavioural variability, but unlike mammals have both a numerically simpler and technically more accessible nervous system. Given the ubiquity of the problem of behavioural variability, it is likely that understanding a simpler system will give us insights into the possible basic mechanisms that operate in more complex systems. "If phenomena such as learning and motivation are emergent properties of aggregates of neurons, then motivation in higher vertebrates may involve no new principles over similar phenomena in insects and molluscs. The processes can be considered homologous entities, albeit in a more elaborate form. Thus motivational concepts are important in simple animals because they act as models for processes in more complex animals" (p. 140, McCleery, 1983). (McCleery, it should be noted, works on vertebrates).

Moreover, although invertebrates are capable of many complex learning tasks (see Mpitsos and Lukowiak, 1985) they are incapable of the type of information integration observed in the rat (Kandel, 1976). This means that there are fewer converging processes determining the

behaviour of invertebrates. This makes invertebrate behaviour less quixotic in its responsiveness to external stimuli. This in turn makes it easier to both define and measure motivational variables, 2 other distinct problems in motivational studies in vertebrates (Hinde, 1970).

For these reasons, molluscan models of motivation have proven to be very useful to neurobiologists in their search to understand the neural basis of behavioural variability (Weiss, et al., 1982). Progress has been steady, but, as was the case with vertebrates, controversy has arisen over the question of the relevance of 'arousal' as a general motivational variable.

Controversy over specific and non-specific 'drives' in molluscs

Despite the unpopularity of the concept of a general motivational variable with behaviourists, Kupfermann and Weiss (1981) postulated the existence of a central arousal system in Aplysia that is capable of affecting diverse behaviours in the animal. Their concept of 'arousal' harks back to the ideas of Hebb (1955) who postulated the existence of a central 'arousal' system which was excited by novel or noxious stimuli and which in turn positively modulated a wide variety of

behavioural responses. For example, Dieringer et al. (1978) define 'arousal' in Aplysia very similarly as "... an intervening variable that influences general activity and responsiveness of the animal." (p. 20).

As in vertebrates, the hypothesis of central 'arousal' in molluscs like Aplysia was partly inspired by the fact that they vary in their responsiveness to stimuli and in their level of activity over a 24 h period (Kupfermann, 1974). Moreover, autonomic functions like heart rate seem to correlate with the level of activity (behavioural state) as well as with responsiveness to external stimuli such as food (Dieringer et al., 1978). Susswein (1984) found that food deprivation, which decreases the time Aplysia spend immobile, increases both food responsiveness (Kupfermann, 1974) and the amount of time spent mating. Therefore, deficits in one motivated behaviour appear to affect the behavioural response thresholds of other motivated behaviours. Presumably some central system is responsible for the correlation of these diverse activities. And, as in vertebrates, noxious stimuli such as tail pinching or handling result in an increase in responsiveness to food stimuli (Kupfermann and Weiss, 1981). Therefore noxious stimuli seem to have 'general' effects which could best be explained by the assumption that they are mediated by a

system that can modulate a wide variety of diverse behaviours.

Weiss et al. (1982) also propose the existence of an executive arousal system to mediate behaviourally specific aspects of 'arousal'. For Weiss et al. (1982) both a central 'arousal' system and an executive arousal system have neural correlates and both are important in the determination of behaviour. Kupfermann and Weiss (1981) compare their executive arousal system to the hypothalamus and peripheral autonomic system in vertebrates. Central 'arousal' in Aplysia has been likened to the sympathetic nervous system in vertebrates (Palovcik et al., 1982).

Over the past 15 years, dedicated researchers have successively uncovered much of the neural basis of the executive arousal system for feeding (reviewed in Weiss et al., 1982). Cells have been found that have the correct input and output functions to serve as mediators of food arousal (Teyke et al., 1990). However, the neural/hormonal components of the central 'arousal' system remain undiscovered. The only suggestion as to the physiological identity of a central 'arousal' system is Palovcik et al.'s (1982) hypothesis that central 'arousal' is mediated by serotonin. Serotonin positively modulates both the level of activity as well as the responsiveness to food stimuli in both Aplysia and

Pleurobranchaea. Serotonin is thought to have a general 'arousing' effect in a wide variety of invertebrates (i.e. Lymnaea, Tuersley and McCrohan, 1988; leech, Willard, 1981; lobster, Kravitz, 1988).

This lack of concrete physiological evidence for a central 'arousal' system has led to the proposal of alternatives. As in vertebrates, some workers rely on specific arousals or drives to account for behavioural variability, and deny the existence of a central 'arousal' system. Leonard and Lukowiak (1986) suggest that Aplysia and Navanax (Leonard and Lukowiak, 1984) have separate and independent drives controlling each behaviour. Their idea strongly resembles the hierarchical assemblies of Baerends (1976). In their hypothesis, behaviours are defined functionally (i.e. feeding, sex,). A behaviour, such as feeding, is recognized as having a number of sub-units (i.e. swallowing) that belong to 'feeding'. These sub-units may be involved in more than one behaviour, but each functionally defined behaviour is centrally (and separately) controlled. It differs from the central 'arousal' model in that the control of and effects of different behaviours are mediated by systems distinct for each behaviour, and not via a central system. For example, the separate 'drive' hypothesis predicts that neurally active substances like serotonin or SCPb will be

behaviourally specific in their effects, and will not affect behaviour globally.

These differing predictions have been examined in the interaction of feeding and sexual behaviour in Aplysia. Susswein (1984) nas postulated that a central 'arousal' system positively modulates both feeding and sexual behaviour in Aplysia, as well as the autonomic responses related to the performance of the 2 behaviours. However, Colebrook et al. (1984) have shown that the suppression of the gill withdrawal reflex (GWR), which can be induced by either food satiation or copulation, is mediated by 2 different peptides, arginine vasotocin (AVT) and metenkephalin. They concluded from this that feeding and sexual behaviour exert effects on other behaviours via separate physiological mechanisms. Therefore they reject the hypothesis of Palovcik et al. (1982) and Weiss et al. (1982) that behaviours exert 'motivational' effects via a common, central system and instead postulate that each behaviour has its own mechanism.

The concept of a central 'arousal' system has suffered other attacks as well. One of the major pieces of supporting evidence for a central system is the ability of nonspecific stimuli to elicit diverse behavioural effects (Kupfermann and Weiss, 1981). However, the same non-specific stimulus, handling, that increases

responsiveness to food stimuli in Aplysia has no effect on feeding in either Navanax (Susswein and Bennet, 1979) or Lymnaea (Tuersley and McCrohan, 1987). This raises the question of the concept's general applicability.

An attempt at synthesis

The following study examines the variability in the sexual responsiveness of the terrestrial mollusc, Helix aspersa. It tests to what extent the concept of central 'arousal' is important in accounting for the observed variability.

Because Helix is a terrestrial mollusc with a dissimilar life history from that of Aplysia, a marine mollusc, it also offers an opportunity to examine the extent to which a central 'arousal' system may be influenced by an animal's ecology.

Moreover, virtually all of the work concerning motivation in molluscs has dealt with feeding behaviour. But feeding behaviour is often viewed as a different kind of motivated behaviour from that of sexual behaviour (Toates, 1986). Feeding behaviour is considered a 'homeostatic' behaviour in that the animal must maintain certain nutritional levels to survive. Food deprivation causes measurable physiological deficits (see Toates, 1986). Not surprisingly the control of feeding

behaviour, and hence its 'motivation', is usually modelled as a negative feedback loop (Toates, 1986; but see Hogan, 1980, for a dissenting view of homeostasis and motivation). Sexual behaviour on the other hand, is usually considered as a typical example of a 'non-homeostatic' behaviour. Sexual deprivation does not result in any physiologically measurable deficit (Halliday, 1983). For this reason, its mechanisms of control may differ from that of feeding, as there is no obvious physiological route for a negative feedback mechanism. This may alter the relevance of a central 'arousal' system to the control of sexual behaviour as opposed to feeding behaviour.

This study begins with a description of the sexual behaviour of Helix aspersa (chapter 2). Chapter 3 explores the effects of dart shooting on courtship and sexual arousal.

Equally important, the slippery concepts of 'arousal', central 'arousal', and other motivational variables are defined and given rigourous operational definitions in the subsequent chapters, particularly in chapter 4. As we will see in the general discussion, much of the controversy over the relevance of specific and non-specific 'drives' or 'arousals' in molluscs is due to a misunderstanding of what is meant by the term central 'arousal'.

The last three chapters explore the effects of different treatments and behaviours on sexual behaviour as well as the effects of sexual behaviour on the expression of other behaviours such as feeding. This allows the assessment of the likelihood that different behaviours are modulated by a common system.

Chapter 2. Courtship and Copulation in the Terrestrial Snail, *Helix aspersa*

Abstract

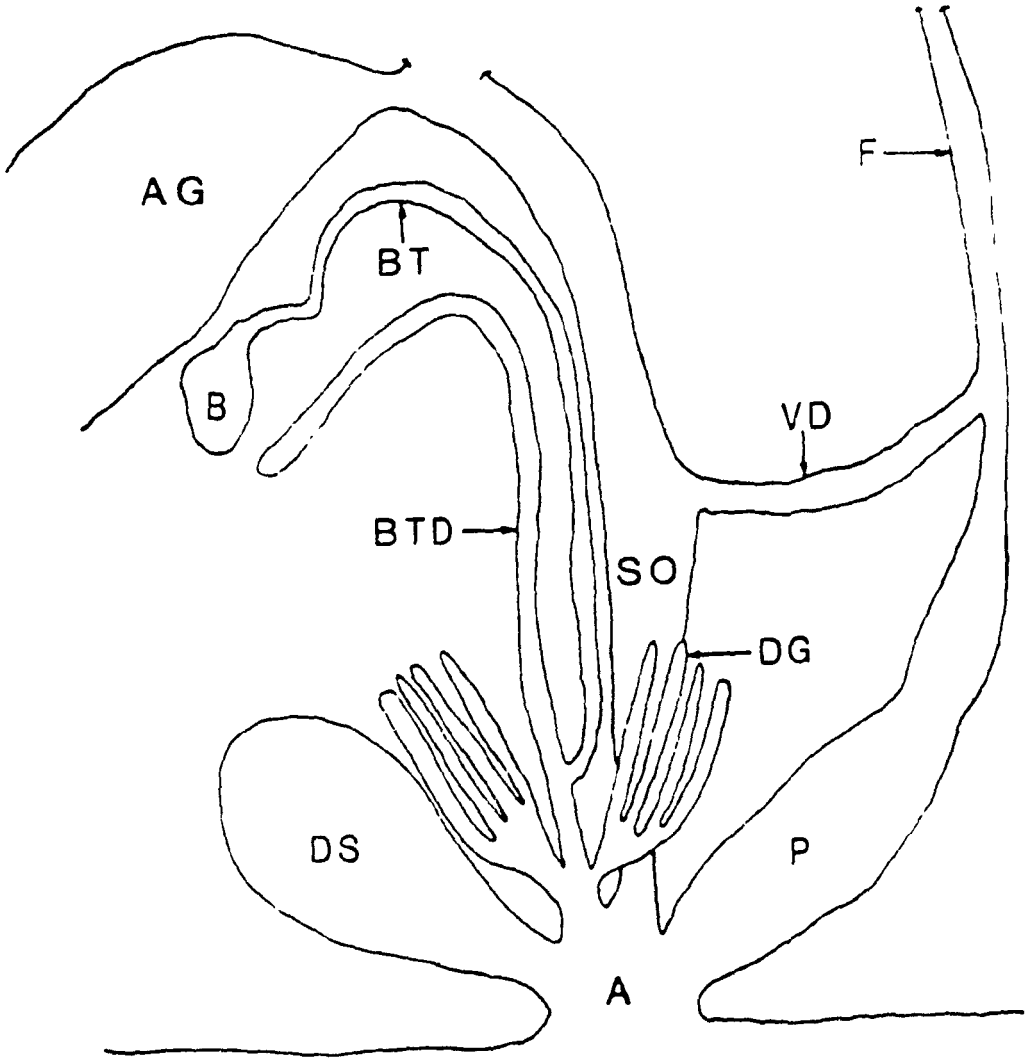
Mating behaviour in Helix aspersa has three major components: introductory behaviour, dart shooting and copulation. Introductory behaviour, which includes reciprocal tactile and oral contacts, lasts an average of 33.7 +/- 23.3 (s.d.) min. Dart shooting, the pushing of a calcareous dart into the mating partner's body, occurs once for each snail per mating sequence. Snails that hit their partners during the first dart-shooting event copulated 14.5 +/- 8.7 min after dart shooting whereas snails that missed their partners took 40.5 +/- 37.5 min to copulate. Dart shooting may facilitate mating by increasing behavioural synchrony. Copulation is reciprocal and has a duration of 421.8 +/- 56.6 min. Spermatophores are transferred approximately 300 min after simultaneous intromission. There are significant differences in the mating behaviours of Helix aspersa and Helix pomatia.

Introduction

Helix aspersa, a simultaneously reciprocal hermaphrodite, has a complicated mating behaviour that has intrigued biologists for over a century. However, previous accounts of courtship and copulation in H. aspersa give an incomplete or poorly quantified description of the behaviour (Tryon 1882; Herzberg and Herzberg, 1962; Giusti and Lepri 1980). This is surprising, given the large amount of work that has been published on the reproductive physiology of this species (see Gomot and Deray (1987) for a review). In the present paper we attempt to provide a more complete description of the mating behaviour of H. aspersa. Such knowledge is especially desirable in view of recent observations suggesting that the neural machinery responsible for mating behaviour in this species may be amenable to experimental analysis (Chase 1986).

Like its behaviour, the reproductive anatomy of H. aspersa is complex (Fig. 1). One of the most enigmatic structures is the dart apparatus, which consists of a muscular sac containing a calcareous, bladed dart. The dart is pushed into the partner during courtship, and is used only once (Tompa 1982). It regenerates once every 6 days under laboratory conditions (Tompa 1982). The dart

Figure 1. Reproductive anatomy of Helix aspersa (adapted from Tompa, 1982). The diagram is schematic and not drawn to scale. Features posterior to the spermoviduct have been omitted. A, atrium; AG, albumen gland; B, bursa; BT, bursa tract; BTD, bursa tract diverticulum; DG, digitiform gland; DS, dart sac; F, flagellum; P, penis; SO, spermoviduct; VD, vas deferens.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is composed of a protein matrix and aragonite, a form of calcium carbonate (Hunt, 1979). Darts used in mating are found in 11 out of 65 pulmonate families (Tompa 1980), and the dart apparatus may have evolved independently in seven major lineages (Chung, 1986). If this is so, it suggests some adaptive value for the dart. Its function, however, remains unclear. Its significance for the mating of H. aspersa is investigated in the present study.

Methods and Materials

Specimens of Helix aspersa were imported from California. After 1 week of acclimation in a colony box (30 x 32 x 36 cm), test snails were individually housed in Lucite containers (7 x 8 x 8 cm). They were fed lettuce and carrots ad libitum. A damp paper towel lining the bottom of each container kept the snails moist. They were maintained on a 16L:8D light cycle. The temperature was kept at 20° +/- 2°C. The containers were cleaned once weekly. Each animal was numbered with nail polish or adhesive tape.

Snails were isolated from one another 3 d - 3 weeks before mating trials, with most snails (76%) being isolated for 10 d. During the trials, 10 - 14 snails were placed together in a Lucite observation chamber (18 x 18 x 8 cm). As a pair began to court, they were

removed from the initial observation chamber and placed in a second container (18 x 18 x 8 cm). The phases of the mating behaviour were timed and recorded. During a trial, 62 +/- 21% of the animals eventually copulated (n=14 trials), though the last pair often started to court more than 1 h after the first pair. There were no significant differences in the mating times of animals that mated first and those that mated last. Also, the number of days the snails had been isolated had no significant effect on mating times. Therefore, the data for all pairs of animals have been treated as equivalent and averaged for statistical purposes. Ten mating sequences were videotaped for a more detailed analysis.

Results

The temporal pattern of the courtship behaviour is shown in Fig. 2. A prominent feature is the repetition of different components of the behaviour. The duration and frequency of the different components varies widely between mating pairs. Some components are always present (i.e., L, LG, C, and P). However, dart shooting and biting do not occur in some sequences. The sequence in Fig. 2 is considered representative because it contains all of the common behavioural components.

Mating can be divided into three distinct phases: introductory behaviour, dart shooting and copulation.

Figure 2. Temporal pattern of a representative mating sequence. The two lines represent a single continuous sequence, showing the behaviours of the mating snails, A and B. Each indicated behaviour continues until the next indicated behaviour. Behaviours lasting less than 30 s have been omitted, except for biting and dart shooting. Most behaviours are expressed reciprocally, but one animals often leads the other by a few seconds. The temporal resolutior shown does not allow for the depiction of such discrepancies. A, apposed atria; B, biting; C, circling; CO, copulation; DS, dart shooting; L, lip-lip; LG, lip-genital atrium; P, pause.

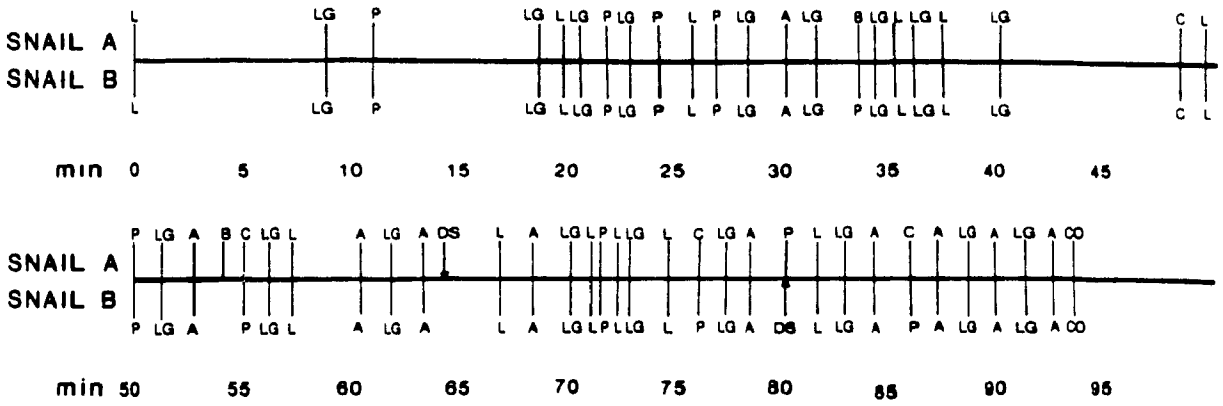

SNAIL A
SNAIL B
min 0 5 10 15 20 25 30 35 40 45
SNAIL A
SNAIL B
min 50 55 60 65 70 75 80 85 90 95

Table 1 gives the average duration of each phase of the mating behaviour. Descriptions of each phase are given below.

Introductory behaviour

When the two receptive animals first make contact, each turns and orients itself head to head with its partner, touching the other with it tentacles. After the initial contact, one or both snails move forward and each raises is forefoot slightly until the mouths touch (lip-lip, Fig. 3A). The head moves slightly from side to side.

Normally, after lip-lip behaviour has continued for 30 s - 3 min, the genital apparatus begins to evert. The eversion has six stages, in each of which an increasing amount of the normally internal genital atrium is extended outside of the body wall. The stages, revised from Chung (1986), are described in Table 2 and illustrated in Fig. 4. During introductory behaviour, the eversion can vary from stages 0 to 5.

After 30 s - 12 min in the lip-lip position, both animals move their heads to their partner's right. Each moves its mouth over the other's partially everted genital atrium (lip - genital atrium, Fig. 3B). In 18 of 55 observed mating sequences, one or both animals bit their partner on the atrium during lip- genital atrium behavior. The bitten animal briefly withdrew, but

TABLE 1. Duration of the phases of mating behaviour and their dependence on the outcome of the two dart-shooting episodes

	Introductory behaviour to dart shooting by first animal	Dart shooting by first animal to dart shooting by second animal	Dart shooting by second animal to copulation	Copulation*
All snails†	33 7 ± 23 3 (n = 36)	25 7 ± 28 7 (n = 36)	8 1 ± 6 2 (n = 34)	421 8 ± 56.6 (n = 22)
First dart shot hits partner	35 7 ± 15 9 (n = 21)	14 5 ± 8 7*a* (n = 21)	7 5 ± 5 6 (n = 21)	417 0 ± 54 1 (n = 16)
First dart shot misses partner‡	32 1 ± 31 4 (n = 14)	40 5 ± 37 5*a* (n = 14)	8 8 ± 6 4 (n = 12)	427 2 ± 82.6 (n = 5)
Second dart shot hits partner	—	—	7 7 ± 5 7 (n = 11)	415.6 ± 61.3 (n = 9)
Second dart shot misses partner‡	—	—	9 4 ± 7 1 (n = 8)	426.8 ± 57.6 (n = 4)

NOTE Values are given as mean duration ± SD (min) Values within a column followed by the same letter are significantly different, $p < 0.05$ (Mann–Whitney test two-tailed or Kruskal-Wallis test two-tailed)

*Measured from the appearance of the copulatory posture to the withdrawal of both penes

†Includes some animals not found in any of the subsequent four rows

‡Includes those couples that did not fire a dart during courtship

Table 2. Stages of genital eversion.

Stage	Description
0	No eversion. Sexually refractory animals have an almost invisible genital pore.
1	Genital pore bud. The genital atrium begins to protrude.
2	Moderate eversion of penial lobe.
3	Full eversion of penial lobe. Penial opening visible.
4	Full genital eversion. Both penial and vaginal lobes are clearly visible.
5	Bulging of the vaginal lobe.
6	The penis is maximally everted from the penial lobe.

Figure 3. Characteristic episodes in the mating behaviour of Helix aspersa. The episodes are arranged in the order of their normal appearance, but the pictures are not all of the same pair. (A) Lip-lip contact. Animal on the left has a stage 2 eversion. (B) Lip-genital atrium contact. (C) Dart shooting. Arrow points to the base of the shot dart. The shaft of the dart has penetrated the recipient's body wall. The tissue that propelled the dart into the recipient is being retracted into the animal. (D) Apposed atria. Arrow points to the artially everted penis. (E) Copulatory posture. The snails are approximately 6 cm in body length when fully extended.

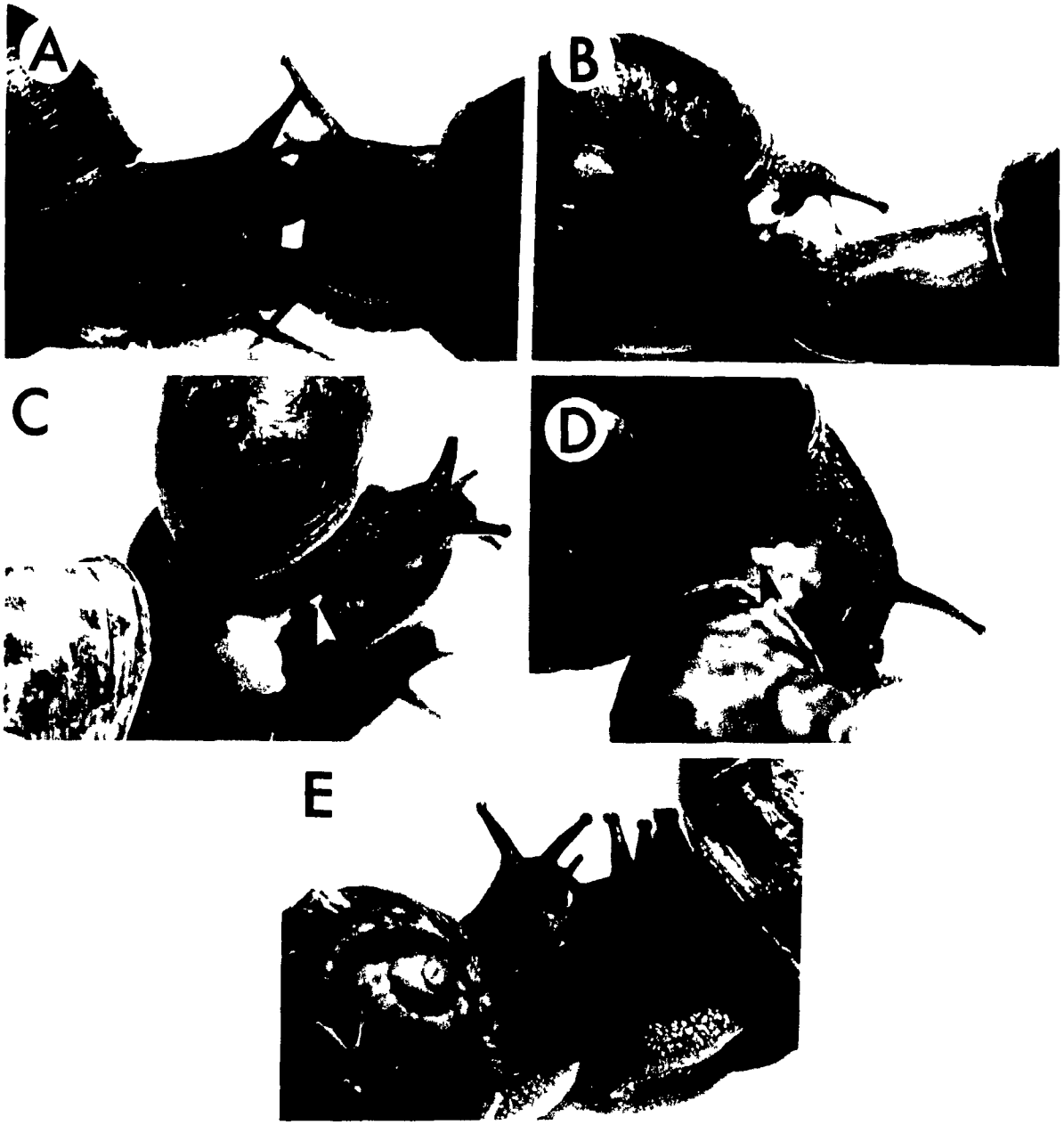
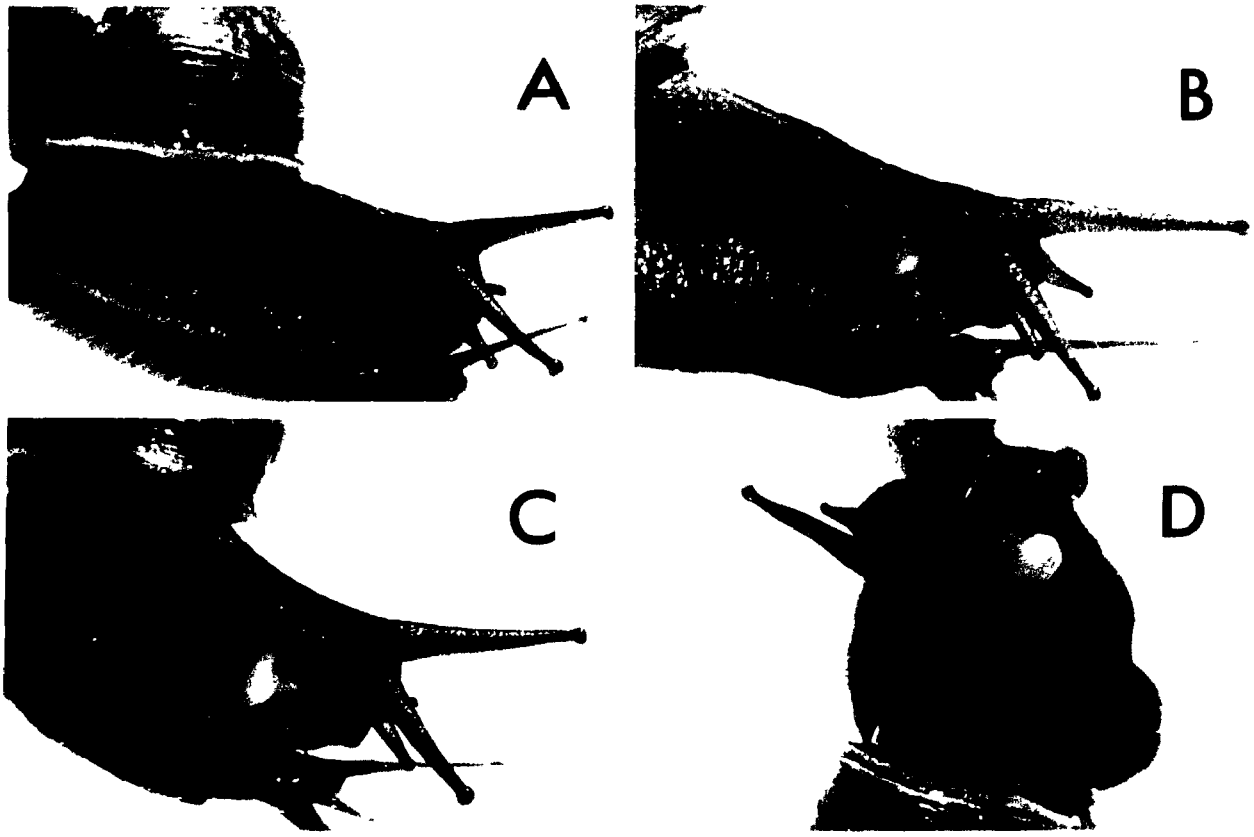

A
B
C
D
E

Figure 4. Genital eversion stages. (A) Stage 1. The genital pore is lighter in colour than the surrounding tissue because it has started to protrude beyond the body wall. (B) Stage 3. The penial lobe, the posterior lobe of the genital apparatus, is fully extended. (C) Stage 4. Both penial (posterior) and vaginal (anterior) lobes are visible. (D) Stage 5. Bulging of the vaginal lobe. The animal is in the pre-dart shooting posture, with a contracted anterior body and a bulging posterior body.


A
B
C
D

reemerged within 10 s and the lip - genital atrium behaviour continued.

Every mating sequence contains brief breaks in mutual contact (pause, Fig. 2). Usually during lip - genital atrium contact, but also during lip - lip contact, the animals withdraw from each other for 5 - 105 s (mean 26.2 +/- 25.2 (s.d.) s n=136). They often remain motionless before re-establishing contact.

Longer breaks also occur during mutual contact. During lip - genital atrium or lip - lip episodes, a snail often turns away from its partner and travels in a full circle or in one or more semicircles (S-shaped path). The circles have a mean radius of 3.0 +/- 1.2 (s.d) cm (n=28) and the radii range from 2.0 to 6.0 cm. The mean duration of the circling behaviour (contact to contact) is 105.0 +/- 54.1 (s.d.) s (n=28). The stage of the genital eversion usually decreases during pause or circle behaviour and increases again when contact is re-established. The temporarily abandoned partner usually remains motionless, except for moving its head from side to side, unless it too is circling. About 14% of the time the partners 'lose' each other while circling and courtship ceases (n=55). Circling behaviour may be one of the methods H. aspersa uses to realign itself while courting.

Usually after lip - genital atrium episodes, but sometimes also after lip - lip episodes, each snail pushes its everted atrium against that of its partner (atrial apposition, Fig. 3D). The animals can copulate only when they are in this position.

Dart shooting

About 30 min after the start of introductory behaviour, the animals adopt a pre-dart shooting posture (Table 1). During this time, the vaginal lobe bulges (stage 5 eversion). A ring of body wall around the dart sac and atrium begins to contract. The body anterior to the genital pore contracts and consequently the posterior portion nearest the shell begins to bulge with accumulated blood (pre-dart shooting posture, Fig. 3D). A midsagittal crease forms along the bottom of the foot just behind the head. (A similar fold is seen during penile eversion.) The animal stops locomoting, then pushes against its mate, and the dart, a hollow calcareous lancehead with four blades (Hunt, 1979), is forced into the body of the partner. The force comes from the eversion of the papilla, a tissue attached to the base of the dart (Meisenheimer, 1912). The everting tissue pushes the dart of out of its sac and into the recipient (Fig. 3C). The dart carries approximately 2 mg of white mucus secreted by the digitiform glands (Chung, 1986). The recipient briefly withdraws (< 10 s) and then

Table 3. Outcomes of dart shooting behaviour in 61 courting snails.

