Current Biology

Neurons in the Endbrain of Numerically Naive Crows Spontaneously Encode Visual Numerosity

Graphical Abstract
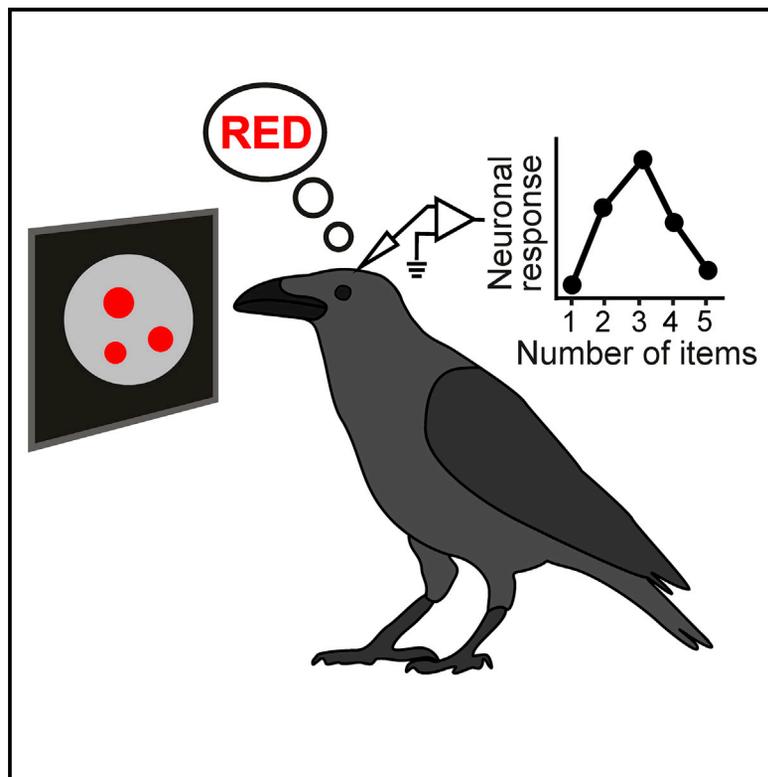
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In Brief

In crows never trained to assess numerical quantity, Wagener et al. found neurons in the nidopallium caudolaterale that were spontaneously selective for the number of items. Hard-wired neuronal connections for numerical information are not only present in the cerebral cortex but also in the avian endbrain that evolved by convergent evolution.

Highlights

- NCL neurons respond to numerosity in numerically naive crows

- Numerosity-selective neurons were tuned to the number of items

- Numerosity-selective neurons are spontaneously present in the avian endbrain

- Convergent evolution of neuronal number code in different vertebrate endbrains

CellPress

Neurons in the Endbrain of Numerically Naive Crows Spontaneously Encode Visual Numerosity

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SUMMARY

Endowed with an elaborate cerebral cortex, humans and other primates can assess the number of items in a set, or numerosity, from birth on [1] and without being trained [2]. Whether spontaneous numerosity extraction is a unique feat of the mammalian cerebral cortex [3–7] or rather an adaptive property that can be found in differently designed and independently evolved neural substrates, such as the avian endbrain [8], is unknown. To address this question, we recorded single-cell activity from the nidopallium caudolaterale (NCL), a high-level avian association brain area [9–11], of numerically naive crows. We found that a proportion of NCL neurons were spontaneously responsive to numerosity and tuned to the number of items, even though the crows were never trained to assess numerical quantity. Our data show that numerosity-selective neuronal responses are spontaneously present in the distinct endbrains of diverge vertebrate taxa. This seemingly hard-wired property of the avian endbrain to extract numerical quantity explains how birds in the wild, or right after hatching, can exploit numerical cues when making foraging or social decisions. It suggests that endbrain circuitries that evolved based on convergent evolution, such as the avian endbrain, give rise to the same numerosity code.

