The Journal of Experimental Biology 205, 241–251 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB3864

The effect of stimulus features on the visual orienting behaviour of the salamander *Plethodon jordani*

Niklas Schülert and Ursula Dicke*
Brain Research Institute, University of Bremen, 28334 Bremen, Germany
*Author for correspondence (e-mail: dicke@uni-bremen.de)

Accepted 29 October 2001

Summary

The effects of the visual features of prey-like objects on the orienting behaviour of the salamander *Plethodon jordani* were studied. Two stimuli (cricket dummies, rectangles), moving in opposite directions, were presented simultaneously on a computer screen. They differed in size, contrast, velocity and movement pattern of the entire body or the body appendages. Size and velocity appeared to be the dominant features; shape was of lesser importance. Contrast and movement pattern were of intermediate importance and local motion of little importance. This rank order was the same when the probability of a response to the different stimuli was estimated by means of the maximum-likelihood method. Cluster analysis revealed that in all animals stimuli could be grouped into five clusters. Among individuals, the rank order of stimuli was similar for high- and low-ranking stimuli and varied for those of intermediate rank; stimuli could be grouped into 3–5 clusters. Our findings favour the view that, in amphibians, prey recognition is guided by a number of visual features acting either alone or in combination and depending on internal motivational or attentional states and individual experience.

Movie available on-line:
http://www.biologists.com/JEB/movies/jeb3864.html.

Key words: orienting response, object recognition, prey scheme, prey experience, plethodontid salamander, amphibian, *Plethodon jordani*.

Introduction

In vertebrates, the appearance of an object in the visual field usually leads to an orienting response consisting of eye, head or body movements towards the object. Such responses provide a gross categorization of objects as important, e.g. dangerous, food-related or relevant for mating, or unimportant. They represent a simple type of sensory-guided attention and are exerted to bring the object into the centre of the visual field for further analysis.

In amphibians, prey-catching behaviour is commonly initiated by such an orienting response towards a prey object followed by approach and snapping. It is suggested that this behavioural sequence is released more-or-less automatically by objects fitting a relatively simple prey scheme, i.e. objects that move and are not too small or too large to be eaten or exhibit simple configural properties (Lettvin et al., 1959; Grüsser and Grüsser-Cornehls, 1976; Ewert, 1984, 1989). However, there is clear evidence that, in amphibians, both visual and non-visual prey recognition is experience-dependent. For example, amphibians can be trained by classical conditioning to ignore prey items and suppress orienting responses as a result of negative experiences with noxious or impalatable prey (Cott, 1936; Sternthal; 1974; Dean, 1980).

In addition, prior to the orienting response, an evaluation of the visual characteristics of the prey item must occur. Such analysis of the visual properties of a potential prey object appears to be more complicated than assumed previously and to include a number of features that appear to influence orienting and feeding behaviour in amphibians (Roth et al., 1998). For example, moving objects usually elicit orienting responses more readily than still objects. Furthermore, the size, shape, contrast and movement pattern of a prey object seem to be important. However, a detailed analysis of the effects of these features, alone or in combination, on the prey-catching behaviour of amphibians is lacking, partly because of methodological restrictions.

To understand better the effects of various visual features relevant for feeding behaviour, orienting behaviour was investigated in the salamander *Plethodon jordani*. This species belongs to the family Plethodontidae (lungless salamanders); it is terrestrial throughout its life. It has a well-developed visual system and is an active hunter; its natural diet comprises a large variety of arthropods (Jaeger, 1972; Fraser, 1976). The method applied in the present study consisted of the simultaneous presentation of two competing stimuli on a screen in front of the animal. The stimuli were taken from a series of a computer-generated variations of the image of a cricket. They differed in velocity, size, contrast and movement pattern of the entire body and of the appendages, and were compared with dummies used in earlier studies (e.g. moving rectangles). The orienting behaviour towards one or the other of the two stimuli was taken

Table 1. *Stimulus parameters of the different image sequences*

Stimulus	Duration (ms)	Velocity ($cm\,s^{-1}$)	Distance (cm)	Length (cm)	Other parameters
Standard cricket (ST)	4800	2.2	9	1	
Small-sized cricket (S)	4800	2.2	9	0.6	
Large-sized cricket (L)	4800	2.2	9	1.5	
Contrast-reduced cricket (C)	4800	2.2	9	1	Contrast reduced to 70 %
Slowly moving cricket (SM)	4800	1.1	4.5	1	
Fast-moving cricket (FM)	1500	7	9	1	
Still-image cricket (SI)	4800	2.2	9	1	
Stepwise-moving cricket (STM)	4800	2.2	9	1	Step frequency 3 Hz
Locally moving cricket (LM)	4800	–	–	1	Local locomotion
Rectangle (R)	4800	2.2	9	1.5×0.5	
Stepwise-moving rectangle (STMR)	4800	2.2	9	1.5×0.5	Step frequency 3 Hz

as an indication of the attractiveness of a particular visual feature. This study attempts to obtain further insight into the mechanisms underlying visually guided orienting responses in amphibians and to provide a basis for studies on the physiology of the neurons involved in visual attention.

Materials and methods

Animals

Salamanders (*Plethodon jordani* Blatchley) were collected from wild populations in the vicinity of Highlands Biological Station, Highlands, North Carolina, USA (collecting permit, Highlands Biological Station). They were housed individually in glass boxes (15 cm×20 cm×20 cm) lined with wet rubber foam and covered with pieces of slate. The glass boxes had removable opposite panes. Salamanders were kept at 20 °C on a 12 h:12 h light:dark cycle and were fed crickets roughly 1 cm in length. Individuals that responded readily to the presentation of crickets with orienting, approaching and snapping were selected for the experiments (N=8; snout–vent length 55–65 mm). The experiments followed the guidelines of the animal welfare laws and also conform to the legal requirements in the UK and the USA.

Stimulation

Visual stimulation was performed by presenting motion pictures on a thin film transistor screen in front of the animals. A movie was produced by recording a cricket (1 cm in body length) running at a characteristic velocity of 2.2 $cm\,s^{-1}$ with a digital video camera at 25 $frames\,s^{-1}$ (Sony TRV900E; Sony Corp.) and read into a computer. The movie was fractionized into bitmaps (Adobe Premiere 5.1; Adobe Systems Inc.); this series of bitmaps was taken as the standard sequence for presentation. In addition, this standard sequence was modified to obtain sequences of cricket images differing in size, contrast, velocity and movement pattern. These sequences were presented on the screen using a program in Labview (Labview 4.0, National Instruments, OptStim 2.0 programmed in our laboratory). The stimuli presented to the salamanders were the following (see Table 1): (i) standard cricket (ST); (ii) large-sized and small-sized crickets (L, S, respectively), in which the ST was modified in size using an image-editing program (Corel Photo-Paint 8.0); (iii) contrast-reduced cricket (C), in which the contrast of ST was reduced to 70 %; (iv) slowly moving and fast-moving crickets (SM, FM, respectively) running at velocities of 1.1 $cm\,s^{-1}$ and 7 $cm\,s^{-1}$, respectively; (v) still-image cricket (SI), moving at a standard velocity of 2.2 $cm\,s^{-1}$, thus simulating a forward-moving cricket without self-motion; (vi) stepwise-moving cricket (STM), in which images were taken out of the standard sequence to obtain a cricket moving at a step frequency of 3 Hz and a basic velocity of 2.2 $cm\,s^{-1}$; (vii) locally moving cricket (LM), in which the standard sequence was presented at the same position on the screen to imitate a moving cricket without forward motion; (viii) rectangle (R) and stepwise-moving rectangle (STMR), which were presented to compare the behavioural responses to prey-like *versus* non-prey-like stimuli.