Outcome	Number of observations
Hit partner	43
Penetrated body wall	22
Dart retracted by shooter	11
Incomplete penetrance of body wall[1]	10
Missed partner	5
Shooting behaviour but no dart fired[2]	2
Pre-dart shooting posture but no dart fired[2]	8
Neither pre-dart shooting posture nor dart fired[3]	3

[1]Darts eventually expelled

[2]Snails were found to have empty dart sacs

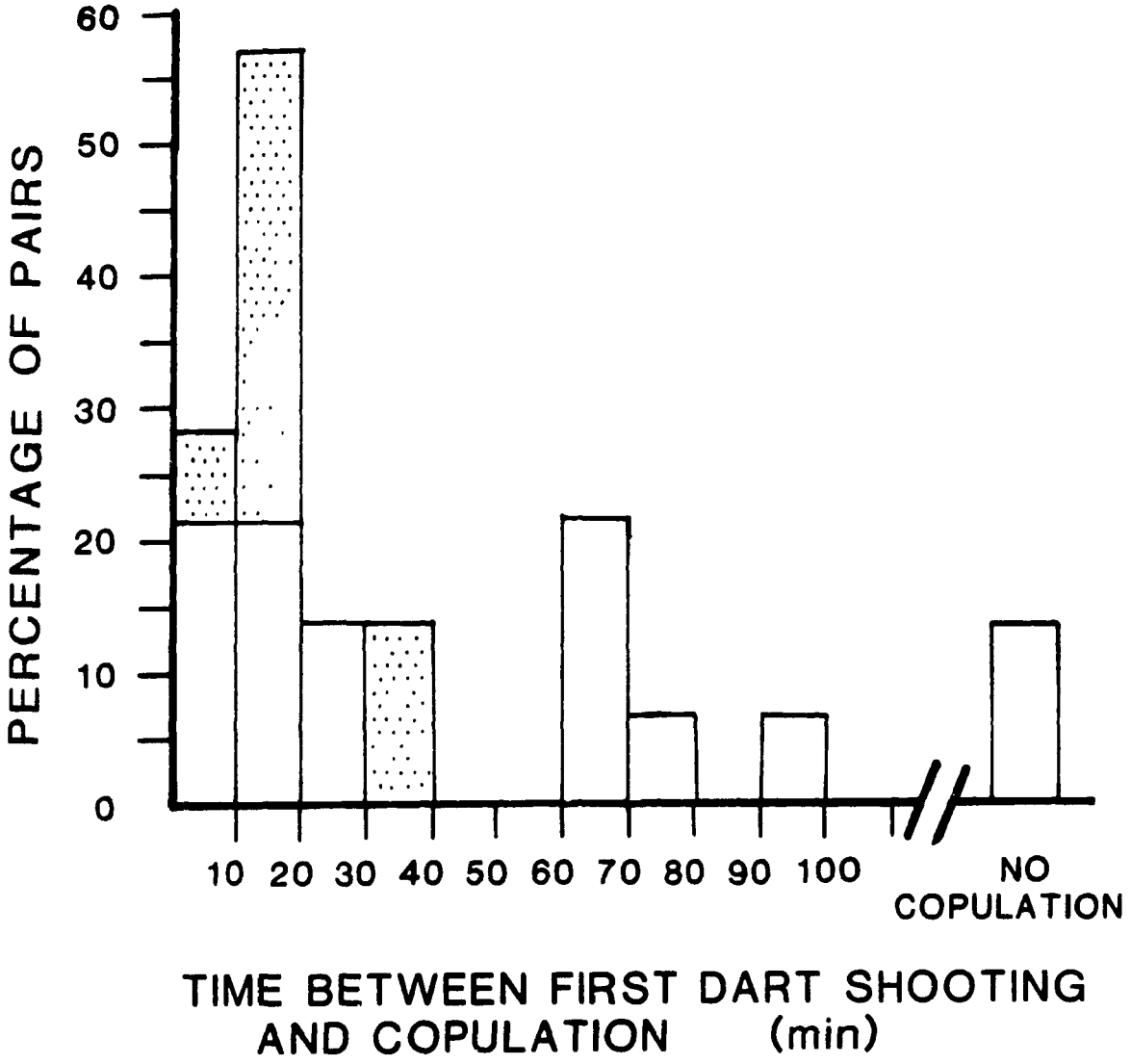
[3]Snails were found to have loaded dart sacs

Table 4. Dart locations in 90 dissected snails. Snails were taken from the colony cage and were not engaged in mating at the time of dissection.

Location	Number of snails
Dart sac	71
Bursa tract	2
Bursa diverticulum	22
Body cavity[1]	4
Underneath albumen gland[1]	57
In mantle cavity but not underneath albumen gland[1]	1

[1]Probably foreign darts

Figure 5. Effect of success or failure of the first dart-shooting event on the latency to copulation after dart shooting. Dotted columns (n=21) show the latencies of couples in which the dart hit the target snail; open columns (n=14) show the latencies of couples in which the dart missed the target snail.


PERCENTAGE OF PAIRS
60
50
40
30
20
10
0
10 20 30 40 50 60 70 80 90 100
NO COPULATION
TIME BETWEEN FIRST DART SHOOTING AND COPULATION (min)

both animals resume mating. The shooter shows full penile eversion (stage 6) simultaneously with, or shortly after, shooting the dart. A stage 6 eversion is never reached before dart shooting.

Tactile stimulation of the atrium is required to trigger the dart-shooting behaviour. Consequently, 42% of dart shootings occur during atrial apposition (n=95). In this position, each animal is exerting pressure on the other's everted atrium. However, in 21 of 95 observations made, while the shooter was pushing against the recipient snail, the recipient crawled over the shooter's everted atrium, causing the dart to be delivered to the bottom of the foot.

Usually, the second animal shoots a dart within 30 min after the first animal has done so (Table 1). Each snail exhibits dart-shooting behaviour only once per mating sequence.

Table 3 reports the fates of 48 darts shot from 61 courting snails. Most (43) penetrated the partner. Ten snails retracted their darts after shooting them into their partners. In 7 of the 10, the retracted dart hindered copulation by blocking the female opening. The retracted dart was never returned intact to the dart sac. Long after copulation, retracted darts could be found in the bursa tract diverticulum, usually ahead of the spermatophore (Table 4). The dart point was towards the

atrium, indicating that it was the animal's own retracted dart. The darts are probably dissolved in the diverticulum.

Eight snails showed no dart-shooting behaviour, even though they did assume a pre-dart-shooting posture (Table 3). All of these snails had empty dart sacs when dissected immediately after copulation. Three snails showed neither pre-dart-shooting posture nor dart-shooting posture, despite the fact that they were found to have darts in their dart sacs when dissected immediately after copulation.

Received darts accumulate in the mantle cavity below the albumen gland. Very few darts are shot into the mantle cavity, therefore used darts must move into the area after entering the animal. How the darts are transported is unknown. The darts appear to be degenerating there. They have a reddish brown coating which resembles in colour the substance found in the bursa, a known site of spermatophore digestion in H. pomatia (Lind, 1973).

Snails that do not shoot a dart, or who miss their partners during the first dart-shooting event, take approximately twice as long to achieve copulation after shooting a dart compared with those that shoot successfully (Table 1, Fig. 5). The difference is due to the fact that animals that fail to receive a dart during

the first dart-shooting event take approximately 3 times as long to fire their own dart compared with those that do receive a dart from their partners (Table 1). Once both animals have shot darts, the time to copulation is short, whether or not the second dart successfully hits its target (Table 1). Therefore, the second dart does not affect the timing of copulation.

Copulation (intromission and spermatophore transfer)

After shooting their darts, the animals evert and withdraw their penes frequently, usually after tactile stimulation of the atrium by the mating partner (Fig. 3D). The behavioural phases exhibited include lip - lip, lip - genital atrium, apposed atria, penile eversion, and pause or circle (Fig. 2). Duration and order can vary considerably. After both animals have shot their darts, pauses become significantly less frequent. During introductory behaviour, pauses occur at a mean interval of 53.8 +/- 46.2 (s.d.) s (n=10 sequences), whereas after dart shooting (within the last 10 minutes before copulation), pauses occur at a mean interval of 372.2 +/- 126 s (n=10) ($p<0.01$).

Courting animals can make as many as 50 intromission attempts, or a few as 1, before achieving copulation (mean 11.0 +/- 8.3 (s.d.) (n=10). Copulation does not occur unless intromission is simultaneous, though often the first simultaneously intromission lasts only a few

seconds and does not lead to spermatophore transfer. Usually only the second or third simultaneous intromission leads to successful spermatophore transfer.

Approximately 30 s after simultaneous intromission, the animals become quiescent and assume a characteristic copulatory posture (Fig. 3E). The tentacles withdraw to half extension and the forefoot is slightly raised. After 30 min - 2 h, the tentacles are completely withdrawn and they remain so for the duration of copulation.

Before copulation, the flagellum and the penis are empty. Two minutes after intromission, dissected animals have a spermatophore in their flagellum, but is does not yet contain sperm. The spermatophore has a foretail 2-3 cm long, a body length of 0.6 - 0.8 cm, and a posterior tail 7 - 11 cm long and thinner that the foretail. After 2 - 6 h, the spermatophore, now containing sperm in the body segment, enters the penis.

Spermatophore transfer is usually 4.5 - 6.0 h after intromission (Table 5). Spermatophores were never observed to be transferred simultaneously, although in most cases the members of a pair transferred their spermatophores within 1 h of each other. The spermatophore moves from the penis into the partner's bursa tract diverticulum. Most snails (15 of 18) have transferred their spermatophore 300 min after

Table 5. Spermatophore transfer. The placement of the letters indicates the location of the body of the spermatophore at the time of dissection. Individual members of a single copulating pair of snails are designated by the same letter.

Time from start of copulation (h)	Location of Spermatophore Body			
	Donor's flagellum	Donor's penis	Recipient's bursa diverticulum, body cavity portion	Recipient's bursa diverticulum, mantle cavity portion
Snails dissected during copulation				
0.05	a,a,b,b			
1.5	c,c,d,d			
2.0	e,e	f,f		
3.0	g	g,h,h		
3.5	i,i			
4.0	j,j,k,k		l	l
4.5	m,n,o,p,p	m,o,q,r	n,q,r,s,s,t,t	
5.0	u		u	
5.5		v	v,w,w	x,x
6.5		y	y,z,z,A,A,B,B,C,C	
Snails dissected after copulation				
15.0				D,D
24.2			E	E,F,F
45.8				G,G,H,H
68.0			I	I

intromission, or about 100 min before copulation ends. The spermatophore moves up the diverticulum and is dissolved (Table 4). Again the speed of transport differs between animals (Table 5).

The duration of spermatophore transfer cannot be stated exactly. From Table 5 it appears that snails take 2 - 4 h to move the spermatophore from the penis into the partner's bursa tract diverticulum.

A full spermatophore contains a yellowish paste filled with sperm. The paste is no longer visible 24 h after intromission, and the spermatophore becomes a hollow shell with white mucus inside. The mucus still contains sperm. After 68 h, the spermatophore is thin and transparent and has begun to dissolve.

The inhibition of locomotion during copulation is not complete. The animals will partially withdraw into their shells if disturbed. Also, one member of a pair of copulating snails may occasionally attempt to locomote away from its partner, but after tugging on the mutual connection, it usually returns to quiescence in less than a minute. In one case, an animal lay on top of its partner, making it possible for the partner to move without tugging on the interlocked penes. The partner locomoted 6 cm. Snails separated before spermatophore transfer can locomote while excreting the spermatophore. If separated after spermatophore transfer, they can

Table 6. Major differences between the mating behaviours of *Helix aspersa* and *Helix pomatia*.

Feature	*H. aspersa*	*H. pomatia*[1]
Duration of introductory behaviour	33.7 ± 23.3 min	70 - 180 min (range)
Frontal upright posture	absent	invariant part of introductory behaviour
Circle behaviour	common	absent
Biting	common	rare
Duration of shooter's pause after dart shooting	< 1 min	typically > 8 min
Posture during copulation	apposed atria	spiral upright
Number of mutual intromissions before copulation	typically < 3	typically > 5
Contact with partner required to maintain immobility[2]	yes	no
Time of spermatophore transfer	approx. 300 min after intromission	approx. 4.5 min after intromission
Copulation duration	421.8 ± 56.6 min	approx. 5 min

[1]Data for *H. pomatia* taken from Lind (1973, 1976) and personal observations.

[2]Immobility in *H. aspersa* lasts as long as copulation; in *H. pomatia* it continues after copulation until the spermatophore tail has become internalized.

locomote while internalising the tail. Animals could usually be separated during copulation without apparent injury, contrary to the reports of Herzberg and Herzberg (1962) and Giusti and Lepri (1980).

At the end of copulation, each animal withdraws its penis. This withdrawal is usually not simultaneous and the pair will often remain unilaterally joined for more than 30 min. Both animals remain quiescent, however, until both penes have been withdrawn. Within a few minutes of decoupling, both snails begin to locomote, even if one or both of the spermatophore tails have not been completely internalized. The 1 - 3 cm of uninternalised tail is taken up within an hour.

Discussion

Because the behaviour of snails is easily observed, and the mating sequences have bizarre features, several earlier studies, such as those of Tryon (1882), Herzberg and Herzberg (1962), and Giusti and Lepri (1980), have described aspects of mating behaviour in Helix aspersa. By contemporary standards, much of the older literature is woefully inadequate. For example, Tryon (1882) reported that the darts are fired at the moment of copulation, but this is clearly not true. Herzberg and Herzberg (1962) pointed out the error, but they failed to specify precisely when the darts are released.

The most detailed of the previous accounts is that of Giusti and Lepri (1980). In general, our observations agree with theirs. They also noted circling behaviour, biting, the tactile contacts during introductory behaviour, and the high percentage of darts that hit their target. In some cases, however, direct comparisons are difficult. For example, they state that copulation ranges between 2 h 10 min and 7 h 8 min in duration. As the average is not given, it is difficult to judge whether the snails that we observed have equivalent copulation times. Similarly, they note the independence of mating times and the receipt or nonreceipt of a dart, but because they do not differentiate between first and second dart-shooting events, a direct comparison with our results is not possible.

Comparison with Helix pomatia

As expected, there are many points of similarity between the mating behaviours of H. aspersa and H. pomatia. For example, during introductory behaviour, both species utilize reciprocal and recurrent tactile contacts and both species exhibit the unusual dart-shooting behavior. However, in several important respects, the mating behaviour of H. aspersa differs substantially from that of H. pomatia, as observed by us and as described by Lind (1976) (Table 6). These differences are probably large enough to serve as an

effective barrier against hybridization where their ranges overlap (Kerney, 1976).

The large difference in duration of copulation between H. aspersa and H. pomatia implies that they differ in the mechanism(s) by which they transfer spermatophores. Whereas the transfer of the spermatophore body in H. pomatia occurs in less than 5 min (Lind, 1976), 300 min are required for H. aspersa. This difference cannot be due to the difference in the time required for formation of the spermatophore, because in both species it is formed in less than 2 min after the beginning of copulation (Table 5; Lind, 1973).

Helix aspersa remain essentially motionless while copulating. Similarly, H. pomatia remain motionless approximately 3 h after copulation while internalising the spermatophore tail. Immobility in H. pomatia decreases the chance that the spermatophore tail will be broken during transfer (Lind, 1973). If the tail is broken, the sperm cannot escape the bursa tract to fertilize the eggs (Lind, 1973). Immobility in H. aspersa probably serves a similar function. Despite this likely similarity of function, the mechanism(s) responsible for the quiescence appears to differ in the two species. In H. aspersa, contact with the partner is essential for immobility. The inhibition in H. aspersa seems to be partly due to a feedback system in the atrium

walls and (or) penis that senses pressure or tension and inhibits locomotion. Copulating pairs return to quiescence when they pull on their mutual connection of interlocked penes. Separated pairs, however, will locomote freely. Therefore, the animals can move while transferring spermatophores but they are prevented from doing so when interlocked. This differs from inhibition of locomotion in H. pomatia, which occurs regardless of whether the other partner is present after copulation and regardless of whether a spermatophore has been transferred (Jeppesen, 1976).

Lind (1973) found that the spermatophores of H. pomatia are digested in the bursa. In H. aspersa, the spermatophores do not enter the bursa, but enter the bursa tract diverticulum instead, as reported by Tompa (1984). The spermatophores appear to dissolve in the diverticulum. Thus the physiological significance of the bursa in H. aspersa remains unknown.

Significance of dart shooting.

At least six theories of dart function have appeared in the literature. (i) The dart may act like a hypodermic needle, injecting digitiform-gland mucus into the recipient snail. It has been suggested that the mucus may act to increase the recipient's 'sexual arousal' (Dorello, 1925). (ii) Goddard (1962), however, postulates that it is the trauma of the dart's

penetration that increases the snail's 'sexual arousal'. (iii) Jeppesen (1976) and Lind (1976) contend that dart shooting, when followed by copulation, decreases the shooter's 'arousal' or 'receptivity' resulting in mating suppression. This gives H. pomatia a periodic mating cycle which my be adaptive. (iv) Tompa (1980) suggests that dart shooting may affect subsequent fertilization but does not primarily affect behaviour at all. (v) Giusti and Lepri (1980) believe that dart shooting tests the 'mating readiness' of the partner. Darts discourage courtship from partners not ready to copulate saving the time and energy of the more aroused partner. (vi) It is also possible that dart shooting has become anachronistic and no longer has a function in H. aspersa. The dart apparatus appears to be degenerating in some terrestrial snails such as Ochtheplaila turricula (Tompa, 1980).

In H. aspersa, however, dart shooting affects the timing of copulation (Fig. 5), and therefore it does appear to have some behavioural function, contrary to Tompa (1980) and contrary to the hypothesis that it is an evolutionary relic. Also Jeppesen's (1976) and Lind's (1976) hypothesis, which posits an effect on the shooter rather than on the recipient, seems unlikely, given the behavioural triggering of dart shooting. Despite the statement of Giusti and Lepri (1980) that dart shooting can occur without any tactile stimulus, we never observed

dart shooting in the absence of pressure being applied to the atrium. This virtually guarantees that a shooting snail will shoot its dart into a tangible object, and indeed few snails miss (Table 3). This suggests that the dart's main effect is on the recipient, not on the shooter. However, Lind (1976) also found that pairs of H. pomatia that receive darts are less likely to copulate, which is the opposite of its effect in H. aspersa. Also, while H. pomatia show a strong withdrawal response when receiving the dart (Lind, 1976), neither we nor Giusti and Lepri (1980) noticed a strong aversive response in H. aspersa. Therefore, there may be a species difference in dart function between H. aspersa and H. pomatia.

The data presented in this paper suggest that the main function of dart shooting in H. aspersa is related to the ideas of Dorello (1925) and Goddard (1962), who suggest that dart shooting increases the sexual arousal of the recipient snail. Thus, snails that hit their partners during the first dart-shooting event take less time to reach copulation than do snails that miss their partners (Table 1). Chung's (1986) recent experiments also support this hypothesis. He found that injections of digitiform gland extract increase genital eversion, and he has evidence suggesting that the active agent is a polypeptide.

In observing snails, one gains the strong impression that they copulate only after reaching some internal threshold. A similar conviction has been expressed by Lind (1976). The data suggest that receiving a dart reduces the time needed for the recipients to reach this threshold. This hypothesis would explain why the second dart-shooting event seems irrelevant to the mating outcome (Table 1); the first animal has presumably already reached the threshold for mating, therefore the dart has no effect on it. This raises the question of why the second dart is fired at all, especially as animals can mate without any dart shooting. The answer may simply be that, given the generally reciprocal nature of the mating behaviours, it would be difficult to selectively suppress the second dart-shooting event.

The repetitive tactile stimulation during introductory behaviour may be the animals's main method of increasing the sexual arousal of itself and its partner, with dart shooting being a mechanism to even out any 'arousal inequality' between the two animals. When no such inequality exists, the effect of the dart should be slight. Thus, in cases where a missed dart had little effect on courtship time (Fig. 5), it may be that the two snails were already approaching the threshold for dart shooting, independently of the occurrence of dart shooting.

In a natural environment, decreasing the time to copulation may be advantageous. Not only would the animals be vulnerable to predators for a shorter time, but the chance of the mating being permanently interrupted would also be reduced. Interestingly, the only instances of mating failure after the occurrence of dart shooting was when the first dart missed its intended target (Fig. 5). In these cases, the second dart shot was never fired. The animals parted before the second animal reached threshold. Peake (1978: p. 497) has pointed out that "considerable effort and risk is expended or encountered by stylommatophoran molluscs during mating, and therefore it may be anticipated that features which reduce the risk or insure successful mating will have a high selective advantage." Dart shooting may be such a feature.

Chapter 3. The 'Love Dart' of the Snail, *Helix aspersa*, Injects a Pheromone that Decreases Courtship Duration

Abstract

During the courtship of Helix aspersa, the snails push calcareous darts into one another. To examine the importance of this unusual action for the normal expression of their sexual behaviour, one group of Helix aspersa was prevented from exchanging darts by surgically removing their dart sacs. Another group of Helix aspersa had their digitiform glands surgically removed. These glands secrete a mucus that coats the dart. Glandless snails were capable of exchanging darts, but the darts lacked their normal mucous coating. Both dartless and glandless pairs required more courtship time to reach copulation than did sham operated controls. Therefore, it appears that it is the mucus from the digitiform gland, not the mechanical action of the dart, which affects courtship duration.

Injections of gland homogenate decreased the courtship duration of sexually receptive, dartless and glandless snails. Gland homogenate also increased the size of the recipient's genital eversion, and retarded locomotion. The mucus was only effective if it entered the circulatory system of a sexually receptive snail at a specific stage of genital eversion.

The active agent in the digitiform gland mucus fulfills the requirements for the substance to be classified as a pheromone. It enters the circulatory

system of the conspecific via inoculation by the dart.

Introduction

The terrestrial snail, Helix aspersa, is a simultaneously reciprocal hermaphrodite with a complicated courtship. Courtship can be divided into three phases: introductory behaviour, dart shooting, and copulation (Chapter 2, Chung, '87). During introductory behaviour, the snails progressively evert their normally internal genital apparatus; the extent of the eversion can be described as consisting of six successive stages (Chapter 2). At stage 0 the snail has no visible eversion, and at stage 5 the eversion is maximal. Stage 5 eversions occur just prior to dart shooting. During dart shooting, the snails push a 1 cm long, calcareous, mucous-coated dart (Hunt, '79) through the body wall and into the hemocoel of their partner. The mucous coating of the dart is secreted by the digitiform glands. Usually, the dart, with its attached mucus, detaches and remains inside the partner's body cavity. Because each snail shoots a single dart in a mating sequence, each courtship contains two dart shooting events (Chung, '87, Chapter 2). The snail can regenerate a new dart in about 6 days under laboratory conditions (Tompa, '82).

Despite speculation for over a century, the effect of dart shooting on courtship behaviour remains uncertain

(see Chung, '87, and Tompa, '84, for reviews). Recently, however, Chung ('86) has found that the injection of boiled digitiform gland extract can elicit a genital eversion in H. aspersa. From this result Chung ('86) has suggested that digitiform gland mucus contains a courtship pheromone. This study investigates the effects of unboiled digitiform gland mucus on courtship behaviour. Specifically, it examines the possibility that digitiform gland mucus contains a pheromone that facilitates mating.

Methods

Specimens of H. aspersa were imported from California (Marinus, Long Beach). The snails had an average weight of approximately 8 g (5.2 - 12.6 g). They were individually housed in humid Lucite containers (7 x 8 x 8 cm) and were fed lettuce, carrots, and oyster shells ad libitum. The light cycle was 16L:8D. The temperature was 17 +/- 2^{o} C. The snails were marked with numbers for individual identification.

Demonstration of mucus transfer

To test if digitiform mucus is actually transferred from the shooting snail to its courting partner, eight snails had their digitiform glands injected with carmine

red. The snails were anesthetized as described below. An incision was made in the body wall and approximately 0.05 ml of 1% carmine red was forced into the lumen at the base of each gland using a syringe and a 26 gauge needle. The glands were stained intensely red. The needle was withdrawn, and the wound was sutured. Three to ten days later, the operated snails shot darts into unoperated animals. The recipient snails were dissected to examine whether the red dyed mucus had entered into their body cavity.

To test how quickly substances can be carried by the snail's circulatory system, five snails were injected with 0.1 ml of 0.2% fast green dye dissolved in snail saline (Kerkut and Meech, '66). The spread of the dye was assessed by observing the snails' change in skin colour.

Lesion experiments

To determine if dart shooting had any effect on subsequent courtship behaviour, either the dart sac or the digitiform glands were removed. Before surgery, snails were injected with an anesthetic (2% $MgCl_2$, 0.01% succinylcholine chloride, 0.005% streptomycin in distilled water; 0.1 mL/ g snail; Chung, '85). An incision was made 3 to 5 mm behind and below the genital pore, transverse to the body axis. To remove the darts,

the dart sac was cut at its base and removed. To remove the glands, both digitiform glands were cut at their bases and removed. Sham operated controls had their reproductive organs manipulated by forceps. The incisions were sutured using Ethicon 6-0 silk and a FS-3 needle. The mortality rate was less than 5% for all operations. Snails were allowed to recover for 2 weeks. When examined six months later, no regeneration of dart sac or digitiform glands was evident.

For the courtship trials, 20 snails were removed from their isolation chambers at the beginning of the light cycle and placed together in a Lucite box (17 x 17 x 7 cm). Dartless and glandless snails were placed in one box, and sham operated controls were placed in another. In four trials control snails and dartless or glandless snails were placed together. When two snails began introductory courtship behaviour, they were separated from the other snails and put together in a smaller Lucite box (14 x 7 x 7 cm). This allowed for easier observation of the behaviour, and prevented its interruption by other snails. If a snail copulated during a trial, it was not tested again for at least 10 days to allow time for the dart to regenerate. Each trial lasted 90 min, after which the snails were returned to their home cages. Courtship duration and the time between the two dart shooting events was recorded.

Effects of digitiform gland mucus

To test the effect of digitiform gland mucus independent of the effect of dart penetration, homogenates of the digitiform glands were injected. Digitiform glands were taken from snails only if they had a complete dart in their dart sac, and had mucus visible in the lumen of the glands. Donor tissue was either used immediately or frozen at -70°C for subsequent use. There was no significant difference between the effects of frozen and fresh samples (Mann-Whitney, two-tailed, $p>>0.05$). Frozen or fresh donor tissue was ground with H. aspersa saline (Kerkut and Meech, '66) in a 1 ml hand homogenizer. The homogenate was spun for 5 min at 3000 g in a refrigerated (4°C) centrifuge to remove solid debris. Homogenates were kept on ice and were used within 3 h. As controls, homogenates of the dart sac and of the pedal gland were prepared in exactly the same manner. The pedal gland is a large mucous gland which is embedded in the foot and secretes the mucus that the snail uses during locomotion.

Two doses of digitiform gland were used. The 'high' dose of 4.5 mg tissue/g snail was approximately equal to injecting the digitiform glands of one animal into another. The 'low' dose was 2 mg tissue/snail.

According to Chung ('86), the amount of mucus carried by a dart is 2 mg. Therefore, 2 mg of digitiform gland (of which only a fraction would be mucus) should not exceed the amount of mucus an animal receives during natural dart shooting.

Before being given an injection, courting snails were separated from their partners. Once their genital eversion level became stable (no changes in eversion level for 10 s), the snails were injected with 0.1 ml of either an homogenate or of saline using a 26 gauge needle in the posterior foot just below the shell. The person scoring the response did not know with what substance the animal had been injected. Snails that secreted a mucous foam, or withdrew for more than 1 min in response to the injection, were assumed to have been injured and were excluded from the analysis.

Snails that have been hit by a dart tend to slow their rate of locomotion (Chung, '86). Therefore, a 'low' dose of digitiform gland homogenate was tested to see if it would decrease locomotion. Snails were placed at one end of an inclined (15°) board covered with paper towel. Because they have a negative geotaxis, the snails crawl along a more or less straight line under these conditions. The distance an active snail travelled was determined by measuring the length of a black thread that was superimposed on the residual mucous trail. A

baseline measure of locomotion was obtained during a 10 min trial prior to any injection. After taking the baseline measurement, snails were then randomly assigned to one of four injection groups: 0.1 ml saline, or 2 mg of dart, digitiform or pedal gland tissue in 0.1 ml saline. The distance the animals crawled during a 10 min period immediately after the injection was compared to the distance travelled prior to the injection.

If digitiform gland mucus normally influences courtship behaviour, injections of the mucus should restore normal courtship durations to dartless and glandless courting pairs. To test this, unoperated snails which had reached genital eversion stages 2 to 5 were randomly assigned to a low dose of either digitiform gland or dart homogenate. Immediately after the injection, these snails were randomly placed with other unoperated snails that had shot a dart within the last 15 min. Post-dart shooting snails were used as partners because they are both consistent and persistent suitors. The time until the injected snail shot its own dart was recorded, as was the time required for the pair to achieve copulation. Snails that secreted mucous foam or withdrew into their shells after the injection were excluded from the results.

Results

Demonstration of mucus transfer

In all eight of the animals injected with carmine red, both the atrium and the digitiform glands were stained red. The dart itself remained unstained.

In cases where the shot dart had fully penetrated the recipient's body wall (7 of 8), the recipient contained red mucus in its body cavity. Some red mucus was still attached to each dart. The mucus appeared to be digitiform gland mucus because it was thick and opaque, whereas mucus from the atrium is thin and transparent. Therefore, these experiments demonstrate that at least some digitiform gland mucus enters the recipient snail during normal dart shooting.

Injections of fast green dye turned the entire snail green, from posterior foot to tentacle tips, in less than 15 s. Therefore, it is clear that once dissolved in the hemolymph, substances can be quickly distributed throughout the animal. The limit to the spread of the active agent(s) of the mucus, then, is the solubility of the agent(s) in the hemolymph.

Lesion experiments

Dartless snails exhibit all the behaviours normally exhibited by intact animals, including characteristic postures before and during dart shooting (Chung, '87, Chapter 2). Therefore, for purposes of comparing the courtship durations of dartless snails and dart shooting snails, the dartless snails' 'phantom' dart shooting was scored as a dart shooting event.

The effect of removing the dart sac or the digitiform glands was to skew to the right the distribution of intervals between the two dart shooting events, relative to sham operated controls ($p<0.01$, Kolmogorov-Smirnov test). This effect was the same regardless of whether a snail had received no dart or a normal dart lacking digitiform gland mucus (a dry dart) (Fig. 1).

It was found that snails that had a stage 2 or stage 3 eversion, and that had also received a mucous coated dart, had significantly shorter courtship durations than did similar snails receiving either no dart or a dry dart (Table 1). Courtship duration decreased because receiving a mucous coated dart decreased the time required for the recipient to fire its own dart (Table 2). After both dart shooting events had occurred, the time required to reach copulation was relatively short

Figure 1. Distribution of latencies from the first dart shooting event to the second dart shooting event. (A) Snails that lack digitiform glands (n=14). (B) Snails that lack a dart (n=16). (C) Sham operated controls (n=20). The sham operated controls differ significantly from both the dartless pairs and the glandless pairs ($p < 0.01$, Kolgomorov-Smirnov test), but the latter two groups do not differ significantly from each other.

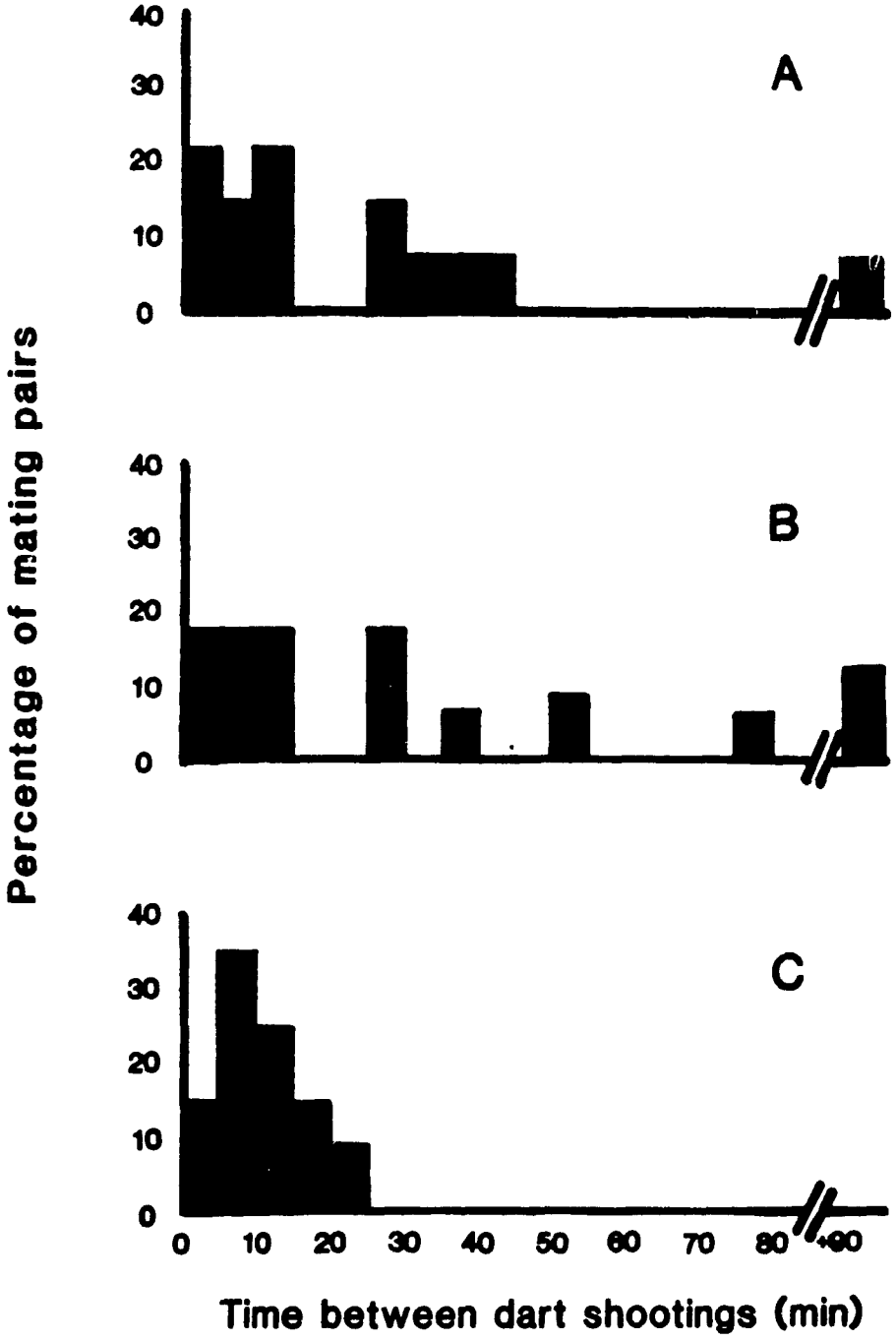

Percentage of mating pairs
40
30
20
10
0
A
B
C
0
10
20
30
40
50
60
70
80
90
Time between dart shootings (min)

Table 1 Courtship and copulation durations of dartless, glandless and sham-operated snails as a function of the stage of genital eversion of the dart recipient during the first dart shooting event Dartless or glandless snail pairs were compared to sham operated control pairs using a 2-tailed Mann-Whitney test Pairs that failed to copulate were given an arbitrary time of 90 min (end of observation period) However, the time from first dart shooting to copulation was calculated using only values from successfully mating pairs Starred values differ significantly ($p<0.05$) At genital eversion stages 2 or 3, mating success was lower for dartless pairs and glandless pairs (X^2, $0.1 > p > 0.05$)

Eversion stage of recipient	Operated or control	Time from first dart shooting to copulation (min + s e m)	Number of pairs that fail to copulate (percent of total)
0 or 1	dartless or glandless	85	14 (93%)
	sham operated controls	-	12 (100%)
2 or 3	dartless or glandless	34 6 +/- 4 5*	3 (14%)
	sham operated controls	25 5 +/- 2 0*	0 (0%)
4 or 5	dartless or glandless	14 1 +/- 2.2	0 (0%)
	sham operated controls	17 3 +/- 2 7	0 (0%)

Table 2 Courtship duration of unoperated dart recipients with genital eversions at stage 2 or stage 3 The difference between the two starred values is significant (p=0 04, 2-tailed Mann-Whitney)

	Dart shooting by first snail to dart shooting by second snail (min + s e m)	Dart shooting by second snail to copulation (min + s e m)
dartless or glandless	27 3 +/- 4 5 * n=21	7 3 +/- 1 6 n=18
sham operated controls	15 9 +/- 1 9 * n=21	9 4 +/- 1 6 n=21

for all pairs. Moreover, snails hit by a dry dart, or by no dart at all, were more likely to fail to copulate (Table 1). No significant effects of receiving a dart were evident in snails that had either minimal eversions (stages 0 and 1) or maximal eversions (stages 4 and 5). In the former cases, mating failure was common regardless of whether darts were received, and in the latter cases, mating followed rapidly and irrespectively of the dart shooting event (Table 1). The behavior of mixed pairs, consisting of a control snail and a dartless or glandless snail, depended on whether the first dart shooting event was performed by the control snail or by the dartless or glandless snail. If the first dart shooting sequence was performed by the control snail, the courtship duration of the pair was the same as if both snails had been controls ($p >> 0.05$, Mann-Whitney 2-tailed test). If the first dart shooting sequence was performed by the dartless or glandless snail and the control operated recipient had a stage 2 or stage 3 genital eversion, courtship duration was increased by the same extent as it was in dartless and/or glandless pairs ($p >> 0.05$, Mann-Whitney 2-tailed test).