RESULTS

Whether humans and animals are endowed with an innate faculty to perceive the number of items in a set (that is, numerosity) is intensely discussed. The idea of a "number sense" [12, 13] argues that numerosity is assessed intuitively as a spontaneous category by hard-wired brain processes, without the need to be learned. Support for the direct and spontaneous assessment of numerosity resulted from psychophysical experiments in humans showing that approximate visual number assessments are subject to adaptation [3, 4]. In addition, recent imaging evidence suggests that the direct and automatic extraction of numerosity also occurs in the human brain [5, 6]. The most direct support for the notion of a "number sense" comes from recordings in monkeys that had not been trained to judge number;

these recordings showed that single neurons in both the parietal and prefrontal cortices spontaneously responded to numerosity and were tuned to preferred numerosities [7].

However, all of these data have been collected in primate species that possess an elaborate six-layered cerebral cortex as highest integration center in the brain. Whether spontaneous numerosity extraction is a special feature of the cerebral cortex or rather an adaptive property that can be found in differently designed and independently evolved endbrains is unknown.

We therefore investigated the question of spontaneous numerosity selectivity in a bird species: the carrion crow. Instead of a cerebral cortex, birds possess nuclear telencephalic areas [8] as highest integration centers that evolved independently since the last common reptilian-like ancestor of birds and mammals lived 320 million years ago [14]. We recently showed that neurons in the endbrain region nidopallium caudolaterale (NCL), a brain area considered to be the avian analog of the primate prefrontal cortex [9–11], respond selectively to the number of visual items in numerically trained crows [15, 16]. In the current study, we explored spontaneous neuronal selectivity to numerosity in crows that had never been trained to discriminate the number of items in a set.

Crows Performed the Color Discrimination Task and Were Ignorant of Numerosity

Two crows (*Corvus corone*) were trained to discriminate color in variable dot displays in a delayed match-to-sample (DMS) task. This ensured that the crows paid attention to the stimulus displays during recording (Figure 1A). The crows saw two colored-dot displays (first sample, then test) separated by a 1 s delay. They were trained to respond by moving their head whenever the (1–5) dots in the sample and test displays were of the same color. Five colors (red, blue, green, yellow, purple) were used (Figure 1B). Importantly, the crows were only trained to discriminate color, not numerosity. All five colors and numerosities were displayed as "standard stimuli," with variable dot sizes and positions, and "control stimuli" equating the total area and the average density of all dots across numerosities. All parameters (color, numerosity, stimulus protocol, match versus non-match trials, etc.) were balanced and pseudorandomly presented in each session.

Both crows performed the color-discrimination task proficiently well above the 50% chance level (crow T: 99% ± 0.2% SEM, n = 50 sessions; crow V: 95% ± 0.3% SEM, n = 43 sessions; Figure 2A) for all sample colors (all binomial tests, p < 0.001). To ensure that the crows had indeed discriminated color and not numerosity, we inserted a small fraction of
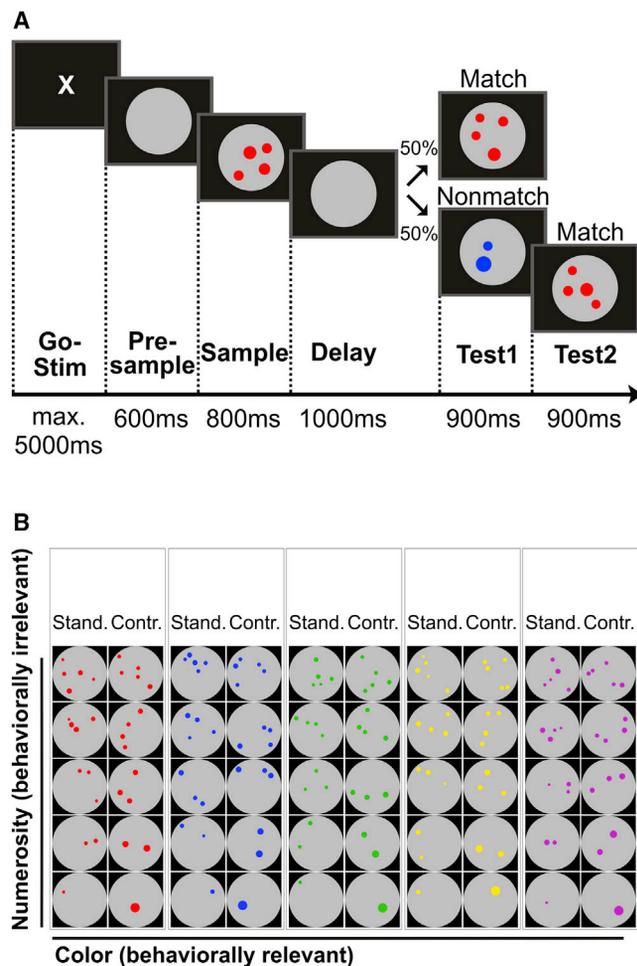
Figure 1. Task Protocol and Example Stimuli

