

## Quantity discrimination in salamanders

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### SUMMARY

We investigated discrimination of large quantities in salamanders of the genus *Plethodon*. Animals were challenged with two different quantities (8 vs 12 or 8 vs 16) in a two-alternative choice task. Stimuli were live crickets, videos of live crickets or images animated by a computer program. Salamanders reliably chose the larger of two quantities when the ratio between the sets was 1:2 and stimuli were live crickets or videos thereof. Magnitude discrimination was not successful when the ratio was 2:3, or when the ratio was 1:2 when stimuli were computer animated. Analysis of the salamanders' success and failure as well as analysis of stimulus features points towards movement as a dominant feature for quantity discrimination. The results are generally consistent with large quantity discrimination investigated in many other animals (e.g. primates, fish), current models of quantity representation (analogue magnitudes) and data on sensory aspects of amphibian prey-catching behaviour (neuronal motion processing).

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### INTRODUCTION

The existence of numerical competence in vertebrates other than primates or birds and the evolutionary root of number-related cognitive abilities is an ongoing matter of interest and debate. Counting, strongly based on learning, and basic arithmetic operations have been investigated in many species. Recently, research has begun to examine what is thought to be part of the underlying mechanism, namely a spontaneously available representation of number. While data from animals in their natural environment are restricted to a few studies, e.g. lions (McComb, 1994), chimpanzees (Wilson et al., 2001) and coots (Lyon, 2003), a wealth of evidence comes from laboratory experiments, which characterize spontaneously available numerical abilities mainly in human infants (Uller et al., 1999; Xu and Spelke, 2000; Feigenson and Carey, 2003; Xu et al., 2005) and non-human primates (Hauser et al., 2003; Flombaum et al., 2005; Lewis et al., 2005; Santos et al., 2005), but also in horses (Uller and Lewis, 2009), birds (Hunt et al., 2008; Rugani et al., 2009), fish (Agrillo et al., 2007; Agrillo et al., 2008), salamanders (Uller et al., 2003) and insects (Carazo et al., 2009; Gross et al., 2009). Results suggest two systems: one system for small sets ( $\leq 4$ ) that is precise but limited, as it works by keeping track of individual entities; and one system for larger sets that is independent of absolute set size, works on imprecise analogue magnitudes and hence is subject to Weber's Law, i.e. discrimination is a function of the ratio between the numbers in question (Feigenson et al., 2004). Interestingly, results from discrimination tasks suggest that 6 month old human infants rather address 'true' number when discriminating larger sets, while in small sets they resort to variables, which usually vary with number such as surface area or contour length (Xu, 2003). Six month old human infants are able to discriminate sets that differ by a 1:2 ratio, but fail with a 2:3 ratio regardless of absolute set size (Xu and Spelke, 2000; Xu et al., 2005).

Precision increases over development up to a 7:8 ratio in adults (Barth et al., 2003). Recently, research in this field was extended by experiments addressing dimensions other than number, namely area (Brannon et al., 2006) or temporal duration (vanMarle and Wynn, 2006), by experiments using auditory stimuli (Jordan and Brannon, 2006), and by experiments addressing discrimination of total amount independent of number (Stevens et al., 2007). Results parallel those from numerical discrimination in threshold and discriminatory success, suggesting either a single system processing all these inputs or several systems working with a similar representational format (Feigenson, 2007). A similar performance pattern to humans has generally been revealed in studies of other vertebrates, mainly primates (Hauser et al., 2003; Flombaum et al., 2005), but also basal vertebrate groups such as fish (Agrillo et al., 2007; Agrillo et al., 2008) and salamanders (Uller et al., 2003). Two recent studies compared monkeys and humans directly: the semantic congruity effect described in humans was also found in *Macaca mulatta* (Cantlon and Brannon, 2005). In this task, monkeys ordered visual arrays based on the number of elements and chose the array containing the larger or smaller number depending on a colour cue. The authors also compared monkeys and humans in an ordinal comparison task (Cantlon and Brannon, 2006), where subjects had to extend a learned numerical rule to a novel set of values. Both studies revealed intriguing consistence in the performance of both monkeys and humans, providing evidence for a single mechanism shared by the primate group. Evidence from vertebrates more distantly related to primates could clarify how 'primitive' this mechanism is, but studies are restricted to the aforementioned work in fish and salamander. Agrillo and colleagues (Agrillo et al., 2007; Agrillo et al., 2008) investigated numerical discrimination in the mosquitofish (*Gambusia hoolbrooki*) and found that they discriminate between shoals that differ by one element in contrasts

up to 3 vs 4 but not 4 vs 5 or larger. Discrimination of large numbers was only successful when the ratios of elements in the shoals were 1:2 or smaller. Additional experiments controlled for either surface area or movement in 2 vs 3 and 4 vs 8 contrasts. Except for the 2 vs 3 movement control, where the fish spent significantly more time near the larger shoal, the fish responded at chance levels. The work on salamanders demonstrated that salamanders of the genus *Plethodon* chose the larger number of fruit flies out of contrasts of 1 vs 2 and 2 vs 3, but failed with 3 vs 4 and 4 vs 6 (Uller et al., 2003). The authors did not control for variables that co-vary with number, and discrimination of larger sets was not investigated.

