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EFFECT OF APPETITIVE FOOD ODOUR STIMULI ON THE GIANT AFRICAN LAND SNAIL *Archachatina marginata*

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The study was carried out to determine the response of *Archachatina marginata* to appetitive food odour. We used multiple learning sessions of appetitive food-reward classical conditioning. The 120 snails were rewarded with the ingestion of unripe carrot as unconditioned stimuli (US) in 4 treatments of unpaired unripe fruit odour of carrot as conditioned stimuli (CS) in control treatment (T1); while paired with pawpaw (T2); combination of pawpaw and pear (T3); and pear (T4) respectively, each replicated 3 times, with 10 snails per treatment. Responses were measured through anterior tentacle lowering to sensory stimuli from CS. Results indicated that while pawpaw elicited highest feeding odour, T3 produced overshadowing effect with least ($P<0.05$) tentacle responses during conditioning. The *A. marginata* can learn olfactory-appetitive tasks and retain them in long-term memory for 12 days. The implication of this study to heliciculture is that, snails learn that food odour is predictive sign which evokes response after conditioning; snails may only eat to satiation appetitive food which has a long-term memory trace.

Key words: Tentacle, olfaction, snail, conditioning, learning

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Animals have the ability to identify odours in a concentration-invariant manner, as olfactory information are used to make appropriate behaviour decisions in order to survive in the wild (Makino and Yano, 2010). Snails from wide geographical distribution in diverse terrestrial ecosystems (Ebenso and Ebenso, 2011) would be prone to food odour during foraging in the wild.

Snail has good learning ability (Teyke, 1995). It has a small and simple brain (Makinae *et al.*, 2008). Snails learn only when they are hungry (Benjamin and Kemenes, 2010). In snails and slugs, olfaction is a dominant sensory modality for recognizing external objects, as visual and auditory systems have not been developed in their brain (Chase, 1982). Snail is a generalist in its feeding habits and it is advantageous for it to learn about potentially useful foods using a variety of sensory modalities (Benjamin and Kemenes, 2010). Stress has been shown to enhance learning as part of a range of changed behavioural response induced by the presence of a predator (Orr and Lukowiak, 2008). Pulmonates use chemotaxis (chemical senses) by directing their movements in concentration gradients of certain chemicals, such as odour or pheromones, in their environment (Cummis *et al.*, 2009).

In snails each pair of tentacle serves a function. The anterior (inferior) tentacles is for trail following, memory retrieval (recall) and odour preference (Kimura *et al.,* 1998), whereas orientation towards odour sources (Chase and Croll, 1981), food attraction learning (Friedrich and Teyke, 1998) depends on the posterior (superior) tentacles. In terrestrial pulmonates, the eyes of snails are located only on the superior tentacle; these eyes are used for phototaxis, evoked by a black and white check pattern, accompanied by a tropotatic strategy to avoid bright and light sources (Matsuo *et al.*, 2011).

Tactile, chemical and visual cues have all been used in conditioning experiments, of which a neutral stimulus (conditioned stimulus or CS), is paired with a strong feeding stimuli (unconditioned stimulus or US). Tactile conditioning requires multiple trial (Alexander *et al.*, 1984), this type of tactile reward-learning shares important characteristics with associative conditioning in vertebrates. It shows stimulus generalization so that training to one site of the body (the mouth) transfers to another site of the body (the tentacles) (Benjamin and Kemenes, 2010).

Stimuli may arouse, they may elicit a response and the may serve to orientate the animal as it responds (Manning, 1980). Associative learning allows for abridging the research on the development of formal learning theories (Acebes *et al.*, 2012).

The aim of this study was to analyse responses of trained *Archachatina marginata* to odour stimuli using the tactile food-reward classical conditioning in the laboratory.

MATERIALS AND METHODS

The *A. marginata* snails (64.00 ± 0.50g) from wild population were housed in plastic snaileries (25.70 x 25.70 x 14.00cm^3) placed on the laboratory bench under controlled laboratory conditions of temperature 25^{o}C, relative humidity 75% and photoperiod of 12 hrs light: 12 hrs darkness. Each Snailery had its lid covered with mosquito netting to keep off predators. Plastic sample bottle with a 1cm diameter hole perforated on the bottle cover was used as apparatus in which a slice of fruit was placed for snails to perceive fruit, but without access to taste/ingestion the fruit.