The rectangle was similar in size and colour to the standard cricket and was moved either continuously (R) or stepwise (STMR) at a step frequency of 3 Hz at the same basic velocity (2.2 $cm\,s^{-1}$). In total, 11 different sequences of bitmaps were used for presentation (Table 1).

Presentation

Stimuli were moved from the centre of the screen either to the left or to the right; the locally moving stimulus was presented on the right or left side of the screen in the middle of the path of the moving stimulus. The stimuli were randomized with regard to type and direction of movement using a computer program (JUMBO 6.2; W. Köpcke, University of Münster, Germany). A pre-test was performed to accustom the salamanders to the test situation and to assess the efficacy of each stimulus. During this test period of 2 months, stimuli were presented singly in each test, and the response of the animal was noted (Fig. 1). The animal remained in its home box and was placed centrally in front of the screen at a distance of 15–20 cm. A pane was removed from the box, and a stimulus was presented. Immediately after the animal had responded to the stimulus with an orienting response, the presentation was stopped. After each animal had been pre-tested, experiments with randomly paired leftward- and rightward-moving stimuli

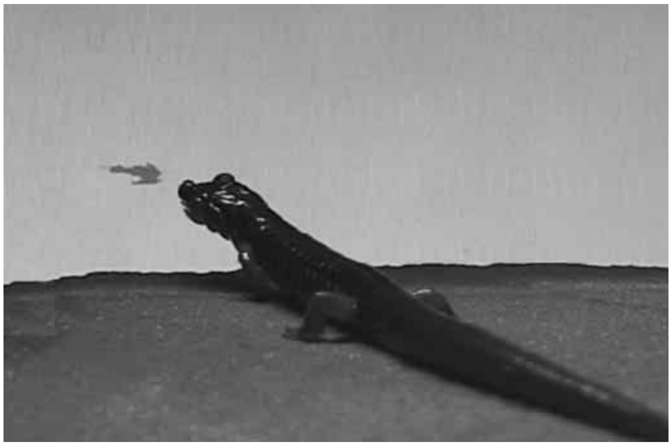

Fig. 1. The salamander *Plethodon jordani* responding to a contrast-reduced cricket presented as a single stimulus. The animal turns its head towards the stimulus and then follows it with its entire body.

were performed once a week with a maximum duration of 30 min per animal. Pairs of stimuli were presented on the screen by moving them from the centre in opposite directions (Fig. 2); the locally moving stimulus was presented on the left or right side of the screen. The minimum interval between two stimulus presentations was 10 s; the next presentation was started only after the animal had turned its head back to the central position. If an animal failed to respond for three consecutive presentations, the experiment was stopped for that day. During the experiments, animals were randomly rewarded with live crickets, which were presented with forceps in front of the screen. The pairing of 11 different stimuli resulted in 121 combinations when the side of the stimulus presentation (left or right) was taken into account and in 66 combinations when the direction of stimulus movement was not considered.

Analyses and statistics

In the experiments, both orienting responses towards one of the stimuli and failures to respond were noted. Statistical analysis was performed to examine intra- and inter-individual differences. The χ^2-test (Microsoft Corp., Excel 2000) was used to test for side preferences and the occurrence of failures of orienting responses. In the latter case, results from presentations of identical *versus* different stimuli were compared. A preference scale was created indicating the probability of an orienting response towards a particular stimulus. The maximum log-likelihood method was applied assuming that the results from different trials are stochastically independent of each other (modified after Bradley and Terry, 1952). The binomial distribution was estimated because only orienting responses to the left or right side, but not failures to respond, were considered. The responses to the standard cricket were taken as baseline. Discriminant analysis was performed to generate a confidence interval for each stimulus. To measure proximity between stimuli, a cluster analysis was carried out according to the complete-linkage method of furthest neighbours (see Jobson, 1992; Fahrmeir et al., 1996).