The general finding that salamanders 'go for more' is consistent with predictions based on optimal foraging theory; with foraging being one of the evolutionary contexts that mechanisms of quantification have been associated with. Such mechanisms are likely to have evolved because they constitute an advantage in fitness, which means they contribute to better survival and successful reproduction. Other contexts include inter-group conflict, parental investment and predator avoidance (Hager and Helfman, 1991; McComb et al., 1994; Wilson et al., 2001; Lyon, 2003; Kitchen, 2004). In salamanders, numerical discrimination may be significant for success in parental care and foraging. The advantages of quantitative assessment of food sources are obvious; optimal foraging theory predicts that animals should attempt to maximize food acquisition per unit time and energy invested, and number usually predicts total amount. The aim of the present study was to investigate numerical discrimination of large sets in the salamander *Plethodon*. Following the study of Uller and colleagues (Uller et al., 2003), we used a two-alternative choice task. Stimuli were crickets, presented either live or videotaped, or images of crickets animated by a computer program. We made use of videos to test whether 2D stimuli presented on a screen would be readily accepted; a positive result is a prerequisite for the use of computer-animated stimuli. The videos also provided a replication of the experiments with live stimuli. Computer-animated stimuli were used to exercise and improve control over stimuli and co-variables and ensure standardization (e.g. movement, distribution, surface and size). Comparison of results from experiments with live and computer-animated stimuli will provide information on underlying processes. Successful discrimination of large quantities that depend on ratios would indicate that for representation of quantities, analogue magnitude-based mechanisms might be a shared feature of the vertebrate group.

## MATERIALS AND METHODS

### Subjects

Adult (>32 mm snout-vent length) (Sayler, 1966) male and female salamanders (*Plethodon shermani*, Stejneger 1906, and *Plethodon metcalfi*, Brimley 1912) were collected in the forests of the Highlands Biological Station, Highlands, NC, USA, and shipped to the Brain Research Institute of the University of Bremen, Germany. In the laboratory, they were housed in groups in terraria with a minimum size of 55 cm × 40 cm × 30 cm. The terraria contained multiple hiding places (potsherds and slate) and a water dish. The ground was composed of gravel, which in some cases was covered by either bark mulch or soil. The salamanders were fed five live crickets (*Gryllus bimaculatus*) per individual dispensed in the terraria once a week. Feeding was stopped at least 10 days before testing. The laboratory rooms were maintained at 18 ± 0.5 °C and 14 ± 0.5 °C with a light:dark cycle of 12 h:12 h. The difference in temperature in the salamander housing rooms (14 and 18 °C) relates to the fact that salamanders used for recording experiments in the

institute need to be kept at a lower temperature. For the purpose of the present experiments, the temperature of the housing condition is irrelevant. Both groups housed at 14 and 18 °C were familiarized to and tested at 20–22 °C, because they are more active at this higher temperature. The salamanders were kept in the laboratory for at least 1 month before being tested. All procedures complied with the German laws on animal research and treatment.

### Testing procedure and stimulus presentation

Salamanders were individually transferred from the terraria into glass boxes (hereafter, test box) measuring 24 cm × 20 cm × 20 cm. The test boxes had removable opposite panes, were lined with moist foam on the floor and contained a potsherd. Animals in the test box were allowed to adapt to the temperature of the testing lab for 1 day before being tested. For a 3D stimulus display, two Petri dishes of 90 mm in diameter were attached to a black rectangular piece of cardboard (30 cm × 23 cm), 12 cm apart from each other and 5 cm from the lower edge. The dishes were moistened to allow size-matched circles of white paper to stick to them. Each dish contained either  $x$  (e.g. 8) or  $y$  (e.g. 16) live crickets of equal size and was closed with a matching dish. The live crickets used in the live cricket experiments were of the same age (to the nearest 14 days) and sorted by size (body length approximately 8 mm). Sorting was double-checked independently by a member of the animal care and use staff. The cardboard with the two attached Petri dishes containing live crickets was positioned vertically, and a moistened foam platform (hereafter, test platform; measuring 23 cm × 18.5 cm × 5 cm) was placed in the centre, perpendicular to the cardboard, so that the lower edges of the Petri dishes touched the foam (Fig. 1). For the 2D stimulus displays, the cardboard with the attached Petri dishes was replaced by an LCD computer monitor (Eizo FlexScan L568, Eizo Nanao Corp., Matto, Ishikawa, Japan) placed before the test platform. The luminous intensity of the monitor (250 cd m<sup>-2</sup>) did not affect light intensity in the presentation to a significant degree compared with the live-cricket presentation. Crickets were moving on an equal white surface with a black background in all displays, and the original colour of the live and videotaped crickets was not changed by the

