

*Hedonic valence of odors in
the locust, Schistocerca
americana*

3.1 Introduction

Behavior is the starting point for any neurophysiological investigation, for it is the function of nervous systems that neurophysiology seeks to explain, and this we call behavior. Thus, before Mark Konishi, and the long series of outstanding scientists he has trained, started work on the neurophysiology of auditory localization in the owl, he first demonstrated that the nocturnal birds were capable of precisely locating a target using sound alone (Konishi, 1973). Analogously, the exploration of the fly's visual system has been guided by the pioneering studies of German ethologists showing what an insect's visual system is capable of (see Wolf and Heisenberg, 1990, for example). More recently, the demonstration that bees are able to learn to discriminate the category of symmetrical images from that of asymmetrical ones (Giurfa et al., 1996) and that of sequentially presented pairs of 'same' objects from that of 'different' objects, even across modalities (Giurfa et al., 2001) will surely pave the way for a neurophysiological investigation of how the bee brain achieves that.

In olfaction, research has been hampered by humans' relative lack of reliance on that sense. Thus, it is only arduously that we can start to piece apart, even at Marr's algorithmic level (Marr, 1982), complex olfactory behavior, such as how dogs are able to determine the direction of a trail (Steen, 1990) or how hamsters detect which over-mark is on top of another (Johnston and Bhorade, 1998). Most research has thus concentrated on the comparatively simple olfactory tasks of odor detection and discrimination.

The rest of this thesis deals with a neurophysiological investigation of the olfactory system of the American locust, *Schistocerca americana*. The first and foremost question when investigating how locusts recognize odors, then, is *whether* locusts indeed recognize odors. This question is made especially important in the light of a report that palpation, which involves contact chemoreceptors rather than olfactory receptors, is involved in all instances of food selection by *Schistocerca americana*, both for acceptance and rejection (Chapman and Sword, 1993). In the past, however,

and despite the abundance of physiological investigations on its olfactory system, this question had remained unanswered, due to the locust's lack of motility (one would almost say its apathy) and the consequent difficulties associated with measuring observable behavior. This chapter deals with the first successful attempt to demonstrate olfactory-guided behavior in *Schistocerca americana*. We set out to test for the presence of innate preferences for or against odors. In later work, Heather Dean and Brian Smith showed that locusts can also be trained to respond to an odor to which they have no innate preference (unpublished observations).

3.2 Failed beginnings

Our initial attempts consisted in placing droplets of an odorant in one corner of a box, placing a locust in the center and measuring the tendency of the locust to go to that corner as compared to the other corners. This experiment failed to reveal a preference or avoidance for any odor tested.

A second approach consisted in placing locusts (both males and females) on sand at the downstream end of a wind tunnel we constructed for this purpose, having odorized air flow along the direction of the tunnel, and observing the position of the locusts as time went by. This too, failed, for the locusts remained mostly static and clung to the walls.

The beginnings of success came using an apparatus constructed by Christine Chee-Ruiter for work with cockroaches (Chee-Ruiter and Laurent, 1995). The apparatus consists of a large cylinder with eight small openings on one plane, around the perimeter of the circle. The openings are large enough for an insect to go through, and each leads to a small compartment that has a smaller hole at the outside end. Through this smaller hole, odorized air can be made to flow into the large cylinder. There is a small hole at the bottom of the large cylinder, to serve as an exhaust. Finally, a cover allows the insects to be restricted to the short section of the cylinder which contains the plane of the openings after they have been introduced in the apparatus. This apparatus serves as a multi-

ple-choice test-bed for odor preferences, allowing an insect to escape through the opening which smells best to it. Preliminary results using locusts in it showed some odor preferences of little significance. We wondered whether the large number of openings made it hard for the locusts to identify the direction from which an odor was coming, and sought to simplify the task.