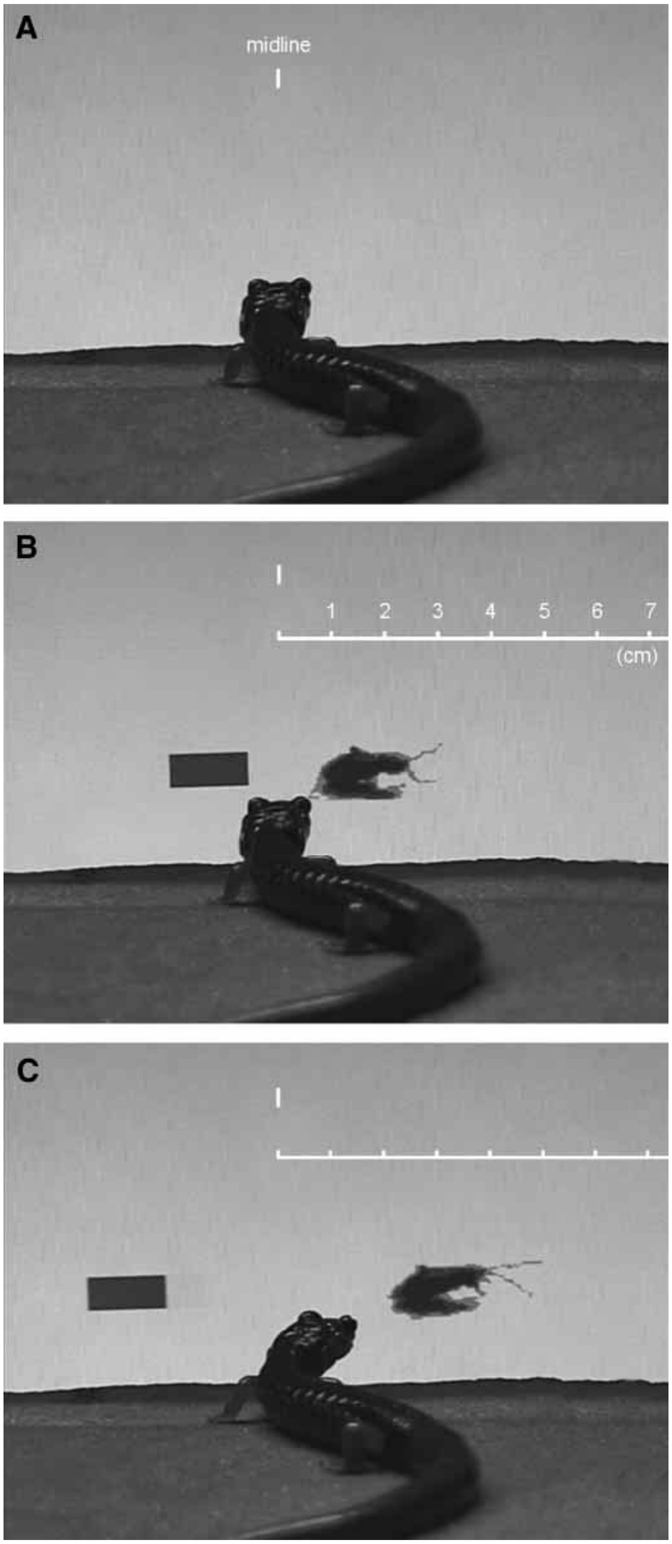

Fig. 2. The orienting response of a salamander. (A) The head of the animal is in a centred position in front of the screen. (B) A pair of stimuli (a leftward-moving rectangle and a rightward-moving large-sized cricket) appear in the centre of the screen. (C) After a delay of 1.7 s, the salamander turns its head towards the large-sized cricket.

Table 2. *Total number of paired stimulus presentations, orienting responses and failures to respond*

Individual	Total number of stimulus presentations	Total number of responses (% of stimulus presentations)	Responses to rightward-moving stimuli (% of total number of responses)	Responses to leftward-moving stimuli (% of total number of responses)	Failures to respond (% of stimulus presentations)
1	396	343 (86.6)	194 (56.6)	149 (43.4)	53 (13.4)
2	660	610 (92.4)	289 (47.4)	321 (52.6)	50 (7.6)
3	264	221 (83.7)	105 (47.5)	116 (52.5)	43 (16.3)
4	528	453 (85.8)	222 (49)	231 (51)	75 (14.2)
5	528	469 (88.8)	205 (43.7)	264 (56.3)	59 (11.2)
6	660	557 (84.4)	271 (48.7)	286 (51.3)	103 (15.6)
7	660	592 (89.7)	280 (47.3)	312 (52.7)	68 (10.3)
8	528	475 (90)	249 (52.4)	226 (47.6)	53 (10)
Mean	528	465 (88.1)	226.9 (48.8)	238.1 (51.2)	63 (11.9)

Data from each individual and pooled data from all individuals were evaluated according to this procedure.

Results

General observations

In the pre-test, all animals investigated readily took part in the experiments and responded to single stimulus presentation with an orienting response of the head at a minimum latency of 1 s. Each individual responded to each of the 11 different stimuli, although in some test sessions not all stimuli were responded to and the readiness to take part fluctuated over time. In the experiments with presentations of paired stimuli, readiness again differed over time among individuals, resulting in different numbers of experiments in which individuals took part. In general, the animal followed the stimulus that it responded to first and did not switch to the other moving stimulus, irrespective of its nature. The animal responded to a stimulus with an orienting response of the head, which was sometimes combined with an approach towards the stimulus. When the stimulus had disappeared, the animal retained its head position for a few seconds before moving it back to the centre. In some cases, animals snapped at the screen, but in most cases snapping occurred only once in a session and at the beginning of the experiment. However, animals fed immediately or with a short delay when live crickets were presented randomly between stimulus presentations. In the following, the results of the paired-stimulus condition are presented.

Frequency of responses and side preferences

Over a period of several months, all paired combinations of different stimuli were presented 10 times (N=660) to three animals, eight times (N=528) to three animals, six times (N=396) to one animal and four times (N=264) to another animal. These differences result from the fact that an experimental session was stopped when an animal failed to respond to three consecutive stimuli and from differences in time intervals between two presentations, which depended on the time when the head was moved back to the centre position. In all individuals, at least 84 %, and maximally 92 %, of stimulus presentations were responded to with head turns towards one of the stimuli, irrespective of movement direction. Accordingly, 8–16 % of stimulus presentations were not responded to; animals remained in their position in front of the screen. The numbers of orienting responses to leftward- or rightward-moving stimuli were approximately equal and ranged from 44 to 57 % of responses to either rightward- or leftward-moving stimuli (Table 2).

Stimulus preferences

The number of orienting responses differed with regard to the different stimuli (Fig. 3A). On average, all animals responded best to the large-sized cricked (mean number of responses 78.6), followed by the fast-moving cricket (69.8 responses), the standard cricket (61.1 responses), the stepwise-moving rectangle (53.8 responses), the continuously moving rectangle (49.4 responses), the still-image cricket (44.9 responses), the stepwise-moving cricket (37.5 responses) the slowly moving cricket (32.6 responses), the small cricket (18.4 responses), the contrast-reduced cricket (12 responses) and the locally moving cricket (seven responses). This rank order was the same when the direction of stimulus movement was taken into account (Fig. 3B) except for the continuously rightward-moving rectangle, which was preferred to the stepwise-moving rectangle.

Among individuals, the rank order of responses to different stimuli varied. All individuals responded best to the large-sized cricket, and in most individuals the fast-moving and standard cricket were the second and third choices, respectively. The least effective stimuli were the locally moving cricket, the contrast-reduced cricket and the small-sized cricket in all individuals; however, the ranking for these stimuli was different among individuals. This was also the case for the five remaining stimuli, the stepwise-moving rectangle, the rectangle, the still-image cricket, the stepwise-moving cricket and the slowly moving cricket. An example of the number of orienting responses and the absence of responses to the different pairs of stimuli for one individual is given in Table 3.