The frequency of mating was recorded for each group of operated snails. Dartless snails (n=18) mated an average of 7.3 +/- 1.8 (s.d.) times over the 23 courtship trials, while the average number of matings for glandless

snails (n=17) was 8.0 +/- 1.7 (s.d.). The average number of matings for sham operated controls (n=21) was 7.0 +/- 2.3 (s.d.). The mating frequencies of dartless and glandless snails were not significantly different from those of controls ($p >> 0.05$, Kruskal-Wallis test). Therefore, removing the dart sac or the digitiform glands did not affect a snail's tendency to mate.

Effects of digitiform gland mucus

Injections of digitiform gland homogenate increased the genital eversions of recipients that had stage 2 or stage 3 eversions prior to injection (Table 3). It had little effect on snails with small or nonexistent genital eversions. The increase in eversion stage caused by an injection of digitiform gland homogenate began 1.2 +/- 0.8 (s.d.) min after the injection (n=22). The effect lasted an average of 8.1 +/- 7.5 (s.d.) min. In six animals, the effect lasted more than 20 min. Injections of dart sac homogenate, or of saline, had no positive effect on genital eversion at any genital eversion stage.

Digitiform gland homogenate caused a significant decrease in locomotion ($p<0.001$, Table 4). No decrease in locomotion was caused by injections of dart sac or of pedal gland homogenate. Therefore, the effect was not

Table 3 Effects of digitiform gland homogenate on genital eversion Data were analyzed using a G-test, with Williams correction, for each eversion stage and dosage level High dosage injections were effective irrespective of the initial eversion stage ($p<0.01$ for stages 0-1 and 2-3) With low doses, the effect was significant only for snails with eversions at stages 2 or 3 ($p<0.05$)

Eversion stage		n	Number responding with an increase in genital eversion (percent of total)
High homogenate dose			
digitiform	0-1	24	4 (16.7%)
	2-3	22	14 (64.0%)
dart	0-1	15	0 (0%)
	2-3	18	0 (0%)
saline	0-1	8	0 (0%)
	2-3	12	0 (0%)
Low homogenate dose			
digitiform	0-1	10	1 (10%)
	2-3	10	4 (40%)
dart	0-1	10	0 (0%)
	2-3	10	0 (0%)
saline	0-1	10	0 (0%)
	2-3	10	0 (0%)

Table 4 Effect of digitiform gland homogenate on snail locomotion Snails were allowed free locomotion on an inclined plane before and after injections The data were tested for normality and homogeneity of variances before using a planned comparison test with the effect of digitiform gland homogenate compared to the three controls The digitiform gland significantly decreased locomotion ($p<0.001$)

Injected material	N	Change in distance travelled (cm +/- s e.m.)
digitiform gland	22	-8 1 +/- 3 1
pedal gland	11	+1 8 +/- 4 4
dart sac	7	+2 9 +/- 6 7
saline	16	+7 6 +/- 3 3

Injections of digitiform gland homogenate also decreased the courtship duration of dartless and/or glandless pairs relative to similar pairs injected with dart sac homogenate. Because dart sac homogenate had no effect on either genital eversion (Table 3) or locomotion (Table 4), it is an appropriate control for the nonspecific effects of injecting a tissue homogenate. Seventeen snails at genital eversion stage 2 or 3 were injected with digitiform gland homogenate. These snails achieved copulation with their post-dartshooting partners in 31.8 ± 28.4 (s.d.) min. Seventeen similar snails injected with dart sac homogenate required 60.2 ± 41.1 (s.d.) min to achieve copulation. The difference in courtship duration between the two treatments was significant at a probability of $p=0.052$ (2-tailed Mann Whitney test). The digitiform gland injections were effective only in snails that had a stage 2 or stage 3 genital eversion prior to the injection. Digitiform gland mucus had no effect on the courtship duration of snails with genital eversions of stage 4 or stage 5 ($p >> 0.05$, 2-tailed Mann-Whitney test).

Discussion

Discussion

Our results demonstrate that dart shooting, accompanied by mucus transfer, decreases courtship duration. Pairs in which neither snail can shoot a dart have longer courtships than controls (Fig. 1). The effective agent is not the dart itself, since snails receiving a dry dart took as long to mate as undarted snails (Fig. 1). The effective agent is the mucus from the digitiform glands which is carried by the dart into the hemocoel of the recipient. Our observations using mucus stained with carmine red confirm that such transfer typically occurs during dart shooting. Once inside the hemocoel, the soluble components of the mucus could be quickly distributed to all parts of the snail, as shown by the injections of fast green. Moreover, the mucus, without the dart, had significant effects on courtship behaviour. When mucus was injected into courting dartless and/or glandless snails, courtship duration was reduced relative to controls.

We found that injections of digitiform gland mucus both decreased locomotion (Table 4) and increased the stage of genital eversion (Table 3). It is likely that both of these effects contribute to the observed decrease in courtship duration. By decreasing locomotion, dart shooting could allow for more tactile contact between

members of a courting pair. Tactile contact is essential for advancing courtship from one phase to the next (Chapter 2). By increasing genital eversion, the dart reduces the number of subsequent stages of genital eversion that the snail must pass through before it can reach its own dart shooting threshold (stage 5) and enter the next phase of courtship. Copulation normally occurs only after both snails have shot their darts (Chapter 2). Therefore, the mucus appears to facilitate mating both indirectly, by increasing the opportunity for the shooter of the dart to court the recipient, and directly, by accelerating the recipient's passage through introductory courtship behaviour. This increases behavioural synchrony by reducing the time that the two snails are in different phases of courtship. Because dart shooting increases behavioural synchrony, pairs that dart shoot tend to be more successful at reaching copulation (Table 1).

It might be argued that the changes in courtship duration are due to an artefact of the surgical procedures. This can be discounted for a number of reasons. First, it has already been established that if normal snails miss their partners during the first dart shooting event, the subsequent courtship is longer (Chapter 2). Second, dartless and glandless animals mated with the same frequency as operated controls;

therefore, the operation did not discourage mating. Third, copulation duration and the time between the second dart shooting and copulation in glandless and dartless pairs were indistinguishable from those of sham operated controls (Table 2). Fourth, when dartless and glandless snails had maximal eversions their latencies to dart shooting did not differ from those of sham operated controls (Table 1). Fifth, mixed control and dartless or glandless pairs had normal courtship durations as long as the first dart was shot by the control snail. Therefore glandless and dartless snails with genital eversions at stage 2 or 3 are capable of normal courtship despite the surgery.

Although a stimulatory role for the dart and its mucus has been implied in the studies of Dorello ('25), Bornchen ('67) and Chung ('86), studies of the mating behaviour in H. pomatia by Lind ('76) and Jeppesen ('76) and in H. aspersa by Chung ('87) failed to find any stimulatory effect of dart shooting on courtship behaviour. Because these authors did not report the eversion level of the recipients, their work cannot be directly compared with ours. Nevertheless, Jeppesen ('76) appears to have found (his Table III) that dartless animals were about twice as likely to fail to copulate after 'phantom' dart shooting as were normal control

snails. Dart shooting may have a similar function in H. pomatia as in H. aspersa.

Giusti and Lepri ('80), working with H. aspersa, and Lind ('76) and Jeppesen ('76) working with H. pomatia, found that snails that copulate within one day of a previous mating do not perform dart shooting behaviour, and have a shorter courtship. This is not what one would predict if dart shooting is an adaptation for facilitating courtship. However, none of the cited authors reported how often the courtship of these snails resulted in copulation. A snail in a dartless mating cannot accelerate the courtship of a slower partner (i.e. a partner at a lower stage of genital eversion) and this may decrease mating success. Secondary courtships may only be successful when both mating partners have large genital eversions simultaneously.

The statistical significance of the difference in courtship duration between snails injected with digitiform gland or dart sac homogenate was slightly less ($p=0.052$) than the usual criterion. One difficulty in performing this experiment was that the injections tended to disrupt the mating behaviour of the injected animal. Both our locomotion studies and our eversion studies suggest that the digitiform gland mucous effects are behaviourally noticeable for only about 10 minutes after the injection. Thus, it is possible that the substance

had lost some of its effectiveness by the time the injected snail resumed courting. Despite these difficulties, the differences between the two groups is not negligible.

Chung ('86) also injected digitiform gland homogenate into H. aspersa but he did not notice an effect on genital eversion using our 'low' dose. In his experiment, however, none of the recipient snails had pre-existing genital eversions. He discovered that boiled digitiform gland homogenate could induce genital eversion in snails without pre-existing eversions, although its effects on courtship behaviour are unknown. He found that a 5000 MW protein in the mucus was responsible for inducing the genital eversions. Since Chung's study used boiled digitiform gland, and since this had a slightly different effect than the unboiled mucus used in our work, further experiments are necessary to determine if the same protein is causing both effects.

Chung ('86) has suggested that digitiform gland mucus induces genital eversion by increasing body wall contractions, which would cause an increase in blood pressure in the head. At the same time, it may also relax the muscle surrounding the genital pore, which would allow the extrusion of the genital apparatus. This idea is supported by Bornchen's ('67) finding that

digitiform gland homogenate increases heart rate, as well as the amplitude of cardiac contraction, thereby increasing blood pressure in the head and the foot.

Topical application of the mucus (Chung, '86), or ingestion of the mucus (our observations), has no effect on genital eversion. Therefore, the substance is only effective when it is injected.

The active agent in the digitiform gland mucus satisfies the requirements for a substance to be classified as a pheromone: it is released to the exterior of one animal and has a specific effect on a receiving animal of the same species (Karlson and Luscher, '59). However, it differs from classical pheromones in that it does not require a specific external receptor to be effective. Instead, the substance is delivered directly into the hemocoel by the dart. Karlson and Luscher ('59) suggested that pheromones that reach the blood stream directly be considered as a separate class of compounds. They suggested the term 'telemones' for such substances. We prefer, however, that the term pheromone be used to describe the active agent in the digitiform gland mucus, as no useful purpose is presently served by a proliferation of terms.

The unusual method of pheromone transfer described here is probably not limited to Helix, though this is the only example known to us in which an inoculate has been

shown to contain a pheromone. Other animals, however, have the necessary behaviours and anatomy to transfer pheromones in this way. In some terrestrial snails, the dart is hollow and shaped like a true hypodermic needle through which gland products are injected directly into the mating partner (Tompa, '84). In some species of Euscopions, a genus of scorpion, the males sting their mates during courtship (Weygoldt, '77). Arnold and Houck ('82) found that the salamander Desmognathus ochrophaeus bites its prospective mate during courtship, allowing secretions from the mental gland to enter the wound. The nudibranchs Palio zosterae and Palio dubia inoculate their partners with sperm during copulation (Rivest, '84), as do some flatworms (Hyman, '51). Further work may reveal that these or other animals also use inoculation as a method of pheromone transfer.

The effects of the digitiform gland on genital eversion, as well as its effects on courtship behaviour, were consistently confined to animals with genital eversions at stages 2 and 3. Comparable results have been obtained in other pheromone systems, where the recipient responds to the pheromone only in certain seasonal or behavioural contexts (Shorey, '77). For example, the sex pheromone of the land snail Euhadra peliomphala is only effective during the snails' breeding period (Takeda and Tsuroka, '79). The contingent

effectiveness of the digitiform gland implies a dependence on synergistic hormonal and/or neuronal conditions. Given that at least part of the neural machinery for the control of mating behaviour is known (Chase, '86), further studies should be able to elucidate the mechanism(s) of this dependence.

Chapter 4. Dissociation of Sexual Arousal and Sexual Proclivity in the Garden Snail, *Helix aspersa*

Abstract

Sexual arousal (intensity of courtship) and sexual proclivity (tendency to court) in Helix aspersa can be reliably measured using externally observable correlates. Snails with sexual proclivity are significantly more likely to turn towards an anesthetized conspecific after contacting it than are sexually unreceptive snails. Sexual arousal can be inferred from the stage of a snail's genital eversion which appears only during courtship. The higher the stage of the eversion, the shorter the time required to complete introductory courtship behaviour, the higher the rate of successful copulation, the fewer the number of breaks and pauses during courtship, and the longer the time a snail will spend in contact with an anesthetized conspecific. Sexual proclivity has no effect on feeding or locomotory behaviour; however, sexual arousal inhibits feeding and increases locomotor activity. Snails that were allowed daily contact with conspecifics required less time to complete introductory courtship behaviour relative to snails that were isolated from conspecifics for 1 week. This suggests that daily contact increases sexual arousal. A greater percentage of isolated snails exhibited courtship behaviour than did snails which had experienced daily conspecific contact. This suggests that isolation increases sexual proclivity. These

differences indicate that sexual arousal is not merely due to an increase in sexual proclivity.

Introduction

Molluscs have proven useful as model systems for the study of the biological basis of simple behaviours (Kandel, 1976). Kupfermann (1974) has argued that molluscs are also appropriate for the study of complex behavioural phenomena, such as 'motivation' (the variability of an animal's response to a given stimulus). Biologists have been chiefly concerned with two types of behavioural variability: the fluctuation in the likelihood of a behaviour and its change in magnitude or intensity (Andrew, 1974, Kupfermann, 1974). In this paper we define the likelihood of an animal to perform a behaviour as the animal's proclivity for that behaviour, and we define the relative intensity with which it performs a behaviour as its level of arousal for that behaviour. In molluscs, these two aspects of behavioural variability have been studied extensively only in the context of feeding behaviour.

Food deprivation has been found to increase both the likelihood of feeding behaviour (Kupfermann, 1974, Tuersley, 1989) as well as its magnitude and intensity (Weiss, Koch, Koester, Rosen & Kupfermann, 1982). Therefore, both proclivity and arousal appear to co-vary

in feeding, suggesting that the physiological control of each is also heavily coordinated (Weiss et. al., 1982).

Proclivity and arousal, however, may not always be as closely associated in other behaviours as they are in feeding. This would suggest a difference in the physiological mechanisms that control feeding behaviour from that in other 'motivated' behaviours. To test whether the degree of association between arousal and proclivity does actually vary in different behaviours, we have examined both phenomena in the sexual behaviour of H. aspersa.

The sexual behaviour of H.aspersa has been previously described (Chapter 2; Chung, 1987). In brief, H. aspersa are simultaneously reciprocal hermaphrodites. Courtship contains 3 basic phases: introductory behaviour, dart shooting and copulation. Introductory behaviour is characterized by repeated conspecific contacts. During introductory behaviour snails progressively evert their normally internal genital apparatus. The extent of the eversion can be unambiguously scored as 6 successive stages by its size and shape (Chapter 2). Towards the end of the introductory phase, snails adopt a stereotypic shape and posture (stage 5). While in this posture, snails push against their partner and then evert their dart sac, thereby thrusting a 1 cm long dart through the body wall of their mate. Each snail fires

only one dart per courtship. After dart shooting the snail reaches stage 6 at which time it everts its penis and attempts to copulate.

We first operationally define sexual proclivity and sexual arousal in H. aspersa. We then examine the degree of independence between sexual proclivity and sexual arousal. The two are distinguished by their differential effects on locomotion and feeding behaviour, as well as by a differential dependency on social deprivation.

General Methods

H. aspersa were imported from California (Marinus, Long Beach). The snails had an average weight of about 8.0 g (range 5 g - 13 g). Those snails that were observed copulating were transferred to individual Lucite containers (7 cm x 8 cm x 8 cm). These snails were maintained at 19° C +/- 2° C and on a 16:8 L:D light cycle. The containers were kept moist and the snails were fed lettuce, carrots and oyster shells ad. lib. The cages were cleaned every day or every other day. All experiments were run at the beginning of the light cycle and continued for a maximum of 2 hours. Each snail was individually marked. Snails were randomly chosen for trials either by a coin flip or by a lottery system based on their identification number.

A snail was used in an experiment only if it had a stable genital eversion (no change in stage for 15 s after being removed from its partner). The genital eversion stage often fluctuated during trials, but snails were classified by their initial genital eversion. Snails were used only once per day. After dart shooting, snails were not used again for another 10 days to allow the dart to regenerate.

Statistical analyses were carried out according to the procedures described by Sokal and Rohlf (1981).

Experiment 1. A Test of Sexual Proclivity

Courtship in H. aspersa begins with reciprocal tentacle touching. Contact with conspecifics results in withdrawal except during courtship. Therefore, to measure sexual proclivity, snails were tested for their response to tactile contact with an anesthetized conspecific.

Methods

Five snails from the laboratory colony were anesthetized by injecting them with 0.1 mL of 2% $MgCl_2$, 0.1% succinylcholine chloride (Chung, 1985). They were placed in small Lucite boxes (8 cm x 8 cm x 10 cm), where

they remained motionless, with extended, but flaccid, bodies.

At the beginning of the light cycle, an isolated snail was placed so as to face the tentacles of an anesthetized snail at a distance of 1 cm. Observations were made as to whether the snail approached (positive response) or withdrew (negative response) from the anesthetized conspecific after the initial tactile contact. A snail was scored as approaching if it continued locomoting towards the anesthetized snail until both bodies were in contact. Snails that appeared to be ambivalent (did not clearly approach or withdraw) were returned to their cages and were not given the second part of the test described below.

After the completion of the initial test with the anesthetized snail, the test snails were placed together in a Lucite 'group box' (20 cm x 20 cm x 8 cm) for 2 h. All courtship behaviour was recorded.

Results

Snails that turned towards an anesthetized conspecific were much more likely to subsequently engage in courtship, compared to snails that withdrew from an anesthetized conspecific (G-test with Williams correction, $p<.01$). 66.7% of positively scoring snails

subsequently courted (54/81 snails) while only 16.8% of negatively scoring snails did so (15/89). Therefore, this test can be used to estimate a snail's sexual proclivity.

Experiment 2. Tests of Sexual Arousal

During courtship, snails evert their genital atrium. The following experiments test whether the stage of the genital eversion, an easily scored variable, can be correlated with the level of a snail's sexual arousal. Sexual arousal can be inferred from the magnitude and intensity of courtship behaviour, which we measure by examining courtship duration, courtship success, and the persistence of courtship behaviour.

Methods

At the beginning of the light cycle, 20 randomly chosen snails were removed from their isolated chambers and were placed in a group box. Thirty to forty-five min later a second group of 20 snails was placed in a second group box. When the snails in group 1 reached a stage 6 eversion, they were allowed to court, but they were prevented from copulating by separating pairs prior to reciprocal intromission. As a snail in group 2 reached a previously assigned stage of genital eversion, it was

placed in a small Lucite container with a snail at stage 6 from group 1. The snails were placed 1 cm apart with the tentacles facing. The duration of introductory behaviour, the number of breaks and pauses during courtship, and the number of pairs that achieved copulation were recorded.

In a second test, 20 isolated snails were placed together in a group box. When snails reached randomly chosen, pre-assigned eversion stages they were individually placed in a small Lucite box, 1 cm away from an anesthetized conspecific. The amount of time the snails were in contact with the anesthetized conspecific was recorded. The snails were considered to be in contact if the bodies of the two snails were touching for at least 10 s. Each trial lasted 5 min. Some of the trials were videotaped, and a naive observer measured the contact times from the video playback. The times scored by the two observers were highly correlated (Spearman rank correlation $r(14)=.92$, $p<.0001$). Since no systematic bias was evident in the behavioural scoring by the first observer, the analysis of data was done using only the scores obtained by this observer.

In a third test snails were given a 5 min trial with a snail model made of rubber tubing. The amount of time snails with different stages of genital eversion spent in contact with the model was recorded.

Results

As genital eversion stage increased, the length of introductory behaviour during courtship significantly decreased (Fig. 1) while courtship success increased (Fig. 2). The number of breaks and pauses during courtship also decreased significantly (Fig. 3). As genital eversion increased, snails spent an increasing amount of time in contact with an anesthetized conspecific (Fig. 4). Snails often exhibited courtship behaviour while in contact with the anesthetized partner (46 of 72 trials). This response was not completely non-specific; snails made no attempt to court a rubber model of the same size and shape, and they spent little time in contact with it irrespective of their initial genital eversion (Fig. 5).

In summary, snails with advanced stages of genital eversion were more intense, faster, and more persistent courters than were snails with lower stage eversions. A snail's stage of genital eversion, then, can be used as an externally observable correlate of its level of sexual arousal.

Figure 1. The duration of introductory behaviour at different stages of genital eversion. The duration of introductory behaviour decreased as the stage of genital eversion of the snail increased. The trend is significant (Kendall's Tau= -.97, $p<.01$).

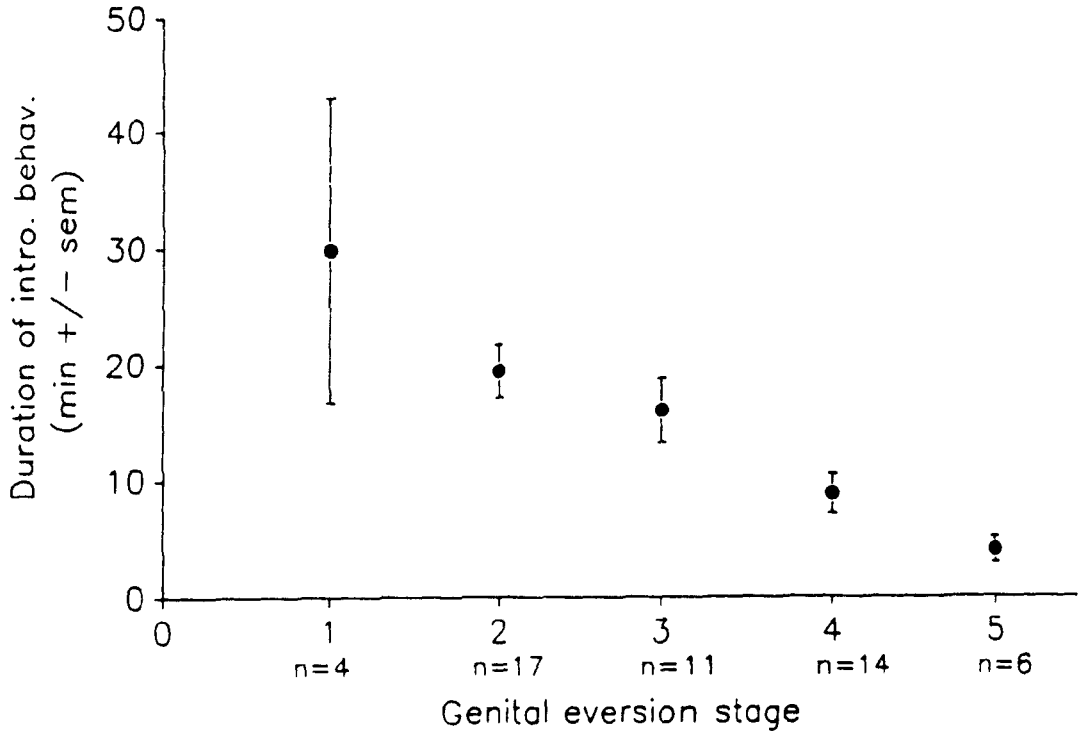

Duration of intro. behav.
(min +/− sem)
50
40
30
20
10
0
0
1
n=4
2
n=17
3
n=11
4
n=14
5
n=6
Genital eversion stage

Figure 2. The percentage of successful courtships at different stages of genital eversion. The percentage of successful courtships increased as the stage of genital eversion increased (Spearman r=.83, p<.05).

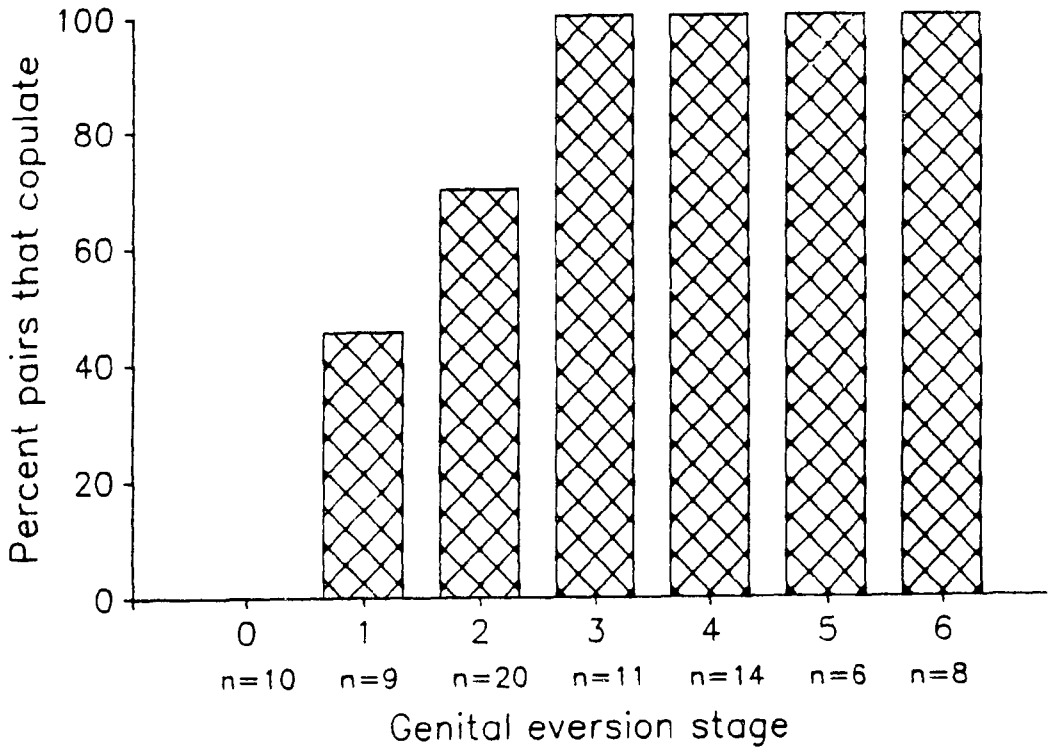

Percent pairs that copulate
100
80
60
40
20
0
0
1
2
3
4
5
6
n=10
n=9
n=20
n=11
n=14
n=6
n=8
Genital eversion stage

Figure 3. The number of breaks and pauses in courtship at different stages of genital eversion. The number of breaks and pauses declined as genital eversion stage increased. The trend is significant (Kendall's Tau=-.97, $p<.01$). Standard errors too small to be drawn to scale are omitted.

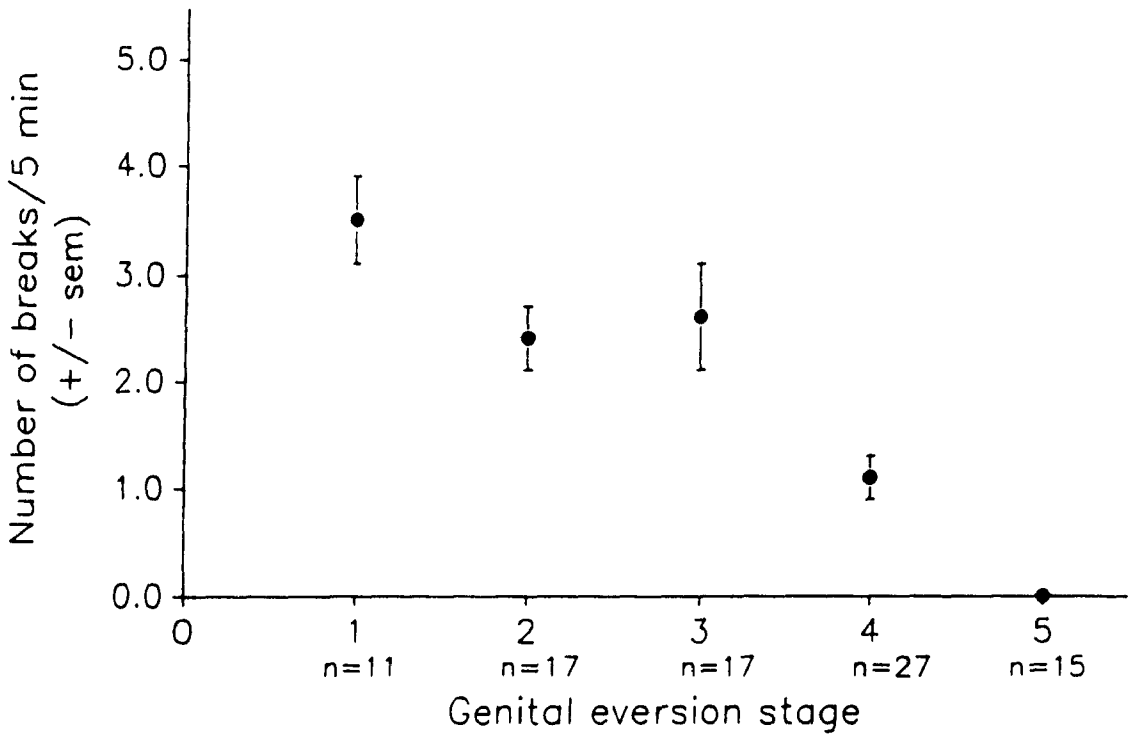

Number of breaks/5 min
(+/- sem)
5.0
4.0
3.0
2.0
1.0
0.0
0
1
n=11
2
n=17
3
n=17
4
n=27
5
n=15
Genital eversion stage

Figure 4. Time in contact with an anesthetized conspecific at different stages of genital eversion. As the stage of genital eversion increased, snails spent an increasing amount of time in contact with an anesthetized conspecific. The trend is significant (Kendall's Tau=.90, $p<.01$).