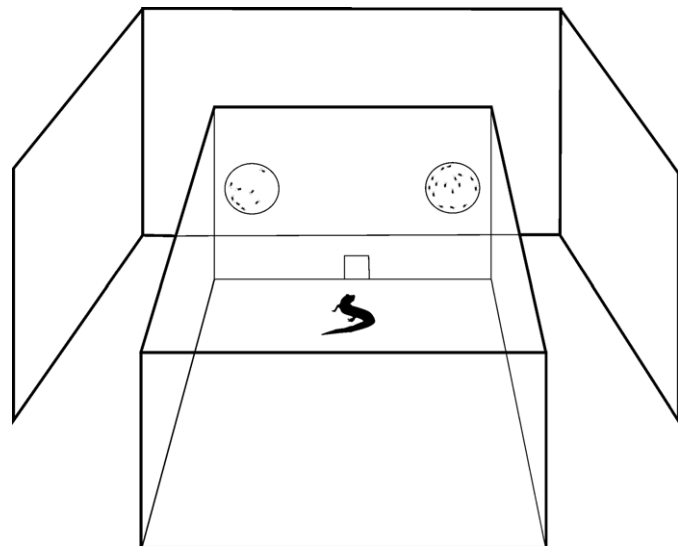


Fig. 1. Schematic illustration of the test set-up. Test box with a salamander in front of the display showing the two sets of stimuli. The test platform is not shown.

program in the computer-animated experiment. 2D stimulus displays were either 16 min high definition video clips (HDV 1080i format) showing live crickets in the 3D stimulus set-up or digital cricket images animated by a computer program. For the video clips, three different videos were recorded using a digital camcorder (Sony Handycam HDR-HC1E, Sony Corp., Tokyo, Japan), and copies of these videos were mirror-inversed using video-editing software (Adobe Premiere Pro 2.0, Adobe Systems Inc., San Jose, CA, USA). For the computer-animated stimuli, the walking cycle of a cricket was filmed with a digital camcorder (Sony Handycam HDR-HC1E, Sony Corp.), and each frame was converted into a bitmap, from which the cricket was extracted into a new digital image. A computer program (programmed in Object Pascal) designed in our lab was used to animate these images. The presentation area of digital crickets was matched to that of the Petri dishes used in the 3D stimulus display. The cricket movement was generated by an algorithm designed to mimic the cricket's natural movement and contained random elements (i.e. new direction after collision with another cricket, probability of stops, duration of stops, probability of change in direction during movement). Cricket speed varied between 0.69 and 2.2 cm s<sup>-2</sup>. The larger or smaller set of live crickets, videotaped crickets or animated crickets on the display was counterbalanced for the side of presentation across trials during the test sessions. The test box was placed in the centre in front of the test platform with one of the panes opposite the display; this pane was then replaced with one that had a small gap in the centre (2.2 cm). The remaining panes were covered with black paper, and the test platform was bordered laterally by walls of black cardboard. Animals were allowed to enter the test platform through the small gap in the centre of the pane facing the display. The distance from the gap to each of the two sets on the display was 20 cm across the foam platform. Before testing, all elements inside the test box were removed, leaving no hiding places for the salamander. A dimmable halogen lamp (50 W) was placed above the set-up, aiming at a low light intensity (~530 lx; Luxmeter MS-1300, Conrad Electronic GmbH, Bremen, Germany) inside the box. A digital camcorder was used to observe the test session and a chronometer to record the time. If the salamander did not leave the box within 5 min, light intensity was maximized (ca. 3700 lx), and after an additional 5 min the distance between the light and the test box was minimized (ca. 5700 lx). Cut-off time was 30 or 10 min for leaving the box and 10 or 5 min for choosing a set of stimuli. (In the live cricket experiments, the cut-off time was set to 30 min for leaving the box and 10 min to make a choice. However, the majority of salamanders left the box within the first 10 min and made a choice within the first 5 min. Therefore, in the video and computer-animation experiments cut-off time was reduced to 10 min for leaving the box and to 5 min for making a choice.)