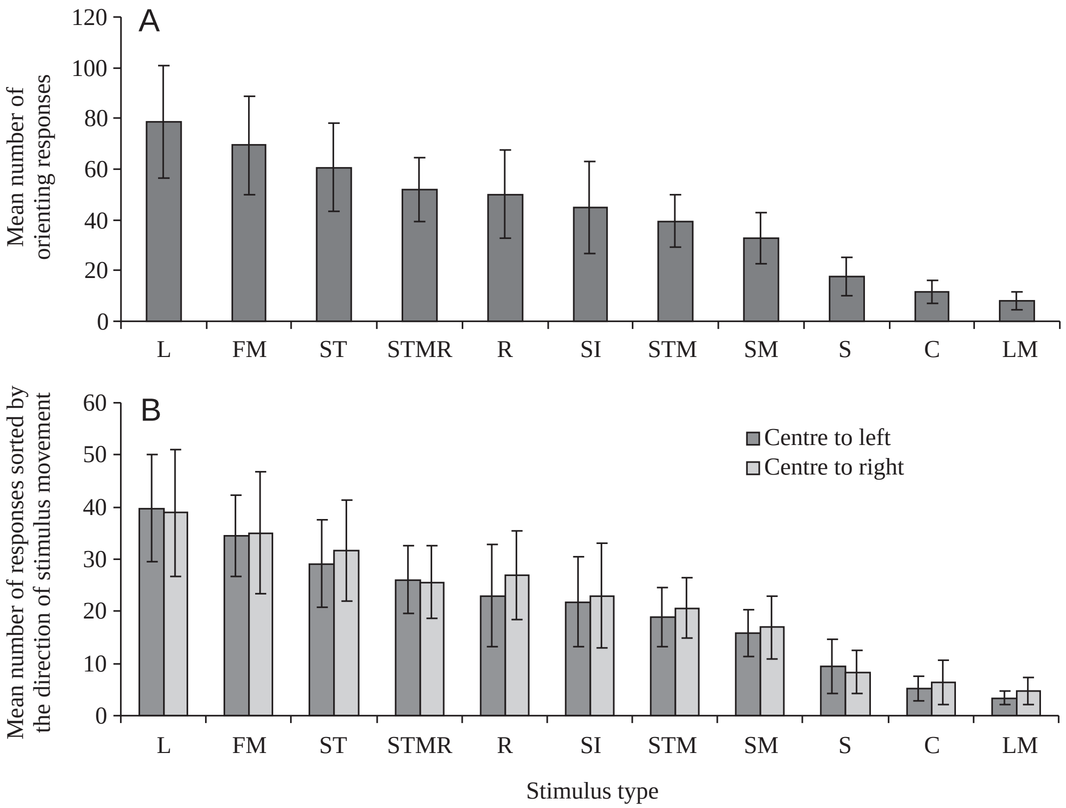

Fig. 3. Frequencies of orienting responses to the different stimuli in all individuals. (A) Mean number of orienting responses for both directions of movement. (B) Mean number of responses for leftward and rightward stimulus movement. Values are means ± S.D., *N*=8. For abbreviations, see Table 3A.

Collectively, animals did not respond to 12 % of presentations under paired-stimulus conditions. For simultaneous presentations of the same stimulus (63 % of all failures), the number of failures was much higher. Within this group, the stimuli responded to least were the locally moving cricket, the contrast-reduced cricket and the small-sized cricket (Table 4). On average, the combinations of identical stimuli were not responded to in 45 % of presentations. When combinations with identical stimuli are excluded, failures occurred in only 5 % of stimulus presentations. For presentations of different stimuli, the number of failures was again highest for the locally moving cricket, the contrast-reduced cricket and the small-sized cricket.

Results from statistical analyses

The statistical analysis of side preference revealed no significant effect for the group of salamanders. When salamanders were tested singly, two individuals showed a significant effect. One individual had a preference for leftward-moving stimuli (no. 1; $P<0.02$) and the other for stimuli moving to the right (no. 5; $P<0.01$). The responses of all individuals taken together were evaluated according to the maximum log-likelihood method and resulted in a preference scale for the different stimuli. A grouped statistical evaluation of all trials was valid, although this was accompanied by loss of accuracy according to the χ^2-test ($P<0.05$). The rank order of stimulus preference for each individual and that for the group of salamanders differed for intermediate-ranking stimuli and concerned only one or two positions (Fig. 4). The ranking of stimuli in all individuals corresponded to the rank order based on the absolute frequency of responses (Figs 3, 4). The stimuli were grouped into five clusters (Fig. 4). The first cluster contained the large-sized and the fast-moving cricket. The

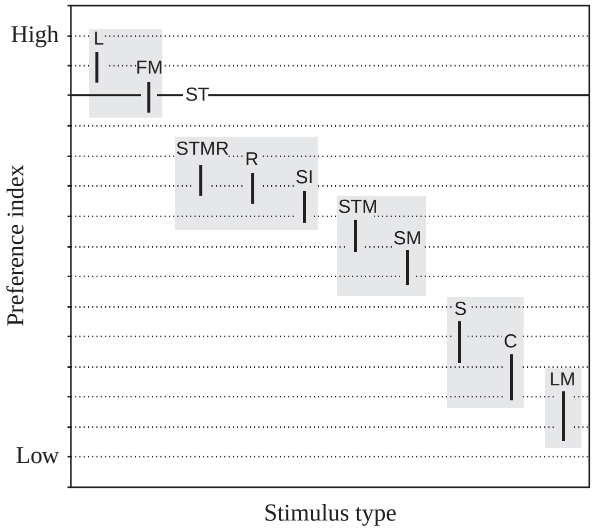

Fig. 4. Preference scale for the different stimuli in all individuals. The thick horizontal line represents the probability of responses to the ‘standard cricket’ (ST) as a baseline for the probability of responses to the other stimuli. Vertical bars indicate the confidence interval for each stimulus; grey rectangles indicate stimulus clusters containing stimuli with similar efficacy. For abbreviations, see Table 3A.

Table 3. *Results of stimulus presentations for one individual*

A

Number of orienting responses

	Stimulus moving from centre to right																						
	L		FM		ST		STMR		R		SI		STM		SM		S		C		LM		Total number of responses to stimuli moving to the left
Stimulus moving from centre to left																							
L	2	2	2	3	4	1	5	0	5	0	4	1	4	0	5	0	5	0	5	0	5	0	46
FM	3	2	0	3	3	2	4	1	4	0	5	0	5	0	5	0	4	1	5	0	4	0	42
ST	1	4	1	3	2	3	2	3	3	2	4	1	4	0	5	0	4	1	5	0	5	0	36
STMR	0	5	3	1	0	4	1	2	4	1	3	2	4	0	4	0	4	0	5	0	5	0	33
R	0	5	0	5	2	3	1	3	2	3	2	2	3	1	5	0	5	0	5	0	5	0	30
SI	0	5	1	4	1	4	3	2	2	3	1	1	2	3	2	3	5	0	4	0	4	1	25
STM	0	5	0	5	1	3	3	2	0	4	0	5	3	2	3	2	3	1	2	1	4	1	19
SM	0	5	0	5	1	4	2	3	1	4	2	3	1	3	0	3	2	0	4	0	5	0	18
S	0	5	0	5	1	3	0	5	0	5	1	4	1	4	0	5	0	3	4	1	4	0	11
C	0	5	0	5	0	5	0	5	0	5	1	4	1	3	0	4	1	3	1	3	4	0	8
LM	0	5	0	4	0	5	0	5	0	5	0	5	0	5	1	4	0	5	2	3	0	0	3
Total number of responses to stimuli moving to the right		48		43		37		31		32		28		21		32		15		8		2	271 286

557 Total number of responses

B

Number of failures

	Stimulus moving from centre to right										
	L	FM	ST	STMR	R	SI	STM	SM	S	C	LM
Stimulus moving from centre to left											
L	6						1				
FM		7			1						1
ST		1	5				1				
STMR		1	1	7			1	1	1		
R				1	5	1	1				
SI						8				1	
STM			1		1		5		1	2	
SM							1	7	2	1	
S			1						7		1
C							1	1	1	6	1
LM		1									10

103 Total number of failures

Each stimulus was tested five times for one movement direction and 10 times in the case of identical stimuli, resulting in a total of 660 presentations. (A) Number of orienting responses. The left-hand number in each column indicates the number of orienting responses to the left (white background), and the right-hand number indicates the number of orienting responses to the right (grey background). (B) Numbers of failures to respond under the same condition as in A.