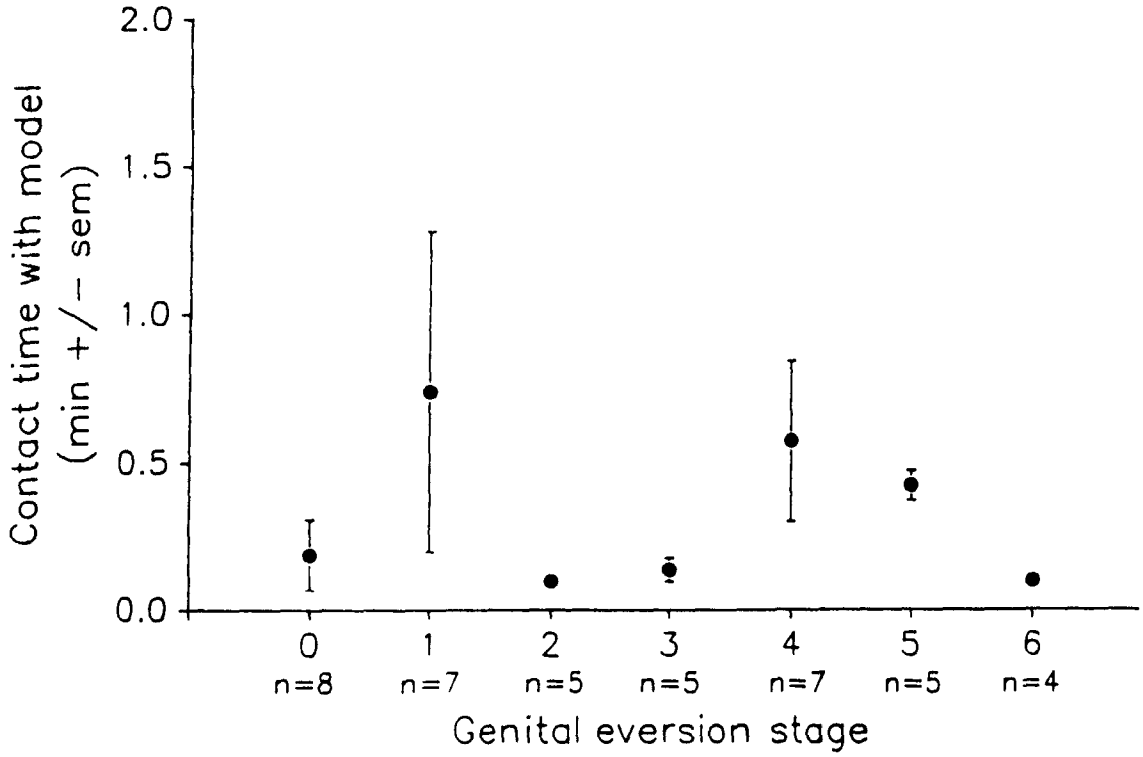
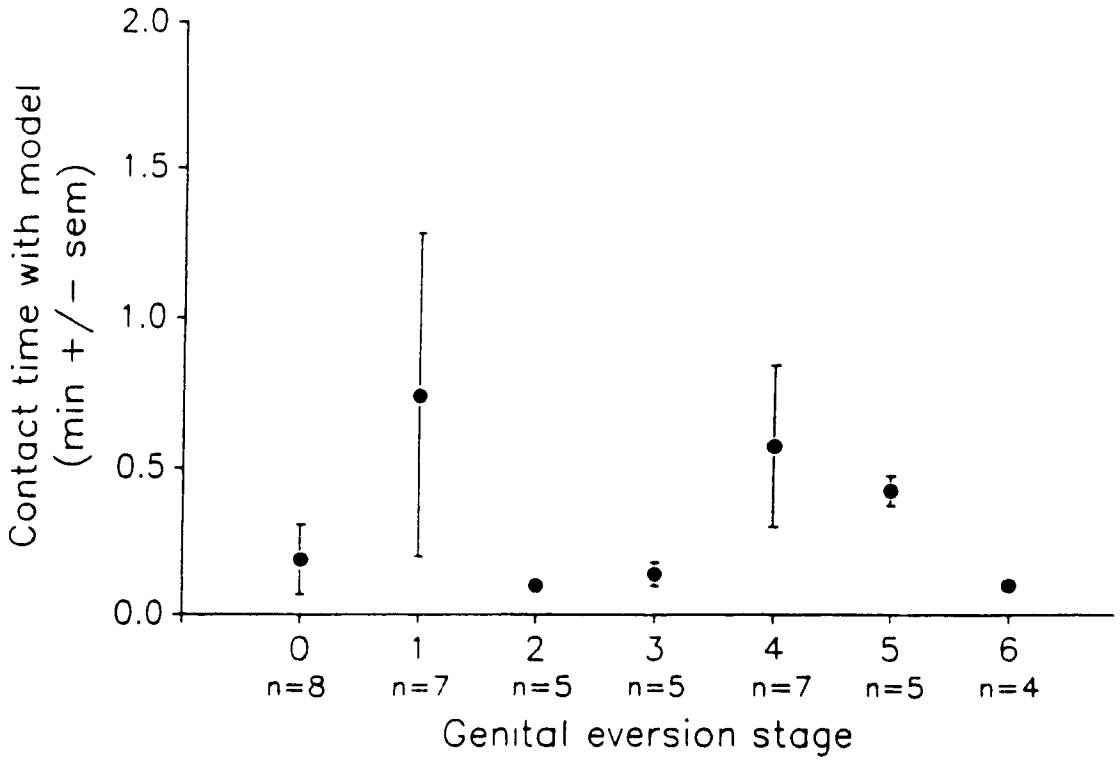

Contact time with model
(min +/− sem)
2.0
1.5
1.0
0.5
0.0
0
n=8
1
n=7
2
n=5
3
n=5
4
n=7
5
n=5
6
n=4
Genital eversion stage

Figure 5. Contact time with a rubber model at different stages of genital eversion. The stage of a snail's genital eversion is not related to the time it spends in contact with a rubber model (Kendall's Tau=-.15, p=.88). Standard errors too small to be drawn to scale are omitted.


Contact time with model
(min +/− sem)
2.0
1.5
1.0
0.5
0.0
0
n=8
1
n=7
2
n=5
3
n=5
4
n=7
5
n=5
6
n=4
Genital eversion stage

Experiment 3. Differential Effects of Isolation on Sexual Arousal and Sexual Proclivity

Methods

Isolated snails were randomly assigned to 2 groups. On day 1 both groups were placed in separate group boxes and the number and identity of courting snails was recorded. The time required for each sexually aroused snail to reach stage 5 was recorded. Once a snail's genital eversion reached stage 5, it was returned to its individual container. This prevented dart shooting, and the snails did not therefore suffer a decline in proclivity (Jeppesen, 1976, Lind, 1976).

Beginning on day 2, snails in group 1 were placed in a group box at the beginning of the light cycle for a maximum of 2 h every day for the next 7 days. Snails were returned to their individual containers when their genital eversions reached stages 3 or 4. The snails in group 2 remained isolated, although they were handled daily. Snails were handled by picking them up by their shells and holding them suspended in the air for 15 s.

On the eighth day, group 1 snails and group 2 snails were placed in separate group boxes. The number of snails showing courtship behaviour was recorded as was the time the snails required to achieve a stage 5 genital

eversion. As before, snails were removed from the group box when they had reached stage 5.

Results

Snails that had daily exposure to conspecifics achieved a stage 5 genital eversion significantly faster than did sexually isolated snails (Table 1). However, significantly fewer snails that had daily exposure to conspecifics displayed a stage 5 eversion than did those that were isolated (Table 1).

Individual snails that had reached a stage 5 genital eversion on day 1 of the experiment, and which were subsequently isolated, required significantly more time to reach stage 5 when they were removed from isolation one week later (Table 2). Conversely, similar snails that were given daily exposure to conspecifics required less time to reach stage 5 on the final test day (day 8) compared to day 1, though this difference was of marginal statistical significance (Table 2). Despite these differences, the time required for the same individual to reach stage 5 on day 1 and on day 8 was significantly correlated for both the daily exposed group and the isolated group (Spearman $\underline{r}(15)=0.63$, $\underline{p}<.01$ for daily exposed snails, Spearman $\underline{r}(18)=0.48$, $\underline{p}<.05$ for isolated snails).

Table 1 The effect of daily contact with conspecifics or social isolation on genital eversion and courtship behaviour The mean times required to reach a stage 5 genital eversion are significantly different between the daily exposed and isolated groups ($p < 02$, 2-tailed Mann-Whitney test) The number of courters in each group is also significantly different ($p < 05$, G-test with William's correction)

	Daily exposure to conspecifics (n=78)	Isolated 1 week from conspecifics (n=70)
Number of courters	38 (49%)	47 (68%)
Number reaching stage 5	23 (30%)	34 (49%)
Time to stage 5 (min +/- s e.m)	44 4 +/- 3 0	63 4 +/- 4 9

Table 2. Time dependent effects of social isolation or daily contact with conspecifics on courtship behaviour. Snails exposed to daily contact with conspecifics achieved a stage 5 genital eversion more quickly on day 8 than on day 1, though the difference was only marginally statistically significant ($p=.07$, 2-tailed Wilcoxon signed ranks test). Isolated snails required significantly more time on day 8 than on day 1 to reach genital eversion stage 5 ($p<.05$, 2-tailed Wilcoxon signed ranks test). The number of courting snails did not significantly change after daily exposure, but the number did increase significantly in the isolated group ($p<.03$, modified G-test for repeated testing of the same individuals).

	Time (min +/- s.e.m.) to reach stage 5	n	Number of courting snails		n
Daily contact					
day 1	49.3 +/-5.6	15	30	(38.5%)	78
day 8	40.3 +/-2.6	15	38	(48.7%)	78
Social isolation					
day 1	47.3 +/-4.2	13	26	(37.1%)	70
day 8	63.5 +/-7.5	13	47	(67.1%)	70

Groups that had daily exposure to conspecifics showed no significant increase in the number of pairs that courted from day 1 to day 8 (Table 2). Snails that were courting on day 1 tended to continue courting every day (11/15 snails). Lind (1976) and Jeppesen (1976) reported similar result in H. pomatia.

In summary, sexual isolation increased the time snails needed to achieve a stage 5 eversion, suggesting that isolation decreased sexual arousal, but it also increased the number of snails that exhibited courtship, suggesting that it increased proclivity in some of the previously non-courting snails. Conversely, daily contact with conspecifics had a slight positive effect on sexual arousal, but did not significantly increase the proclivity of non-courting snails.

Experiment 4. Differential Effects of Digitiform Gland Homogenate on Sexual Arousal and Sexual Proclivity

The digitiform glands of H. aspersa secrete the mucus that coats the dart as it is shot. Homogenates of these glands can increase the genital eversion stage when injected into snails with genital eversions at stage 2 or 3 (Chapter 3). There is also evidence that the homogenate can decrease courtship duration times (Chapter 3). These data suggest that the mucus can increase

sexual arousal. In the present experiment, the homogenate was tested for its effect on sexual proclivity.

Methods

Digitiform gland homogenates of 2 different concentrations (2 mg digitiform gland/snail and 4.5 mg digitiform gland/ g snail) were prepared as previously described (Chapter 3). Digitiform gland homogenate was injected into randomly chosen, non-mating, previously isolated snails. Non-mating snails were those that had shown no mating behaviour during the previous 5 trials in the group box and which had tested negative on a proclivity test immediately prior to injection. Another group of similar snails received saline injections. After the injections, the snails were given a second proclivity test. They were then collectively placed in a group box and their courtship behaviour was recorded. Daily tests and injections were given for 8 consecutive days.

The above test was repeated using snails that had tested positive on an initial proclivity test and that had exhibited courtship behaviour during at least one of the last 5 courtship trials.

Results

Digitiform gland homogenate injections at either dose had no effect on sexual proclivity irrespective of the initial sexual tendency of the recipient (Table 3). Therefore, although digitiform gland homogenate appears to increase sexual arousal at the delivered doses (Chapter 3), in the present experiment it neither increased nor decreased sexual proclivity.

Experiment 5. Differential Effects of Sexual Arousal and Sexual Proclivity on Locomotory Behaviour

Methods

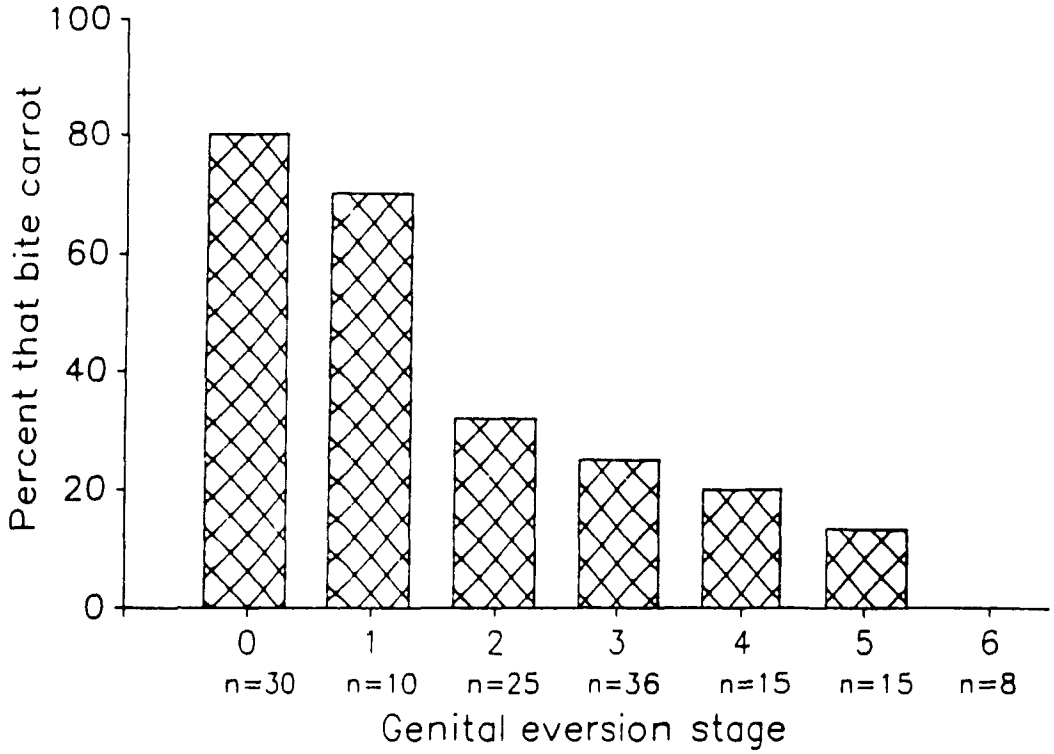
Snails were removed from their individual containers and placed in a group box. During a trial a randomly chosen snail was placed on a vertical paper treadmill. The presence or absence of a genital eversion was noted. Snails have a negative geotaxis and therefore tend to crawl upwards under these conditions. Snails were allowed to locomote freely for 5 min. Afterwards, the distance the snail travelled was measured by laying a black thread over its mucous trail.

In a second experiment, snails were given a proclivity test prior to the locomotory trial. These snails were not placed in a group box prior to the trial. After the trial, snails were placed in a group box and any courtship behaviour was recorded.

Table 3. The effect of digitiform gland homogenate on sexual proclivity. Digitiform gland homogenate had no effect on proclivity at either a low dose (2 mg digitiform gland/ snail) or a high dose (4.5 mg digitiform gland/g snail) (in each case, $p>>.1$, ^{G}-test with William's correction). The values below are the number of snails that tested positive on a proclivity test (proclivity column) and the number of snails that exhibited courtship behaviour (courtship column) (+/- s.e.m.) averaged over the 8 daily trials. N=10 for all groups.

digitiform gland homogenate				saline	
low dose		high dose			
proclivity	courtship	proclivity	courtship	proclivity	courtship
Non-maters					
0.3±0.2	0±0	0.3±0.2	0±0	0.3±0.2	0±0
Maters					
10.0±0.0	5.8±1.3	10.0±0.0	6.5±1.1	10.0±0.0	5.9+1.2

Figure 6. Percentage of snails that bite a carrot at different stages of genital eversion. As genital eversion stage increased, the percentage of snails that took at least one bite during the trial decreased. The trend is significant (Spearman r=.97, p<.001).


100
80
60
40
20
0
Percent that bite carrot
0
n=30
1
n=10
2
n=25
3
n=36
4
n=15
5
n=15
6
n=8
Genital eversion stage

Results

Snails with genital eversions locomoted significantly further (29.5 +/- 8.1 cm, n=16) than did unaroused controls (21.7 +/- 8.7 cm, n=16, 2-tailed Mann-Whitney test, $p<.05$).

Snails that scored positively on the proclivity test (n=23) locomoted the same distance as those that scored negatively (n=27; 2-tailed Mann-Whitney test, $p>>.10$). As expected, there was no difference between the locomotion of snails that subsequently courted (n=19) and those that did not (n=31; 2-tailed Mann-Whitney test, $p>>.10$, corroborating the proclivity test results.

Therefore, under conditions in which sexually aroused snails exhibited increased locomotion, snails with sexual proclivity showed neither an increase nor decrease in their locomotory behaviour.

Experiment 6. Differential Effects of Sexual Arousal and Sexual Proclivity on Feeding Behaviour

Methods

Previously isolated snails were placed in a group box. Randomly selected snails were removed from the group box and were placed in a small box (8 cm x 8 cm x 8 cm) facing a piece of carrot 1 cm away. The initial genital eversion of the snail, if any, was recorded. The time

the snail spent in contact with the carrot was recorded. Contact was defined as touching the carrot with the head or body. Touching the carrot with only a tentacle did not count as contact. Snails in contact with the carrot were also observed to see if they bit the carrot (i.e. scraped it with their radula).

A similar trial was conducted with isolated snails after they had been given a proclivity test. After the trial, snails were placed in a group box and their courtship behaviour was recorded.

Some trials were videotaped, and the times were scored by an independent observer. The correlation between the two scorers was high (Spearman $r(28)=.92$, $p<.01$), therefore only the observations of the initial scorer were analyzed.

Results

Snails with a higher stage of genital eversion spent less time in contact with the carrot than did those with lower stage eversions (Kendall's Tau $n(7)= -.68$, $p<.03$). The proportion of snails that took at least one bite was also less when they exhibited high stage eversions than when they exhibited low stage eversions (Fig. 6). Snails that had scored positively on the proclivity test (n=16) did not differ in the time they spent in contact with the carrot or in the proportion of snails that took at least one bite when compared to snails that had scored

negatively on the same test (n=34). Similarly, there was no difference in carrot contact time, nor in the proportion that fed, between snails that subsequently courted and those that did not.

In summary, although sexual arousal inhibited feeding behaviour, under the same conditions sexual proclivity had no measurable effect.

General Discussion

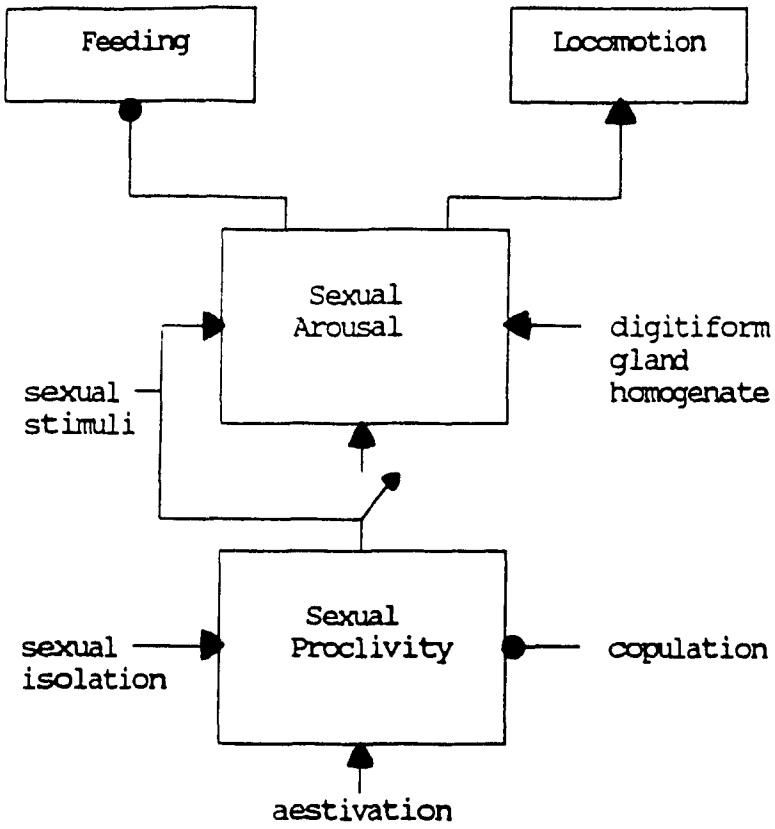
Table 4 summarizes the differences between sexual arousal and sexual proclivity, based on the results of this study. Though sexual proclivity is necessary for the expression of sexual arousal, conditions which increase proclivity (such as social isolation) do not increase sexual arousal. This suggests that arousal is not simply due to an increase in the same physiological conditions that create proclivity. Fig. 7 illustrates this hypothesis as part of a model for Helix sexual behaviour.

The conclusion that arousal and proclivity are distinct phenomena differs from that which would have been suggested if similar experiments had been conducted using feeding behaviour. For example, we would expect starvation to increase the number of snails that would feed, but we would also expect starvation to have a

Table 4 Summary table of the experimentally determined differences between sexual proclivity and sexual arousal +, increase, -, decrease, ? marginally significant

	Proclivity	Arousal
Dependent variables		
feeding	none	-
locomotion	none	+
Independent variables		
social isolation	+	-
daily contact	none	+?
digitiform gland homogenate	none	+

Figure 7. A schematic summary of the organization of sexual behaviour in Helix aspersa. Sexual proclivity results in sexual arousal, and hence sexual behaviour, only in the presence of sexual stimuli. The box labelled 'sexual proclivity' represents the sum of all the neural and/or hormonal components which contribute to an increase in the animal's responsiveness to sexual stimuli. The box labelled 'sexual arousal' represents the neural components responsible for the execution, and the speed of execution, of sexual behaviour. The filled arrows represent excitatory effects, while the filled circles represent inhibitory effects.


Feeding
Locomotion
Sexual
Arousal
digitiform
gland
homogenate
sexual
stimuli
Sexual
Proclivity
sexual
isolation
copulation
aestivation

positive effect on their level of food arousal. Indeed, feeding experiments done using other molluscs have found that starvation increases both the tendency to feed, usually measured as the latency to the first bite or the number of spontaneous rasps, as well as the intensity of feeding, as expressed by the frequency and intensity of biting (Susswein, Weiss, & Kupfermann, 1978, Weiss, et. al., 1982, Tuersley, 1989). Therefore, sexual behaviour in Helix differs from feeding behaviour in these other molluscs, and probably also in Helix, in that arousal and proclivity appear to be more independent of each other.

In the pond snail, Lymnaea, however, isolation both increases the number of copulating snails and decreases their latency to intromission (Van Duivenboden and Ter Maat, 1985). Therefore, in this snail, isolation has similar effects on both proclivity and arousal. The relationship between arousal and proclivity for a particular behaviour probably depends on the selective forces present during the animal's evolution, and not on whether the behaviour is feeding or sex.

The differences between the isolated and daily contact groups in the time required to complete introductory behaviour (Table 1) could occur by at least 3 different (and not necessarily mutually exclusive) mechanisms. First, because exposure to conspecifics appeared to affect behaviour 24 hours later, contact

with conspecifics may have residual effects that cause the snails to be already slightly aroused when they are placed in the group box, thus decreasing the time they need to reach stage 5. The effect cannot last longer than a week, however, since isolated snails show no increase in arousal. This conclusion is also indirectly supported by the fact that the correlation between courtship times on day 1 and day 8 is stronger for daily exposed snails than for isolated snails. The daily exposed snails seemed to maintain their relative courtship speeds, suggesting some carry-over effect. If this were not the case, one would expect the distribution of courtship speeds amongst individuals to be more or less random each day. Of course, this would not be the case if the relative courting speed of a snail is merely due to some factor intrinsic to an individual snail, such as age, but this seems unlikely because then one would expect isolated snails to be equally well correlated. Other animals have shown a similar residual effect after sexual arousal (Toates, 1980), and the phenomenon is also found after food arousal (Susswein et. al., 1978).

A second possible explanation for the differences in courtship times between the two groups is that the daily exposed snails show a learning, or practice, effect which increases their speed relative to isolated snails.

A third possibility is that isolation inhibits sexual arousal. Lack of sensory stimuli from conspecifics may cause a decrease in the 'excitability' of the system that controls courtship behaviour, thereby requiring a longer period of sexual stimulation before the snail can reach a stage 5 genital eversion.

Because snails continued to court with daily exposure to conspecifics, it does not appear that exposure to conspecifics decreases proclivity. However, social isolation is even more effective than daily exposure in inducing or increasing proclivity (Table 1). Because both groups are sexually deprived in that neither group had an opportunity to copulate, the only difference between the 2 groups is in the amount of conspecific contact each group receives. Conspecific contact is absent during aestivation, during which sexual proclivity also increases (Bonnefoy-Claudet & Deray, 1984). This suggests that sensory contact with conspecifics and/or their mucus can depress the development of sexual proclivity. Sensory contact with conspecifics and/or their mucus has been shown to depress growth and egglaying in H. aspersa (Herzberg, 1965, Dan & Bailey, 1981).

It seems likely that whatever initiates sexual behaviour, a different process controls sexual arousal. Proclivity biases the snail's response to specific

stimuli (Fig. 4 and Fig. 5), but it develops without sensory stimuli from conspecifics, and without any specific motor response. Courtship involves receiving sensory stimuli from a conspecific, as well as producing the appropriate motor responses. These concomitant activities in the central nervous system could be used as cues to raise or lower the intensity of courtship behaviour. Proclivity, lacking these cues, would need to be controlled differently (i.e. hormonally). Sexual behaviour in vertebrates is also thought to be made up of mechanistically distinct components (Beach, 1976).

One of the fundamental difficulties facing any attempt to rigorously study the biological basis of 'motivation', especially sexual 'motivation', is the lack of externally observable correlates. However, sexual behaviour in H. aspersa has 2 distinct, externally verifiable components: sexual arousal (behavioural intensity) and sexual proclivity (the tendency to mate). Moreover, sexual behaviour in H. aspersa has the potential to be studied using the same standard neurophysiological techniques that have been successful in the study of feeding behaviour (Chase, 1986, Balaban and Chase, in press). This makes H. aspersa a convenient animal in which to study the biological basis of sexual arousal and sexual proclivity.

Chapter 5. The interactions of sexual behaviour, feeding behaviour and locomotion in the behavioural hierarchy of the snail Helix aspersa

Summary

1. Starvation both increased the number of snails that exhibited feeding behaviour (i.e. increased feeding proclivity) and decreased the latencies of response to a food stimulus (i.e. increased food arousal) (Fig. 3). Feeding behaviour inhibited locomotion, although starvation itself had no effect (Table 3).

2. Sexual arousal increased locomotive behaviour (Table 5), but only in the absence of a mating partner. Sexual proclivity had no effect on locomotion. In general, proclivity and arousal had different effects on behaviour (summarized in Fig. 9).

3. Food deprivation did not alter the preference of sexually aroused snails for sexual stimuli over food stimuli (Fig. 7). Both starved and fed courting pairs responded to a food stimulus only during periods of low sexual arousal (Table 4), although when not sexually aroused, starved snails usually increased the amount of time they spent in contact with a food stimulus. These results suggest that sexual behaviour is dominant over feeding behaviour and that the expression of feeding behaviour is dependent upon the occurrence of low levels of sexual arousal.

4. The interaction between sexual behaviour and feeding behaviour can best be described as a time-sharing arrangement.

Introduction

Snails, like other animals, normally perform only one behaviour at a time. This 'singleness of action' requires that a snail must make a 'choice' when stimuli for two mutually incompatible behaviours are presented simultaneously. The resolution of this seemingly simple problem depends upon such less than simple concepts as 'arousal', i.e. the ability of hunger and other internal states to bias behaviour.

Given the actual complexity of the problem, one fruitful approach has been to study an animal that exhibits behavioural choice, but which also has a minimum number of elements involved (neurons and hormones) and a minimal behavioural repertoire. One such animal, the marine mollusc, Pleurobranchaea, has been found to make behavioural 'decisions' according to a behavioural hierarchy, with some behaviours dominant over others (Davis, et al., 1974a). Because of the relative simplicity of the nervous system of Pleurobranchaea, Davis and co-workers have been able to elucidate some of the physiological mechanisms underlying this hierarchy (Davis et al., 1974b, Kovac and Davis, 1980a).

Like Pleurobranchaea, Helix aspersa has relatively simple nervous and endocrine systems which have been used to study physiological mechanisms of behavioural control

(Chase, 1986; Balaban and Chase, in press; Chapter 3). This paper examines behavioural choice in Helix aspersa, adding to results published previously by Everett et al. (1982). Everett et al. (1982) found that withdrawal behaviour inhibits feeding, which in turn inhibits righting, rearing and negative geotaxic locomotion. We further investigate the relationships between sexual behaviour, locomotion, and feeding.

We also examine the effect of 'arousal' on behavioural choice. In Pleurobranchaea, food 'arousal' alters the relative positions of some of the behaviours in the hierarchy (Kovac and Davis, 1980b). In this paper we discriminate between two different usages of the term 'arousal'. 'Arousal' has been used to describe the intensity or vigour with which an animal performs a response, but it has also been used to describe the likelihood that a particular stimulus will evoke an appropriate response (Andrew, 1974). In this paper, the intensity of a response will be called arousal, while the tendency of an animal to perform a given behaviour will be called the proclivity for that behaviour. A similar differentiation has already been described for sexual behaviour in H. aspersa (Chapter 4). Sexual deprivation increases a snail's sexual proclivity, i.e. it is more likely to initiate mating, but it has no effect on a snail's sexual arousal, i.e. the intensity with which it

courts. Proclivity and arousal are here tested for their separate effects on the behavioural hierarchy.

The sexual behaviour of H. aspersa has been previously described (Chapter 2, Chung, 1987). Briefly, snails are simultaneously reciprocal hermaphrodites. Courtship has three main phases: introductory behaviour, dart shooting and copulation. During introductory behaviour snails evert their normally internal genital apparatus. Prior to dart shooting, the degree of the eversion can be reliably classified into six successive stages from its size and shape (Chapter 2).

Materials and Methods

General Methods

H. aspersa were imported from California (Marinus, Long Beach) and were placed in a colony box (30 cm x 32 cm x 35 cm). The snails had an average weight of about 8.0 g (range 5 g - 13 g). Snails that were observed copulating in the colony box were individually numbered for subsequent identification. Fifty-six numbered snails were transferred to individual Lucite containers (7 cm x 8 cm x 8 cm) to sexually isolate them. Forty other numbered snails were randomly divided into two groups and each group was placed in one half of a large divided plastic container (group cage) (16 cm x 30 cm x 35 cm).

The container was divided by a Lucite sheet which had been drilled with holes (diameter 3 mm) to allow the passage of air, but not snails. The remainder of the numbered snails was left in the colony box. The snails were maintained at 19° C ± 2° C on a 16:8 L:D photoperiod. The snails were fed lettuce, carrots and oyster shells ad lib. unless otherwise noted. All containers were kept moist and clean.

The experiments were run at the beginning of the light cycle and continued for a maximum of 3 h. Snails were randomly chosen for trials either by a coin flip or by a lottery system based on their identification number. Snails were used only once per day. Only snails with a Behavioural State Score (BSS) of 4 or 5 (see below) were used for experimental trials unless otherwise indicated.

Statistical analysis was done according to the procedures described in Sokal and Rohlf (1981) unless otherwise indicated.

Behavioural State Score (BSS) Measurements

Behavioural State Scores (BSS), derived from an animal's stereotypic postures, have been found to form a scale of increasing levels of 'alertness', or activity, in Aplysia (Preston and Lee, 1973) and in Pleurobranchaea (Lee and Palovcik, 1976). Because the BSS is derived

from body posture, the BSS estimates snail activity without disturbing the animals. We have divided the behavioural state of H. aspersa into six categories from 0 to 5 (Table 1). To test whether the BSS correlated with any other independently measured aspect of activity, randomly chosen, sexually mature (i.e. numbered) snails were removed from the colony box and placed on a large tiled surface covered with fine sand. The BSSs were taken 5 min later, and the distance each snail locomoted over the next 5 min was recorded. The distance was measured by laying a black thread over the mucous trail and measuring the length of the thread.

To test the relationship between feeding and BSS, a randomly chosen, sexually mature snail was removed from the colony box and placed behind a line drawn on a glass plate. A carrot root was attached to a force transducer 1 cm away from the snail. The carrot was suspended 5 mm below and 5 mm away from the edge of the glass plate. The BSS of the snail was recorded 10 s after placing it on the glass plate. The time required for the snail to take a bite of the carrot, as evinced by the transducer records, was recorded.

Table 1. Criteria for Behavioural State Scores (BSS).

State	Description
0	Body completely withdrawn into shell, foot not attached to substrate
1	Body not visibly extended beyond shell, but foot attached to substrate
2	Body partially extended from shell, tentacles unextended
3	Body partially extended from shell, tentacles visible
4	Body fully extended from shell, movement of head and/or tentacles
5	Body fully extended from shell, whole body movement (locomotion)

Assessment of Proclivity and Arousal

Sexual proclivity was determined by a test given immediately before a trial, as described in Chapter 4. Briefly, the test consisted of observations as to whether a snail turned towards or away from an anesthetized conspecific after initial contact. Levels of sexual arousal were inferred from the stage of the snail's genital eversion (as defined in Chapter 2). Higher stages of genital eversion correlate with higher levels of sexual arousal (Chapter 4).

To demonstrate that food deprivation could increase both feeding proclivity and feeding arousal, individually housed snails were starved from 0 to 5 days. They were placed behind a line drawn across a 10 cm x 8 cm x 8 cm Lucite box, facing a carrot root 5 cm away. The carrot was connected to a force transducer. The average feeding proclivity of the group was measured as the number of snails that bit the carrot. The level of feeding arousal was measured as the latencies of individual snails to come into contact with the carrot (as evinced by the transducer output). Contact was defined as touching the carrot with some part of the head for more than 15 s. Touching the carrot with a tentacle did not count as contact. Trials were 5 min in length. Since H. aspersa is able to orient to food sources using olfactory cues

(Farkas and Shorey, 1976), it was assumed that hungry snails would exhibit directed locomotion towards the food source, whereas fed snails would move more randomly. We show later that starvation alone does not increase locomotion (Table 3).

The Effect of Food Deprivation on BSS

Snails in one half of the divided group cage were given food ad lib. Snails in the other half of the cage were given no food for 5 days. Small Petri dishes of water were supplied to help maintain the snail's hydration since they were unable to extract water from their usual food source. After the 5 days of food deprivation, all snails were fed ad lib. for 5 days, after which the previously ad lib. fed snails became the starved group and the procedure was repeated. BSSs and copulation rates were recorded daily for each snail. Data were collected every day at the beginning of the light cycle for 100 days.

The Effect of Food Deprivation on Locomotion

Randomly chosen fed and starved snails were taken from the group box described above and placed on a large tiled surface covered with fine sand. A snail was

allowed to locomote freely for 10 min after which the distance it had travelled was measured by placing a black thread over the mucous trail and measuring the length of the thread.

In a second experiment, starved or fed snails were placed on a vertical paper treadmill. Snails have a negative geotaxis and therefore tend to crawl upwards under these conditions. Prior to a trial the paper was lightly brushed with 'V-8' juice along a distance extending 20 cm above the point at which the snail was to be placed on the paper. The distance each snail travelled was measured as described above. Both experiments were scored without knowing whether a given snail was from the starved or fed group.

