Role of Bag Cells in Egg Deposition of Aplysia brasiliana

I. Comparison of Normal and Elicited Behaviors

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Summary. Egg deposition behaviors are analyzed from time-lapse recordings during which “spontaneous” discharges of the neuroendocrine bag cells are recorded with chronically implanted cuff electrodes. In the laboratory, Aplysia brasiliana normally deposit long egg cordons on the substrate in a characteristic “figure 8” pattern similar to the configuration of egg masses observed in the natural environment. The overt behaviors associated with egg deposition are rhythmic head movements consisting of three components that overlap with characteristic relative latencies: up-and-down “undulations”, side-to-side “weaves” and in-and-out “tamps”. The characteristics of the three behaviors and their time courses relative to the appearance of eggs on the substrate suggest that undulations prepare the substrate, weaves distribute the egg cordon and tamps attach the cordon to the substrate. The same rhythmic head movements are also elicited by injections of homogenized abdominal ganglia (HAG) containing bag cell clusters, with comparable “relative” latencies and maximum frequencies but for shorter total durations. The overt behaviors begin earlier for normal than for triggered egg laying, often before the “spontaneous” release of bag cell hormones. This suggests that the head oscillations in intact animals are not normally initiated by bag cell activity. The mean latency to the appearance of the egg cordon on the substrate is the same (about 34 min) following either HAG injections or spontaneous bag cell discharges, confirming previous suggestions that the bag cell discharge triggers ovulation. Furthermore, the head movements appear to terminate at the same time following release or injection of hormone. The accompanying paper demonstrates that the full expression of the behavioral effects of bag cell injections depend upon normal movement of eggs in the reproductive tract.

Introduction

Invertebrates have provided model systems for studying neurosecretory dynamics (Berlind 1977). A well studied system for neurosecretory control of reproductive behavior is egg laying in the hermaphroditic marine gastropod, Aplysia. Aplysia is a prolific egg layer (MacGinitie 1934) depositing a long string of eggs in a characteristically shaped mass onto the substrate (Fig. 1). Egg laying behaviors have “overt” components involving end organs and behaviors that can be observed in the intact animal as well as “covert” components that can be observed only by exposing internal organs. The effector organs for the covert components of egg laying comprise the internal reproductive tract. Ripe eggs (see Fig. 1C) are released from the ovotestis (located caudally within the body cavity) and are fertilized and packaged as they wind rostrally through the complex reproductive tract (Thompson and Bebbington 1969; Coggeshall 1972). After exiting the body cavity and travelling along the external genital groove to the head region, the egg cordons are released onto the substrate from near the mouth at the base of the right tentacle. The overt behaviors associated with egg deposition involve rhythmic movements of the head and neck that distribute the egg string and attach it to the substrate. The animal does not typically locomote during deposition, so the final configuration of the egg mass is determined by the precise three-dimensional pattern of head oscillations. Thus the overt (head) and the covert (egg) movements involve different end organs that must be coordinated both spatially and temporally. Coordination of reproductive behaviors typically involves hormonal as well as neuronal control systems that are programmed developmentally to trigger the appropriate behavioral sequences when specific environmental cues are present (e.g., Bünning 1964). In this and the accompanying paper, we analyze the coordination of the overt and covert components of...
egg deposition of *Aplysia* in the laboratory. As a background for laboratory studies, it would be best to examine coordination of egg laying behaviors in the natural environment with all the cues present. However, field studies of *Aplysia* reproductive behaviors (e.g., Audesirk 1979; Kupfermann and Carew 1974) have not described in detail the behaviors involved in egg deposition. In lieu of direct field observation of egg laying animals, the present paper includes a comparison of the configuration of deposited egg masses in the field and in the laboratory.