L, large-sized cricket; FM, fast-moving cricket; ST, standard cricket; STMR, stepwise-moving rectangle; R, continuously moving rectangle; SI, still-image cricket; STM, stepwise-moving cricket; SM, slowly moving cricket; S, small-sized cricket; C, contrast-reduced cricket; LM, locally moving cricket.

second cluster included the stepwise-moving rectangle, the continuously moving rectangle and the still-image cricket. The third cluster comprised the stepwise-moving and the slowly moving cricket and was separated from a fourth cluster containing the small-sized and contrast-reduced cricket. The locally moving cricket represented the fifth cluster. This rank

Table 4. *Number of failures of orienting responses for all individuals*

	L	FM	ST	STMR	R	SI	STM	SM	S	C	LM
L	18										
FM	0	30									
ST	1	3	19								
STMR	2	2	3	29							
R	0	5	2	3	18						
SI	0	9	1	0	2	29					
STM	1	1	4	5	5	1	27				
SM	0	0	3	1	7	5	5	25			
S	0	1	2	3	5	2	2	8	33		
C	0	2	1	0	3	7	8	9	20	44	
LM	0	2	3	5	4	5	0	1	10	12	46

504
Total

In each cell, the number of failures to respond to paired stimulus presentations is given. Note that the number of failures is highest for the presentation of two identical stimuli.

L, large-sized cricket; FM, fast-moving cricket; ST, standard cricket; STMR, stepwise-moving rectangle; R, continuously moving rectangle; SI, still-image cricket; STM, stepwise-moving cricket; SM, slowly moving cricket; S, small-sized cricket; C, contrast-reduced cricket; LM, locally moving cricket.

Table 5. *Preference scale and clustering of values for each individual and for all individuals*

Individual	1	2	3	4	5	6	7	8	1–8
First cluster	L	L FM	L FM STMR	L	FM L	L FM	L FM	L FM	L FM
Second cluster	FM R STMR STM SI	SI R STMR STM	R STM SI	FM R SI STMR	STMR SI STM R SM	STMR	STMR SI R STM SM	STMR	STMR R SI
Third cluster	SM S C	SM	SM C S	STM SM	C LM S	R SI STM	S LM C	R SI STM	STM SM
Fourth cluster	LM	S C LM		S C LM		S C LM		S	S C
Fifth cluster								C LM	LM

L, large-sized cricket; FM, fast-moving cricket; ST, standard cricket; STMR, stepwise-moving rectangle; R, continuously moving rectangle; SI, still-image cricket; STM, stepwise-moving cricket; SM, slowly moving cricket; S, small-sized cricket; C, contrast-reduced cricket; LM, locally moving cricket.

order varied when responses of individuals were evaluated singly (Table 5). However, the most- and least-effective stimuli were found in a similar rank order compared with the grouped data for all animals taken together. Stimuli were grouped into 3–5 clusters among individuals. In five individuals, the large-sized cricket and the fast-moving cricket formed the first cluster, although the preference for these two stimuli was reversed in one individual. In two individuals, the first cluster consisted only of the large-sized cricket, whereas in one individual the large-sized cricket, the fast-moving cricket and the stepwise-moving rectangle were grouped in the first cluster.

The second cluster contained five stimuli in three individuals, four stimuli in two individuals, three stimuli in one individual and one stimulus in two individuals. Here, the stepwise-moving rectangle was found in seven individuals, the still-image cricket and the continuously moving rectangle in six, the stepwise-moving cricket in five and the slow- and fast-moving crickets in two individuals. The third cluster comprised four or three stimuli in three individuals, and two stimuli or one stimulus in one individual. In six individuals, this third cluster contained the slowly moving cricket, while the small-sized and contrast-reduced cricket were found here in four individuals. In three individuals, the stepwise-moving rectangle and the locally moving cricket were included in this cluster, and the continuously moving rectangle as well as the still-image cricket were included in two individuals.

A fourth cluster was found in only five individuals. The small-sized cricket, the contrast-reduced cricket and the locally moving cricket were grouped in this cluster for three individuals, while the locally moving cricket was the only stimulus in the fourth cluster of one individual and the small-sized cricket in that of another individual. A fifth cluster was present in only one individual and contained the contrast-reduced and the locally moving cricket. Failures to respond to identical stimulus combinations were much higher than those to different stimuli. The differences are highly significant ($P<0.0001$) for each individual and for all individuals combined.

Discussion

The goal of the present study was to analyze the effects of different visual features of prey-like objects on the orienting behaviour of woodland salamanders. In eight *Plethodon jordani*, visual stimulation was performed by simultaneously presenting on a computer screen two out of 11 stimuli (nine cricket dummies, two rectangles). The stimuli differed in size (small, S; large, L; standard, ST), shape (rectangle, R), contrast (reduced at 70 %, C), velocity (fast, FM; slow, SM) and movement pattern of the entire body or body appendages (stepwise-moving cricket, STM; stepwise-moving rectangle, STMR; still-image cricket, SI; locally moving cricket, LM). Under paired-stimulus conditions, 88 % of all presentations led to an orienting response of the head to one of the stimuli in all individuals. The absolute frequency of responses was highest for the stimuli L, FM and ST, intermediate for the stimuli STMR, R, SI , STM and SM and lowest for the stimuli S, C and LM. This rank order of stimuli was the same when the probability of a response was estimated by means of the maximum log-likelihood method. Cluster analysis revealed that in all animals stimuli could be grouped into five clusters. When individuals were considered singly, the rank order of stimuli was similar for high- and low-ranking stimuli, but varied for those of intermediate rank. Among individuals, stimuli could be grouped into 3–5 clusters. Failures were exceptionally high in number when the rightward- and leftward-moving stimuli were of the same type. Side preferences were found in two individuals.

Side preferences

Six of the eight tested animals showed no side preferences, whereas in the remaining two a side preference for the right or left side was observed. However, these side preferences were not highly significant. The paired stimuli were presented in equal numbers with equal combinations of stimuli for both sides and did not contribute to the rank order of stimuli. Side preferences would only have effects on small numbers of responses. Because side preferences were of minor extent and occurred in only two animals, and since the rank order for the different stimuli based on frequency of responses was similar on both sides, the data for all animals were pooled for further analysis. In a study on *Rana pipiens* (Stull and Gruberg, 1998), side preferences of orienting responses were found in some frogs when living crickets were presented simultaneously at 90 ° to the right or left in the frontal visual field. Roughly equal numbers of frogs showed a preference for the right or left side or no significant side preference. In biased situations, when prey objects were presented on one side repeatedly, a preference for the opposite side occurred. In amphibians, no further studies on side preferences of orienting behaviour exist, and the present study provides little evidence for such preferences in salamanders. Here, the stimuli were presented in the frontal binocular field of the salamanders, whereas in the study of Stull and Gruberg (1998) presentation was in the monocular field, and this might make a significant difference to the results.