3.3 *Schistocerca gregaria*

At this point, frustrated by our poor luck getting *S. americana* to demonstrate odor-induced behavior, we resorted to a cousin, *S. gregaria*, which inhabits Africa. *S. gregaria* exists in two states: solitary and gregarious, depending on the amount of aggregating pheromone segregated by cohorts into the medium. *S. americana*, instead, exists in a single state that appears visually similar to *S. gregaria*'s solitary phase. Odor-mediated behavior of *S. gregaria* has been demonstrated before (Loher, 1958; Haskell et al., 1962), and we decided to use the African locust as a positive control for the apparatus. We obtained male specimens of *S. gregaria* from Dr. Ahmed Hassanali at the ICIPE, in Kenya. In our hands, *S. gregaria* exhibited significantly more locomotion than their American counterparts in our colony. With these locusts, we were able to refine a protocol to demonstrate olfactory preferences in the locust, and then were able to apply the same protocol to *S. americana* and show that they, too, had innate preferences for odors.

3.4 *Naive locusts show preferences for cherry and grass odors*

The design that finally served our purpose had several improvements over our previous attempts. Most importantly, the task was simplified to a two-alternative forced choice by placing locusts—several in preliminary experiments, one at a time in later experiments—at the bottom of a vertical Y-maze, at the top of which we placed a light source. Locusts are attracted by the heat and

the light of the bulb, and move upwards. Upward movement was further facilitated by the placement of mosquito netting along the inside surface of the Y-maze. Locusts like to cling on to the netting (somewhat like they cling to grass) and tend to move more when they can grab onto it. A locust can choose, then, between one of two symmetrical arms to climb into. Each arm had a hole at the top through which air could be made to flow in, and there was an exhaust at the bottom. One of the arms had air going into it, while the other had an equal flow rate of odorized air. Experiments were counterbalanced by switching which arm received odorized air after every experiment. Following Simpson's (1990) demonstration of the importance of food deprivation in observing odor preferences, our locusts were starved for at least 2 hours, usually several hours, before each experiment. An experiment then consisted of placing an adult locust in the maze while odor flowed into one arm and air into the other, and recording which arm the locust went into first. If the locust went into neither arm within 5 minutes, the experiment was aborted and not included in the statistics, since we were not interested in the probability that a locust would move, but rather the probability that a locust which did move did so toward the odorized air significantly more frequently than toward the non-odorized control.

For odors that appeared to cause repulsion using the above setup (i.e., through a preference of locusts *not* to enter the odorized chamber), we confirmed repulsion by using a different setup which more directly tested repulsion: we placed the Y-maze upsidedown and compared the tendency of locusts to exit the odorized arm with their tendency to exit the control arm with pure air¹, by placing 3 locusts on each arm of the inverted Y-maze and recording which branch the locusts

1. A simultaneous test of attraction and repulsion can be implemented by using a modification of the elevator maze used by Tully and Quinn (1985), where the insect is placed between two arms, one of which leads toward an odor source and the other leads away from it. Heather Dean and I built such a maze, which was used in experiments by Natalia Caporale (unpublished results).

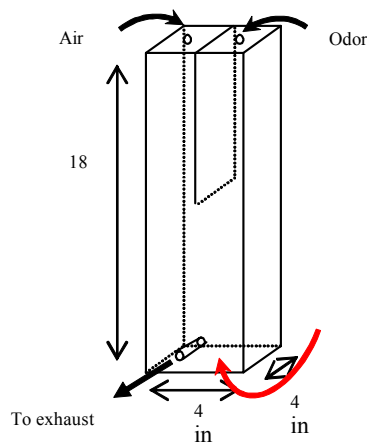


Figure 3.1. The Y-maze. The odor and air input as well as the exhaust are indicated by black arrows. The location of the air and odor tubes was alternated between experiments. The red arrow indicates the place in which the animal was introduced into the device (diagram courtesy of Natalia Caporale).

were more likely to exit within 5 minutes.

In preliminary experiments, 6 locusts were placed at the base of the Y-maze and each trial was scored by counting the number of locusts to climb each branch during the course of 5 minutes. These experiments unveiled a significant preference of *Schistocerca gregaria* for wheat grass ($p < 0.008$, binomial test, $n = 8$ experiments).

