

Influence of Photoperiod on the Behavioral Response to Olfactory Stimulation in the Snail *Helix pomatia* L.

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Summary

An ethanol vapor concentration of 1.6 mmol/l was used to test the diurnal variations of the olfactory response in two groups of snails, which were adapted to different light-dark cycles. The results revealed that the olfactory sensitivity to stimulation with ethanol was significantly increased during the day-time, which corresponds to the scotophase of the light-dark cycle, to which the animals had been adapted (χ^2 -test, $P < 0.01$).

Key words

Chemosensitivity • *Helix pomatia* • Olfaction • Snail • Diurnal

Introduction

As was revealed by behavioral and electrophysiological studies, terrestrial pulmonates possess a separate olfactory sense apart from the gustatory sense (Croll and Chase 1980, Croll 1983, Voss 2000). In this connection, the importance of the posterior tentacles was emphasized in a number of behavioral and neurophysiological studies (Schulz 1938, Kieckebusch 1953, Chase 1981). Olfaction is used for the detection of distant objects in the environment and allows precise discrimination between various substances. Furthermore, the behavioral repertoire of most of these animals is influenced by olfactory stimuli.

It has also been reported that behavioral reactions and metabolic processes in snails may be governed by the light regimen to which the animals are exposed (Mounzih *et al.* 1988, Dorlöchter 1989). Behavioral reactions as well as the sensitivity to olfactory stimulation may underlie diurnal variations as shown for

other physiological processes, e.g. the heart rate (Wünnenberg 1994). Regarding the chemical sense, it was demonstrated that the neuronal response to gustatory stimulation was influenced by the photoperiod, to which adult specimens of *Helix pomatia* L. had been adapted (Voss *et al.* 1997).

The present study was performed to obtain evidence of a correlation between the photoperiod and olfactory sensitivity. For this purpose, we used behavioral studies, because we wished to ascertain whether light-induced modulation of chemosensitivity could also be observed in intact animals.

Methods

Adult specimens of *Helix pomatia* L., collected in the field, were used in this study.

For determination of the avoidance threshold of vaporized ethanol, snails, which had been maintained under constant conditions were used and a 12:12 LD

light-dark cycle with photophase from 8:00 to 20:00 h for at least three weeks, as described previously (Voss *et al.* 1997). A second group of snails was adapted to an inverted photoperiod with photophase from 20.00 to 8.00 h.

Prior to testing, all animals had been starving for 2 days, because the nutritional status might influence the olfactory sensitivity in snails (Croll and Chase 1980). The experiments were performed at constant ambient temperature ($T_a = 23 \text{ }^\circ\text{C} \pm 0.4$) during the day, between 12:00 and 16:00. This means that the test period correlated with the photophase of the first group and with the scotophase of the second group, respectively. All experiments were performed under red light. Since light of this wavelength is not visible to *Helix pomatia* L. (Tischler 1974), the possible influence of light induced orientation was excluded.

For the tests, a rectangular plastic chamber (32 x 6.5 x 4.5 cm) was connected to an application system, similar to that described previously (Voss 2000). The airstreams, produced by two membrane-pumps, were first led through activated carbon filters. Then each airstream was directed through a plastic-container, containing either a swab soaked in 1 ml of 96 % ethanol or a swab soaked in 1 ml of distilled water. Both containers were closed airtight by a lid. The flow rates were controlled by means of two flow meters in order to produce different saturation levels of ethanol in the airstream of the test chamber. The different relative

saturation of ethanol and their molarity, used in these experiments, are shown in Table 1. Calculations were based on an ambient temperature (T_a) of $20 \text{ }^\circ\text{C}$, using the gas law. The sum of both flow rates was always kept constant. The overall flow rate was always 40 l/h.

Table 1. Molarities of ethanol vapor saturation, used in this study. Calculations are based on an ambient temperature of $20 \text{ }^\circ\text{C}$, using the gas law

Saturation of air with ethanol (%)	Molarity of ethanol (mmol/l)
15	1.2
20	1.6
25	2.0

A thoroughly cleaned chamber was used in each single experiment. Afterwards it was cleaned again, rinsed several times, and ventilated for at least six hours before reusing. Experiments were performed as described previously (Voss 2000). The locomotor activity of each snail was observed for 10 min. If the snail moved at least 5 cm in the direction of the odor source, the response was evaluated as positive. Movement of at least 5 cm in the opposite direction or withdrawing into the shell were assessed as avoidance behavior. If the snail did not move but remained outside its shell, the stimulus was assessed as neutral.