Failures to respond

The number of failures to respond to paired-stimulus presentations with identical stimuli was significantly higher than to presentations of different types of stimulus. The difficulty of animals in responding to one of two identical stimuli may indicate a conflict of interpretation. Whether this conflict takes place at the processing level of the sensory system, i.e. the optic tectum, or during the ‘decision-making process’ in the limbic system is unclear. The sensory information about the two stimuli is identical and can lead to equivalent excitation in the two tectal hemispheres, which in turn blocks a visuomotor response to the stimulus. Such inhibition effects based on an interhemispheric, crossed-inhibitory mechanism in the optic tectum were proposed by Ingle (1976) and Ewert et al. (1970) when the presentation of two synchronously moving prey dummies to frogs and toads led to a delay in snapping responses. However, in the present study, half the animals responded with a turn of the head in response to one of these stimuli. One possible explanation could be that the motivational system ‘overrides’ the equivocal sensory information signalling ‘no differences in properties of either stimulus’. Another explanation is that the neuronal network in one tectal hemisphere has a higher level of activation as a result of preceding activation or of attentional effects and, thus, initiates a response to one of the two competing stimuli. When combinations of identical stimuli are excluded, no correlation was found for the combinations of pairs with different types of stimulus to the occurrence of a failure. This suggests that other parameters, such as motivation or the attentional state, can indeed contribute to the occurrence of an orienting response.

Rank order of stimulus preferences

The rank order of preferred stimuli was the same when absolute numbers of responses were considered or the maximum-likelihood method was applied. Nevertheless, the choice of method is important for the evaluation of the data. By using the maximum-likelihood method, the relative probability of an orienting response to a given stimulus is determined by comparing data for each stimulus pair with all other stimulus pairs. For example, the large-sized stimulus was responded to best by each individual, but in one individual the fast-moving stimulus was placed before the large-sized one when the maximum-likelihood method was applied. Analysis of the data from each individual compared with pooled data from all individuals revealed a significantly improved accuracy for the data for individuals, which was expressed in different locations of intermediate-ranking stimuli among individuals. However, these differences concerned maximally one or two positions on the preference scale and did not substantially alter the rank order. Because the loss of information is small, data from all individuals were pooled for analysis, resulting in smaller confidence intervals and thus contributing to the formation of more distinct clusters. The cluster analysis revealed that the probability of an orienting response towards the different stimuli does not decrease continuously, but is distinct among the high-ranking stimuli of the first cluster, the intermediate-ranking ones of the second and third clusters, and the low-ranking ones of the fourth and fifth clusters.

Size

Prey size seems to be one of the most important features eliciting orienting responses: the large-sized cricket (15 mm) was most preferred by all individuals and the small-sized cricket (6 mm) was among the least-preferred stimuli. Earlier studies on natural diets in salamanders of the genus *Plethodon* reported that the size of prey ranged from 0.5 to 7 mm in length in approximately 90 % of stomach contents (Jaeger, 1972; Fraser, 1976). Roth (1987) tested a variety of plethodontid salamanders by presenting them with pieces of blackboard and found a preference for snapping at smaller stimuli (2 or 5 mm) in *Plethodon jordani*, although it was reported that the salamanders also responded to larger objects 10 mm in length. On the basis of these facts, one could assume that large objects induce orienting, but not snapping, responses. However, in the present study, crickets up to 2 cm in length were presented after the experiments and were immediately eaten by the salamanders. The preference for larger prey observed in our study could be due to the higher motivational state of our animals. In a neural model based on behavioural, anatomical and physiological data in anurans and subserving prey–predator discrimination and size preference, simulations were performed under normal conditions and under a variety of motivated states (Cervantes-Pérez et al., 1985). The authors postulate that, in states of high feeding motivation, toads show preferences for larger objects. This was also attributed to frogs with moderate ethanol intoxication, which showed altered size preferences towards larger objects compared with a normal control group (Ingle, 1973). In *Bufo fowleri*, the upper size threshold of prey eliciting feeding behaviour was reduced when the toad fed and became satiated (Heatwole and Heatwole, 1968), although this threshold is not fixed and fluctuates with changes in the internal state of the animal. What speaks against a strong influence of feeding motivation is the fact that the stepwise-moving rectangle and the continuously moving rectangle were of the same size and orientation as the large-sized cricket but elicited fewer responses by each individual. Here, differences in ‘*Gestalt*’ between the rectangles and the large-sized cricket probably had an effect.

Velocity

The frequency of orienting towards the fast-moving cricket was high in all individuals and ranked just behind that for the large-sized cricket moving at standard velocity. The slowly moving cricket was found lower on the preference scale, and the locally moving cricket was least preferred by all individuals. These results suggest that fast forward movement increases the likelihood of an orienting response. In most studies on feeding responses or orienting behaviour of salamanders, different velocities of prey object have been tested by presenting artificial dummies such as squares and rectangles: for feeding, see Himstedt (1967) and Roth (1976); for orienting behaviour, see Finkenstädt and Ewert (1983). An optimum response was found at $0.5\text{–}2.5\,\mathrm{cm\,s^{-1}}$. Only in salamanders with fast projectile tongues such as *Hydromantes italicus* or *Bolitoglossa subpalmata* were much higher velocities of $6\text{–}10\,\mathrm{cm\,s^{-1}}$ preferred (Roth, 1976, 1987). Although, in the present study, orienting but not snapping behaviour was studied, the preferred stimulus velocity of $7\,\mathrm{cm\,s^{-1}}$ fits these data nicely. Salamanders of the genus *Plethodon* have fast, but not free, projectile tongues. Generally, movement seems to be one of the main features used to classify objects as prey, and amphibians usually do not pay attention to non-moving objects as long as no other sensory information, such as olfaction, is available. However, frogs and salamanders can be trained to accept stationary objects as prey (Himstedt et al., 1978; Roth and Wiggers, 1983).

Movement pattern

On the basis of a number of studies, movement pattern has been assumed to play an important role in prey recognition (Roth, 1978; Luthard and Roth, 1979a,b) (see also Roth, 1987). In the present study, the stepwise-moving cricket and stepwise-moving rectangle, the still-image cricket, the continuously moving rectangle and the locally moving cricket all differed in movement of the entire body or of the body appendages (legs and antennae). However, in contrast to earlier findings, these stimuli did not exhibit large differences in position on the preference scale, except for the stimulus LM, which was the least effective stimulus. Of the five stimuli mentioned above, STMR and R evoked the most responses just behind the standard cricket in the same cluster. They moved at the same average velocity, and differences in movement pattern were irrelevant. However, in contrast to the other stimuli with altered movement pattern, they were larger in size, and this fact is the most plausible explanation for their high rank in the preference scale. At the same time, forward movement of an object appears to be important for eliciting an orienting response, and stepwise movement does not necessarily improve the ‘attractiveness’ of such an object. In *Salamandra*

salamandra, the occurrence of prey-catching responses to stepwise or continuously moving rectangles oriented perpendicularly or parallel was velocity-dependent (Luthard and Roth, 1979a). A horizontally oriented rectangle (4 mm×16 mm) presented at a velocity of 3 cm s^{-1} had no positive effect on the probability of feeding responses when step frequencies of 0.25–8 Hz were tested. At a velocity of 0.5 cm s^{-1}, low step frequencies of 0.5–2 Hz had a negative effect compared with continuously moving rectangles. In the case of *Salamandra salamandra*, we have to bear in mind that this salamander shows a strong preference for worm-like stimuli, while *Plethodon jordani* and most other plethodontid salamanders prefer compact prey object such as insects (Roth, 1987).

