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Comparison of single cell sensitivities to acetone, 1-octen-3-ol and 3-methylphenol in the riverine tsetse species *Glossina fuscipes fuscipes and G. palpalis palpalis*

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ABSTRACT

Action potentials from individual cells were recorded from antennae (funiculi) of living tsetse flies, *Glossina p. palpalis and Glossina f. fuscipes* using a "surface-contact" recording technique. Stimuli were vapours of 1-octen-3-ol, acetone and 3-methylphenol. Of the 101 and 128 olfactory cells tested for their sensitivity to odour stimuli in *G. p. palpalis* and *G. f. fuscipes*, respectively, the majority (83 and 77%) were activated by more than one chemical. The numbers of these "generalist" cells were 20 and 15% higher in females than in males. Response intensity increased with increasing odour dose. Temporal patterns of excitation were phasic-tonic and showed cells with relatively rapid cessation of spike activity after the end of stimulation and cells which continued firing for several seconds or even minutes after stimulation. Inhibition by odours only occurred in a minority of cells and was dose-dependent. For each of the three substances the excitatory response was significantly higher in *G. f. fuscipes* than in *G. p. palpalis*, whereas no significant differences between inhibitory responses were found. In *G. f. fuscipes* each stimulus evoked equal excitatory responses. In *G. p. palpalis*, however, acetone induced significantly higher responses than 1-octen-3-ol and 3-methylphenol. Response intensities to each of the three chemicals did not differ between male and female *G. p. palpalis*, whereas in *G. f. fuscipes* 1-octen-3-ol evoked significantly higher responses in males. Possible mechanisms of receptor cell odour coding and behavioural effects of the various cell type activities are discussed.

1. Introduction

Over the last 80 years control of tsetse flies has been an important tool to fight African trypanosomiasis. In the beginning of the 20th century, the most prevailing way of tsetse control was removal of the habitat of tsetse or of their wild hosts. Clearing of the vegetation to turn woodland into grassland and destruction of game animals, which are potential reservoirs of trypanosomes, were widely practiced. Soon after World War II the availability of DDT and other insecticides induced the onset of chemical control of the flies. However, these substances did not only kill tsetse but also many other organisms. Although nowadays insecticides are more selectively applied, many non-target animals are still killed. Moreover, high levels of insecticide residues accumulate in food chains, endangering future generations.

As a result of the increased awareness of environmental pollution by insecticides, the interest to control tsetse flies with less destructive methods expanded. In various parts of Africa, tsetse control now largely depends on the attraction of flies to traps or screens ("targets") treated with insecticides. These targets have a low environmental impact and are relatively cheap. In addition, they can be adopted by local communities on a self-help basis.

The fight against trypanosomes by control of the flies has primarily been based on the exploitation of their response to visual cues. Shape, orientation, colour, brightness, contrast and movement are all important properties in the attraction of tsetse flies to targets (see review by Green, 1994). Thus, the role of odours has been underestimated for a long time. An important advance in this field was made by Vale (1974), who showed that the odour of a single ox considerably increased the catches of the savannah species of the *morsitans* group, *Glossina morsitans morsitans* and *G. pallidipes*; about 10 times more males and almost 20 times more females were caught in the presence of attracting volatiles than in their absence. Since then, it has become evident that

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Table1
Percentages and numbers (between parentheses) of cells excited (Exc), inhibited (Inh) or not responding (No) to 0.5 mg doses of acetone, 1-octen-3-ol and 3-methyl phenol. Numbers of cells tested: *G. p. palpalis* males 13, females 28; *G. f. fuscipes* males 19, females 29.

Stimulus	*G. p. palpalis*						*G. f. fuscipes*					
	Male			Female			Male			Female		
	Exc	Inh	No	Exc	Inh	No.	Exc	Inh	No	Exc	Inh	No
Acetone	77 (10)	0	23 (3)	78 (22)	4 (1)	18 (5)	84 (16)	0	16 (3)	83(24)	3 (1)	14 (4)
1-Octen-3-ol	69 (9)	0	31 (4)	75 (21)	4 (1)	21 (6)	79 (15)	0	21 (4)	83 (24)	3 (1)	14 (4)
3-Methylphenol	77 (10)	8 (1)	15 (2)	86 (24)	14 (4)	0	74 (14)	5 (1)	21 (4)	90(26)	7 (2)	3 (1)

Table 2
Numbers of cell types in samples of antennal olfactory cells in *G. p. palpalis* and *G. f. fuscipes* responding to 0.5 mg doses of acetone (Ac), 1-octen-3-ol (O) and/or 3-methylphenol (3MP). unknown No response to any of the three stimuli.