The Effects of Feeding and Food Deprivation on Sexual Behaviour

Snails in the individual containers were randomly divided into two groups. One group was given food ad lib.; the other group was starved for 22 h prior to the trial. Pairs of either fed or starved snails were placed in small Lucite boxes (8 cm x 8 cm x 8 cm). Fed or starved pairs were randomly assigned to either an empty Lucite box or to one which contained a leaf of lettuce covering the bottom of the container. The two snails

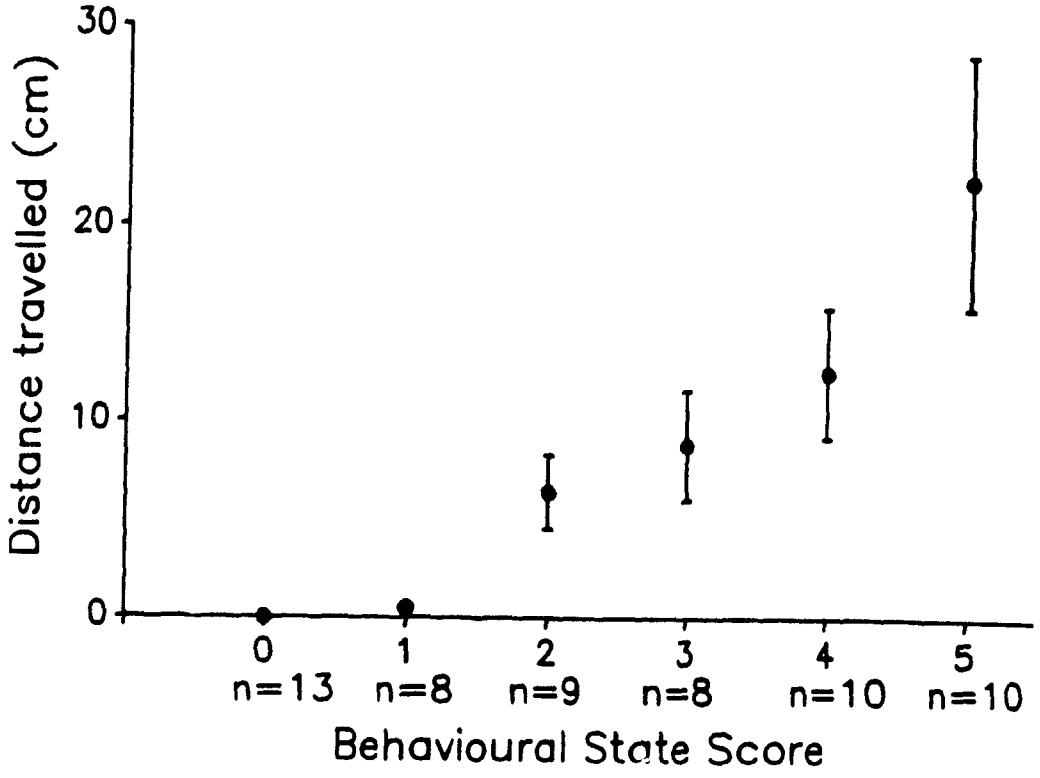
were placed 1 cm apart, facing each other's tentacles. The time required for each pair to begin introductory behaviour, and the time required for at least one member of the pair to reach a genital eversion of stage 5 was recorded. Snails that failed to reach stage 5 were given an arbitrary score of 120 min, which was the length of the trial. The number and lengths of courtship breaks (> 15 s) were observed and recorded. The amount of food consumed during the 2 h trial was determined by weighing the lettuce before and after the trial.

The Effect of Sexual Arousal on Feeding

Snails were taken from their individual containers and placed in a large group box (20 cm x 20 cm x 8 cm). As a snail reached a previously assigned genital eversion stage, it was placed behind a line drawn in a small Lucite box, facing a carrot 1 cm away. The time the snail spent in contact with the carrot was recorded.

In a second experiment, socially isolated snails were placed in a group box. When a snail reached a previously assigned genital eversion stage, it was placed 1 cm equidistant from an anesthetized snail and a carrot. An anesthetized snail was assumed to act as a sexual stimulus. Snails were anesthetized by injecting them with 0.1 mL of 2% $MgCl_2$ and 0.1% succinylcholine

Figure 1. The relationship between a snail's Behavioural State Score and its subsequent locomotive behaviour. As the Behavioural State Score increased snails travelled significantly farther during a 5 min trial (Spearman's $r=0.99$, $p<0.05$). Error bars denote ± standard error of the mean.


30
20
10
0
Distance travelled (cm)
0
n=13
1
n=8
2
n=9
3
n=8
4
n=10
5
n=10
Behavioural State Score

chloride dissolved in H. aspersa saline (Chung, 1985). The anesthetized snails remained motionless, with extended, but flaccid, bodies. Whether the test snail made first contact with the anesthetized conspecific or the carrot was recorded, as was the time the snail spent in contact with both objects. In both experiments the trials were videotaped and an observer otherwise uninvolved in this study scored the snails.

The Effect of Sexual Arousal on Locomotion

Chapter 4 has shown that sexually aroused snails travel farther on a vertical treadmill than do unaroused snails. In a further experiment, the treadmill was again used, except that the snails were placed 1 cm below and facing a snail with a stage 6 genital eversion. Snails with stage 6 genital eversions are both consistent and persistent courters (Chapter 3). The effect of a potential mating partner on the distance locomoted was measured.

Results

Behavioural State Score (BSS) Measurements

The distance a snail travelled in the locomotor test was positively correlated with the snail's BSS (Fig. 1).

As in Pleurobranchaea (Lee and Palovcik, 1976), BSSs also negatively correlated with the latency of contact with a food stimulus (Fig. 2). Given these results, BSS will be used in the following experiments to estimate snail activity.

Assessment of Proclivity and Arousal

Twenty-two h of food deprivation increased feeding proclivity. The number of snails that responded to (i.e. bit) a food stimulus was greater in starved snails (29/30) compared to fed snails (22/30, $p<0.05$, G-test using William's correction).

Food deprivation also increased feeding arousal. It increased the amount of time snails spent in contact with the carrot during the 5 min trial (Spearman's $r(6)= 0.97$, $p<0.05$). Food deprivation decreased the time required for a snail to come into contact with the carrot (Fig. 3), even after one day of deprivation ($p<0.05$, one sided Wilcoxon sign test).

The Effect of Food Deprivation on BSS

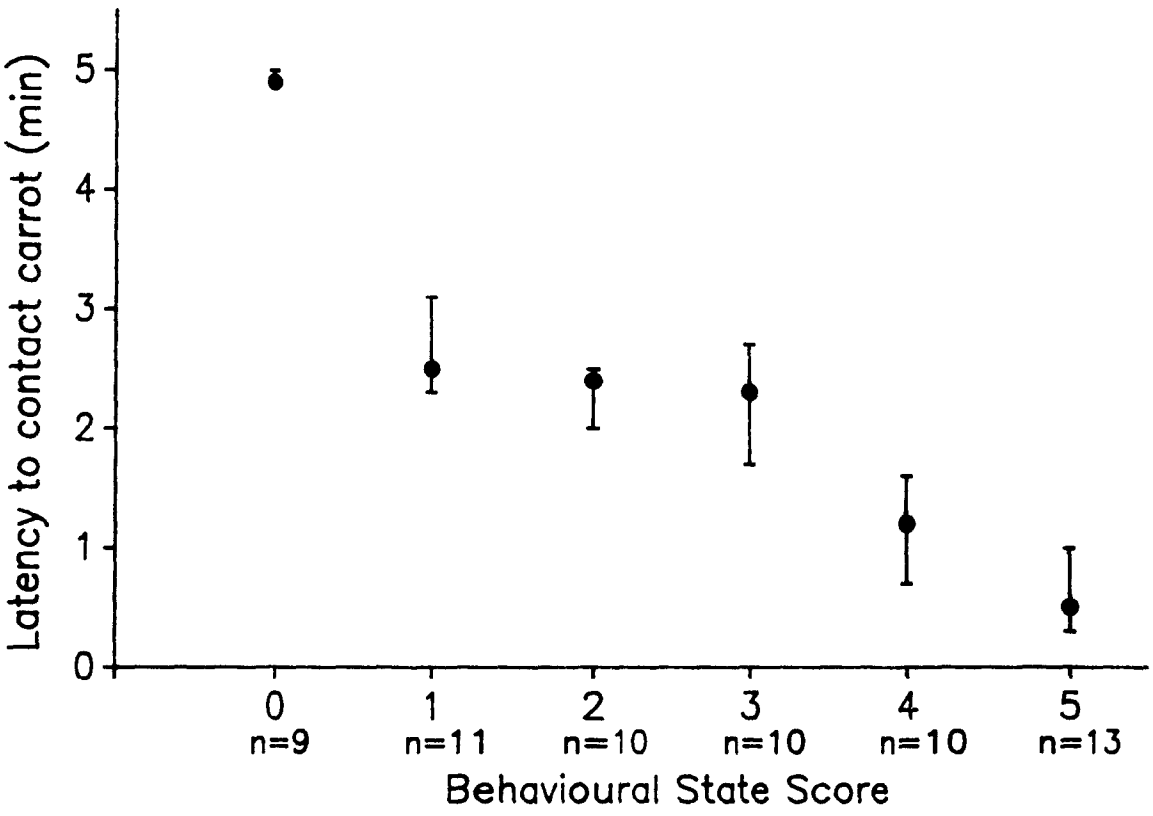
As the number of days of starvation increased, activity declined (Table 2). After 2 or 3 days of food deprivation, many snails withdrew into their shells.

Table 2. The effect of starvation on Behavioural State Score.

	Behavioural State Score	Number of 5-day trials
Fed (n=20)	2.2 ± 0.2	20
Starved (n=20)		10
Number of days starved		
1	2.3 ± 0.2	
2	2.0 ± 0.1	
3-5	1.4 ± 0.2	
Post-starved (n=20)		10
Number of days after starvation		
1	2.9 ± 0.2	
2	2.8 ± 0.2	
3-5	2.4 ± 0.2	

Values are given as the mean ± standard error. The difference in BSS between starved, fed, and post-starved snails depended significantly on the number of days starved ($p<0.01$, nonparametric factorial analysis, $H= 20.7$; Meddis, 1984). Post-starvation snails were more active than fed snails, and this difference decreased progressively with the time after starvation ($p<0.01$, nonparametric factorial analysis, $z= 2.73$).

Figure 2. The relationship between a snail's Behavioural State Score and its latency to contact a carrot root. As Behavioural State Score increased, fed snails contacted the carrot faster (Spearman's $r=-0.96$, $p<0.05$). Latency to carrot contact is plotted as the median with error bars denoting the first and third interquartile values. Medians were used due to the large number of snails that did not contact the carrot and which were given arbitrary scores of 5 min (length of trial).


Latency to contact carrot (min)
0
1
2
3
4
5
0
n=9
1
n=11
2
n=10
3
n=10
4
n=10
5
n=13
Behavioural State Score

When starved snails were again given food, not only did the average BSS increase and the number of active snails increase, but both parameters rose significantly above the values for fed snails (Table 2).

The Effect of Food Deprivation on Locomotion

In the absence of food stimuli, there were no significant differences between the distances locomoted by fed snails, 22 h starved snails, or snails recovering from starvation (Table 3). This lack of effect is not because 22 h of food deprivation is insufficient to create any change in the snail's behaviour; starvation at this level causes a significant increase in food arousal (Fig. 3). Moreover, when a food stimulus was present ('V-8' juice), fed snails locomoted significantly farther than did 22 h starved snails ($p<0.01$, 2-tailed Mann-Whitney test). Fed snails locomoted 20.7 cm ± 9.9 cm (s.d., n=20), while starved snails locomoted 10.8 cm ± 11.5 cm (s.d., n=20). The difference between the two groups can be accounted for by the fact that significantly more starved snails (14/20) stopped to feed on the paper than did fed snails (7/20) ($p<0.01$, G-test with William's correction). If the data from all the snails that bit the paper at least once are excluded from the analysis, then there was no difference in locomotion

Figure 3. Effect of food deprivation on latency to contact a carrot. As the number of days of food deprivation increased, latency to carrot contact decreased (Non-parametric 2 x 2 factorial design, Meddis (1984), $n=10$, $p<0.01$). Data are plotted as medians with error bars denoting first and third interquartile values.

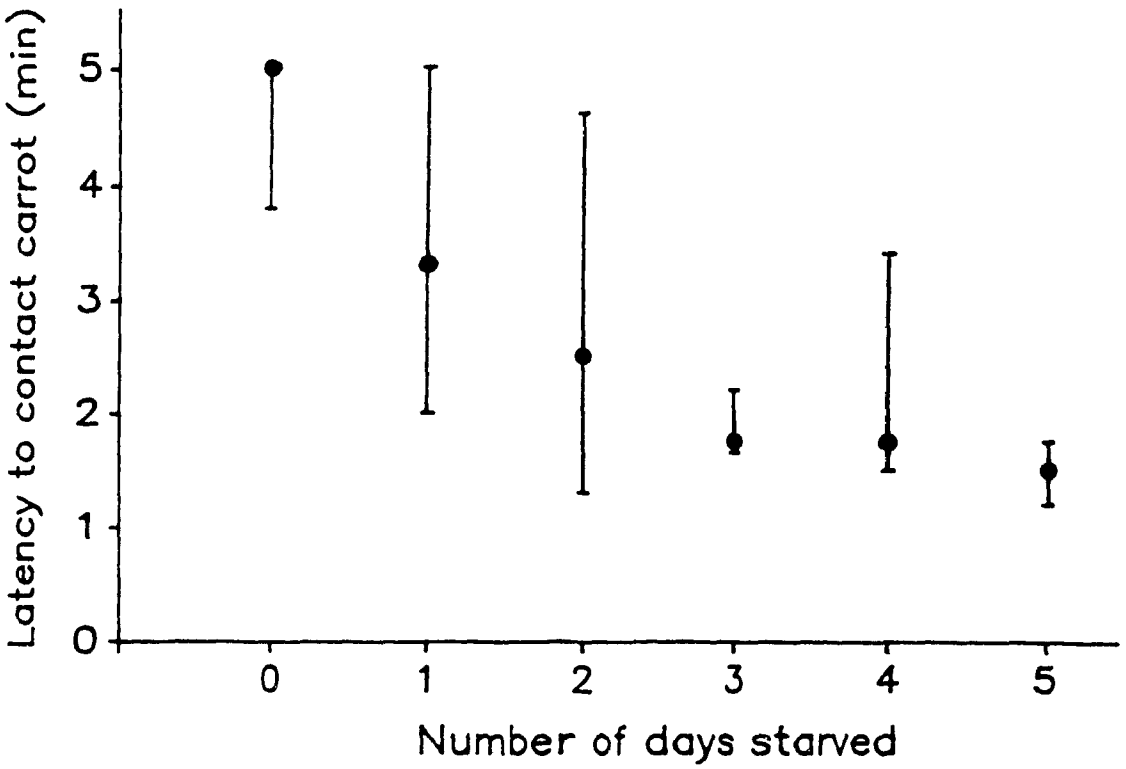

Latency to contact carrot (min)
0
1
2
3
4
5
0
1
2
3
4
5
Number of days starved

Table 3. The effect of starvation on locomotion in the absence of food stimuli.

Fed	Starved	Post-starved
Distance locomoted in 5 min (cm)		
17.3 ± 3.0	20.8 ± 3.0	21.8 ± 5.3

There were no significant effects of starvation on locomotion (2-way ANOVA, repeated measures on individual snails, $F(2,38)$. Since there was no difference over the five days of starvation, the mean distance locomoted (± standard error) is shown for each treatment. N=20 for all groups.

between the two groups ($p>0.1$, 2-tailed Mann-Whitney test). Therefore, although food deprivation increased the likelihood that snails would stop locomoting to feed, it had no effect on the speed of locomotion.

The Effects of Feeding and Food Deprivation on Sexual Behaviour

The presence of food stimuli affected sexual behaviour even in snails that had not been food deprived. Fed snail pairs that mated on lettuce took longer to reach a stage 5 genital eversion (Table 4) than did those that mated on bare Lucite, although the latency to start courtship was unaffected. Fed snails courting in the presence of lettuce took significantly longer breaks than did fed pairs courting in a box without lettuce (Table 4). During 39% of these breaks snails bit the lettuce (Table 4). Therefore, snails ate lettuce during courtship even if they were not food deprived.

In contrast to fed courting snails, starved snail pairs required more time to begin introductory courtship behaviour when they performed their courtship on a bed of lettuce as opposed to in an empty Lucite box (Table 4). Prior to courtship, 100% (24/24) of the starved snails took bites from the lettuce. Further, unlike fed courting snails, starved snails required the same length

Table 4. The effect of food stimuli on courtship behaviour

Courtship Parameter	Fed Snails		22h Starved Snails	
	No Lettuce	Lettuce	No Lettuce	Lettuce
Latency to courtship (min) n=24	17 4 ± 2 5	22 3 ± 3 3	20.5 ± 4.5[a]	32 8 ± 3 9[a]
Time to stage 5 (min) n=24	35 (27-43)[b]	59 (40-82)[b]	50 (43-61)	44(32-49)
Number failing to reach stage 5 n=24	3	6	5	6
Percentage that eat during breaks n=10	-	39% (22/56)	-	38% (20/52)
Number of breaks > 15 s n=10	5 6 ± 0 8	7 0 ± 0.7	6.5 ± 0 6	6 5 ± 0 6
Length of breaks (min) n=10	1 9 ± 0 7[b]	5 6 ± 2 8[b]	2 5 ± 2 1	3 2 ± 1 ɔ

[a] The indicated values are significantly different, p<0 02 (2-sided t-test).

[b] The indicated values are significantly different, p<0 05 (2-sided Mann-Whitney test)

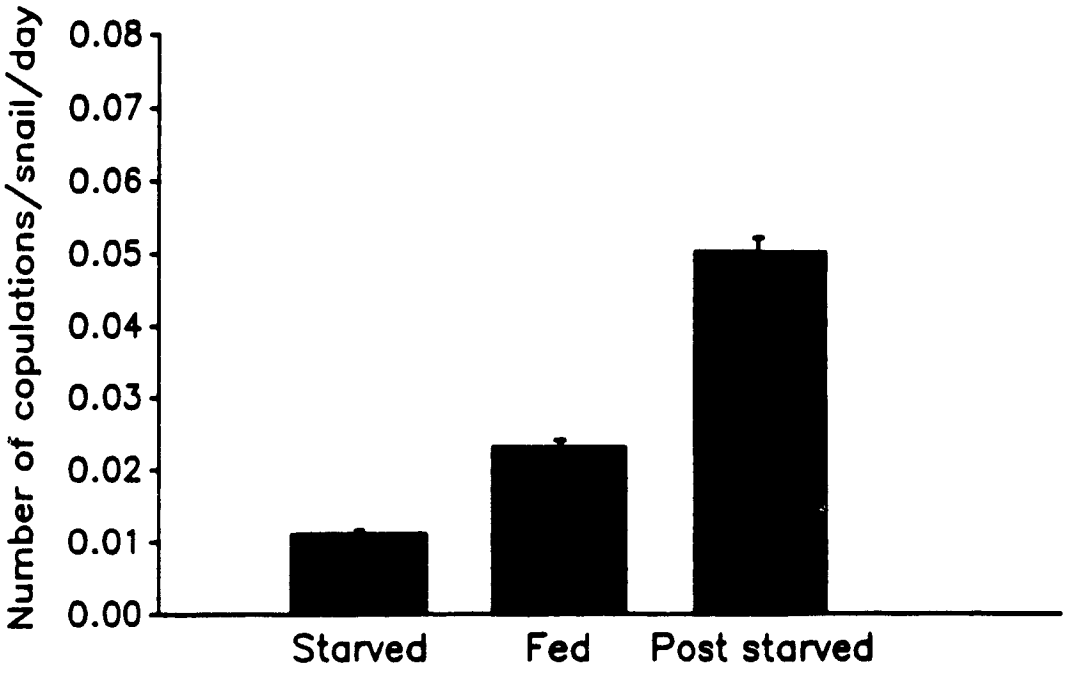
Latency to courtship, number of breaks and length of breaks are shown as the mean ± standard error Time to stage 5 is shown as the median with the first and third quartile values given in brackets. The median was used because snails that failed to reach stage 5 were given an arbitrary score of 120 min (length of trial)

Table 5. The effect of sexual arousal on locomotion.

	Distance travelled (cm ± sem)	
	Alone	With mating partner
Sexually aroused	29.5 ± 2.0	19.6 ± 2.3
Sexually unaroused	22.7 ± 4.9	19.9 ± 3.0

Snails were given 5 min to locomote. The depression of locomotion by the presence of a mating partner is dependent upon whether the locomoting snail is sexually aroused (2-way ANOVA repeated measures, $F(1,15)$ $p<0.05$). N=16 for all groups.

Figure 4. The effect of starvation on the frequency of copulation. Starved snails copulated less frequently than fed snails, while post-starved snails copulated more frequently than fed snails ($p<0.01$, G-test). Error bars denote ± 1 standard error of the mean. N=20 for all groups.


Number of copulations/snail/day
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Starved
Fed
Post starved

of time to reach dart shooting threshold (stage 5) irrespective of the presence or absence of lettuce (Table 4). Therefore, food stimuli affect mating behaviour, but the effect depends on the length of food deprivation. With little or no food deprivation, the presence of food stimuli increases the length of breaks during courtship, and deprivations of 22 h inhibit the initiation of courtship behaviour. Snail pairs that did not mate ate significantly more lettuce (0.71 g ± 0.33 g, n=8 pairs) than did mating pairs (0.21 g ± 0.13 g, n=10 pairs) during the trial whether fed or starved for 22 h ($p<0.05$, 2 tailed t-test). Therefore, the expression of sexual behaviour coincides with reduced feeding in both starved and fed snails.

Starved snails copulated significantly less frequently than did fed controls (Fig. 4). However, snails fed after the 5 day starvation period copulated significantly more frequently than did fed snails (Fig. 4). Starvation, therefore appears to decrease proclivity, but, as with the level of general activity (Table 2), there was a rebound effect such that levels increased to above baseline once food was again available. However, proclivity tests performed on fed snails and snails starved for 22 h showed no difference in the number of snails that exhibited a positive response ($p>0.1$, G-test, n=20 for both groups). The

number of snails that courted when starved and fed snails were placed together in a group box after the proclivity test was also no different for starved and fed snails ($p>0.1$, G-test, $n=20$ for both groups).

The Effect of Sexual Arousal on Feeding

As sexual arousal increased, the food stimulus was less able to elicit a response (Fig. 5; Chapter 4). Also, the number of snails that chose (i.e. turned towards) an anesthetized conspecific, rather than a carrot, increased in a two choice test (Fig. 6). Further, as sexual arousal increased, snails spent an increasing amount of time in contact with the anesthetized conspecific (Fig. 7). These three results (Figs. 5-7) were not due to mating snails being food satiated, and thus having reduced feeding proclivities, because those snails that courted ate no more carrot than did those snails that did not court (0.98 g ± 0.52 g, for courting snails, $n=14$; 0.78 ± 0.54 g, for non-courting snails $n=12$; $p>0.1$, 2-tailed t-test).

Food deprivation did not alter the percentage of sexually aroused snails that chose an anesthetized snail instead of a carrot (Fig. 6). Moreover, 22 h of food deprivation did not change the amount of time a snail spent in contact with an anesthetized partner (Fig. 7). However, food deprivation did increase the amount of time sexually aroused snails spent in contact with the carrot

Figure 5. The relationship between the stage of a snail's genital eversion and the length of time a snail spent in contact with a carrot during a 5 min trial. As genital eversion stage increased, snails spent significantly less time in contact with the carrot (Spearman's $r=-0.94$, $p<0.05$). Error bars denote ± standard error of the mean.

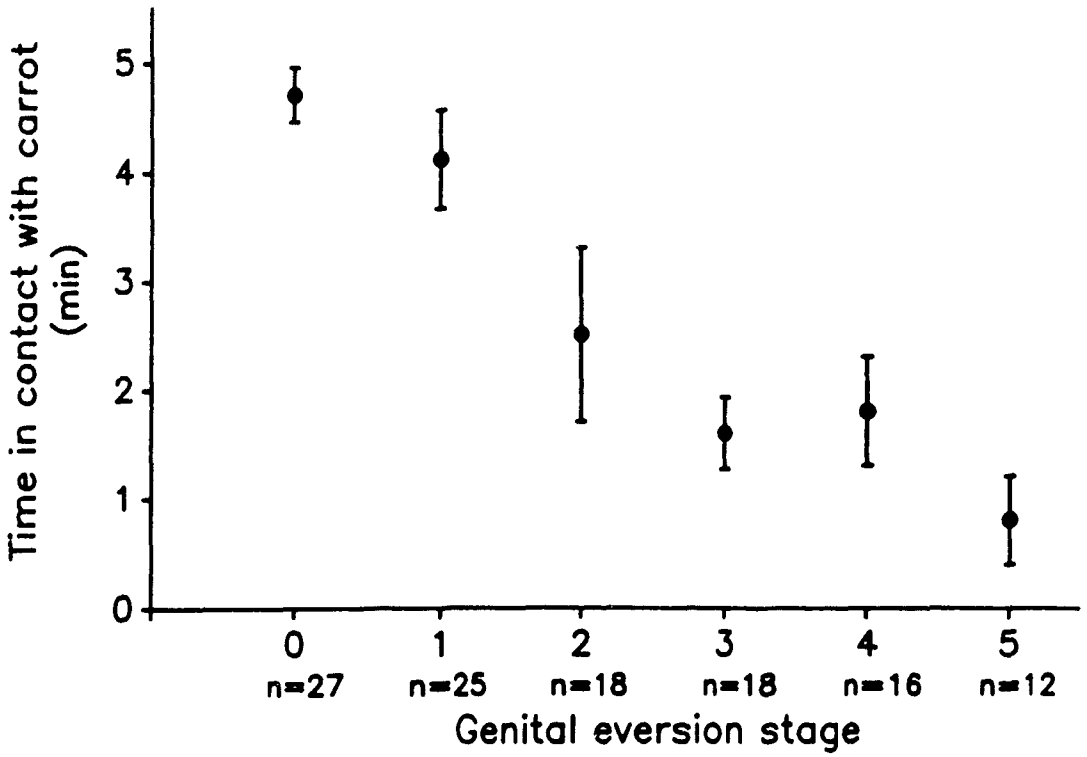

Time in contact with carrot (min)
0
1
2
3
4
5
0
n=27
1
n=25
2
n=18
3
n=18
4
n=16
5
n=12
Genital eversion stage

Figure 6. The effect of starvation on a snail's choice between a carrot and an anesthetized conspecific. Sexually aroused, starved snails choose an anesthetized snail over a carrot as often as did sexually aroused, fed snails ($p>0.1$, G-test). Both starved and fed snails chose an anesthetized snail more often as their genital eversion stages increased (Spearman's rank $r(fed)=0.99$, $p<0.05$, $r(starved)=0.94$, $p<0.05$).

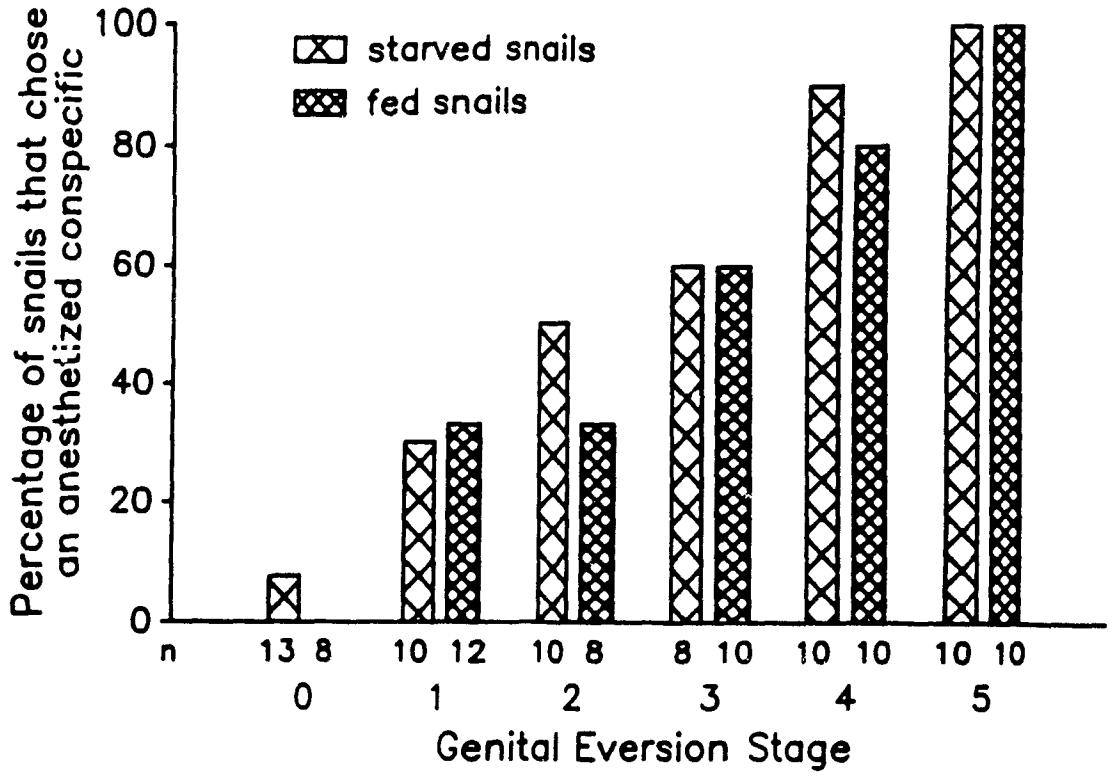

Percentage of snails that chose an anesthetized conspecific
100
80
60
40
20
0
starved snails
fed snails
n
13 8
10 12
10 8
8 10
10 10
10 10
0
1
2
3
4
5
Genital Eversion Stage

Figure 7. The effect of starvation on the time snails spent with an anesthetized conspecific during a two choice test. All snails spent more time with an anesthetized conspecific as their genital eversion stages increased ($p<0.01$, $F(5,5)$ 2-way split plot ANOVA). There was no significant difference between starved and fed snails ($F(1,5)$). Error bars denote ± standard error of the mean. The numbers next to each data point denote sample size.

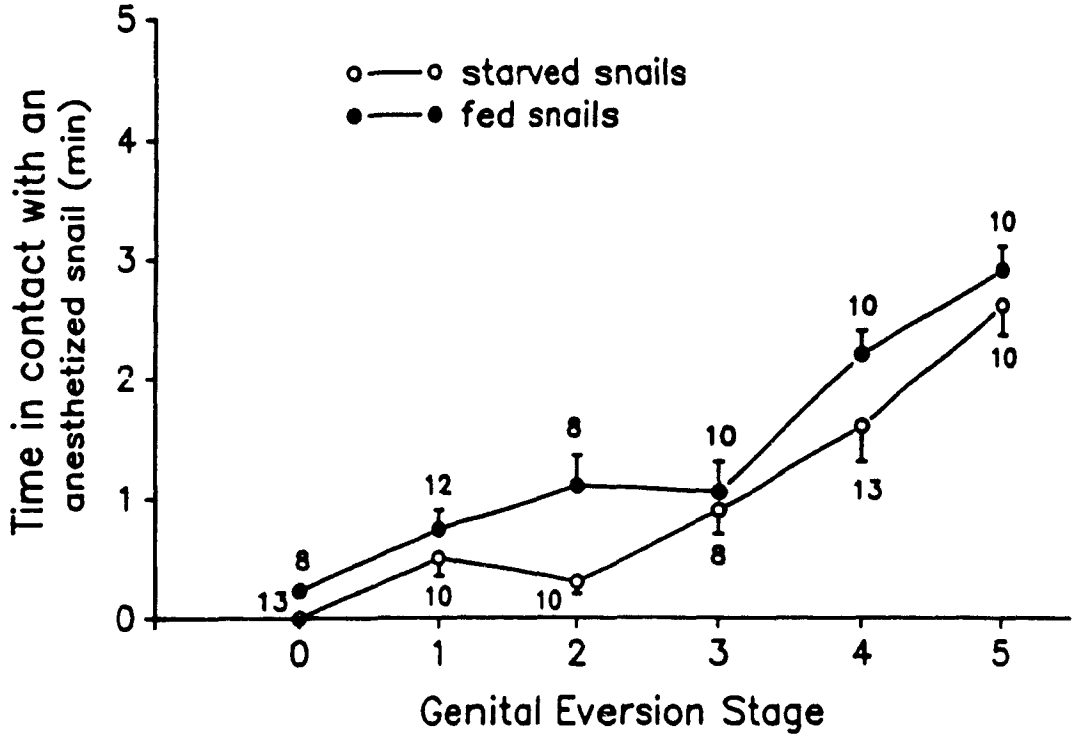
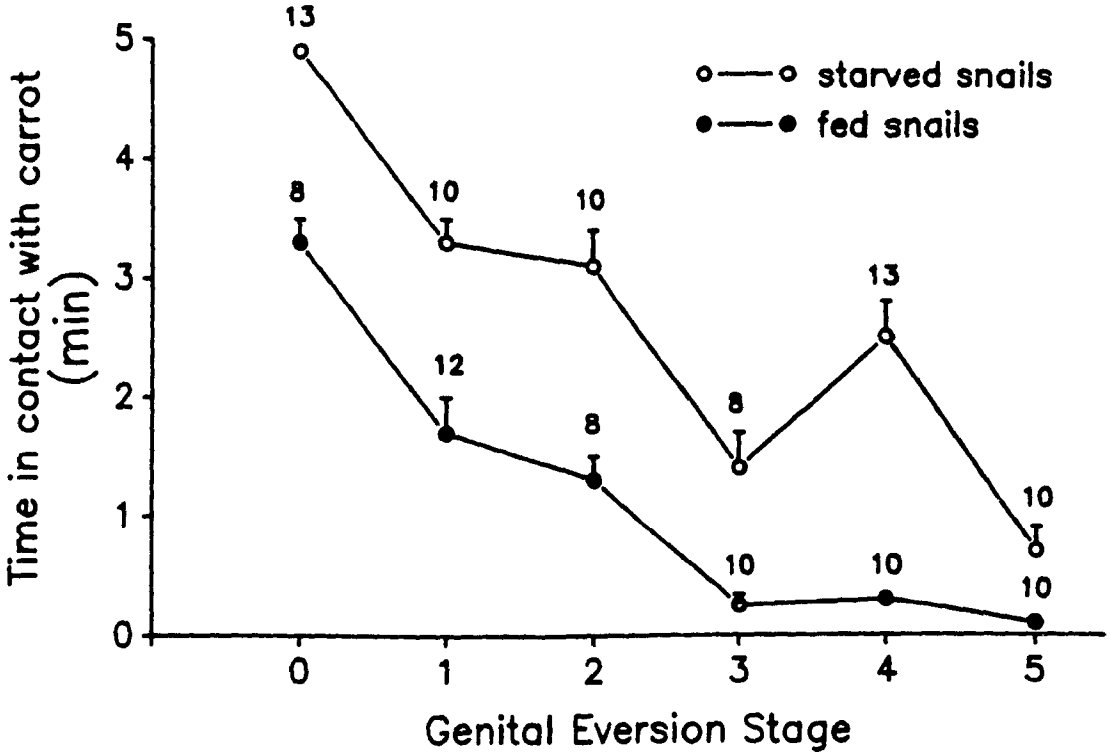

Time in contact with an anesthetized snail (min)
starved snails
fed snails
Genital Eversion Stage
0
1
2
3
4
5
13
8
12
10
8
10
10
8
10
13
10
10

Figure 8. The effect of starvation on the time snails spent in contact with a carrot during a two choice test. All snails spent less time in contact with the carrot as their genital eversions increased ($p<0.01$, $F(1,5)$, 2-way split plot ANOVA). However, fed snails consistently spent less time in contact with the carrot than did starved snails ($p<0.01$, $F(1,5)$, 2-way split plot ANOVA). Error bars denote ± standard error of the mean. The numbers above each data point denote the sample size.