Prior to commencement of experimentation snails were starved for 7 days, while at the same time subjected to a sensory pre-condition stimulus effect of unripe pawpaw odour. At the commencement of experimentation, *A. marginata* were randomly assigned to 4 treatments with 3 replicates, each replicate consisted of 10 snails. On day 8, immediately prior to conditioning, the snails were dipped in water for 1 min. and then returned into the snailery, while being placed on their sides to induce activity. According to Ebenso and Ikon (2007), snails become active upon sensory stimuli of contact with water.

An appetitive food-reward classical conditioning was employed in which odour from unripe fruit was used as the condition stimuli (CS), presented in plastic sample bottle, while taste/ingestion of carrot was used as the unconditioned stimuli (US). Control treatment (T1) had carrot; T2 had pawpaw; T3 pawpaw + pear; T4 had pear as CS respectively. All snails were allowed to taste/ingest US (10cm in diameter) 5 minutes after learning of the CS and learning sessions repeated 3 times at 3hrs intervals/day. The conditioning lasted 5 days.

On the 12th day, all snails were subjected to memory retention test with unripe pawpaw as CS following same procedure as above.

Each response was scored as the movement of the anterior tentacles below an imaginary line over the top of the snail’s head. The number of responses was recorded and data are presented as means using descriptive statistics, according to methods of Steel and Torrie (1980).

RESULTS and DISCUSSION

The use of appetitive food-reward conditioning in this study demonstrated inferior tentacle responses to stimuli in olfactory-attraction conditioning during learning sessions, this result is comparable to report of Shaley *et al.*, (1981), these authors suggested that low response was because snails were naïve to food odour. However, in terrestrial slugs *Limax* there was no differentiation of roles of each part of tentacles (Yamagishi *et al* 2008).

Snails presented with paw paw odour showed higher ($P<0.05$) feeding response in this study, which means that paw paw elicited increased activity within the cerebral buccal interneuron (CBI) than other food odours. It was suggested by Kemenes *et al.*, (2002) that the CBIs act as "command cells" for the feeding network so that when they fire the feeding central pattern generator is activated followed by rhythmic ingestion movement.

In figure (1), the mean value of tentacle in control group which received similar stimuli (CS and US) in explicitly unpaired fashion as compared to pre-learning session exposure recorded similar value. According to Kemenes and Benjamin (1989), similar rhythmic feeding movement is observed in control groups which CS and US are applied alone.

Post-conditioning result (Fig 1), indicates higher ($P<0.05$) tentacle lowering than during pre-conditioning, this is similar to results of Nikitin and Balaban (2000), they observed an increase of 56% in tentacle lowering after learning session. It was emphasized by Jones *et al.*, (2003) that underlying this increase in tactile response after conditioning is a membrane depolarization that persist for as long as the electrophysiological and behavioural memory trace last.

Among non-control snails, T3 (figure 1) recorded the least response.

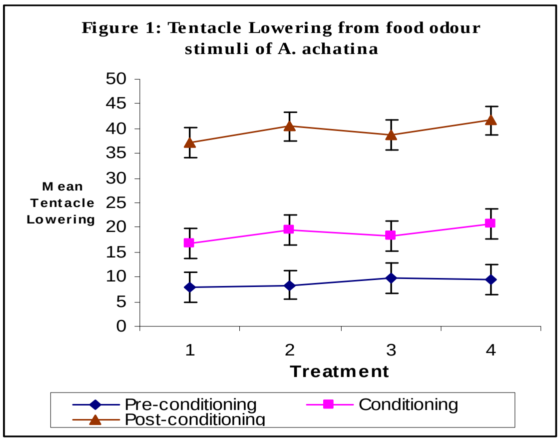
Figure 1: Tentacle Lowering from food odour stimuli of A. achatina

This compares to previous study by Ebenso and Adeyemo (2011) involved pairing of pear and pawpaw odour with *Achatina achatina*. It appears that the odour of pear overshadowed the memory of paw-paw odour, hence snails showed less appetitive response than the control snails.

The *A. marginata* in this study recorded high ($P<0.05$) feeding attraction memory from 15 learning sessions in 5 days to classical appetitive odour. Benjamin and Kemenes (2010) reported that 15 behavioural trials over 3 days induced a significant pattern of feeding response with pond snail *Lymnaea stagnalis*. A long-term memory of 12 days was recorded in this study after 5 minutes multiple learning sessions, whereas Ebenso and Adeyemo (2011) with *A. achatina* had a memory retrieval of 7 days 6hrs multiple learning sessions. Using slug *Limax marginatas* Teyke and Gelperin (1999), reported no impaired recognition of conditioned odours after learning sessions.

CONCLUSION

Data of this study confirm that olfaction arising from appetitive food odour is essential and complementary in snail nutrition and production.

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