#### Scoring and analysis

A test session was considered over, and the video camera and the chronometer were switched off, when the salamander either (a) successfully chose one quantity over the other by snapping at a cricket stimulus in an attempt to feed on it (see supplementary material Movie 1) or (b) did move out of the box, but did not make a choice within the cut-off time or left the test platform. We measured the salamander's choice for one quantity (*x*) over the other (*y*). For each experiment, 30 animals that successfully made a choice were used, and those that did not were discarded. A binomial test was performed to test for significance of choices (SPSS 15, SPSS Inc., Chicago, IL, USA). The latency between leaving the test box and snapping at a stimulus within the 5 min cut-off time was recorded

and compared between choices of individuals using an unpaired *t*-test (Prism 4, Graph Pad Software Inc., La Jolla, CA, USA). In order to assess differences in movement of the cricket stimuli, a randomly chosen minute from each of the three videotapes and the computer-animated 2D stimulus display was analysed. The duration of movement was determined for each individual stimulus (i.e. every single cricket of the larger and smaller set), and the number of simultaneous moving stimuli was identified. Time of local movement such as movement of body appendages was also included, because salamanders readily respond to locally moving objects (Schuelert and Dicke, 2002). Differences were analysed using Mann–Whitney *U*-test (Prism 4). Video analysis was performed frame by frame with a computer program (Virtual Dub 1.6.11, © Avery Lee, www.virtualdub.org). Graphics were created with Corel Graphics Suite X3 Version 13.0 (Corel Corp., Ottawa, Ontario, Canada) and Prism 4.

## RESULTS

### Choices

#### Live crickets: 8 vs 16

Fifty-two salamanders were tested for the discrimination of 8 vs 16 live crickets. Twenty-two individuals were discarded because they failed to make a choice within the cut-off time (*N*=21) or because they did not leave the test box (*N*=1). Out of the 30 animals that successfully made a choice, 25 chose the larger quantity, and 5 chose the smaller one. Statistical analyses revealed a significant effect (binomial test, *P*<0.001, two-tailed). The salamanders reliably chose 16 over 8 (Fig. 2).

#### Live crickets: 8 vs 12

Fifty-five salamanders were used to investigate the 8 vs 12 contrast. Seventeen out of 30 chose the larger quantity, while 13 chose the smaller. Another 25 animals were discarded because they failed to make a choice within the cut-off time. Statistical analyses revealed no significant effect (binomial test, *P*=0.585, two-tailed). When this experiment was replicated, 18 out of 30 salamanders chose the larger and 12 the smaller quantity. Salamanders failed to choose the larger quantity when challenged with a 2:3 ratio (Fig. 2).

#### Video of crickets: 8 vs 16

Sixty-one salamanders were used to investigate the 8 vs 16 numerical contrast using video stimuli. Thirty-one were discarded for not

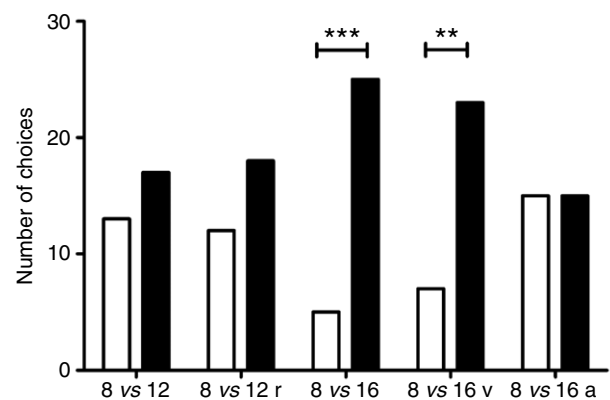


Fig. 2. Results of the two-alternative choice task. White columns represent salamanders choosing 8-cricket sets and black columns represent salamanders choosing 12- or 16-cricket sets. \*\**P*<0.01, \*\*\**P*<0.001. r, replicate; a, animation; v, video.

making a choice within the cut-off time ( $N=12$ ) or not leaving the test box ( $N=19$ ). Out of the 30 individuals that successfully made a choice, 23 chose the larger and 7 chose the smaller quantity. Statistical analyses revealed a significant effect (binomial test,  $P=0.005$ , two-tailed). Salamanders reliably chose 16 over 8 crickets (Fig. 2).

#### Computer-animated crickets: 8 vs 16

Eighty-two salamanders were tested in the 8 vs 16 numerical contrast using computer-generated stimuli. Fifty-two animals were discarded for not leaving the test box ( $N=29$ ) or not making a choice within the cut-off time ( $N=23$ ). Thirty animals successfully made a choice; 15 chose the smaller and 15 the larger number. Statistical analyses revealed no significant effect (binomial test,  $P=1.0$ , two-tailed). Salamanders failed to choose the larger quantity when challenged with a 1:2 ratio of computer-animated crickets (Fig. 2).

#### Latencies of choices

Data were pooled by significance of choices (pool 1: 8 vs 16 live crickets and videotaped crickets, and pool 2: 8 vs 12 live crickets and 8 vs 16 computer-generated crickets) and the latencies of choices for the larger quantity were compared with those for the smaller one. Pooling was performed because data from the unsuccessful experiments would obscure statistical effects. In addition, the number of salamanders choosing 8 over 16 live crickets was too small to analyse the data singly.