Time in contact with carrot (min)
Genital Eversion Stage
starved snails
fed snails
0
1
2
3
4
5
13
10
10
8
13
10
8
12
8
10
10
10

during the same two choice-paradigm (Fig. 8). To summarize, both the food deprived, and the fed, sexually aroused snails spent the same amount of time responding to a sexual stimulus, but food deprived snails spent more time in contact with the carrot when not in contact with the sexual stimulus.

In contrast to sexual arousal, sexual proclivity had no effec. on whether snails turned first towards an anesthetized snail or a carrot. Snails that tested positively for sexual proclivity were equally likely to choose an anesthetized conspecific over a carrot (10/40) as were snails that scored negatively on the proclivity test (6/22, G-test, $p>0.1$).

The Effect of Sexual Arousal on Locomotion

Sexual arousal increased the distance snails travelled on a paper treadmill (Table 5; Chapter 4). The snails tended to travel along circular paths, which is common during courtship when mating pairs become separated (Chapter 2). However, when a snail at genital eversion stage 6 (post dart shooting snail) was placed 1 cm away from the sexually aroused snail whose locomotion was being tested, the sexually aroused snail locomoted significantly less (Table 5). The presence of snails at genital eversion stage 6 had no effect on the locomotion

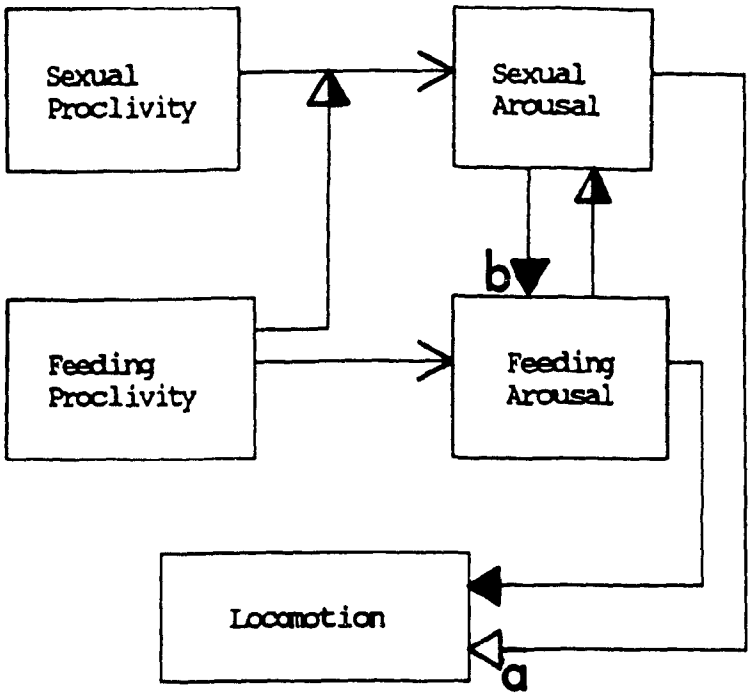
of unaroused snails (Table 5). Therefore, sexually aroused snails locomote faster only if they are without a partner.

Discussion

Figure 9 summarizes the results of this study by illustrating the interactions between locomotion, sexual behaviour, and feeding behaviour in H. aspersa. These behaviours do not form a rigid, inflexible hierarchy, but rather the position of each behaviour is dependent upon the behavioural context in which it is expressed and upon the relative levels of 'arousal' for each of the behaviours.

Sexual behaviour dominates feeding behaviour under most conditions, with feeding only occurring during periods of low sexual arousal. The nature of the relationship between sex and feeding closely resembles a time-sharing arrangement (Brown and McFarland, 1979). The evidence for time-sharing, which does not seem to have been previously demonstrated in an invertebrate, is summarized below. Sexual stimuli were chosen over food stimuli by a sexually aroused snail even if that snail was food deprived (Fig. 7). Moreover, starved, sexually aroused snails spent as much time with a sexual stimulus (an anesthetized snail) as did fed, sexually aroused

Figure 9. Schematic summary of the relationships between locomotion, sexual behaviour and feeding behaviour in the snail, Helix aspersa. Unshaded arrow denotes an excitatory effect; shaded arrows denote an inhibitory effect; half-shaded arrows denote a partial inhibitory effect; open arrows signify that proclivity will produce arousal when the appropriate stimuli are present. The boxes for sexual arousal or feeding arousal signify that the behaviour can be performed at different levels of intensity. (a) signifies excitatory effect only when there is no sexual partner; (b) signifies inhibition is incomplete during high levels of feeding proclivity or feeding arousal.


Sexual
Proclivity
Sexual
Arousal
b
Feeding
Proclivity
Feeding
Arousal
Locomotion
a

controls. These results demonstrate that when given a choice, sexually aroused snails will chose a sexual stimulus over a food stimulus. When not in contact with the anesthetized snail, however, starved, sexually aroused snails spent significantly more time with the carrot than did fed, sexually aroused controls (Fig. 7 and 8). Therefore sexual behaviour only partially suppresses feeding. Fed, sexually aroused snails also responded to a food stimulus, and the amount of time they spent in contact with the carrot increased as sexual arousal decreased (Fig 5). This pattern was also seen during courtship behaviour. Eating only occurred before courtship, or during breaks in courtship in both fed and starved mating pairs. During such periods, sexual arousal is relatively low (Chapter 2). Therefore, in both starved and unstarved pairs, eating only occurs during courtship sequences of low sexual arousal. This is similar to rats, where starved male rats also eat only during periods of low sexual arousal (Brown and McFarland, 1979).

During periods of low sexual arousal, elevated levels of feeding proclivity can inhibit sexual behaviour, reversing the usual relationship between the two behaviours. Fed snails take longer courtship breaks in the presence of food stimuli, and in a starved snail, food stimuli can delay the initiation of courtship

behaviour (Table 4). This is also seen in the rat where starvation can increase the latency to courtship initiation (Brown and McFarland, 1979).

Nevertheless, starved snails mating either on lettuce or in empty containers have the same number of breaks during courtship as do fed mating pairs. It does not, therefore, appear that a strong feeding proclivity can increase the periods of low sexual arousal that occur during mating. In other words, the length of time a snail spends in contact with a food stimulus during courtship is not determined by the length of food deprivation, but by the level of sexual arousal.

The effects of food stimuli on the courtship of snails at different levels of food deprivation are complex (Table 4). The snails starved for 22 h responded immediately to the lettuce, unlike fed controls. This would suggest that a high proclivity for feeding can delay the expression of a proclivity for sex. It is possible that once the starved snails had eaten, the snails' feeding proclivity and feeding arousal declined, and the food stimulus was no longer able to inhibit sexual behaviour. The fed snails were more influenced by the food stimulus (Table 4), perhaps because they began to court before they ate and they still had a relatively high proclivity for feeding. They therefore had a tendency to take longer breaks during mating in the

presence of lettuce. This suggests that in some circumstances, sexual proclivity can inhibit the expression of a proclivity for feeding.

Feeding proclivity appears to inhibit sexual proclivity, because food deprived snails showed a decline in copulation frequency (Fig. 4). However, our test for sexual proclivity failed to detect any effect of food deprivation. This apparently contradictory result can be explained by the fact that only active snails were tested for proclivity, but since starvation decreases the number of active snails, it also decreases the number of snails available for mating (Table 2). This could explain the decline in the copulation frequency. Therefore it appears that a proclivity for feeding does not affect sexual proclivity per se, but that feeding proclivity reduces the opportunities for mating by lessening the number of active snails.

The increases in mating that occur during the starvation recovery period may be caused by more than just increased activity. Inactive snails are also sexually isolated snails, and sexual deprivation has been shown to enhance sexual proclivity (Chapter 4).

Everett et al. (1982) constructed a partial behavioural hierarchy for H. aspersa and found that feeding in H. aspersa has a less dominant position than in Pleurobranchaea. They speculated that this was due to

a difference in food strategies (carnivore vs. herbivore; patchy vs. relatively continuous food distributions). They predicted that mating would be dominant to feeding in H. aspersa, contrary to the situation in Pleurobranchaea. This prediction is supported by our results. The same domination of mating over feeding is found in another herbivorous mollusc, the marine opisthobranch, Ercolamia nigra (Jensen, 1987). However, in Aplysia, which is also a herbivorous marine opisthobranch, the interaction between mating and feeding is less easily characterized. In Aplysia, mating and feeding can occur at the same time (Susswein et. al., 1983). This suggests that the organization of behaviour will not necessarily be the same even for related animals, but will depend upon the details of the life history of the animal. This issue has been further, discussed by Everett et al. (1982), Kovac and Davis (1980), and McCleery (1983).

Although proclivities for either sex or feeding affect behavioural choice, they do not have the same effect on the behavioural hierarchy as the actual expression of the behaviour. For example, sexual proclivity has no effect on locomotion, but sexual arousal can increase locomotion (Table 5). Sexual proclivity, unlike sexual arousal, does not affect the amount of time a snail spends in contact with a carrot.

Increased feeding proclivity has no effect on locomotion (Table 3). In these cases, changes to the hierarchy are dependent on the receipt of the appropriate sensory stimuli and/or the initiation of the motor program for the behaviour. Pleurobranchaea exhibits a similar phenomenon; the inhibition of withdrawal by feeding is dependent on the activation of feeding behaviour. Starvation alone has no effect (Davis et al., 1977).

Given the differences in the effectiveness of arousal and proclivity in altering an animal's behavioural hierarchy, the two aspects of 'arousal' should be kept explicitly separate in any behavioural hierarchy. The mechanisms that bias a snail's response to a given stimulus (proclivity) may be very different from the mechanisms by which an aroused snail suppresses competing behaviours once the motor program for a behaviour has been initiated.

Chapter 6. 'Central Arousal' and Sexual Responsiveness in the snail, *Helix aspersa*

Abstract

In molluscs, a 'central arousal' system is thought to positively modulate both an animal's level of activity and its behavioural responsiveness. This hypothesis is examined in Helix aspersa by testing the relationships between activity, feeding, and sexual behaviour. Activity, feeding and mating exhibit parallel daily rhythms. Snails are most active, and eat and mate most frequently, during scotophase and the first 3 h of photophase. Handling and injections of serotonin (5 x 10^{-7} m/kg body wt) increase general activity. Inducing activity during late photophase increases food consumption, but it does not induce sexual activity. Moreover, serotonin and handling have no effect on sexual arousal; treated snails show no increase in genital eversion stage and require the same length of courtship as controls. If snails are sexually deprived, increasing activity in late photophase does increase copulation frequency, but, snails do not copulate more frequently than do unhandled snails tested during early photophase. These results suggest that 'central arousal', as estimated by the snail's level of activity, has a permissive or 'gating' effect on sexual behaviour, but does not directly affect sexual proclivity or sexual arousal. Helix appears to differ from Aplysia in the effect of 'central arousal' on sexual behaviour. This

difference between the two species may be related to the differences in their reproductive strategies.

Introduction

A snail, like other animals, varies in its responsiveness to external stimuli. Often this variability follows a predictable pattern. For example, in Aplysia, responsiveness to food stimuli waxes and wanes in parallel with the daily activity rhythm (Kupfermann, 1974). An underlying variable called 'arousal' has been proposed to account for this positive correlation between an animal's level of activity and its level of behavioural responsiveness (Andrew, 1974; Dieringer, Koester, & Weiss, 1978). Palovcik, Basberg and Ram (1982) have postulated that a 'central arousal' system drives increases in both behavioural responsiveness and behavioural state (the animal's level of activity) in Aplysia. This idea is supported by the finding that serotonin can increase both behavioural state and the responsiveness to food stimuli, and Palovcik et al. (1982) have suggested that 'central arousal' may be mediated by serotonin. In order to explore the extent to which 'central arousal' is a meaningful variable in Helix, we have examined whether increases in 'central arousal' are accompanied by increases in behavioural responsiveness to food and sexual stimuli. Helix aspersa exhibits a circadian

activity rhythm (Bailey, 1981), and therefore we have tested the concordance of the daily patterns for feeding, sexual behaviour and activity.

Susswein (1984) has hypothesized that, in Aplysia, not only feeding, but also sexual behaviour is positively modulated by a 'central arousal' system. (Susswein (1984) uses the term 'common arousal', but this appears to be equivalent to 'central arousal'). However, whether or not a single system can positively modulate both feeding and sexual behaviour is likely to depend on the feeding and reproductive strategies of the species. Aplysia exhibits a reproductive strategy in which it lives through only one reproductive season (Kandel, 1979) and spends 25% of its time engaged in mating activity (Susswein, Gev, Feldman & Markovich, 1983). Helix, on the other hand, typically lives through more than one reproductive season and mates infrequently (Potts, 1975; Moulin, 1980). Tuersley (1989) has argued that an animal's ecology shapes the way in which its behaviour is modulated. This suggests that the neural and hormonal mechanisms controlling an animal's mating behaviour are adaptations to its reproductive strategy (Crews & Moore, 1986). For this reason, it is probable that the relationship between 'central arousal', feeding and sexual behaviour will differ between Helix and Aplysia.

A comparison between Helix and Aplysia is desirable because Helix, like Aplysia, has proven amenable to neurophysiological analysis (Chase, 1986; Balaban & Chase, in press). The opportunity therefore exists to examine, and to compare, the physiological mechanisms responsible for modulating feeding and sexual behaviour in animals with very different life histories. To execute this test, handling of the animals and injections of serotonin will be used to increase behavioural state in Helix. If behavioural state, feeding and sexual behaviour are all positively modulated by a 'central arousal' system, then feeding and sexual behaviour should increase in parallel with increases in behavioural state.

Sexual behaviour in Helix aspersa has been previously described (Chung, 1987; Chapter 2). It consists of 3 main sequences: introductory behaviour, dart shooting and copulation. During introductory behaviour snails evert their normally internal genital apparatus. The degree of the eversion can be categorized as six different stages, and these stages have been found to correlate with the level of sexual arousal (Chapter 4). During dart shooting, a snail pushes a calcareous dart into its partner. The dart has a mucous coating which is able to increase sexual arousal (Chapter 3). Increases in sexual arousal can be estimated not only from the stage of the

genital eversion, but also by the time a snail requires to reach stage 5 (dart shooting threshold) (Chapter 4).

General methods

H. aspersa were imported from California (Marinus, Long Beach). The snails had an average weight of 9 g (range 6 g - 11 g). Those snails that were observed copulating were transferred to one of three types of containers: individual Lucite containers (7 cm x 8 cm x 8 cm), group containers (18 cm x 18 cm x 8 cm; 10 snails per box) or a colony container (35 cm x 30 cm x 30 cm; 40 snails per box). All snails were individually marked. Colony snails were maintained at room temperature (24° C ± 2° C), all other snails were maintained at 19° C ± 2°. The light cycle was 16:8 L:D. The containers were kept moist, and the snails were fed lettuce, carrot roots and oyster shells ad lib. The cages were cleaned and the snails were fed every day or every other day at the start of photophase. All experiments were run at the beginning of photophase unless otherwise noted, and they were continued for a maximum of 2 h. Snails were used only once per trial. After dart shooting, snails were not used again for another 10 days to allow the dart to regenerate (Tompa, 1982).

Snails were randomly chosen for trials by either a coin flip or by a lottery system based on their identification number. As the data often did not fit the assumptions for ANOVA, nonparametric analyses were performed (Meddis, 1984) unless otherwise noted. When testing specific hypotheses after completing the initial `nonspecific' analysis, the statistic $\underline{z}^2=\underline{H}$ was used.

Experiment 1. Determination of Daily Rhythms in Activity, Feeding and Sexual Behaviour

The following experiment examines whether behavioural responsiveness to food and mates follows a daily rhythm paralleling the daily rhythm in activity.

In many molluscs, including Helix, the level of activity can be quantified by a behavioural state score (BSS) derived from the animal's posture (Preston & Lee, 1973; Lee & Palovcik, 1976, Tuersley & McCrohan, 1987; Chapter 5). In Helix, the scale consists of 6 ascending levels based on the amount of extrusion from the shell, ranging from completely withdrawn, to completely extended and locomoting (Chapter 5). Total activity in the colony at any given time was measured by averaging the BSSs of all the snails in the colony. BSSs were

collected every hour. After inspecting the activity results, the day was divided into three periods: the first 3 h of photophase, the rest of photophase, and scotophase; subsequent statistical analyses justified these divisions (see below).

Food consumption was determined by measuring the carrot weights in the 4 group boxes at the beginning and end of each of the 3 time periods, as well as at 2 h intervals during photophase. The weights for each time period were averaged over the 4 boxes for 10 consecutive days, and weights were converted to hourly rates to allow comparisons between each time period.

The number of copulations that occurred during each time period in the group boxes was also recorded. During scotophase, copulations were assessed from photographs taken at hourly intervals under red light. As copulation typically lasts 6 hours (Chapter 2), all cases of copulation were recorded.

Replicates were found not to differ significantly ($p>0.1$, nonparametric factorial analysis, related samples) and therefore the data were pooled for each behaviour.

Results

Snails showed a prominent daily rhythm in their activity (Fig. 1). They were most active during scotophase and the first 3 hours of photophase ($p<0.01$, nonparametric factorial analysis, related samples).

Mating and feeding rates paralleled the activity rhythm (Fig. 2). Snails ate most when they were most active ($p<0.01$, nonparametric factorial analysis, related samples). Snails were also most likely to copulate when they were most active ($p<0.01$, nonparametric factorial analysis, related samples).

There were no significant differences in BSSs, mating or feeding rates between early photophase and scotophase, but the 2 time periods are analyzed separately in the following experiments, because the effects of other factors, such as handling, may depend on the light phase. There were no significant differences between hourly measures within each time period ($p>0.1$, nonparametric factorial analysis, related samples) suggesting that there were no large trends within the time periods. Therefore, there is no justification for any further divisions of the day (Fig. 1).

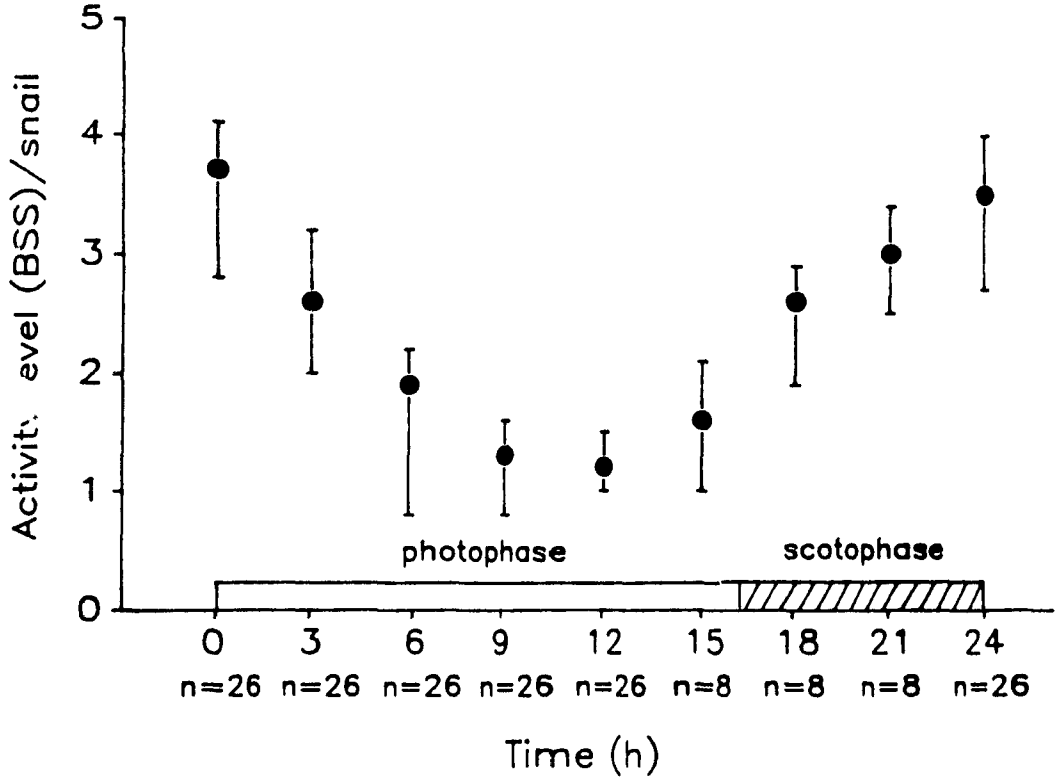

Activit: evel (BSS)/snail
5
4
3
2
1
0
photophase
scotophase
0
3
6
9
12
15
18
21
24
n=26
n=26
n=26
n=26
n=26
n=8
n=8
n=8
n=26
Time (h)

Figure 1. The daily rhythm of behavioural state scores (BSSs) in Helix aspersa. Snails were significantly more active during early photophase and scotophase than they were during mid and late photophase ($p<0.01$ nonparametric factorial analysis, related samples). The BSSs of 40 colony snails were averaged over (n) days to give the mean score at each time point. Values during scotophase were collected under red light. Values are medians ± 1 quartile.

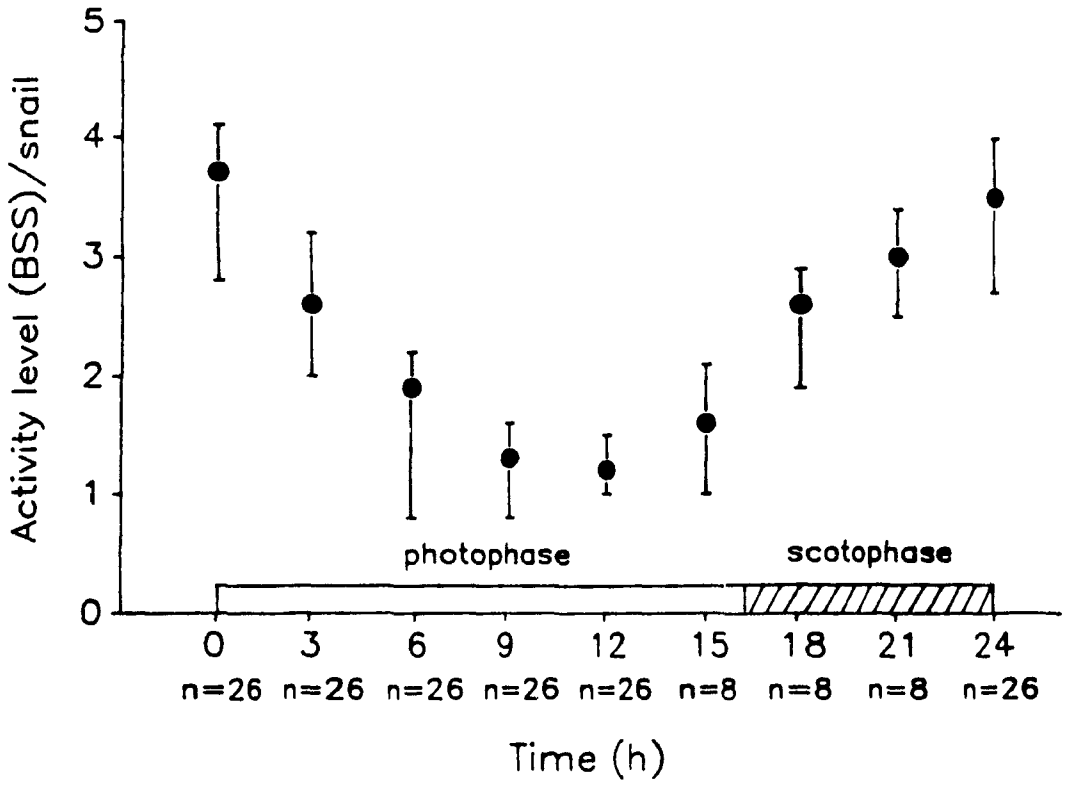
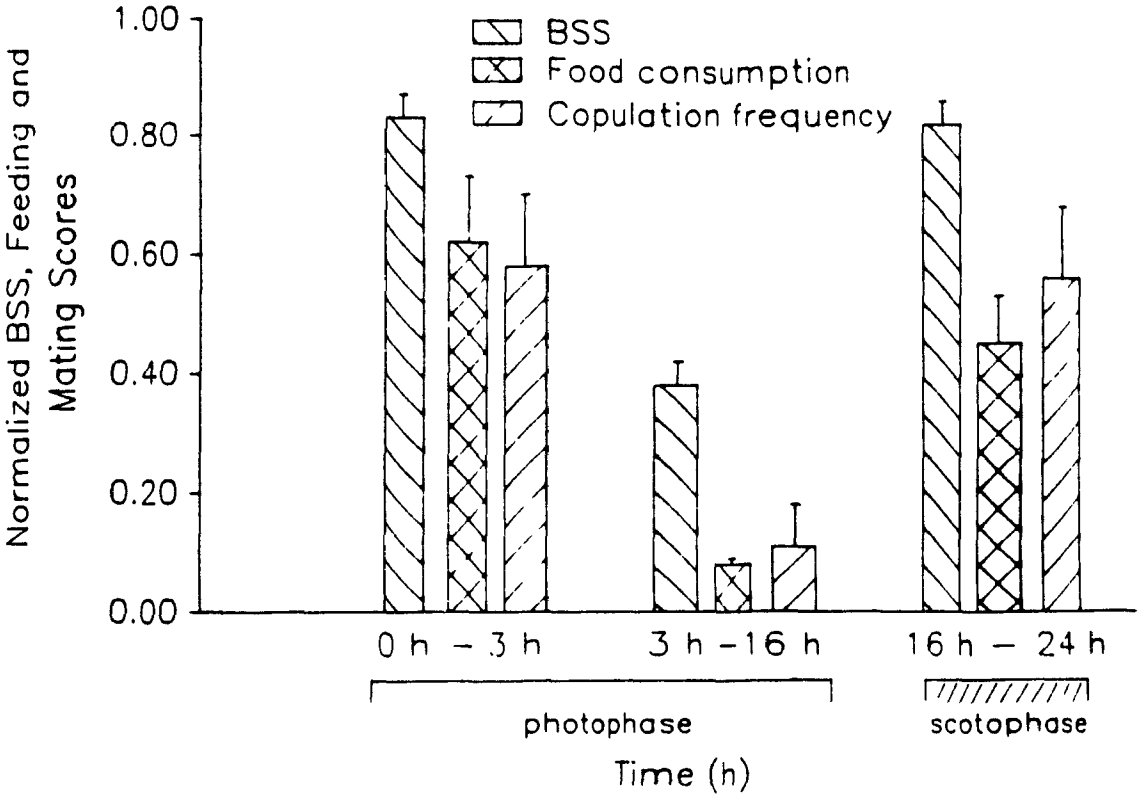

Activity level (BSS)/snail
5
4
3
2
1
0
photophase
scotophase
0
3
6
9
12
15
18
21
24
n=26
n=26
n=26
n=26
n=26
n=8
n=8
n=8
n=26
Time (h)

Figure 2. The daily rhythms of BSSs, food consumption and mating. All scores were normalized to the largest data point value for each of the 3 behaviours. A score of 1.00 is equivalent to a BSS of 4.0, a food consumption rate of 0.8 g carrot/h/10 snails, and a copulation rate of 0.15 matings/h/10 snails. There were no significant differences in the daily rhythm for each activity ($p>0.1$, nonparametric factorial analysis, related samples). Matings were assigned to the time period in which copulation began. All values are means ± standard error.


1.00
0.80
0.60
0.40
0.20
0.00
Normalized BSS, Feeding and Mating Scores
BSS
Food consumption
Copulation frequency
0 h – 3 h
3 h – 16 h
16 h – 24 h
photophase
scotophase
Time (h)

Experiment 2. Effects of Serotonin and Handling on Activity Levels

Handling (Kupfermann & Weiss, 1981) and serotonin (Palovcik et al., 1982) increase behavioural state in Aplysia. Before the same methods could be used to increase behavioural state in Helix, it was necessary to confirm an equivalent effect in this species.

Snails were randomly assigned to 8 group boxes. Each group was randomly assigned to one of 4 treatments: injections of serotonin (0.1 mL, 5×10^{-5} M; approx. 5×10^{-7} moles/kg body weight); 0.1 mL of snail saline (Chase, 1986, the vehicle for serotonin); handling; or unhandled and uninjected (controls). Handled animals were flipped onto their backs, inducing a righting reflex and an increase in activity. Half of the groups received treatments daily at the beginning of photophase, while the other half received treatments 10 h into photophase (late photophase), when snails were typically inactive (Fig. 1).

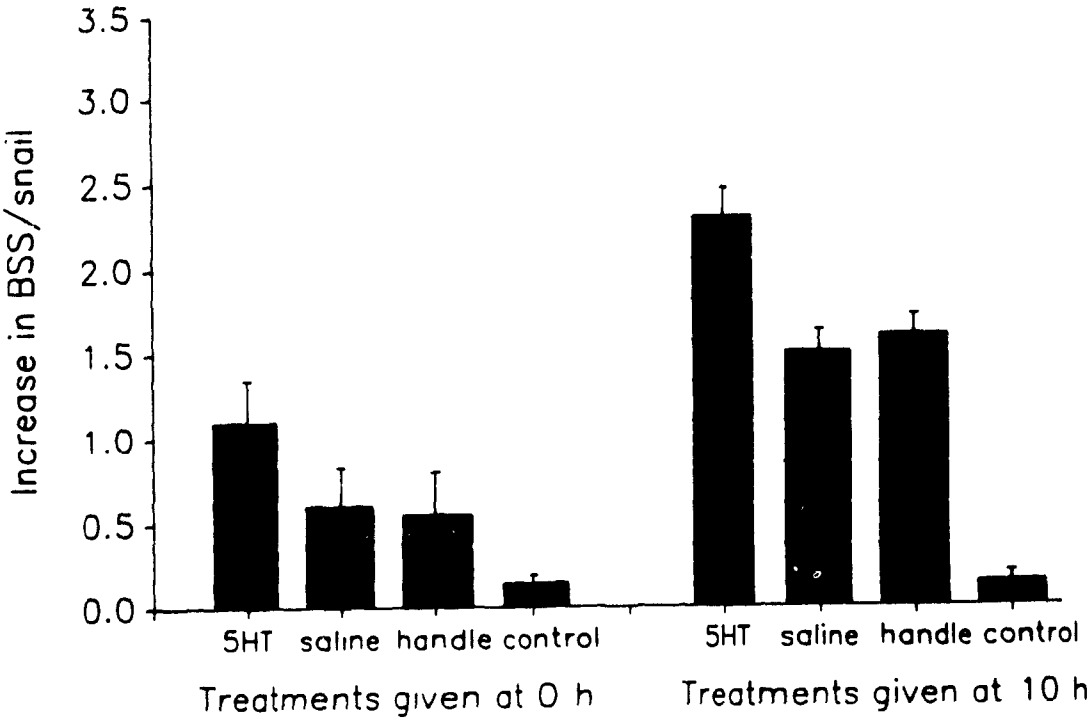
BSSs were recorded immediately before treatment as well as 5 min and 1 h after treatment.

Results

Five minutes after treatment, the average BSSs of the serotonin injected snails increased irrespective of the photophase in which the injection was given (Fig. 3, $p<0.01$, nonparametric factorial analysis, related samples). Serotonin increased BSSs to a greater extent than did handling or saline, although handling and saline did increase BSSs significantly above that of controls (Fig. 3, $p<0.01$). There was no difference between the saline effect and the handling effect. The effects were largest when the treatments were given during late photophase (the inactive phase; $p<0.05$). Five minutes after treatment, snails were equally active (i.e. had similar BSSs) in both early and late photophase.

One hour after treatments given in early photophase, handled, saline and serotonin injected snails were not significantly more active than controls. However, 1 h after treatments given in late photophase, serotonin injected snails remained more active than saline and handled snails, which were more active than controls ($p<0.05$, nonparametric factorial analysis, related samples). Nevertheless, snails treated in late photophase were significantly less active 1 h after treatment than were snails treated in early photophase ($p<0.01$). Therefore, although all the treatments

Figure 3. The effects of serotonin, saline and handling on BSS. Serotonin caused a significantly larger increase in BSS than did either saline or handling at either treatment time ($p<0.01$ nonparametric factorial analysis, related samples). BSS scores represent the mean increase in BSS measured from immediately before treatment to 5 min after treatment. The daily means were averaged over the 10 day trial. Values are means ± standard error.


Increase in BSS/snail
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
5HT
saline
handle control
5HT
saline
handle control
Treatments given at 0 h
Treatments given at 10 h

increased activity, the magnitude and duration of the effect was dependent on the time of treatment.