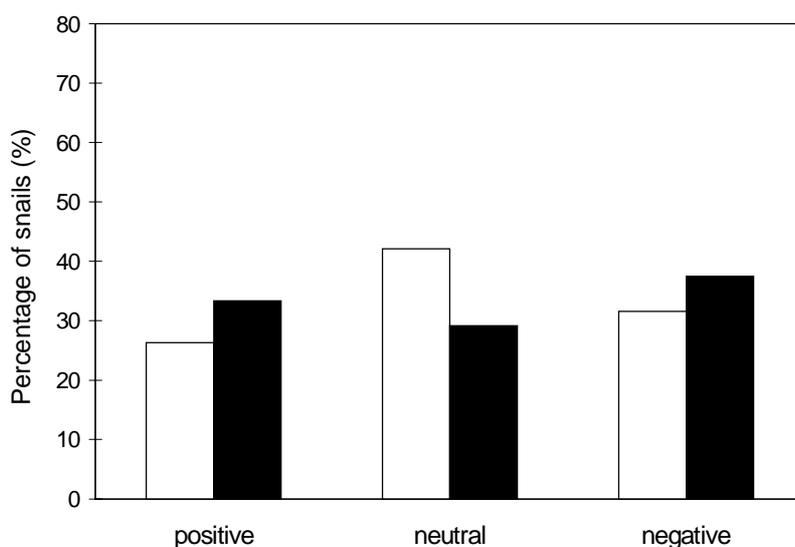


Fig. 1. Behavioral reactions to stimulation with pure air. White columns: During the test the snails were in the photophase of the light-dark cycle they were adapted to ($n = 20$). Black columns: During the test the snails were in the scotophase of the light-dark cycle they were adapted to ($n = 20$).

Results

First, controls with pure air were performed in both groups of differently adapted snails. The results are shown in Fig. 1. A qualitative analysis of the snail movements shows a nearly equivocal distribution of negative, positive, and neutral behavior in both groups. Thus, it can be assumed that in the experimental setup, besides olfactory stimulation, there was no factor influencing the behavior of the animals. Furthermore, it is obvious that the locomotor behavior in both groups is the same in the absence of an olfactory stimulus.

The results of determining the avoidance threshold are summarized in Fig. 2. These tests were performed on those animals that were in the photophase of the light-dark cycle to which they were adapted. At a saturation of 15 % ethanol, about 37 % of the snails (N=40) showed a negative reaction, whereas the number increased to 55 % at 20 % saturation. Consequently, the threshold for eliciting a behavioral avoidance reaction is in the interval between 15 % and 20 % ethanol saturation under these experimental conditions, which corresponds to a molarity of 1.2 and 1.6 mmol/l, respectively.

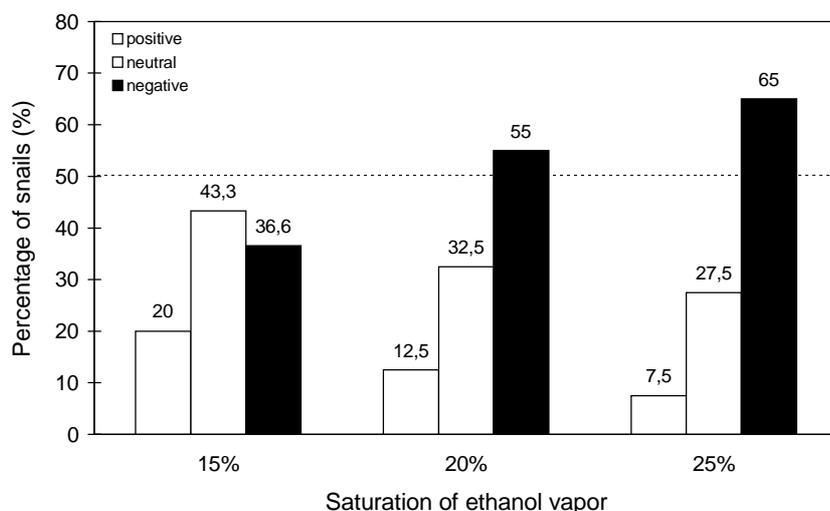
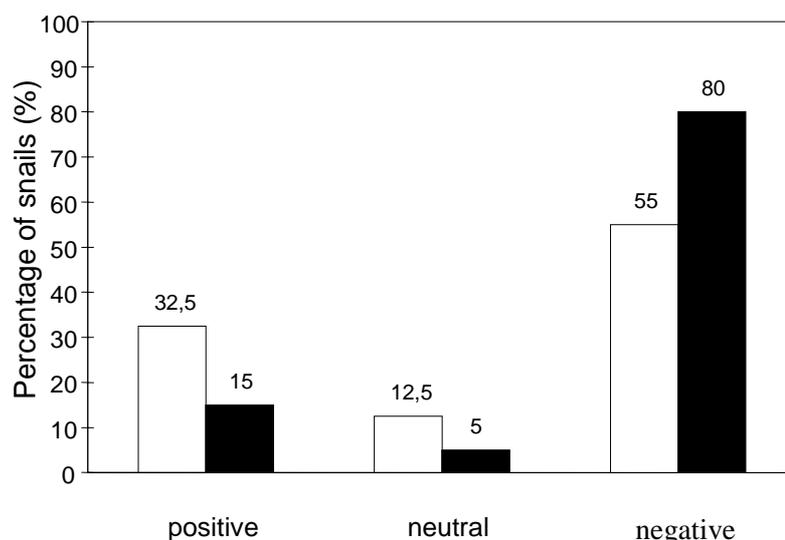