In pool 1, mean ( $\pm$ s.d.) latency was  $135.92 \pm 75.85$  s ( $N=12$ ) for animals that chose 8 crickets and  $87.58 \pm 65.39$  s ( $N=48$ ) for those choosing 16 crickets. In pool 2, mean ( $\pm$ s.d.) latency for animals choosing 8 crickets was  $113.11 \pm 71.68$  s ( $N=38$ ) and  $99.88 \pm 72.54$  s, ( $N=48$ ) for animals that chose the other set (i.e. 12 or 16 crickets). Statistical analyses revealed a significant difference (unpaired  $t$ -test,  $t=2.22$ , d.f.=58,  $P=0.03$ , two-tailed); salamanders of pool 1 that chose 16 live or videotaped crickets over 8 were significantly faster than those choosing 8 over 16 (Fig. 3).

#### Analysis of stimuli

Analysis of videotaped and computer-animated stimuli was performed because salamanders failed to choose the larger quantity in the 8 vs 16 experiments only when confronted with computer-

animated stimuli. Speed, shape and surface of the computer-animated crickets matched those of live crickets, and hence ratios of the associated continuous variables (e.g. cumulative surface area, brightness, contour length) were identical. Therefore, the duration of movement and simultaneous movement of stimuli in the computer-animated stimulus set were compared with those of videotaped stimuli.

#### Duration of stimulus movement

In 1 min randomly chosen in each of the videos and in the computer-generated presentation of stimuli, the time spent in motion was determined for each individual stimulus in the larger and smaller set (Table 1). The movement duration of naturally behaving crickets in the videos did not differ significantly between stimuli in the 8 and 16 stimulus set and was on average  $20.36 \pm 13.15$  s  $\text{min}^{-1}$  for a single stimulus (mean  $\pm$  s.d.;  $N=72$ ). Also, movement duration between stimuli of the 8 and 16 stimulus set did not differ significantly in the computer-animated stimuli. On average, the duration of movement amounted to  $29.33 \pm 3.23$  s  $\text{min}^{-1}$  ( $N=24$ ). Statistical analysis showed a significant difference in the duration of movement between live and computer-animated crickets (Mann-Whitney  $U$ -test,  $U=380.5$   $P<0.001$ , two-tailed).

#### Simultaneous stimulus movement

The number of simultaneously moving stimuli was determined every 5 s within the sample minute. Numbers of simultaneously moving crickets in the computer-animated sets were roughly twice as high as those in the video sets. In the 8-cricket sets, the mean number of stimuli moving at the same time was  $2.58 \pm 1.24$  in video 1,  $2.75 \pm 0.97$  in video 2,  $1.67 \pm 1.07$  in video 3 and  $4.08 \pm 1.93$  in the computer animation, while in the 16-cricket sets means were  $6.25 \pm 1.76$  for video 1,  $6.25 \pm 2.05$  for video 2,  $3.5 \pm 1.56$  for video 3 and  $10.41 \pm 3.2$  for computer-animated stimuli. Statistical comparison between the videos and the animation revealed significant differences between the 8-stimuli sets (Mann-Whitney  $U$ -test,  $U=90.5$ ,  $P<0.003$ , two-tailed) as well as between the 16-stimuli sets (Mann-Whitney  $U$ -test,  $U=39$   $P<0.001$ ) (Fig. 4).

## DISCUSSION

The results of our experiments reveal that: (1) salamanders successfully discriminate between contrasts of 8 vs 16 crickets when live crickets or videos of live crickets are presented, (2) salamanders fail to reliably choose the larger number in an 8 vs 12 contrast of live crickets, and (3) salamanders fail to discriminate in an 8 vs 16 contrast when cricket stimuli are digital images animated by a computer program. When able to discriminate between presented quantities, salamanders choosing the larger number were significantly faster than those choosing the smaller one. Analyses of stimulus movement revealed substantial differences between live and computer-animated crickets. Duration of movement was significantly longer for computer-animated stimuli, and number of simultaneously moving crickets was roughly twice as high for computer-animated stimuli compared with live crickets. Quantity discrimination is dependent on ratio, and since ratios of co-variables (cumulative surface area, brightness or contour length of stimuli in the small and large set) did not differ between live and computer-animated stimuli except for movement, we conclude that salamanders assess quantities on the basis of movement cues. Consequently, we did not perform experiments controlling for co-variables but investigated differences in movement-related cues.