Self-motion of an object, i.e. movement of the legs or antennae, seems to play a lesser role in the detection of objects in situations in which a forward-moving stimulus is presented. This is demonstrated by the fact that the locally moving stimulus occupied the lowest position in the preference scale. However, this does not mean that local motion is irrelevant because, in the single-stimulus presentations, all stimulus types were responded to with a turn of the head.

Shape and contrast

The rectangle was the only stimulus that differed in shape from the other stimuli, i.e. crickets. Salamanders often responded to the stimuli STMR and R. The horizontal rectangle is similar in size to the large cricket dummy. Certainly, a larger number of differently shaped stimuli are needed to test more adequately the shape parameter. The fact that amphibians respond to square or rectangular dummies at all is known from other studies and is often cited as an argument for the existence of stereotyped prey-catching behaviour and of simple prey-recognition mechanisms in amphibians (Ingle, 1968). A possible explanation for the response to artificial stimuli, which fulfils the prey scheme, is that amphibians lack feeding experience with this kind of ‘prey’ because they are prevented from ingesting these dummies. Within their feeding behaviour, amphibians – as well as most other vertebrates – when confronted with unfamiliar, but interesting objects appear to follow the rule ‘take and try it and store the result’. Accordingly, when amphibians are allowed to ingest cardboard dummies, they spit them out and lose interest in these objects (Göckel, 2001) (U. Dicke, unpublished observations).

The fact that the contrast-reduced cricket evoked fewer responses than most of the other stimuli suggests that contrast is another important feature in eliciting orienting responses. The stimuli STMR and R were of higher contrast than the cricket dummies because of their uniform grey tone and distinct boundaries. This fact, in combination with their larger size, may be another reason for their higher rank in the preference scale.

Functional considerations

The results obtained in this study corroborate the view that, in amphibians, prey recognition is not based on the fulfilment of a ‘simple’ prey scheme, but is driven by a number of visual features that, alone or in combination, influence the attentional state of the animal and lead to orienting, approach and snapping behaviour to various degrees. Size, shape, contrast, velocity and movement pattern of the entire body and of body appendages such as the legs or antennae turn out to be the most important features. Different quantitative and qualitative combinations of these features characterize different prey types. They appear to be processed within the visual system relatively independently; accordingly, a complex prey item activates several visual subsystems simultaneously. Such a view fits data from studies on the morphology and functional organization of the visual system of a number of salamander and frog species. Different types of neurons in the optic tectum, the main visual centre for object recognition, have been demonstrated to receive different retinal inputs (Wiggers, 1998). Electrophysiological recordings have revealed different classes of retinal ganglion cells that terminate in different tectal layers and respond to changes in either contrast or size of small objects, to moving objects, to slow motion or to overall illumination (Grüsser and Grüsser-Cornehls, 1976; Mandon, 1997). Consequently, the different types of tectal neuron are assumed to process different prey features such as size, contrast, velocity, luminance and movement pattern. These populations of neurons project through anatomically separate ascending and descending pathways to different targets in the diencephalon, tegmentum and medulla oblongata and spinalis, where the premotor and motor centres related to orienting and feeding responses are situated (Dicke and Roth, 1996; Dicke et al., 1998; Dicke, 1999; Roth et al., 1999). At the same time, inside the tectum there is an interaction between these different pathways constituting a ‘super-population’ of neurons, which in its activity represents the specific combination of features characteristic of the prey item under consideration (Schübert and Dicke, 2001). We must also assume that the activity of tectal neurons in the context of sensory-driven attention is modulated by centres outside the tectum, such as the nucleus isthmi and nuclei of the reticular formation. Both centres have reciprocal connections with the optic tectum (Weber et al., 1996; Dudkin and Gruberg, 1999; Wiggers and Roth, 1991; Dicke and Mühlenbrock-Lenter, 1998).

Our data show that different visual features have different importance in eliciting an orienting response. Size and velocity appear to be the dominant features, acting either alone or in combination. Thus, a large and fast-moving object is expected to be the most effective stimulus. Shape seems to be of lesser importance because the rectangles were almost as effective as the stepwise-moving and the still-image cricket. Contrast and movement pattern were of intermediate importance, whereas local motion was of little importance.

However, this rank order determined in our experiments with *Plethodon jordani* does not necessarily indicate a fixed order of relevant visual prey features. Not only do different amphibian species possess different rank orders of preferences for prey features – which may explain the differences between our findings and the results of other studies – but this rank order is influenced both by the actual state of motivation and by prey experience. It has been shown that different individual experiences contribute to modified patterns of prey preference. For instance, for *Salamandra salamandra*, prey experiences during juvenile development had an effect on the prey preferences of adults (Roth, 1987; Luthard and Roth, 1979b; Luthard-Laimer and Roth, 1983). Furthermore, it is possible that, even during adulthood, diet may influence amphibian preferences. In our case, the test animals had been fed exclusively with crickets. This important attribute needs to be tested in greater detail.

This research was supported by grant SFB 517 from the Deutsche Forschungsgemeinschaft to U.D. We thank Ulrike Schmonsees and Heiko Stemmann for assisting in the behavioural experiments, Werner Wosniok, Institute for Statistics, University of Bremen, for his expert advice and processing in statistics and Dr Christian Eurich, Institute for Theoretical Neurophysics, University of Bremen, for helpful remarks on statistical data. We are grateful to Dr Gerhard Roth, Brain Research Institute, University of Bremen, for criticism.

References

Bradley, R. A. and Terry, M. E. (1952). Rank analysis of incomplete block designs. I. The method of paired comparisons. *Biometrika* **39**, 324–345.

Cervantes-Pérez, F., Lara, R. and Arbib, M. (1985). A neural model of interactions subserving prey–predator discrimination and size preference in anuran amphibia. *J. Theor. Biol*. **113**, 117–152.

Cott, H. B. (1936). The effectiveness of protective adaptations in the hive-bee, illustrated by experiments on the feeding reactions, habit formation and memory of the common toad (*Bufo bufo*). *Proc. Zool. Soc., Lond.* **1**, 113–133.

Dean, J. (1980). Encounters between bombardier beetles and two species of toads (*Bufo americanus*, *B. marinus*): Speed of prey-capture does not determine success. *J. Comp. Physiol.* **135**, 41–50.

Dicke, U. (1999). Morphology, axonal projection pattern and response types of tectal neurons in plethodontid salamanders. I. Tracer study of projection neurons and their pathways. *J. Comp. Neurol.* **404**, 473–488.

Dicke, U. and Mühlenbrock-Lenter, S. (1998). Primary and secondary somatosensory projections in direct-developing plethodontid salamanders. *J. Morphol.* **288**, 307–326.

