

Effects of Isolation and Crowding on Nociceptive Sensitization in the caterpillar, *Manduca sexta*



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Introduction

The tobacco hornworm *Manduca sexta* belongs to the order Lepidoptera. The larval *M. sexta* has been used to demonstrate short-term habituation of the proleg withdrawal reflex (Walters et al., 2001). Nociceptive sensitization, the sensory component of pain, was studied in larval *Manduca sexta*.

Social crowding in rats has been demonstrated to result in higher threshold responses to nociceptive stimuli such as electroshock and paw pinch pressure (Pilcher and Browne, 1982). This has been shown to be under hormonal control (Bereiter and Barker, 1979).

Behavioral responses to mechanical stimulation have also been studied in insects such as the cockroach, locust, and cricket (Hiraguchi and Yamaguchi, 2000). Withdrawal and escape behaviors were demonstrated in response to wind stimuli and tactile stimulation (Hiraguchi and Yamaguchi, 2000).

Manduca sexta has been a strong model for studying hormonal regulation in insects (Fuse and Truman, 2002) and has recently become a model for studying nociception as well (Walters et al., 2001). Interestingly, larvae are reared in isolation, and are relatively nonmotile. We were interested in whether social crowding would have a similar alteration in threshold responses to pain. Nociceptive sensitization, the induction of the sensory component of pain, on the larval *Manduca sexta* was used to establish the difference between the larval withdrawal reflex of motile, crowded animals and isolated nonmotile animals.

The results of this study suggest that the nonmotile larva had a greater sensitization to pain than did the motile larva. This indicates that social crowding has a direct role in nociception.

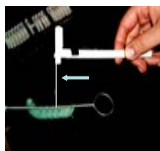
Hypothesis

Manduca sexta maintained in crowded conditions, and therefore more motile, will demonstrate a decreased sensitivity to nociceptive stimulation compared to the nonmotile, isolated larva.

Materials & Methods

Paired locomotory appendages on the abdomen, termed prolegs, were stimulated using nociceptive sensitization. Planta hairs are located on the tip of each proleg. There are 40-50 of these mechanosensory hairs on each proleg (Wiel and Weeks, 1996). The withdrawal reflex is induced by the principal planta retractor muscle (PPRM) and the accessory planta retractor muscle (APRM). When the retractor muscles relax, the proleg returns to its original position passively by hydrostatic pressure (Wiel and Weeks, 1996).

1. The larvae were separated into two groups: Free range and cupped.
2. They were kept in an incubator under a thermal and photoperiodic cycle, consisting of 27°C: 25°C / Light:Dark (17h:7h)
3. The larvae had their food inside of the containers they were living in.
4. The food consisted of wheat germ, vitamins, and antibiotics (Bell and Joachim, 1976).



Materials & Methods

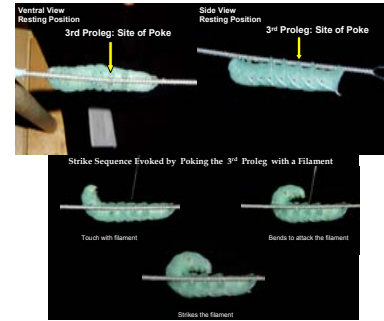
•The "Touch-Test™ Sensory Evaluator" was used to present a non-invasive method of sensory evaluation (arrow).

•The "Touch-Test™ Sensory Evaluators" are individual filaments, calibrated to provide its targeted force within a 5% standard deviation.

•The calibration strengths ranged from 0.4-10.0g.

•The filaments were pressed into each larva's third proleg at a 90° angle.

•Each monofilament was held in place for 1.5 seconds, and then switched to a higher calibration when no response was demonstrated.



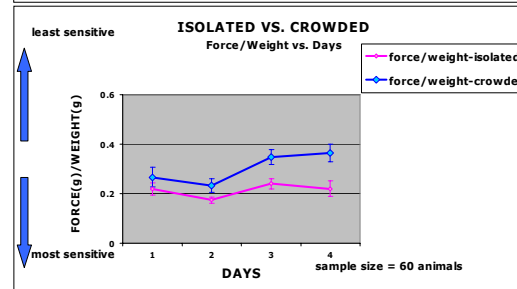
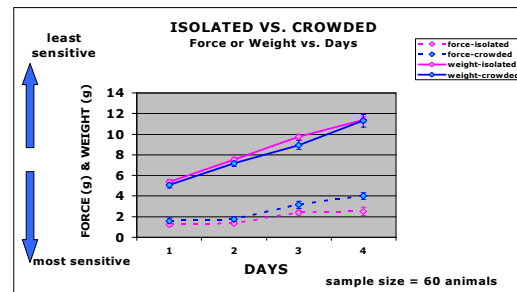
Isolated & Nonmotile (Cupped)



Crowded & Motile (Free Range)



Results



Discussion

The insect model, *Manduca sexta*, can be particularly useful in studying neural regulation of behaviors. This species is easy to manage, and large sample sizes are available to test sensitization, habituation, and social crowding. The results of this study demonstrated that crowded, motile animals required a greater amount of pressure to respond and were therefore less sensitive to the noxious stimuli. The isolated, nonmotile animals required less force and therefore were considered more sensitive to the noxious stimuli. These data support our hypothesis. Social crowding appeared to have an effect on nociceptive responses in *Manduca sexta*. Differences in motility between free range and cupped animals, however, could also have contributed to the changes in threshold sensitivity.

Similar changes are observed in locusts, which are highly sensitive to changes in population density. Densities may dramatically alter their behavioral, morphological and physiological characteristics. One of the key behavioral changes is the more intense flight behavior and improved flight performance of the gregarious locust compared to the solitary locust (Fuchs et al., 2003).

Behaviors exhibited by the caterpillar, *Manduca sexta*, and the gregarious locust may apply to real life situations because of competition and foraging behavior. Constant contact with each other, or stimulation by neighboring plants or wind, may lead to desensitization to these different stimuli. It is in the animal's best interest to conserve their energy by not responding to each stimulus and thus improve foraging behavior.

Future Directions

- Examine if crowded animals increase in muscle mass or fat mass due to constant muscle movement.
- Examine if crowded animals begin to wander faster than cupped animals.
- Examine if temperature of rearing has an effect on sensitization and habituation.
- Explore various noxious stimuli and see whether you get the same response. If not, it suggests that behaviorally there are different neural mechanisms participating in the perception of noxious stimuli.
- Examine the hormonal or neural basis for the altered thresholds.

References

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