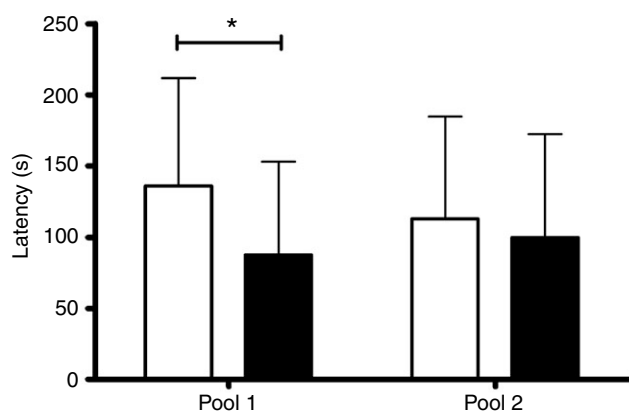


Fig. 3. Mean latencies of choices. Data were pooled by significance of choices. Pool 1 includes data from 8 vs 16 live and videotaped experiments (significant results) and Pool 2 includes 8 vs 12 and 8 vs 16 computer-animated experiments (non-significant results). White columns represent salamanders choosing the smaller quantity; black columns represent salamanders choosing the larger quantity. \* $P<0.05$ .

Table 1. Duration of movement (s) for each individual cricket stimulus determined from one randomly selected sample minute of the videos and of the computer animation

Stimulus number	Video 1		Video 2		Video 3		Animation	
1	12	24	20	58	18	4	26	27
2	17	24	31	18	22	25	26	27
3	37	24	13	13	35	26	30	33
4	7	19	5	4	16	26	27	30
5	22	38	17	23	6	5	36	31
6	32	30	25	40	0	11	25	31
7	7	10	10	24	0	23	28	25
8	32	14	10	52	0	22	25	28
9		44		29		0		27
10		41		9		0		32
11		30		50		5		34
12		27		16		0		27
13		15		18		24		33
14		19		24		11		31
15		15		31		22		33
16		32		39		14		32
Mean	20.75	25.37	16.37	28	12.12	13.62	27.87	30.06

### Live cricket and video experiments

Our data provide evidence for the first time of ratio-dependent, spontaneous discrimination of large quantities in an amphibian species, i.e. salamanders of the genus *Plethodon*. Salamanders were able to discriminate large numbers in sets that differed by a 1:2 ratio and failed in sets that differed by a 2:3 ratio.

### Comparison with other vertebrates

The findings of the present study indicate that the mechanism of large number discrimination works on imprecise analogue magnitudes that are subject to Weber's Law. This assumption is consistent with results obtained from species investigated so far, namely human infants, rhesus monkeys, fish and cotton-top tamarins. By means of a preferential-looking paradigm, 6 month old human infants discriminate between displays of dots with contrasts of 8 vs 16 and 16 vs 32, but fail with 8 vs 12 and 16 vs 24 (Xu and Spelke, 2000; Xu et al., 2005); in these experiments total filled area, array size and density, item size and brightness were controlled. By use of the same paradigm, Lipton and Spelke demonstrated that 6 month old human infants discriminate 16 vs 8 sounds but fail with 12 vs 8 sounds, when stimuli were controlled for element duration, sequence duration, interelement interval and acoustic energy (Lipton

and Spelke, 2003). Flombaum and colleagues found that rhesus monkeys challenged with addition tasks of large numbers to look longer at the incorrect of two possible results, when the ratio between the two was 1:2 but not 2:3 (Flombaum et al., 2005). Agrillo and colleagues investigated mosquito fish using a shoal preference paradigm (Agrillo et al., 2008). The fish chose the larger shoal of conspecifics out of contrasts of 1 vs 2, 2 vs 3, 3 vs 4, 4 vs 10, 4 vs 8 and 8 vs 16 while failing in 4 vs 5, 5 vs 6, 6 vs 7, 7 vs 8, 4 vs 6, 4 vs 7, 6 vs 8 and 8 vs 12 contrasts. Additional experiments controlled either surface area or movement in 2 vs 3 and 4 vs 8 contrasts, and the fish failed in all but the 2 vs 3 movement control. Cotton-top tamarins were investigated by a habituation–discrimination paradigm using speech syllables as stimuli, which were controlled for sequence duration, item duration, inter-stimulus interval and acoustic energy (Hauser et al., 2003). Tamarins discriminated between 4 vs 8, 4 vs 6 and 8 vs 12 while failing with 4 vs 5 and 8 vs 10. Altogether, results from large number discrimination experiments are robustly consistent across a variety of stimuli (dots, sounds, conspecifics and food), experimental paradigms (preferential looking, shoal preference, food choice) and species as diverse as fish and humans. Evidence from primates is clearly pointing to sensitivity to 'real number'. In contrast, fish when challenged with large numbers, do