Stimulus	*G. p. palpalis*		*G. f. fuscipes*	
	male n = 13	Female n = 28	male n = 19	female n = 29
Ac	2	1	1	0
O	1	0	0	1
3MP	1	0	1	1
Ac, O	1	4	3	3
Ac, 3MP	0	5	0	3
O, 3MP	1	3	0	0
Ac, O, 3MP	7	13	10	18
unknown	0	2	4	3
Percentage of cells responding to more than one stimulus in male and female *G.p. palpalis* and *G. f. fuscipes*	69	89	68	83
Percentage of cells responding to more than one stimulus in each species	83		77	

odours play an important role in host finding by tsetse flies, and that the attractiveness of targets can considerably be increased by baiting them with host odours (Vale et al., 1986). A large part of the attractiveness of ox odour could be attributed to substances present in ox breath: carbon dioxide (Vale, 1974), acetone (Vale, 1980) and 1-octen-3-ol (Hall et al., 1984; Vale and Hall, 1985a, b). It was also demonstrated that buffalo urine is a powerful attractant for *G. pallidipes* (Owaga, 1985). This attractiveness was attributed to a fraction of seven phenols (Hassanali et al., 1986; Bursell et al., 1988), which includes phenol itself, and 3-methyl-, 3-ethyl-, 3-*n*-propyl-, 4-methyl-, 4-ethyl- and 4-*n*-propylphenol. Adding a combination of acetone, 1-octen-3-ol, 4-methylphenol and 3-*n*-propylphenol to a visual target in Zimbabwe increased catches of *G. m. morsitans* 5 times and of *G. pallidipes* 20 times (Vale et al., 1988). In addition, Syed and Guerin (2004) found that terpenes identified in volatiles collected from the invasive plant *Lantana camara*, used as refuge by tsetse flies during the high daytime temperatures of Africa (Okoth and Kapaata, 1987), attracted tsetse flies. More recently, Harraca et al. (2009) performed gas chromatography coupled to electroantennography in order to study the response of three tsetse species, *G. pallidipes* (*morsitans* group), *G. fuscipes* (*palpalis* group) and *G. brevipalpis* (*fusca* group), to rumen fluid odours from the acidic, mildly acidic and neutral fractions. It was found that these flies can detect terpenes, ketones, carboxylic acids sulphides, phenols, indoles, and aliphatic aldehydes. It soon appeared, however, that marked interspecific differences in the responses to chemicals occur in these flies and that, for example, riverine species of the *palpalis* group poorly respond to the odours which are highly successful in catching savannah species. Furthermore, Merot et al. (1988), Mwangelwa et al. (1995) and Späth (1995) reported that the attractants used for savannah species are hardly or not attractive to *palpalis* species, and sometimes even repel them. Consequently, in practice, trapping of the *palpalis* group flies still relies on visual cues (Green, 1994). Yet, in the Central African Republic (Gouteux et al., 1995) and in Kenya (Omolo et al., 2009), trapping experiments showed that catches of the *palpalis* species *G. fuscipes fuscipes* significantly increased with traps associated as bait the monitor lizard *Varanus niloticus*. In Democratic Republic of Congo the latter authors also found that *G. f. quanzenzis* flies were attracted to the odour of pigs. This suggested that odours may also play an important role in host-finding for some *palpalis* species.

So far, it is unclear if the reason of this difference is peripheral responsiveness or central treatment; in other words, if the variation in behaviour reflects species-specific differences in the sensitivity of the olfactory system, or in the behavioural responsiveness of the various species to odours. Electrophysiological studies by Den Otter et al. (1988, 1991), Den Otter (1991) and Van der Goes van Naters et al. (1996), revealed that flies of the *palpalis* group do also sense the substances used for attracting *morsitans* species. In both the *morsitans* and *palpalis* species, the majority of the olfactory cells appeared to respond to one of the chemicals 1-octen-3-ol, acetone, carbon dioxide and the phenols ("specialist" cells), while a minority was activated by more than one of these ("generalist" cells). In addition, a large group of cells was present that did not respond to any of these stimuli (Den Otter et al., 1988, 1991; Den Otter, 1991).

Voskamp et al. (1999a) suggested that the differences in behavioural responsiveness to these attractants could be due to differences in the relative numbers of specialist and generalist cells activated by these substances. Comparing the responses of individual olfactory cells of *G. f. fuscipes*, *G. m. morsitans* and *G. pallidipes* on stimulation with 1-octen-3-ol, acetone, 3- methylphenol and carbon dioxide, these authors found that in the former two species, the minority (16 and 20%, respectively) of the cells were activated by more than one of these odours, while the remaining cells responded to one odour only. They also found that the antennae of *G. pallidipes* differed from those of the other two species in that the relative number of generalist cells was significantly higher (53%).

In order to shed additional light on the mechanisms behind interspecific differences, we measured and compared the responses of individual olfactory cells of the *palpalis* tsetse, *G. f. fuscipes* and *G. p. palpalis* to 1-octen-3-ol, 3-methylphenol and acetone. We report a survey of receptor cell types present in the funiculi of *G. p. palpalis* and *G. f. fuscipes*, the level of the sensitivity of these cells to the three chemicals, and the temporal patterns of excitation shown by the various cell types. In addition, we speculate about how the olfactory cells detect chemicals.

2. Materials and Methods

2.1. Insects

Pupae of *Glossina fuscipes fuscipes* Newstead and *G. palpalis palpalis* (Robineau-Desvoidy) were obtained from a colony at The International Atomic Energy Agency, Seibersdorf, Austria. Pupae were kept individually in glass tubes until emergence, whereupon they were grouped in cages by sex and age. Pupae and flies were kept in a LD