In later experiments, we restricted each trial to a single locust and changed the locust on every trial, to ensure that we were sampling decisions by every locust and that each of them was an independent choice. The results with single locusts were found to be similar to those using several locusts. In these experiments, *Schistocerca americana* showed attraction towards fresh crushed wheat grass ($p < 0.04$, binomial one-sided test, $n = 8$) and cherry extract (Lorann oils) ($p < 0.006$, $n = 27$), a significant avoidance of pentanol ($p < 0.001$, $n = 14$), and no significant effect by apple, hexanol, octanol, citral, strawberry, lavender, cineole, spearmint, parsley, cilantro, apple-blossom and geran-

iol ($p > 0.05$, $n = 21$ to 53 experiments for each odor, Table 1).

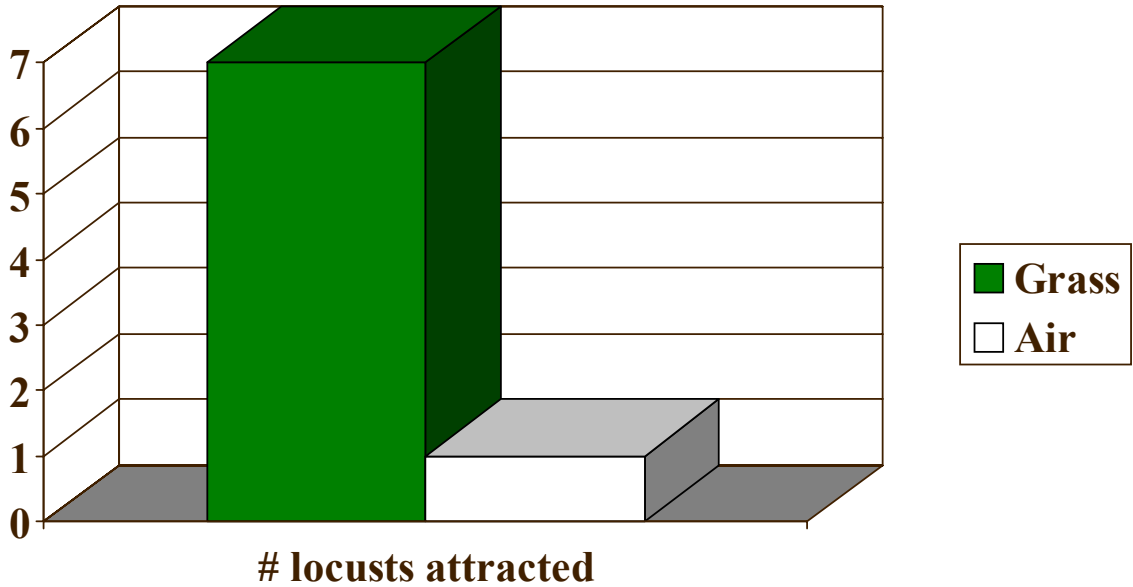


Figure 3.2. The odor of crushed wheat grass attracts *Schistocerca gregaria*.

TABLE 1. Olfactory preferences of *Schistocerca americana*

Odor	Chose odor	Chose air	No response	p-value
grass	38	23	16	0.04
cherry	16	4	7	0.006
apple	23	13	11	0.066
strawberry	14	17	12	0.76
lavender	10	12	8	0.42
cineole	23	13	17	0.06
citral	13	16	27	0.356
geraniol	5	4	19	0.5
spearmint	11	7	10	0.24
parsley	8	6	14	0.395
cilantro	8	6	14	0.395
apple-blossom	10	12	13	0.416
octanol	6	7	8	0.5
hexanol ^a	14	16	8	0.428
pentanol ^a	13	1	0	0.0009

- a. Hexanol and pentanol were tested using the inverted Y-maze.

3.4 Conclusions

In summary, we have demonstrated that the American locust, *S. americana*, does indeed smell, as evidenced by olfactory preferences. Furthermore, we have showed that at least one of these preferences (cherry) and one of these deterrences (pentanol) are innate, since the animals had not been exposed to the odorants previous to testing. This paves the way for continued studies to explain the physiological basis that allows locusts to perform discrimination between odors and that grants some odorants a positive or negative valence.

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