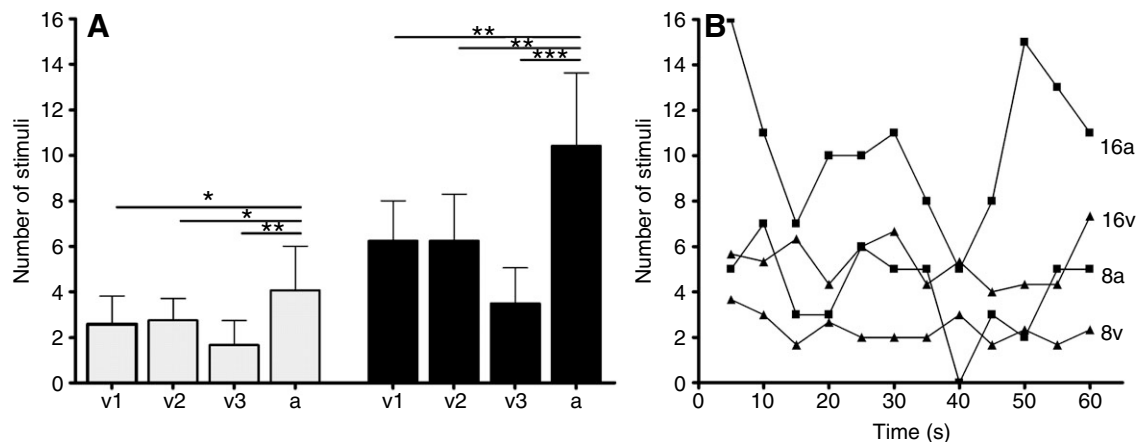


Fig. 4. Simultaneous movement of stimuli in one randomly selected sample minute of the 8 vs 16 videos ( $N=3$ ) and of the 8 vs 16 animation of stimuli. (A) Average number. Grey columns represent 8-cricket sets and black columns 16-cricket sets. Error bars indicate standard deviation. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ . (B) Variation over time. Average number of moving stimuli per set sampled every 5 s in the sample minute. a, animation; v, video.

not seem to respond to number and may instead combine cues from dimensions that usually vary with number (i.e. movement and surface) to discriminate between shoal sizes (Agrillo et al., 2008). Salamanders in the live and video experiments of the present study are able to discriminate quantities depending on ratio, but conclusions on cues utilized cannot be drawn on the basis of these experiments alone. On a larger scale, as results are robust across a variety of species, a shared mechanism seems plausible. However, the variety of stimuli that have been used in the studies mentioned may pose a problem for direct comparison – the various stimuli differ greatly in biological relevance (e.g. dots vs conspecifics, food), and hence discrimination may recruit different networks and mechanisms in the brain. This fact may account for minor differences in discriminatory success (e.g. 8 vs 12 in cotton-top tamarins) and for the use of different cues for discrimination (surface and movement controls in fish). It remains unclear whether experiments can be designed with large number as the salient cue for species aside from the primate group. Also, the different studies did not control for co-variables in a comparable manner, and the type of stimulus that can be used for some species is restricted. Finally, not all stimuli allow control for all co-variables.

#### Discrimination in salamanders

While simple or virtual stimuli can be manipulated with little or no limitation, live stimuli allow only certain manipulations and natural behaviour poses problems of its own. Live crickets pause during walking for significant periods of time, and crickets that do not move while a salamander is attending the sets are presumably not part of the numerical contrast evaluated. Therefore, some individuals might be challenged with contrasts other than 8 vs 16 or 8 vs 12. On the other hand, the average contrast ratio should be preserved, as crickets behave similarly, and numbers should be sufficiently high to ensure a large number challenge. Indeed, analyses of simultaneous movement of stimuli revealed average ratios of about 1:2 and absolute set size exceeded the theoretical threshold for the small number system (Feigenson et al., 2004). Uller and colleagues tested salamanders with a 4 vs 6 contrast using live *Drosophila* as stimuli (Uller et al., 2003). Consistent with the 2:3 ratio limit, salamanders failed. The movement pattern of fruit flies was not controlled, and real contrasts might have been different, because – parallel to the present study – not all of the live stimuli might have moved constantly. Latencies from salamanders' successful discrimination of larger numbers were significantly shorter than those of smaller numbers. On average, the salamanders going for the larger of the two sets were roughly 50s faster than their conspecifics. Latency includes the time spent approaching the set until strike of a stimulus on the screen, but also comprises time moving around on the test platform, which limits strict conclusions on underlying processing. However, the robust and significantly shorter latency indicates the fundamental advantage of the 1:2 contrast in larger sets to positively influence object recognition, discrimination and/or decision. Whether this is related to a simple 'pop-out' effect upon first perception of the sets or is part of a more complex evaluation process remains to be clarified. *Vice versa*, long latency indicates that salamanders were confronted with two very similar or non-distinguishable sets of stimuli, i.e. regardless of stimulus number choices were of equal relevance for the salamanders, and thus did not lead to a preference in decision.

#### Computer-animated experiment

When confronted with computer-animated stimuli, salamanders made random choices. Compared with results of the live and video

experiments and other studies investigating large number discrimination in a 1:2 ratio contrast (Xu and Spelke, 2000; Hauser et al., 2003; Lipton and Spelke, 2003; Flombaum et al., 2005; Xu et al., 2005; Agrillo et al., 2008), a positive result would have been expected. However, a comparison across studies is limited because of different conditions; only two of these studies used either a comparable paradigm (shoal preference) (Agrillo et al., 2008) or stimulus (food) (Flombaum et al., 2005).