Neurobiological analyses of egg laying in *Aplysia* have focused primarily on the bag cells (Kupfermann 1967; Stuart et al. 1980), a bilateral cluster of secretory neurons that have been studied electrophysiologically, biochemically and behaviorally (see review of Blankenship 1980). Electrical stimulation or high-potassium perfusion (Arch 1972a; Loh et al. 1975) of isolated bag cell clusters causes release of several peptides, the major one similar in molecular weight and isoelectric focus point to egg laying hormone (ELH) purified from bag cell homogenates. Bag cell hormones have a variety of central and peripheral target organs (Blankenship 1980, Table 1). Centrally, they produce prolonged modulation of electrical activity of identifiable neurons in the abdominal, buccal, pedal and cerebral ganglia (Mayeri et al. 1979; Stuart and Strumwasser 1980). Changes in activity of some of these neurons are known to modulate behavior directly, however none of the identified target neurons in these studies have an established role in egg deposition. Since the neuronal populations that mediate egg deposition have not yet been identified, it is not possible to specify the central neuronal events relevant to egg deposition that follow hormone release or administration. Peripherally, injection of bag cell extract into intact animals causes a short-latency (< 1 min) release of mature eggs from the ootestis in vivo (Coggeshall 1970). In vitro release can also be used as a bioassay (Dudek and Tobe 1978). Thus, the basic covert component of egg laying, ovulation, is presumably initiated by bag cell activity under normal conditions, although direct evidence is lacking. Most behavioral studies of bag cell function have used injections of crude or purified bag cell extracts which initiate egg laying in intact animals (Kupfermann 1967). Overt behavioral changes have been described with extract injection (Strumwasser et al. 1969; Arch and Smock 1977). *Aplysia californica* shows an early constriction of the mouth followed by swelling of the external common genital groove and a series of head movements (nodding, weaving and tucking) as the eggs are deposited (Arch and Smock 1977). No attempt has been made to quantify the sequences of rhythmic head movements during “spontaneous” egg laying and to correlate them with the actual deposition of eggs on the substrate. Locomotion and feeding also decrease prior to and during egg deposition (Arch and Smock 1977; Strumwasser et al. 1969; Stuart and Strumwasser 1980). However, injection experiments cannot determine whether bag cell activity initiates overt egg laying behaviors under normal conditions.

In isolated ganglia, the bag cells are silent and must be stimulated artificially to fire. Little is known about the events that normally lead to “spontaneous” bag cell activation in intact animals. In previous studies, we elucidated the role of the bag cells in normal egg laying of intact *Aplysia* by removing or deafferenting them and also by recording their activity chronically (Pinsker and Dudek 1977; Dudek et al. 1979). Removal or bilateral deafferentation of the bag cells produces a marked decrease in the frequency of egg laying episodes across days, indicating that the bag cells play an important role. Alternate mechanisms exist since egg laying occurs occasionally in the absence of bag cell activity. “Spontaneous” bag cell discharges are invariably associated with episodes of egg laying, again suggesting that the bag cells play a critical role. An implicit assumption underlying many published studies is that the bag cells are the central neurosecretory “command” system for initiating not only the covert behaviors (release of eggs from the ootestis) but also the overt behaviors (head movements) associated with egg deposition. To establish a causal link between release of bag cell hormone and the initiation of the overt behaviors, a minimal requirement is to show that the onset of bag cell activity normally occurs before the onset of the behaviors. This, in turn, requires a quantitative monitor of the ongoing behaviors so that their temporal relationship with the spontaneous neurosecretory discharge can be determined.

In this paper we quantify by means of time-lapse video recording three distinct types of rhythmic head movements (“undulations”, “weaves” and “tamps”) involved in spontaneous and elicited egg laying whose spatial and temporal interactions determine the configuration of the deposited egg mass of *Aplysia brasiliana*. Egg masses in the field are compared to egg masses laid normally in the laboratory. The onset of the three head oscillations and the initial appearance of eggs on the substrate are compared for normal egg laying involving “spontaneous” bag cell activity and for injection of homogenized abdominal ganglia (HAG) containing bag cell hormones. The findings confirm the suggestion that bag cell activity is responsible for ovulation. However, the overt behaviors mediating egg deposition are normally initiated prior to a “spontaneous” bag cell discharge. In the accompanying paper we examine the contribution of egg move-