Fig. 2. Behavioral responses to olfactory stimulation with ethanol vapor of different saturations. During the test the snails were in the photophase of the light-dark cycle they were adapted to. N=40

Fig. 3. Behavioral reactions to stimulation with 20 % saturated ethanol vapor (1.6 mmol/l). White columns: During the test the snails were in the photophase of the light-dark cycle they were adapted to (n= 40). Black columns: During the test the snails were in the scotophase of the light-dark cycle they were adapted to (n= 20).



Furthermore, Fig. 2. shows that a further rise in saturation to 25 % increased the number of animals which responded with an avoidance behavior, correlating with the results of stimulation with pure ethanol. In addition,

this behavior was sometimes accompanied by tentacle retractions, probably indicating the onset of irritation at this concentration.

The inhibitory effect of ethanol did not only result in a change of the snail's movement, but influenced the frequency of tentacle retraction. Whereas saturation of 15 % and 20 % did not cause any tentacle retraction, an additional increase of 5 % did so in nine animals (22.5 %) during the first 5 seconds of stimulation.

On the basis of these results, a 20 % saturated ethanol vapor was used for the second group of snails tested during the period corresponding to their scotophase. Here, 16 (80 %) of a total of 20 snails showed a stimulus-induced avoidance reaction, whereas three animals moved towards the stimulus source, and only one single snail showed no clear orientation.

These values differ significantly from those of the first group of snails (χ^2 -test, $P < 0.01$). The results of both differently adapted groups of *Helix pomatia* L. are compared in Fig. 3.

Discussion

Our experiments show that the photoperiod may influence behavioral reactions to olfactory stimulation with ethanol vapor. The effect of olfactory stimulation with ethanol on the behavior of *Helix pomatia* L. was already described by Schulz (1938). His studies also revealed different types of avoidance behavior when a capillary, containing ethanol at a concentration of 40 %, was brought near different sections of the snail's body. The strongest reactions could be evoked by stimulation of the anterior or posterior tentacles. The increase of neuronal activity on stimulation with vaporized ethanol and the retraction of the tentacles at higher saturations confirmed the importance of the olfactory nerve of the tentacles in this context.

This is in agreement with similar data observed from other species, e.g., *Limax maximus*, where neurophysiological recordings from the procerebrum and from the tentacle nerve in response to stimulation with 2-ethyl-3-methoxypyrazine (potato odor) and air puffs showed stimulus-specific changes in neuronal activity

and procerebrum-oscillations, respectively (Gelperin and Tank 1990).

It is known that not only the light-dark regimen, but also other parameters, such as ambient temperature, may have an influence on certain periodicity, e.g. locomotor activity (Beiswanger *et al.* 1981). In the environment of *Helix pomatia* L. not only the light, but also ambient temperature (T_a) and relative humidity are changing regularly. Therefore, it is likely that activity rhythms and other periodic life processes are also influenced by other parameters. One example is the connection between temperature and the activity of slugs (Dainton 1954).

Since the animals used in the present study were maintained at constant T_a and constant relative humidity for at least three weeks, the photoperiod was the only parameter exerting a possible influence in controlling certain rhythmicities. This result correlates with the finding, observed from neurophysiological recordings, that the gustatory sensitivity of *Helix pomatia* L. is influenced by the light-dark cycle, to which the animals were adapted (Voss *et al.* 1997) and emphasizes the importance of the photoperiod as the main controlling parameter.

The biological meaning of the increased olfactory response during the scotophase is obvious. The scotophase is correlated with the maximum locomotor activity of these snails and is the time of the day when foraging takes place (Gelderloos 1979). In this context, increased olfactory sensitivity facilitates the detection of common food sources or potential sources of irritation.

The acceptance or avoidance of certain odors cannot sufficiently be explained by sensory adaptation. They are rather the result of learned associations between odor and nutritional value (Croll and Chase 1980). Ethanol, which is produced by fermentation during decomposition of sugars, could, at higher concentrations, indicate an inadequate food resource with reduced nutritional value.

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