Experiment 3. Effects of Serotonin and Handling on Food Consumption

Serotonin and handling have been shown to increase BSSs (experiment 2). In the following experiments, we test their effects on the snail's responsiveness to food stimuli (experiments 3 and 4) and sexual stimuli (experiments 5 and 6). If 'central arousal' has a direct, positive, effect on both feeding and sexual behaviour, then increases in 'central arousal' should result in increases in both feeding and sexual behaviour.

The snails were randomly reassigned to the 8 groups described above. The food consumption of each group was determined by measuring the difference in carrot weight from immediately prior to a treatment to 3 h later. The number of snails observed scraping the carrot at least once with their radula was also recorded. The results were averaged over 10 consecutive days. Each snail was weighed before and after the 10 day trial.

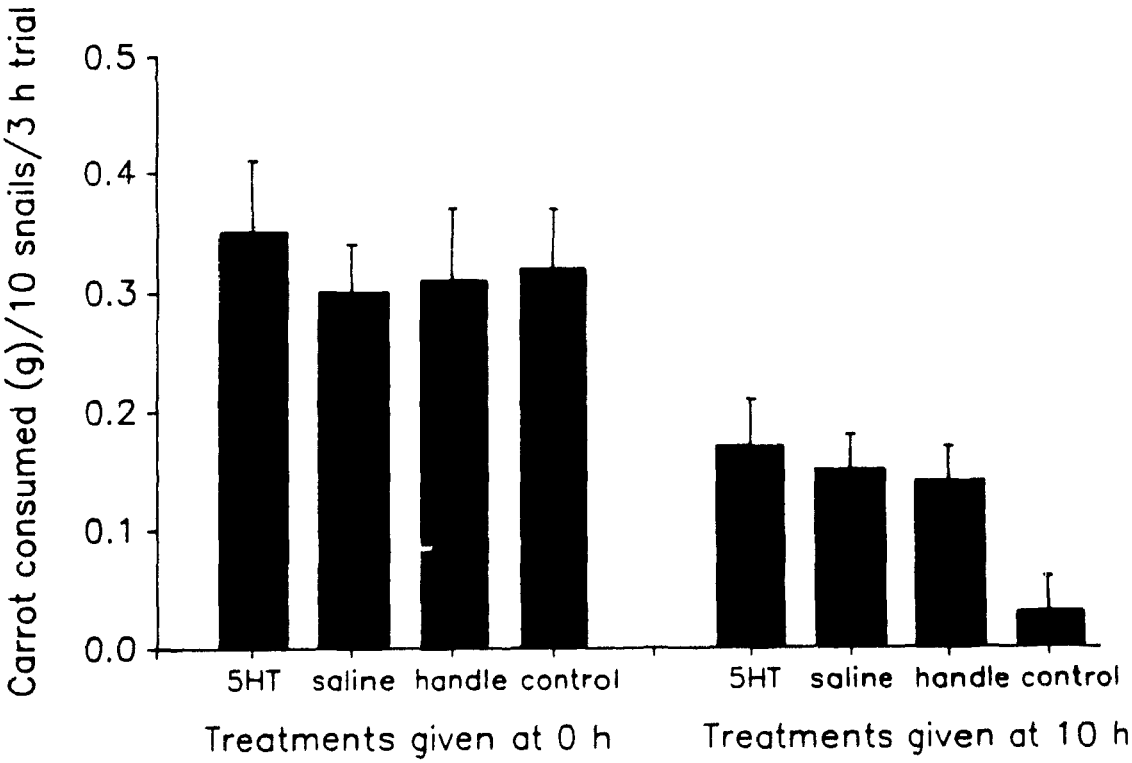
Results

The effects of serotonin and handling on food intake did not exactly parallel their effects on BSSs. Trials given at the start of photophase had no effect on food intake, even though activity was significantly increased (Fig. 4, $p>0.1$, nonparametric factorial analysis, related measures).

Trials given at late photophase did significantly increase food intake over controls (Non-parametric factorial analysis, related samples, $p<0.02$), though the effect of serotonin was no larger than the effect of handling and saline injections (Fig. 4). Also, while treated snails ate more than controls during the late photophase trial, they ate less than did similarly treated snails during the 3 hour early photophase trial ($p<0.01$). The number of snails that ate also increased significantly over controls (1.2 ± 0.2) in the serotonin (4.8 ± 1.2), saline (5.1 ± 1.4) and handled (4.5 ± 1.1) groups, but only during late photophase ($p<0.01$, G-test).

Despite the increase in food intake in late photophase, there was no net weight gain over the 10 day trial in the handled, serotonin or saline injected snails compared to controls (Kruskall-Wallis, $p>0.1$).

Figure 4. The effects of serotonin, saline and handling on carrot consumption. Serotonin, saline and handling significantly increased carrot consumption compared to controls, but only when treatments were given in late photophase ($p<0.02$, nonparametric factorial analysis, related samples). Scores represent the amount of carrot consumed, per group, averaged over 10 trials. Values are means ± standard error.


Carrot consumed (g)/10 snails/3 h trial
0.5
0.4
0.3
0.2
0.1
0.0
5HT
saline
handle control
5HT
saline
handle control
Treatments given at 0 h
Treatments given at 10 h

Experiment 4. Effects of Serotonin and Handling on Bite Frequency

Increases in BSS may have an effect on the speed with which food is eaten even in the absence of an effect on the amount consumed. Feeding speed is one parameter used to estimate food arousal (Susswein, Weiss & Kupfermann, 1978). The following experiment examines the effect of increasing the BSS on bite frequency.

Colony snails were gently placed 1 cm away from a piece of carrot attached to a force transducer. The number of bites (scraping the carrot with the radula), recorded visually, over 10 randomly selected 30 s segments was identical to the number of bites recorded for the same time period on a chart recorder by means of the force transducer. This indicated that the force transducer gave an accurate measure of bite frequency.

Prior to each trial, snails were randomly assigned to one of 4 groups, 5 snails per group: injections of serotonin (0.1 mL, 5 x 10^{-5} M); 0.1 mL snail saline; handled; and unhandled, uninjected (controls). Baseline bite frequencies were determined during the first 5 min. Snails that did not respond to the food stimulus within 30 s were discarded. Snails were then subjected to one of the 4 pre-assigned treatments. Bite frequency was recorded for the next 5 min, and for 1 min periods every

5 min for the next 15 min. Because injections and handling interrupted feeding, the 5 min post-treatment trial was not started until the snails had spontaneously resumed feeding.

Results

Snails averaged 4.6 ± 0.5 bites per 10 s during baseline trials, 4.6 ± 0.6 bites per 10 s after handling, 4.6 ± 0.6 bites per 10 s after saline injections, and 4.6 ± 0.9 bites per 10 s after serotonin injections. There were no significant trends in the number of bites as time progressed after treatment ($p>0.1$, nonparametric factorial analysis, related samples).

Therefore, neither serotonin injections nor handling had any significant effect on bite frequency (nonparametric factorial analysis, related samples $p>0.1$).

Experiment 5. Effects of Serotonin and Handling on Sexual Proclivity

In these experiments we examine the effects of increasing behavioural state on the responsiveness to sexual stimuli. If 'central arousal' potentiates responsiveness to sexual stimuli, then increasing

'central arousal' should correlate with increased responsiveness to sexual stimuli. Feeding behaviour is also monitored to examine the interactions between 'central arousal', feeding, and mating. We differentiate between 2 types of responsiveness: proclivity (the likelihood of a response), and arousal (the intensity of the response). In this experiment we consider proclivity, in the next, arousal. There is evidence that, in Helix, sexual proclivity and sexual arousal are controlled by 2 distinct mechanisms (Chapter 4).

As sexual proclivity increases, the number of snails that display courtship behaviour and which eventually copulate also increases (Chapter 4). Therefore, proclivity was estimated from either the number of courting snails or by the number of copulating snails. The effect of increasing BSSs on sexual proclivity was examined by following the same procedure used to test for an effect of increased BSSs on feeding behaviour (experiments 3 and 4). Treatments were given daily for 5 days to the 8 groups described above (experiment 3), 10 snails per group. Snails were sexually isolated for 10 days between 3 replicate trials. The number of copulating snails in each group was recorded for 5 days before trials as well as during the trial. If increasing BSS correlates with increased mating, then the

serotonin, saline, and handled groups should copulate more than the controls.

In the above test, snails had constant access to mates, except between trials. Because copulation decreases sexual proclivity, their sexual proclivity was progressively declining (Chapter 4). To determine the effects of the 4 treatments on snails with high sexual proclivity, treatments were given to snails that had been aestivated for 2 months prior to trials. Aestivation in H. aspersa increases the likelihood of subsequent mating (Bonnefoy-Claudet & Deray, 1984). Chapter 4 shows that sexual isolation also increases sexual proclivity. The snails were therefore sexually isolated after being removed from aestivation. At the start of each trial, snails were randomly divided into 8 groups of 12 snails each. During the trials, snails were gently placed into a Lucite observation box (20 cm x 20 cm x 8 cm) at either early photophase or late photophase. Thirty minutes after being placed in the observation box, each group was given one of the 4 preassigned treatments: injections of serotonin (0.1 mL, 5 x 10^{-5} M); injections of 0.1 mL saline; handling; or no injection and no handling (controls). The number of snails that displayed courtship behaviour was recorded. The snails were prevented from copulating by returning them to their individual containers when their genital eversions

reached stage 5. The time required for each snail to reach dart shooting threshold (stage 5) was also recorded.

Because placing the snails in the observation box involved handling them, and as this may have influenced the results, a third test was performed in which snails were not handled prior to trials. There were 3 groups: handled, unhandled, and control. Each group contained 12 snails. During the trials, the barriers between snails in the handled and unhandled groups were removed without disturbing them. The control group remained isolated throughout the trial. By comparing the BSSs of the control group and the unhandled group, the effect of conspecific contact on BSSs could be measured. After the barriers were removed, the snails in the handled group were flipped onto their backs. The BSSs of the snails were recorded before treatment, 5 min after, 1 h after and 2 h after treatment. In the handled and unhandled groups the number of snails that courted as well as the time required for each snail to reach dart shooting threshold was recorded. The number of snails that were observed biting the carrot in all 3 groups was also recorded. Snails were removed once their genital eversions reached stage 5. The above procedure was repeated during late photophase to determine whether the handling effect was dependent on the time of day.

Results

Despite clear effects on activity and feeding (Fig. 2), neither serotonin nor handling had any significant effect on the copulation frequency of snails that were not sexually isolated, irrespective of the time of treatment (Fig. 5, $p>0.1$ nonparametric factorial analysis, related measures). Even though snails treated in late photophase were active for more hours of the day than were controls (Fig. 3), they did not use this time to mate (though they did use it to eat, Fig. 4). Mating tends to inhibit feeding, and appears to be the more dominant behaviour (Chapter 5). Therefore, it is unlikely that feeding during late photophase inhibited any putative stimulation of mating behaviour. In summary, there is no evidence that increasing BSSs can induce sexual behaviour.

However, in sexually deprived snails, mating occurred in both early and late photophase in all animals (Table 1). There was no significant difference in the number of snails that courted as a function of treatment, during either early or late photophase (Table 1, $p>0.1$, G-test). Nor did the treatments affect the time that snails required to reach dart shooting threshold, again regardless of the time of treatment (Table 1, $p>0.1$,

Figure 5. The effects of serotonin, saline and handling on copulation frequency. None of the treatments had any effect on copulation frequency ($p>0.1$, G-test). Each score is the mean copulation rate, per day, averaged over the 10 day trial. Each score represents the median value of the three replicates while the error bars represent the remaining 2 values.

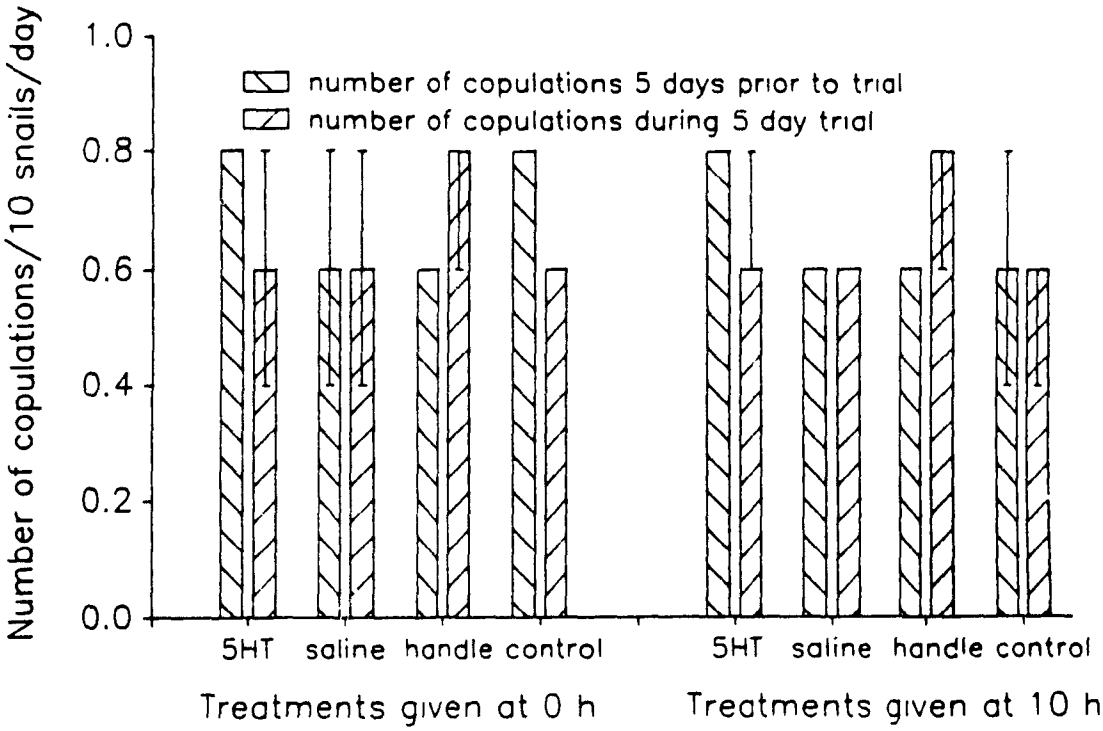

Number of copulations/10 snails/day
1.0
0.8
0.6
0.4
0.2
0.0
number of copulations 5 days prior to trial
number of copulations during 5 day trial
5HT
saline
handle control
5HT
saline
handle control
Treatments given at 0 h
Treatments given at 10 h

Table 1. Serotonin, saline, and handling have no effect on the number of courting snails or on the time required to achieve dart shooting threshold (ds) in sexually deprived snails Each group contained 12 snails. There were no significant differences generated by any experimental treatment ($p>0.1$, G-test; $p>0.1$, nonparametric factorial analysis, unrelated samples).

	Treatment			
	Control	Handling	Saline	Serotonin
Early photophase				
time to ds (min ± sd)	31.0 ± 24.2	35 2 ± 24.8	42.8 ± 25.9	36.9 ± 23.4
Number of courters	7	8	7	6
Late photophase				
Time to ds (min ± sd)	35.8 ± 25.6	37.9 ± 22.8	33.4 ± 26.6	38.7 ± 23.5
Number of courters	8	6	6	7

Kruskall-Wallis). Therefore, increasing BSSs with serotonin, saline or handling did not increase sexual proclivity in sexually deprived snails.

When the barriers between sexually isolated snails were removed, thus allowing exposure to conspecifics, the BSSs increased relative to those of continuously isolated controls. However, this increase only occurred when the barriers were removed in late photophase (Table 2, nonparametric factorial analysis, related measures, $p<0.05$). This result suggests that when the BSS is low, exposure to conspecifics can induce an increase in BSS when sexual proclivity is high. Although removing barriers increased BSS in late photophase, the unhandled snails were still less active than were unhandled or isolated snails in early photophase (Table 2, G-test $p<0.05$).

During early photophase, handling sexually isolated snails immediately prior to removing the barriers between them had no significant effect on the number of snails that subsequently courted, nor on the time required to reach dart-shooting threshold, compared to unhandled controls (Table 2, $p>0.1$, G-test, $p>0.1$, nonparametric factorial analysis, related samples). However, handling immediately prior to trials in late photophase had a highly significant effect on the number of snails that courted and on the number that reached dart shooting

Table 2. The effects of removing barriers between sexually isolated snails, and the dependence of these effects on the time of day. Each group contained 36 snails. Behavioural state scores (BSSs) were taken 1 h after treatment.

	Percentage courting	Percentage feeding	BSS/snail ± sd
Barriers retained			
early photophase	n/a	50[a]	3.3 ± 0.5[a]
late photophase	n/a	28[b]	1.8 ± 0.3[b]
Barriers removed (unhandled)			
early photophase	72[a]	56[a]	3.5 ± 0.2[a]
late photophase	28[b]	28[b]	2.5 ± 0 3[c]
Barriers removed (handled)			
early photophase	78[a]	61[a]	3.4 ± 0.6[a]
late photophase	78[a]	42[c]	3.3 ± 0 3[a]

n/a not applicable

Column scores that differ significantly from one another are denoted by different letters (percentage courting, $p<0.01$, G-test; percentage feeding, $p<0.05$, G-test; BSSs, $p<0.05$, nonparametric test, related samples.)

threshold, compared to unhandled controls (Table 2, $p<0.001$, G-test). Therefore, handling only increased mating when BSSs were low prior to treatment.

In the experiment reported in Table 1, serotonin and handling had no effect on sexual behaviour. This result is seemingly at odds with the results reported in Table 2 which show that handling increased sexual behaviour in late photophase. Because handling itself can affect BSS (Table 2, Fig. 3), it is likely that handling the snails to perform the experiment reported in Table 1 increased the activity of the controls, thus masking the effects of the treatments.

Neither handling nor serotonin had any significant effect on courtship duration ($p>0.1$, nonparametric factorial analysis), suggesting that these treatments had no effect on sexual arousal. This inference is tested more directly in the following experiment.

Experiment 6. The Effects of Serotonin and Handling on Sexual Arousal

Sexual arousal in *Helix* is characterized by a reduction in courtship duration, an increase in the stage of a snail's genital eversion and an increase in the time a snail will spend in contact with an anesthetized

conspecific (Chapter 4). The effects of serotonin and handling on each of these parameters were examined.

In the first test, mating snail pairs were randomly assigned to one of 4 treatments: serotonin; saline; handling; and unhandled, uninjected controls. The genital eversion stage at the time of injection was recorded. The time required for each snail to reach dart shooting threshold was also recorded.

In the second test, 4 previously isolated snails were placed in an observation box. At preassigned genital eversion stages, all snails except the target snail were removed from the box. Target snails were randomly given one of 4 treatments as above. The genital eversions at 30 s, 1 min, 2 min, 3 min and 5 min were recorded.

In the third test, snails, at preassigned stages of genital eversion, were confronted with an anesthetized partner placed at a distance of 1 cm. The procedure has been previously described (Chapter 4). The time that snails remained in contact with the conspecific during the 5 min trial was recorded.

Results

Increasing snail activity by injecting serotonin or saline, or by handling the snails, did not reduce courtship duration (Fig. 6, $p>0.1$, Kruskall-Wallis). None of the treatments significantly increased the stage of the snail's genital eversion ($p>0.1$, G-test), although

Figure 6. The effects of serotonin, saline, and handling on the latency to dart shooting, and their dependence on the stage of genital eversion at the time of treatment. There was no effect of any of the treatments at any genital eversion stage ($p>0.1$, Kruskall-Wallis). Each score represents the average latency to dart shooting for 10 snails. Values are median ± 1 quartile. Snails that failed to mate after courting received a maximum score of 60 min.

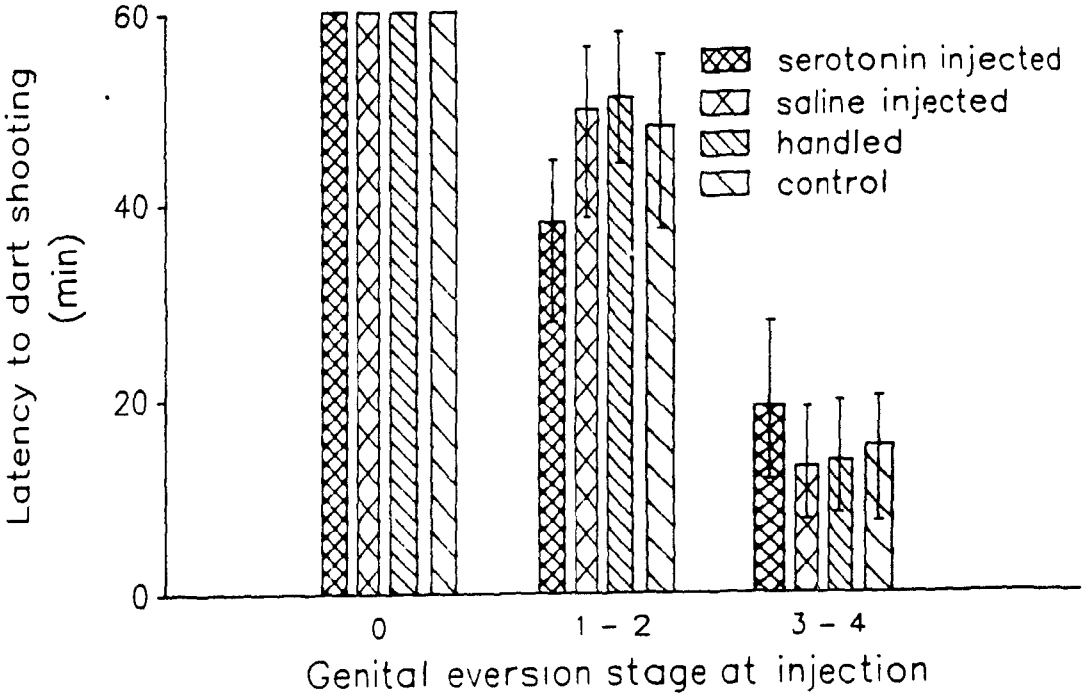
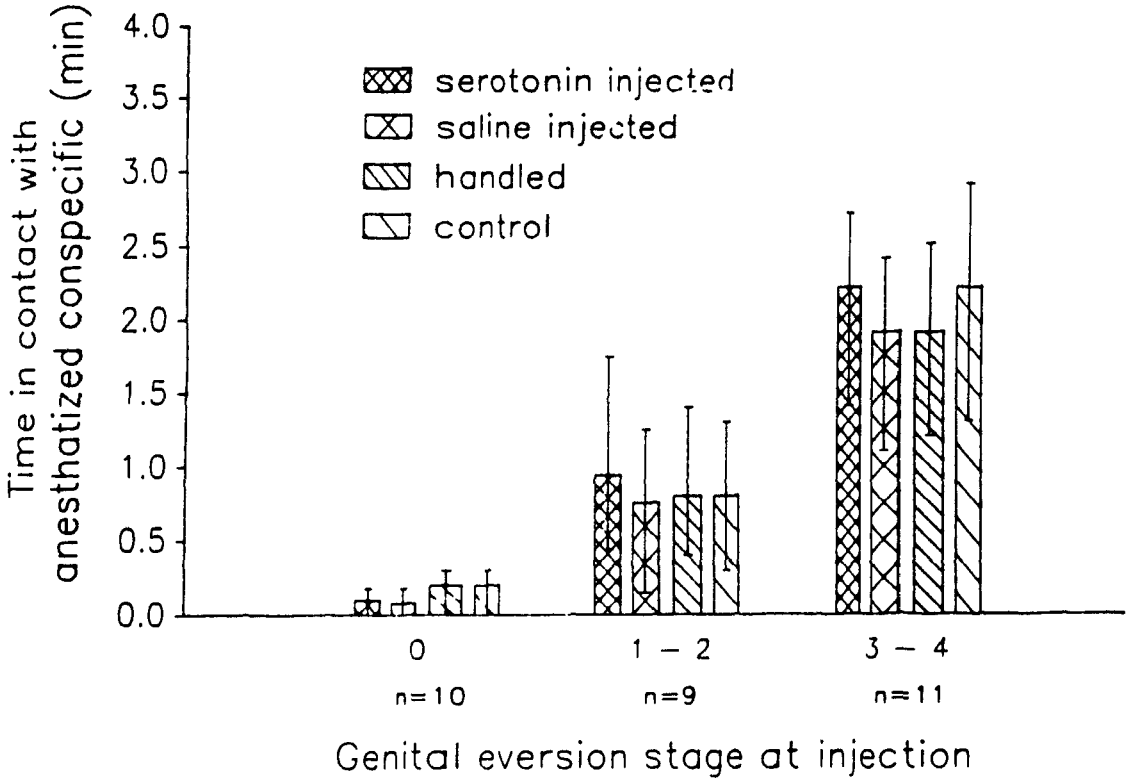

60
40
20
0
Latency to dart shooting (min)
serotonin injected
saline injected
handled
control
0
1 - 2
3 - 4
Genital eversion stage at injection

Figure 7. The effects of serotonin, saline, and handling on the time spent in contact with an anesthetized conspecific, and their dependence on the stage of genital eversion at the time of treatment. Treatments had no effect on contact time at any stage of genital eversion ($p>0.1$, Kruskall-Wallis). Each score represents the average of 10 snails. Values are median ± 1 quartile.


Time in contact with anesthatized conspecific (min)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
serotonin injected
saline injected
handled
control
0
1 – 2
3 – 4
n=10
n=9
n=11
Genital eversion stage at injection

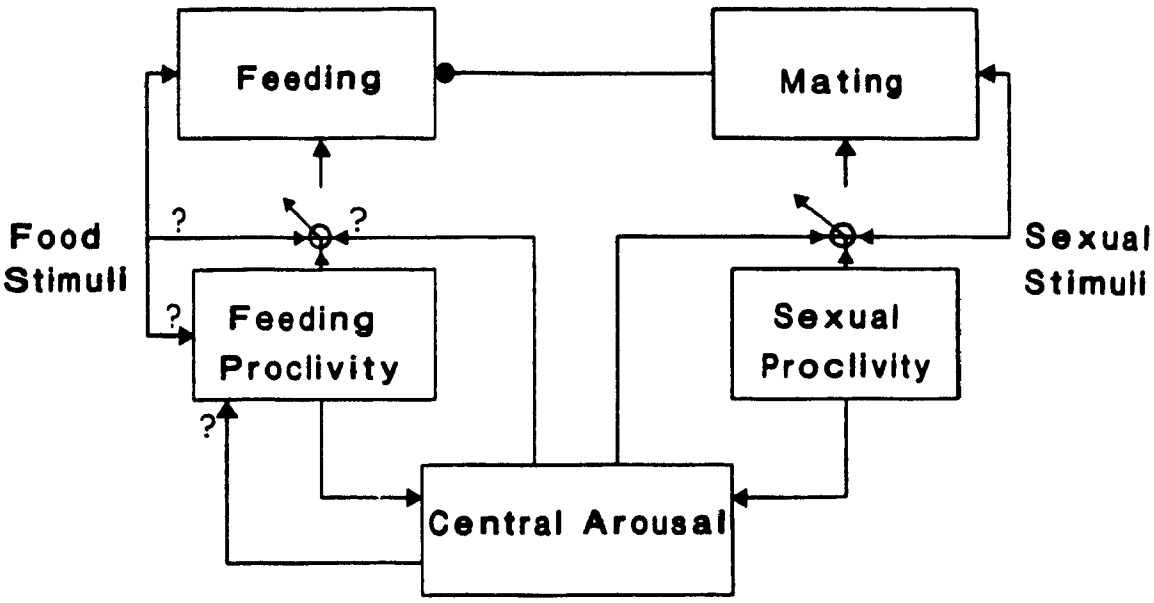
of the snail's genital eversion ($p>0.1$, G-test), although saline and serotonin sometimes increased the size of the penial lobe. This change may have been due to increased liquid volume in the hemocoel, and hence increased blood pressure in the cephalopedal area. None of the treatments increased the time snails spent in contact with an anesthetized conspecific (Fig. 7, $p>0.1$, Kruskall-Wallis).

Therefore, there is no evidence that either serotonin or handling increased sexual arousal.

Discussion

Helix exhibits a variability in its responsiveness to food and mates which follows a daily rhythm paralleling that of activity (Fig. 2). This finding superficially supports the hypothesis that a 'central arousal' system positively modulates activity, feeding and sexual behaviour. However, in *Helix*, particularly in the case of sexual behaviour, the effect of 'central arousal' appears to be only a permissive one, allowing the expression of sexual proclivity and arousal without directly influencing the mechanisms underlying sexual proclivity and sexual arousal. This conclusion for *Helix* contrasts with the case in *Aplysia*, where the mechanism(s) underlying 'central arousal' is thought to

Figure 8. Schematic summary of the relationships between 'central arousal', feeding and sexual behaviour. BSS was used to estimate 'central arousal'. The boxes labelled 'sexual proclivity' and 'feeding proclivity' represent the sum of the neural and hormonal factors that contribute to creating the change in responsiveness to sexual or food stimuli. The boxes labelled 'mating' and 'feeding' represent the neural and hormonal components responsible for the execution, and the speed of execution (i.e. arousal), of either feeding or sexual behaviour. Although the boxes are separate, some of the same components may contribute to both proclivity and arousal for either behaviour. 'Central arousal' is shown as having a permissive, or gating, effect on sexual behaviour, but it has no direct effect on sexual proclivity. Sexual proclivity leads to sexual behaviour only when sexual stimuli are present and when the snail is at some minimum level of 'central arousal'. Whether increased 'central arousal' can directly affect the components responsible for determining feeding is not known. Arrowheads represent excitatory relationships; a filled circle represents a relationship that is predominantly inhibitory. Question marks denote plausible, but not yet demonstrated, relationships.


Feeding
Mating
Food
Stimuli
Sexual
Stimuli
?
?
?
?
Feeding
Proclivity
Sexual
Proclivity
Central Arousal

positively modulate the mechanism(s) responsible for proclivity and arousal in both feeding and sexual behaviour (Ziv, Benni, Markovich, & Susswein, 1989).

Although behavioural state was used to estimate the relative level of 'central arousal', the 2 terms are not synonymous. 'central arousal' is characterized by behavioural responsiveness as well as by activity. Behavioural state can be used as an estimate of 'central arousal' only if it is assumed that behavioural state never increases except when 'central arousal' also increases. Given that increases in behavioural state do seem to correspond to increases in behavioural responsiveness (Preston & Lee, 1973; Andrew, 1974; Chapter 5) the assumption appears to be valid.

'Central arousal', as estimated by the BSS, plays a permissive role in that it is a necessary precondition for mating. In terrestrial snails, like Helix, high cephalopedal blood pressure is presumably required not only for locomotion and feeding, but also for such courtship behaviours as dart shooting and penile extrusion (Dale, 1973). High cephalopedal blood pressure results in extrusion from the shell (Dale, 1973). Since extrusion from the shell is equivalent, by definition, to a high BSS, a high BSS is required before mating can occur. However, activity is not a sufficient precondition for mating because increasing activity has

no effect on copulation frequency (Fig. 5). The inability of an increase in behavioural state to increase copulation frequency is contrary to what would be expected if a 'central arousal' system directly modulated sexual proclivity. However, it is consistent with the idea that 'central arousal' is simply correlated with the necessary physiological conditions for mating.

In apparent contradiction to the above, increasing activity in late photophase led to increases in both feeding and mating behaviour in sexually deprived snails (Table 2). However, copulation rates in these snails were identical to those found in the early photophase group (Table 2). Assuming snails with sexual proclivity were equally likely to be in either the early or late photophase group, then increasing 'central arousal' did not increase proclivity above that already present and capable of being expressed when the snails were naturally active in early photophase. Therefore, it appears that increased levels of 'central arousal' did not result in increased mating behaviour, but merely allowed for the expression of the underlying sexual proclivity.

It is likely that sexual behaviour requires specific internal conditions before it can be initiated (Chapter 4). Moreover, evidence suggests that changes in these conditions occur only over a period of days (Chapter 4),

not minutes, as might be expected if increasing activity could induce sexual behaviour.

Although the mechanism(s) responsible for 'central arousal' appear to have no direct effect on sexual proclivity, snails isolated so as to increase their sexual proclivity, increased their BSS when exposed to sexual stimuli in late photophase, a time when they are usually inactive (Table 2). Exposure to food causes a similar increase in BSS in hungry Helix (Chapter 5). Food stimuli can also increase BSS in food deprived Pleurobranchaea (Palovcik et al., 1982). Therefore, a positive proclivity for either food or sex can, in the presence of the appropriate stimuli, induce an increase in 'central arousal'.

Sexually deprived, unhandled snails are less active when exposed to conspecifics in late photophase than when they are exposed in early photophase. Moreover, sexually deprived snails mate during late photophase less frequently than they do in early photophase (Table 2). Therefore, only a proportion of sexually deprived snails are capable of mating during late photophase. During early photophase, when 'central arousal' is high, lower levels of sexual proclivity are required for sexual stimuli to elicit courtship. This may explain why more snails mated in early photophase and why the rhythm of

mating in Helix followed the daily activity rhythm (Fig. 2).

Increasing activity not only failed to affect sexual proclivity, but it also had no effect on sexual arousal (Fig. 6, Fig. 7, Table 1). 'Central arousal' also has no effect on bite frequency, which was the only parameter of food arousal that was measured. A lack of effect on sexual arousal and feeding arousal would be expected if a 'central arousal' system has a permissive effect, and not an active effect, on the neural mechanisms that control these behaviours.

The evidence from the experiments on feeding behaviour is consistent with the hypothesis that 'central arousal' serves a permissive function for feeding, similar to that for sexual behaviour, though the evidence does not exclude the possibility that 'central arousal' exerts a more direct effect. We have already reported that latency to bite is negatively correlated with BSSs (Chapter 5). In the present study, we found that increasing activity increased feeding (Fig. 5), and snails in late photophase were both less active, and fed less, than snails in early photophase (Figs. 3 and 4). These results support the hypothesis that a 'central arousal' system directly modulates the system responsible for feeding proclivity. During late photophase, however, snails are inactive and incapable of eating. As with

sexual behaviour, it is possible that increasing activity during late photophase simply allowed the expression of feeding proclivity, rather than directly affecting feeding proclivity. BSSs and feeding will appear as tightly coupled behaviours if feeding proclivity is normally present and satiation is rare.