#### Failure of discrimination

The positive results for the video experiments of the present study provide a valid basis for analysis of the salamanders' failure in discrimination of computer-animated stimuli. Both types of experiments used stimuli on an LCD screen and these lack depth cues derived from stereopsis, accommodation and motion parallax (Oliveira et al., 2000; Zeil, 2000). Salamanders use information from stereopsis and accommodation for prey capture (Roth, 1987; Wiggers et al., 1995). Since salamanders readily responded to the cricket stimuli of the video, we infer that neither of these factors contribute to the failure of large-number discrimination. The same holds for spectral sensitivity, which in salamanders is comparable to that of other vertebrates including humans (Roth et al., 1998). Furthermore, previous studies demonstrated that salamanders and their visual neurons readily respond to images of prey presented on an LCD screen (Schuelert and Dicke, 2002; Schuelert and Dicke, 2005). Analyses of computer-animated stimuli yielded an average ratio of 1:2.5 for simultaneously moving stimuli in the 8:16 sets. With increasing numerical distance, discrimination between the two sets should be facilitated. Consequently, discrimination success should at least equal that of the live and video experiments. As this is not the case, we conclude that it is not ratio of stimulus number that is used for discrimination by salamanders. The failure must, therefore, be related to the stimulus itself or the arrangement of parameters performed by the program. Perceptual features that have been shown to be relevant for numerical representations include differences in contour length, brightness, cumulative surface area, movement and element density (Xu and Spelke, 2000; Brannon, 2002; Xu, 2003; Xu et al., 2005; Agrillo et al., 2008). In the computer-animated condition, ratios for all these features equalled those in the live and videotaped cricket conditions except for movement-related dimensions. In order to limit variance of parameters that go along with the use of live, naturally behaving crickets, the image used for animation was cut out from a video and was copied by the program. Hence, ratios of contour length, of object brightness and of cumulative surface area of stimuli were the same in all 8 vs 16 experiments. We conclude that contour, brightness and surface are not used as the key feature for discrimination of large numbers in salamanders.

#### Role of stimulus movement

Analysis revealed that movement parameters differed substantially between animated and live or video stimuli. Salamanders are known to be sensitive to prey features such as size, speed, movement pattern, orientation and shape (Roth, 1987; Schuelert and Dicke, 2002; Schuelert and Dicke, 2005). Furthermore, spatial distribution of stimuli might also influence the performance of salamanders. Ideal stimuli should be evenly distributed within the available space, whereas distribution of natural cricket stimuli was far from that. The program used for the computer-animated stimuli distributed stimuli evenly across the area and moved them at a rather homogeneous speed. This different spatio-temporal distribution pattern of prey objects might require greater attention and probably

exceeds the salamanders' attention capabilities. In addition, the considerably higher number of simultaneously moving stimuli and the equality of stimuli might have disturbed performance. Although stimulus uniformity is required for experimental standardization, it is known that uniformity in groups of prey makes the choices of predators more difficult (Landeau and Terborgh, 1986). However, prey uniformity is inherently high in the computer-animated experiment of the present study. It remains open whether discrimination and/or attention were impaired in salamanders. Latencies of feeding responses were distributed over the same range as those of live or video stimuli. Also, the fact that salamanders readily attempted to feed on the computer-animated stimuli indicates that they recognized them as familiar prey. It likewise implies that processing of visual features like shape was not affected.

### CONCLUSIONS

In plethodontid salamanders, cues of movement-related features are good candidates to form the basis of quantity judgements. In salamanders, visual neurons processing motion possess the largest dendritic trees among neuron types (indicating input from large areas of the visual field) and readily respond to movement (Dicke, 1999; Roth et al., 1998; Roth et al., 1999). Hence, it is plausible that the decision is based on this dominant information in naive salamanders. In the live and video experiments, the set containing non-homogeneously distributed and higher overall movement appears to be a strong attractor to capture attention. Movement of stimuli might act as a 'pop-out' and enables the salamander to be faster in its choice of the larger set of stimuli. Sets with computer-animated stimuli contained many uniformly moving stimuli, probably to such an extent that the salamander's visual system was unable to extract differences between the sets. This leads to random choices of large vs small number sets. The evidence for salamanders' assessment of large quantities *via* movement indicates that anamniotes – taking results from fish into account – use movement as an important cue for representation of relevant entities in their environment. Our study further indicates that salamanders make use of a two-number system currently under debate. The underlying mechanism seems to be shared across vertebrates suggesting evolutionary ancient origin and great adaptive power. Actual information (movement, cumulative surface or other features) used by this mechanism might differ substantially between vertebrate groups. However, several vertebrate groups (e.g. reptiles) have not been investigated so far, and future research needs to clarify aspects of analogue magnitude-based mechanisms and quantification abilities.

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