Dicke, U. and Roth, G. (1996). Similarities and differences in the cytoarchitecture of the tectum of frogs and salamanders. *Acta Biol. Hung.* **47**, 41–59.

Dicke, U., Roth, G. and Matsushima, T. (1998). Neural substrate for motor control of feeding in amphibians. *Acta Anat*. **163**, 127–143.

Dudkin, E. A. and Gruberg, E. R. (1999). Relative number of cells projecting from contralateral and ipsilateral nucleus isthmi to loci in the optic tectum is dependent on visuotopic location: horseradish peroxidase study in the leopard frog. *J. Comp. Neurol.* **414**, 212–216.

Ewert, J. P. (1984). Tectal mechanisms that underlie prey-catching and avoidance behaviors in toads. In *Comparative Neurology of the Optic Tectum* (ed. H. Vanegas), pp. 247–416. New York: Plenum Press.

Ewert, J. P. (1989). The release of visual behavior in toads: Stages of parallel/hierarchical information processing. In *Visuomotor Coordination, Amphibians, Comparisons, Models and Robots* (ed. J. P. Ewert and M. A. Arbib), pp. 39–120. New York: Plenum Press.

Ewert, J. P., Speckhardt, I. and Amelang, W. (1970). Visuelle Inhibition und Exzitation im Beutefangverhalten der Erdkröte (*Bufo bufo* L.). *Z. Vergl. Physiol.* **68**, 84–110.

Fahrmeir, L., Hamerle, A. and Tutz, G. (1996). *Multivariate Statistische Verfahren*. Berlin, New York: Springer.

Finkenstädt, T. and Ewert, J. P. (1983). Processing of area dimensions of visual key stimuli by tectal neurons in *Salamandra salamandra*. *J. Comp. Physiol.* **153**, 85–98.

Fraser, D. F. (1976). Empirical evaluation of the hypothesis of food competition in salamanders of the genus *Plethodon*. *Ecology* **57**, 459–471.

Göckel, M. (2001) Der Einfluss visueller und olfaktorischer Merkmale auf das Beutefangverhalten von Amphibien. Dissertation, University of Bremen.

Grüsser, O. J. and Grüsser-Cornehls, U. (1976). Neurophysiology of the anuran visual system. In *Frog Neurobiology* (ed. R. Llinas and W. Precht), pp. 297–385. Berlin, Heidelberg: Springer.

Heatwole, H. and Heatwole, A. (1968). Motivational aspects of feeding behavior in toads. *Copeia* **4**, 692–698.

Himstedt, W. (1967). Experimentelle Analyse der optischen Sinnesleistungen im Beutefangverhalten der einheimischen Urodelen. *Zool. Jb. Physiol*. **73**, 281–320.

Himstedt, W., Tempel, P. and Weiler, J. (1978). Responses of salamanders to stationary visual patterns. *J. Comp. Physiol.* **124**, 49–52.

Ingle, D. (1968). Visual releasers of prey-catching behavior in frogs and toads. *Brain Behav. Evol.* **1**, 500–518.

Ingle, D. (1973). Size preference for prey-catching in frogs: Relationship to motivational state. *Behav. Biol.* **9**, 485–491.

Ingle, D. (1976). Spatial vision in anurans. In *The Amphibian Visual System. A Multidisciplinary Approach* (ed. K. V. Fite), pp. 119–140. New York: Academic Press.

Jaeger, R. G. (1972). Food as a limited resource in competition between two species of terrestrial salamanders. *Ecology* **53**, 535–546.

Jobson, J. D. (1992). *Applied Multivariate Data Analysis*, vol. II, *Categorical and Multivariate Methods*. New York, Berlin: Springer.

Lettvin, J. Y., Maturana, H. R., McCulloch, W. S. and Pitts, W. H. (1959). What the frog's eye tells the frog's brain. *Proc. Inst. Radio Engrs NY* **47**, 1940–1951.

Luthard, G. and Roth, G. (1979a). The relationship between stimulus orientation and stimulus movement pattern in the prey catching behavior of *Salamandra salamandra*. *Copeia* **3**, 442–447.

Luthard, G. and Roth, G. (1979b). The influence of prey experience on movement pattern preference in *Salamandra salamandra* L. *Z. Tierpsychol.* **51**, 252–259.

Luthard-Laimer, G. and Roth, G. (1983). Reduction of visual inhibition to stationary prey by early experience in *Salamandra salamandra* (L.). *Z. Tierpsychol.* **63**, 294–302.

Mandon, S. (1997). Funktionelle Klassifizierung retinaler Afferenzen im Tectum opticum von lungenlosen Salamandern (Fam. Plethodontidae). Dissertation, University of Bremen.

Roth, G. (1976). Experimental analysis of the prey-catching behavior of *Hydromantes italicus* Dunn (Amphibia, Plethodontidae). *J. Comp. Physiol.* **109**, 47–58.

Roth, G. (1987). *Visual Behavior in Salamanders*. Berlin, New York: Springer.

Roth, G., Dicke, U. and Grunwald, W. (1999). Morphology, axonal projection pattern and response types of tectal neurons in plethodontid salamanders. II. Intracellular recording and labeling experiments. *J. Comp. Neurol.* **404**, 489–504.

Roth, G., Dicke, U. and Wiggers, W. (1998). Vision. In *Amphibian Biology. Sensory Perception*, vol. 3 (ed. E. Heatwhole), pp. 783–877. Chipping Norton, UK: Beatty & Sons.

Roth, G. and Wiggers, W. (1983). Responses of the toad *Bufo bufo* to stationary prey stimuli. *Z. Tierpsychol.* **61**, 225–234.

Schülert, N. and Dicke, U. (2001). The effect of stimulus features on the visual orienting behavior and on spike characteristics of tectal neurons in the salamander *Plethodon jordani*. In *Proceedings of the Fourth Göttingen Conference of the German Neuroscience Society*, vol. II (ed. N. Elsner and G. W. Kreutzberg), p. 125. Stuttgart: Thieme.

Sternthal, D. E. (1974). Olfactory and visual cues in the feeding behavior of the leopard frog (*Rana pipiens*). *Z. Tierpsychol*. **34**, 239–246.

Stull, A. K. and Gruberg, E. R. (1998). Prey selection in the leopard frog: Choosing in biased and unbiased situations. *Brain Behav. Evol.* **52**, 37–45.

Weber, B. C., Waldeck, R. F. and Gruberg, E. R. (1996). Seeing beyond the midline: the role of the contralateral isthmotectal projection in the leopard frog. *Vis. Neurosci.* **13**, 467–476.

Wiggers, W. (1998). Projections of single retinal ganglion cells to the visual centers: An intracellular staining study in a plethodontid salamander. *Vis. Neurosci.* **16**, 435–447.

Wiggers, W. and Roth, G. (1991). Anatomy, neurophysiology and functional aspects of the nucleus isthmi in salamanders of the family Plethodontidae. *J. Comp. Physiol.* **169**, 165–176.