It is paradoxical that increasing activity had an effect on food consumption only in late photophase (Fig. 4). Possibly, the initial increase in activity after treatment was due to a `stress' or `startle' response. There is evidence that tactile contact initially inhibits feeding in Helix (Everett, Ostfeld & Davis, 1982), and serotonin, saline and handling treatments all involve tactile contact with the animal. Once this effect decays, snails are no more active during early photophase trials than are controls (experiment 2). However, during late photophase, snails are `aroused' from an inactive state. Snails may initially be `stressed' or `startled' as during early photophase, but as this effect decays, the snails slowly return to dormancy. As the snails return to dormancy, they pass through levels of activity that are compatible with eating. Therefore, when compared to completely inactive controls, they exhibit an increase in food consumption. A similar `stress' or `startle' phenomenon is thought to occur in Aplysia, which shows decreased feeding immediately after handling

but increased feeding 4 min later (Kupfermann & Weiss, 1981).

Fig. 8 summarizes the interactions between 'central arousal' (estimated by BSS), feeding and sexual behavior. It should be noted that a 'central arousal' system has yet to be identified in any mollusc. Nevertheless, some mechanism(s) must exist to account for the coordination of behavioural responses to diverse internal and external stimuli (Kupfermann & Weiss, 1981). Whether this system exists as a set of neural/neurosecretory cells with a specialized 'central arousal' function, or whether 'central arousal' exists by virtue of the parallel connections of sensory cells and endogenous oscillators to each of the different behavioural centres, remains to be discovered. A recent report, however, describes neurons which appear to be specialized for exerting a `motivational effect' when activated (Teyke, Weiss, & Kupfermann, 1990).

In contrast to Helix, in Aplysia both sexual behaviour and feeding appear to be positively modulated by a 'central arousal' system (Ziv et al., 1989a). Food deprivation decreases the amount of time Aplysia spends immobile and increases mating activity and spontaneously occurring appetitive feeding behaviours (Susswein, 1984; Weiss, Koch, Koester, Rosen, & Kupfermann, 1982). In Helix, however, food deprivation decreases both BSS and

mating activity (Chapter 5). The differences in the interactions between activity, sexual behaviour and feeding in these 2 gastropod molluscs are interesting from an ethological viewpoint and underscore the importance of comparative studies. Aplysia and Helix have very different reproductive strategies. Aplysia lives only one reproductive season, and during the breeding season spends over 25% of its time mating (Kandel, 1979; Susswein, et al., 1983). It produces tens of thousands of eggs in a single bout of egglaying (Kandel, 1979). Helix, on the other hand, typically lives more than one reproductive season, but even with surplus food and access to mates, these snails mate less than once every 28 days during the breeding season (Potts, 1975; Moulin, 1980). Helix lays less than a hundred eggs per oviposit (Potts, 1975). Therefore, for Helix, which feeds far more often than it mates, a system that enhanced responsiveness to sexual stimuli whenever feeding behaviour was facilitated, would be inappropriate. The feeding and reproductive strategies of Helix require that feeding and sexual behaviour be capable of independent modulation. The different reproductive strategies of Helix and Aplysia appear to have led to differences in how sexual and feeding behaviours are related to 'central arousal'.

Other molluscs also differ from Aplysia in the way their behaviours are related to 'central arousal'. In Lymnaea, handling has no effect on food arousal (Tuersley and McCrohan, 1987). Again, this difference is thought to be a reflection of the differences between the feeding strategies of the two species (Tuersley & McCrohan, 1987; Tuersley, 1989). Lymnaea is a herbivorous grazer which feeds continuously, and satiates rarely. Aplysia feeds in bouts, and altering the level of food arousal is one mechanism by which the duration and intensity of the bouts can be controlled. Such control is not required in Lymnaea.

Although serotonin had a significant effect on activity, it was not more effective than injections of saline or handling in potentiating feeding and mating. Nevertheless, Kemenes and S.-Rozsa (1987) have evidence that serotonin is involved in food arousal in Helix. It is possible that injections, being invasive, and handling, which may be 'stressful', activate the 'central arousal system' postulated by Palovcik et al. (1982). This may make the injected serotonin superfluous. It is also possible that systemically delivered serotonin does not mimic the effect of serotonin in vivo in the sexual and feeding neural systems. The role of serotonin in 'central arousal',

feeding and sexual behaviour in Helix remains to be determined.

Chapter 7. Thesis Discussion

Summary and model of Helix aspersa sexual behaviour

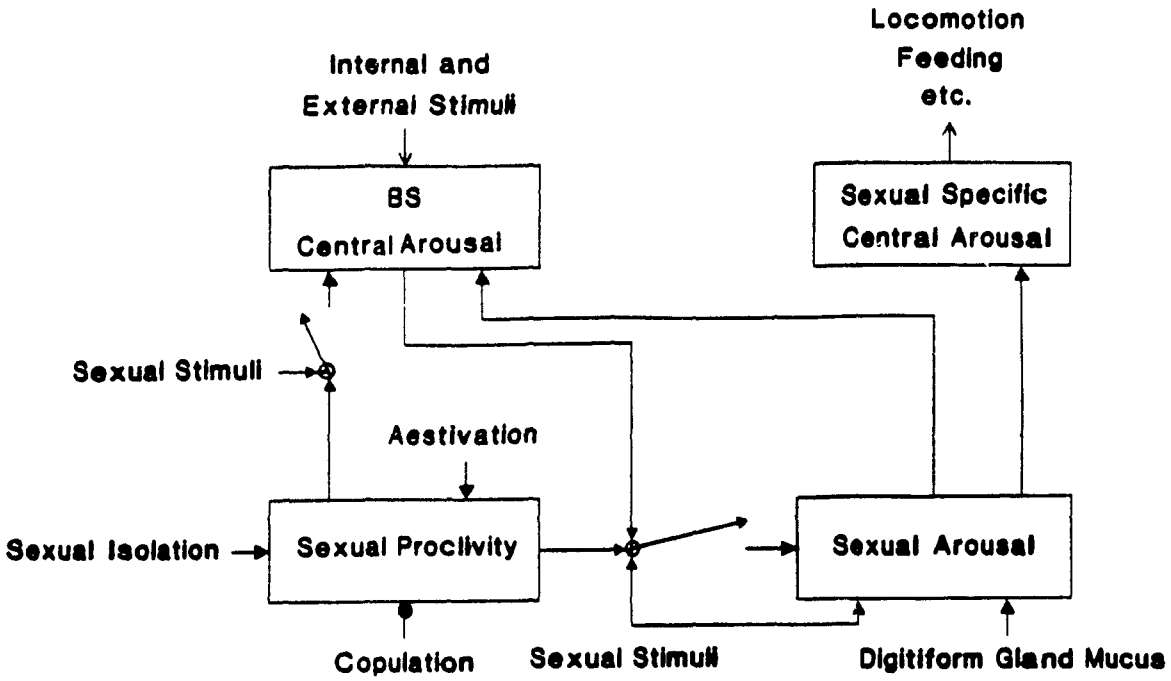
Fig. 1 illustrates a schematic model of Helix sexual behaviour that is consistent with the results presented in the previous chapters. The following sections give a summary of Helix aspersa sexual behaviour based on this model.

Sexual proclivity

In a constant environment with moderate temperatures, abundant food and a summer light cycle, sexual isolation, aestivation, or simply the passage of time since the last copulation, will increase a snail's sexual proclivity (Chapters 4 and 6). The increase in sexual proclivity is gradual and occurs over several days, suggesting that there is a hormonal component in its control.

Sexual proclivity cannot be induced by increasing behavioural state (BS) (Chapter 6). This suggests that a central 'arousal' system responsible for increasing BS (modelled in Fig. 1) has only a permissive effect on sexual behaviour. In other words, it does not interact directly with the mechanisms controlling either sexual arousal or sexual proclivity (Chapter 6). However, in the presence of sexual stimuli, snails that have been sexually isolated for several days will increase their BS, if it is below the level needed for locomotion

Figure 1. Schematic model of Helix aspersa sexual behaviour emphasizing its relationship with 'central arousal'. Shaded arrows denote excitatory relationships while shaded circles denote relationships that are inhibitory. Open arrows signify that both inhibitory and excitatory relationships are possible. Points at which two or more inputs converge denote that all inputs are required before there is an effective output.


Locomotion
Feeding
etc.
Internal and
External Stimuli
BS
Central Arousal
Sexual Specific
Central Arousal
Sexual Stimuli
Aestivation
Sexual Isolation
Sexual Proclivity
Sexual Arousal
Copulation
Sexual Stimuli
Digitiform Gland Mucus

(Chapter 6). Under these conditions, sexual stimuli are capable of activating the BS central arousal system (BSCAS).

Gomot and Deray (1987) list various physical factors such as humidity, temperature, food availability, time since previous aestivation, time since previous copulation, and photoperiod, that appear to be important in Helix in controlling sexual proclivity. Of these factors, the time since the previous aestivation appears to be the most important, followed by the time since last copulation (personal observations). Humidity, temperature, and food availability are likely to be permissive factors, but are unlikely to interact directly with the mechanisms involved in sexual proclivity. Snails must be active to court, and physical conditions that promote aestivation obviously prevent courtship.

The effect of photoperiod on sexual proclivity is not completely clear. Despite the evidence that a long photoperiod cues sexual proclivity in this species (Bonnefoy-Claudet et al., 1987), in California, Helix aspersa tends to mate and reproduce during short, not long, light periods (Ingram, 1947; Potts, 1975). Moreover, Guemene and Daguzan (1982) have found that Helix will breed in both constant light and constant darkness, indicating that photoperiod is not a necessary cue for the development of sexual proclivity.

Sexual proclivity appears to have a circannual cycle (Bailey, 1981), therefore given favourable conditions, its onset may be controlled by an endogenous clock.

Sexual proclivity declines after copulation. This decrease is not dependent on spermatophore exchange (i.e. on factors from the dissolving spermatophore) (Jeppesen, 1976). Dart shooting alone will decrease sexual proclivity (Jeppesen, 1976; Lind, 1976). The actual performance of dart shooting is not required for the decline of sexual proclivity; the same decline in proclivity is seen in snails that are missing dart sacs (Jeppesen, 1976). Snails that are missing dart sacs perform 'phantom' dart shooting, suggesting that the neural circuits involved in dart shooting continue to function despite the loss of the dart sac (Chapter 3). This suggests that the decline in sexual proclivity may be triggered directly or indirectly by the neurons in the mesocerebrum which activate the firing of the dart.

The frequency of sexual proclivity differs widely amongst molluscs. In Helix, sexual proclivity occurs rarely. It has a time course very different from that of feeding which occurs more or less daily (personal observations). The estimates for mating frequency in Helix vary between different reports. Guemene and Daguzan (1982) found that snails mated an average of once every 15 days. They checked the snails for mating

behaviour twice a day for 8 weeks during the breeding season. Moulin (1980) found that snails mated an average of once every 30 days over the entire 5 month breeding season. In this study, snails were checked once per day. Madec and Daguzan (1987) found that their snails mated once every 30 days, and snails were checked twice per day for 15 weeks during the breeding season. Chapter 6 reports that the snails used in this study mated an average of once every 12 days, and the snails were monitored hourly over the 24 h cycle for 3 weeks after being removed from aestivation. Despite the variation amongst the estimates, all of these studies show that Helix courtship and copulation is a much rarer event than it is in the marine mollusc, Aplysia (Susswein et al., 1983).

This naturally raises the question of why periods of sexual proclivity should occur more often in Aplysia than in Helix. Though the ecological information on both species is rather scanty, one can speculate on some of the forces that may have contributed to the difference. In Aplysia, frequent mating has been hypothesized to increase the genetic variability of offspring. Given their highly changeable habitat, this increase is thought to be adaptive (Susswein et al., 1983). However, Helix tends to spend its entire life in small colonies with virtually no immigration (Potts, 1975). Selander and

Kaufman (1975) estimate that the deme size for Helix is about 15 individuals. Therefore, highly promiscuous mating in Helix may not substantially increase the variability of its offspring, even if variable offspring were also advantageous for Helix. Perhaps it is not surprising then that snails do not seem to have evolved any behavioural mechanisms to encourage outbreeding. Snails appear to mate at random, and neither favour nor disfavour newly introduced individuals from a different population (Woyciechowski and Lomnicki, 1977).

Nevertheless, as Baur (1988) points out, fertilizing other snails decreases the chance that a snail will leave no genetic offspring if it is preyed upon. However, because the cost of mating is not known, it is difficult to determine what the optimal rate of copulation may be for Helix.

The cost of copulation may be especially high in Helix aspersa because of the concomitant risk of parasitism. In Helix aspersa there are a number of parasites that are spread exclusively by sexual behaviour (Lind, 1973; Morand, 1988). At least one of these parasites, the nematode, Nemhelix bakeri decreases egg output (Morand, 1988). Although the rate of parasitism is not known, this pressure may have contributed to the low mating rate of Helix, as low maters would have a decreased chance of being parasitized. As Helix can store viable

sperm for 4 years (Taylor, 1900) frequent copulation may not be required for a typical season's egg production.

If the physiological basis of sexual proclivity could be elucidated in both species, it would be interesting to compare how the control of sexual proclivity has been shaped by evolution to give the 2 species their different rates of copulation.

Sexual arousal

In snails with sexual proclivity and a relatively high BS, sexual stimuli lead to courtship behaviour (Chapter 6). Sexual arousal does not occur unless the snail can engage in tactile contact with a conspecific. An anesthetized conspecific can induce sexual arousal but it is less effective at inducing arousal in its partner than is an active snail (Chapter 5). A rubber snail model is ineffective in inducing sexual arousal. Attempting to induce sexual arousal by stroking the animal with a brush is equally ineffective (Syzmanski, 1913- discussed by Lind, 1976). This suggests that a combination of the appropriate tactile and chemosensory stimuli are required to elicit sexual behaviour in Helix. Visual cues are unlikely to be important because Helix has poor form vision (Hamilton and Winter, 1984).

Sexual arousal is probably partly controlled by a positive feedback mechanism, initiated by tactile and chemosensory cues. As sexual arousal increases, the size of the genital eversion increases, which increases the amount of tissue available for stimulation (Chapter 4). This would increase the amount of incoming sexual sensory stimuli. The dependence of sexual arousal on this type of positive feedback mechanism would help to explain why courtship in Helix relies so extensively on mutual lip-genital contact (Chapter 2).

This reliance on sensory stimulation illustrates another fundamental difference between sexual arousal and sexual proclivity. Sexual arousal is controlled predominantly by exogenous stimuli, while sexual proclivity is more dependent on internal cues. This again suggests that each is controlled by a separate mechanism.

As well as tactile and chemosensory stimulation, snails also use a courtship pheromone found in the digitiform gland mucus to increase the sexual arousal of a partner (Chapter 3). During dart shooting, the dart carries the digitiform gland mucus into the body cavity of the recipient. Presumably the pheromone is then dispersed by the circulatory system to its target tissues.

Sexual arousal exerts specific effects on other behaviours such as feeding and locomotion (Chapter 5). Since these effects do not occur with sexual proclivity, presumably activating the neural circuits responsible for sexual behaviour coincidentally influences circuits controlling feeding and locomotion. These effects are modelled as occurring via the sexual specific central arousal system (Fig. 1). A similar phenomenon is observed in Pleurobranchaea. In Pleurobranchaea chemostimulation alone does not inhibit withdrawal behaviour; the animal must actively feed (i.e. exhibit food arousal) to inhibit withdrawal (Davis, et al., 1977).

The level of arousal determines the strength of its effects on other behaviours (Chapter 5). This, too, is observed in Pleurobranchaea (Kovac and Davis, 1980b). This implies that the sexual specific central arousal system must be capable of a graded effect.

Proclivity and arousal in molluscs

Fig. 1 shows sexual 'arousal' divided into 2 components, sexual proclivity and sexual arousal.

Chapters 4, 5, and 6 illustrated the utility of this separation. Given that the 2 components are likely to be mediated by separate mechanisms, the search for the

physiological basis of 'arousal' would probably benefit by the division being made explicit in motivational studies done in other molluscs. The following section summarizes work on both sex and feeding in molluscs, but restates the results in terms of proclivity and arousal to demonstrate the general applicability of the 2 terms.

Sexual 'arousal'

The functional division of sexual 'arousal' into separate components is well established in vertebrates and these distinctions appear to reflect real mechanistic divisions in both females (Beach, 1976; Aous et al., 1988) and males (Oomura et al., 1988).

These differences have been included in models of rat male sexual behaviour as different types of 'arousability' (Toates and O'Rourke, 1978). Proclivity is modelled as the 'state of arousability' and is controlled by the level of sexual satiation (Toates, 1986).

In Aplysia, too, it is likely that sexual proclivity and sexual arousal are to some extent separate entities. Aplysia has a courtship pheromone that appears to effect sexual proclivity, but there is no evidence that it affects sexual arousal (Audesirk, 1977; Painter et al., 1989).

As in Helix, sexual proclivity can be estimated in Aplysia dactylomeda by the number of previous non-sexual contacts between animals (Zaferes et al., 1988). This suggests that, as in Helix, sexual proclivity exists prior to any sexual stimulation.

However, sexual proclivity and sexual arousal may be less distinct in some molluscs than they are in Helix. In Lymnaea, for example, higher levels of sexual proclivity also correlate with higher levels of sexual arousal (Van Duivenboden, 1984).

Food 'arousal'

The division between feeding proclivity and food arousal has been made implicitly in Aplysia (Kuslansky et al., 1987), in which feeding proclivity has been modelled as having a control mechanism that is separate from that controlling food arousal (Weiss et al., 1982). The control of feeding proclivity is not addressed in the papers which examine food arousal (i.e. Weiss et al., 1982), suggesting that many molluscan workers see food arousal as separate enough from feeding proclivity that the 2 can be studied in isolation. Nevertheless, the fact that the 2 aspects of feeding are distinct behavioural phenomena, likely to be subserved by different physiological systems, is never made explicit.

In the molluscan feeding systems studied, however, the division between arousal and proclivity is less complete than it is in Helix sexual behaviour. For example, gut stretch in Aplysia not only decreases feeding proclivity, but it can both increase and decrease food arousal depending on the amount of stretch (Susswein et al., 1978; Susswein et al., 1984). In Limax, gut distension also decreases both feeding proclivity and food arousal (Reingold and Gelperin, 1980). In these systems, both arousal and proclivity utilize similar sensory information.

Nevertheless, a separate term for responsiveness to food stimuli, such as proclivity, would still be useful for studies on feeding. Presently the term satiation is sometimes used to differentiate between the responsiveness to food stimuli and the intensity of the response (Susswein et al., 1984). However, the term becomes cumbersome when trying to describe the state of an unsatiated animal. For example, feeding proclivity in Pleurobranchaea is increased by non-food stimuli such as serotonin injections (Palovcik et al., 1982). If the term satiated were used to describe these results, we would need to say that the animal became more unsatiated after the serotonin injection.

Moreover, satiation in the sense of gut stretch does not control feeding proclivity in all molluscs. Haminoea

zelandiae respond to food continuously, and as intake increases over the processing limits of the digestive system, undigested food is excreted out the anus (Kohn, 1983). Therefore changes in food responsiveness are unlikely to involve factors that would be well described by the term satiation. Lymnaea, too, appears to respond to food almost continuously, though they were only tested for their responsiveness to food during the day (Tuersley and McCrohan, 1987). In both species, feeding proclivity is a better term than satiation to describe any variations in their food responsiveness.

In Achatina, release of gut distension does not result in increased feeding proclivity as might be expected if feeding proclivity were controlled by a simple negative feedback system such as is envisioned controlling feeding in Pleurobranchaea (Davis et al., 1977). Two days of starvation has little effect on the snail's responsiveness to food, and responsiveness is not increased until after 7 days of food deprivation. However all feces have passed from the animal by the second day of food deprivation, therefore the gut is unlikely still to be extended after 2 days of starvation (Croll and Chase, 1980). Presumably feeding proclivity in Achatina is controlled by other internal processes, with gut distension being only part of the control system. Even in Aplysia, other factors, such as blood

glucose concentration, contribute to the control of feeding proclivity (Bentivegna et al., 1988).

These results suggest that it is possible that satiation (the decline in feeding proclivity) is controlled by a mechanism separate from that which initiates feeding (increases feeding proclivity). By giving separate terms to arousal and proclivity, these various hypotheses can be more clearly stated.

Some of the neural mechanisms responsible for changes in feeding proclivity have been studied in Aplysia, as well as in other molluscs. In Aplysia, one group of cerebral mechanoreceptors have been found which excite the motorneurons for feeding. Various endogenous substances, such as serotonin, FMRFamide, SCP_b, buccalin, and myodulin have varying effects on spike width (and hence, potentially on neurotransmitter release) of the mechanoreceptors (Rosen et al, 1989a). These substances are likely to be involved in modulating food responsiveness, and hence proclivity.

In Tritonia, feeding proclivity is low during an escape swim. Audesirk and Audesirk (1980) found that during an escape swim, complex mechanoreceptors become less responsive to food stimuli. Work on Pleurobranchaea has also found neurons that are responsible for feeding. As in Aplysia, these cell's responses are modified by

decreases in feeding proclivity (Davis and Gillette, 1978; Gillette and Gillette, 1983).

The physiological control of food arousal is better understood than that underlying feeding proclivity. In Aplysia, and other molluscs, neurons involved in food arousal have been identified (Pentreath et al., 1982). The best studied system is that of food arousal in Aplysia. In this animal the control of bite speed and bite magnitude is intricate, involving both central and peripheral mechanisms (Weiss et al, 1982). Food arousal is not controlled by any one cell (Rosen et al., 1989b), but is a highly distributed system. However some cells appear to be dedicated to an 'arousal' function. The cell CPR probably affects the activity of 2000 other neurons and can also affect heart rate, as well as other aspects of food arousal (Teyke, et al., 1990). It appears to be involved in coordinating the animal's whole response to facilitate feeding behaviour.

Therefore, in Aplysia, feeding proclivity is controlled primarily by gut distension and blood glucose levels (internal signals), while food arousal is initiated by sensory stimuli and is mediated primarily by specialized cells like CPR and MCC. This is similar to one of the differences between sexual proclivity and

sexual arousal in Helix, in that sexual arousal is dependent primarily on external stimuli, while sexual proclivity is cued by internal signals.

By dividing 'arousal' into arousal and proclivity, possible relationships between different behaviours may appear more clear. For example, Susswein (1984) and Ziv et al. (1989b) found that food deprivation, which increases feeding proclivity, positively modulates sexual proclivity. Sexual proclivity, induced by sexual isolation, has no positive effect on feeding, while sexual arousal increases feeding proclivity. The possible mechanisms of these interactions are less obvious if proclivity and arousal are lumped into a single entity.

'Central arousal' in Helix

In Fig. 1, the concept of 'central arousal' has been divided into 2 separate components. Based on the results from this work and from others in the molluscan literature, it seems likely that these 2 components are mechanistically distinct.

'Central arousal' in the sense of a variable describing the relative level of the animal's 'alertness', is often estimated by behavioural state (BS) (Lee and Palovcik, 1976). In Helix it has a gating

or permissive effect on sexual proclivity (Chapter 6). Behavioural state fluctuates in a daily rhythm and can be increased by both handling and serotonin (Chapter 6). It does appear to have a global, if indirect effect on other behaviours such as sex and feeding (Chapter 6).

It is conceivable that this type of 'central arousal' is controlled by a unitary system, that is a system in which the components are primarily dedicated to that particular function. This system would receive inputs from the circadian pacemaker as well as other internal and external stimuli (i.e. noxious stimuli). Its output would influence heart rate (necessary to generate the blood pressure needed to increase BS), body wall tone, and perhaps locomotory circuits as well.

In Helix, BS is decreased by mucus from conspecifics (Cameron and Carter, 1979; Dan and Bailey, 1982). This leads to a decrease in their food consumption and reproduction (Cameron and Carter, 1979; Dan and Bailey, 1982; Lucarz, 1984). The most parsimonious explanation for this effect is that the mucus negatively modulates the system that controls BS in Helix.

The second, and probably mechanistically distinct, meaning of 'central arousal' is also shown in Fig. 1. This type of 'central arousal' consists of a system which mediates the effects of a behaviour, like feeding, on

other behaviours and autonomic functions to insure the animal's ability to perform the activity in a coordinated fashion. It may be mediated by specialized cells like CPR in Aplysia (Teyke, et al., 1990). Each behaviour will need a different orchestration of suppressed and facilitated behavioural responses. These behaviour-specific arousal systems (BSAS) are likely to be unique. This suggests that each behaviour would have its own system (Leonard and Lukowiak, 1986).

Indirect support for the existence of different BSASs for each behaviour in Helix comes from the finding that it has at least 5 endogenous compounds that can affect heart rate (Lloyd, 1982) If all 'arousing' effects were mediated by the same system, only one or two cardioactive substances would be required.

'Central arousal' in the molluscan literature

The subsequent section summarizes the current theories concerning 'central arousal' in molluscs. It shows that much of the controversy surrounding different theories of 'central arousal' is largely due to the ambiguities in the terminology. This confusion would be lessened by adopting the practice of splitting 'central arousal' into 2 separate components, as has been done in the model of the sexual behaviour of Helix aspersa (Fig. 1).

Weiss et al.'s (1982) model of 'central arousal' refers to 'central arousal' only in its behaviour specific sense. 'Central arousal' is the neural/neurohormonal system which coordinates the animal's other behaviours and autonomic systems so that feeding can occur. It is shown as activating the executive feeding arousal system, (equivalent to the food arousal system in this thesis' terminology). The place of a BS central arousal system (BSCAS) is not explicitly modelled, though the feeding specific central arousal system is shown as integrating a variety of inputs, one of which could be information dealing with BS. Weiss et al. (1982) do not imply that their feeding specific central arousal system mediates sexual specific central arousal, or the effects of any other behaviour. It is specific for feeding.

Palovcik et al. (1982), on the other hand, clearly mean 'central arousal' in the sense of behavioural state. As with virtually all work on molluscs, motivational variables are constructed with the explicit hypothesis that they are mediated by a real physiological system. Palovcik et al. (1982) propose, "Thus, by activating a central neuronal mechanism, serotonin may bring about the changes associated with the more active behavioral states, namely muscle tone, locomotion, and spontaneous behaviour, as well as increased responsiveness to

external stimuli such as food stimuli." (p. 391). In Palovcik's BSCAS hypothesis, unlike the BSCAS hypothesis for Helix, the BSCAS system is thought to directly influence the mechanisms controlling a variety of behaviours.

Kupfermann and Weiss (1981) also postulate the existence of a 'central arousal' system in the behavioural state sense, except that they do not hypothesize that it is necessarily involved in BS. Instead they see this system as positively modulating a variety of behaviours, but it may not necessarily be involved in increasing BS. The system is activated by diverse sensory stimuli.

Leonard and Lukowiak (1986), however, deny the existence of a BSCAS. They argue that each behaviour, which they define functionally, is controlled by its own 'drive', and mediates its effects via its own separate system. Therefore they only admit the existence of behaviour specific arousal systems (BSASs). In support of their hypothesis, they have found that although both food satiation and copulation suppress the gill withdrawal reflex (GWR), the suppression is mediated by a different peptide in each case (Colebrook et al., 1984).

Leonard and Lukowiak (1986) are opposed to the hypothesis that different behaviours would share the same BSAS. Notice that neither Palovcik et al (1982), nor

Kupfermann and Weiss (1981) are suggesting this. Kupfermann and Weiss (1981) and Palovcik et al. (1982) are suggesting that there exists a system which can modulate both the proclivity and subsequent arousal of a variety of different behaviours. Leonard and Lukowiak (1986) would not deny the obvious importance of circadian rhythms in behaviour, but their hypothesis does not deal with behavioural proclivity, as do the postulates of Kupfermann and Weiss (1981) and Palovcik et al. (1982), but instead with what mediates behavioural effects.

To add to the confusion, the hypotheses of Kupfermann and Weiss (1981) and Palovcik et al. (1982) could be redefined in such a way that there would be no disagreement between their hypotheses and that of Leonard and Lukowiak (1986). If Kupfermann and Weiss (1981) define that the positive modulation of different behaviours by noxious tactile stimuli is a stress behaviour, controlled by its own 'drive', and if Palovcik et al. (1982) hypothesize that activity (high BS) or inactivity (low BS) is also a functional behaviour controlled by a 'drive', then these theories will now agree with the general hypothesis of Leonard and Lukowiak (1986). Their ideas would clash only if someone suggested that the BS 'drive' uses the same system as the stress 'drive'. The differences between the various theories would be clearer if a distinction between the

two forms of 'central arousal' were made, and if the distinction between proclivity and arousal were adopted.

Heart rate and a BS central arousal system in molluscs

Many workers have suggested that the increase in heart rate that is observed as the animal becomes more 'active' is evidence of a general 'arousal' state (Dieringer et al., 1978; Koch and Koester, 1982; Koch et al., 1984). However, because molluscs require high blood pressure in the cephalopedal area to perform any behaviour, especially feeding, sex, and locomotion, it is hardly a surprise that performing these behaviours correlates with increases in heart rate. However, this increase will only be required if the resting heart rate is incapable of generating the required blood pressure to perform the behaviour. For this reason the correlation of heart rate with BSASs is less than perfect. For example, in Aplysia, food arousal causes no increase in heart rate unless the animals have been previously starved (Dieringer et al., 1978). Why should this be? Perhaps it is because starvation severely decreases their resting heart rate (Dieringer et al., 1978). Once an animal has been 'aroused' by a behaviour such as feeding, its heart rate remains high for some time afterwards (Dieringer et al., 1978). Therefore it is 'easier' for

it to perform other behaviours, as it has the necessary preconditions for any other active behaviour. It may now show a decreased time to respond to other stimuli, but this may be because it does not need to wait for blood pressure to build up for it to be capable of responding, and not because a specialized neural system has been activated.

For example, Kupfermann and Weiss (1981) found that handling Aplysia increases their responsiveness to food 4 min later. They concluded from this that a 'central arousal' system positively modulates a wide variety of behaviours. However, previous to being handled, the animals had been quiescent. After being handled the animals became 'aroused' in the BS sense, and had high BSSs. This would have increased their heart rate. The heart rate would still have been elevated even after the animals returned to their baseline BSSs 4 min later (Dieringer et al., 1978). The faster feeding found by Kupfermann and Weiss (1981) may have been partly due to the residual effects of having an increased BSS on the cardiovascular system.

Therefore, although these experiments suggest that a BSCAS exists, they do not necessarily show that the animal has a special neural system mediating this 'arousal'. Performing any active behaviour has effects on the cardiovascular system and it may be that it is

through these effects that behavioural responsiveness is increased. Only time, and more neuroethology, will tell.

Evidence for a BSCAS in molluscs

Because a BSCAS is not the only determinant of behaviour, the correlation between BS and behavioural responsiveness is not likely to be perfect. For example, Advocat (1980) found that animals that had recently been fed were more active (had a higher BSS) than did starved animals, but that the starved animals remained more responsive to stimuli, both food and tactile. The observed decrease in responsiveness could be induced simply by chemostimulation; the ingestion of food was not required. Therefore although BS correlates with responsiveness (Chapter 5), responsiveness is also modulated by previous activity. In other words, mechanisms such as those mediating a decline in feeding proclivity are also likely to influence the proclivity for other behaviours. This makes conclusive evidence for a BSCAS difficult to glean from behavioural data alone.

The strongest evidence for the existence of such a BSCAS comes from experiments by Kupfermann and Weiss (1982). By monitoring the output of the metacerebral cell (MCC), a cell intimately involved in food arousal, they found that handling the animals caused an increase

in its firing rate. This suggests that a central system that responds to non-food stimuli directly interacts with the feeding specific arousal system and may be partly responsible for the increase in food arousal observed in handled animals (Kupfermann and Weiss, 1981).

Possible mechanisms mediating the BSCAS in Helix

As in Palovcik et al.'s (1982) hypothesis, other workers have suggested that serotonin is involved in mediating the BSCAS (leech, Willard, 1981; Aplysia, Parsons and Pinsker, 1989). However, although 5,7 DHT, a neurotoxin which decreases serotonin levels in Helisoma by 85%-90%, did decrease activity in Helisoma for the first 3 days, but the snails showed normal feeding, swimming and mating behaviour long before any significant recovery in their serotonin levels had occurred (Gadotti et al., 1986). Moreover, very young snails appear to have no serotonin in their systems, yet these animals still feed (Zakharov and Balaban, 1987) and have typical daily activity rhythms (personal observations). These results suggest that serotonin is not a necessary component of the BSCAS in snails.

In Helix, BS is associated with physiological mechanisms that require some degree of coordinated control (Dale, 1973). It is not unrealistic to assume

that there may be a system in which certain neural/neuralhormonal units are dedicated to this task. For example, the snail's emergence from the shell is thought to be controlled, at least in part, by the peptide pQDPFLRF which also increases heart rate (Lehman and Greenberg, 1987).

Final remarks

As Weiss et al. (1981) have pointed out, any motivated behaviour cannot be completely understood until its underlying physiological basis is elucidated. This will not be possible without clear definitions of the behaviours involved. Any definition that is so broad as to encompass phenomena that appear to be distinct even at the behavioural level will not be very helpful to neuroethologists. Future neuroethological studies dealing with motivated behaviours would benefit from an explicit division of the concept of 'arousal' into proclivity and arousal and from a similar division of the concept of 'central arousal' into behavioural state central arousal and behaviour-specific central arousal. The resulting clarity in behavioural terminology will also reduce much of the confusion in the literature surrounding the concepts and theories concerning 'arousal' and 'central arousal'.

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