(A) The crows performed a delayed match-to-sample task in which they discriminated the color of dot arrays. A trial was initiated by moving the head into a light barrier in front of the screen and keeping it in this position. After a short pre-sample phase, a sample stimulus (colored-dot array) was presented for 800 ms, followed by a delay of 1,000 ms. In the subsequent test phase, a match stimulus (same color as the sample) was shown as test 1 in 50% of the trials, in the other half a non-match stimulus (different color as the sample) was presented first and followed by a match stimulus. The crow was rewarded for responding by moving its head out of the light barrier whenever the color of a test stimulus matched the color of the sample.

(B) Example stimulus displays. Each of the five colors was presented in five different numerosities and two different stimulus sets (standard and control).

Figure 2. Behavioral Performance of Both Crows

(A) Performance in the color discrimination task during recording sessions (crow T: n = 50; crow V: n = 43). Chance level is 50%. Error bars indicate SEM across the sessions.

(B) Performance in the numerosity-discrimination task in the generalization test sessions (crow T: n = 283 trials; crow V: n = 270 trials). Chance level is 50%.

generalization trials during the ongoing color-discrimination task. In generalization trials, the dots of both sample and test stimuli were all black. If the crows were ignorant of numerosity and relied on color, they would perform at chance level for the all black dot arrays. Indeed, both crows performed at chance level in black-dot trials (crow T: 52%, n = 283 trials; crow V: 52%, n = 270 trials; both binomial tests, p ≥ 0.5; Figure 2B).

Neurons Spontaneously Tuned to Numerosity

We recorded the activity of 403 single neurons (crow T: 289; crow V: 114) in the NCL (Figure 3A) while the crows performed the color-discrimination task with colored-dot stimuli. We found cells that responded differently to specific numbers of dots (i.e., numerosities) during the sample presentation. Figure 3 shows the activity of three exemplary neurons. The example neuron in Figure 3B showed the highest activity to numerosity 1, whereas the other neurons responded strongest to numerosity 2 (Figure 3C) and 5 (Figure 3D).

A three-factor ANOVA (numerosity × color × protocol) was used to statistically test the neurons' selectivity to the different stimulus parameters. Neurons that showed a significant main effect for numerosity (p < 0.01), but no significant main effect for protocol or any interaction, were identified as numerosity-selective neurons and considered for further analyses. The behaviorally irrelevant parameter "numerosity" significantly modulated the activity of 12% (48/403) of the NCL neurons. Of those 48 numerosity-selective cells, 19 neurons (39.6%) showed an additional main effect for color. All neurons depicted in Figure 3 were numerosity selective according to this criterion. Table S1 shows the proportions of neurons that were significant to each of the main factors and interactions. These proportions of significant neurons are well beyond the chance level of about 1% of selective cells that we got when the spike rates of individual neurons were shuffled and analyzed in the same way (Table S2).

These neurons were tuned to the number of dots; they showed the highest discharge rates to a specific numerosity, its preferred numerosity, and a progressive decay of activity for neighboring numerosities (see tuning curve insets in Figures 3B–3D). Most of the selective neurons preferred numerosity 1 and 5; fewer neurons were tuned to the other intermediate numerosities (Figure 4A). Note that an increased frequency count for preferred numerosity 5 is even expected as the tested numerosity range was truncated to numerosity 5, and few neurons assigned to this class may, in fact, have been tuned to numerosities larger than 5.

To create average neural filter functions, activity rates were normalized by setting the maximum activity to the most preferred numerosity as 100% and the activity to the least preferred numerosity as 0%. Tuning functions to each of the sample numerosities were constructed by averaging the normalized spike rates
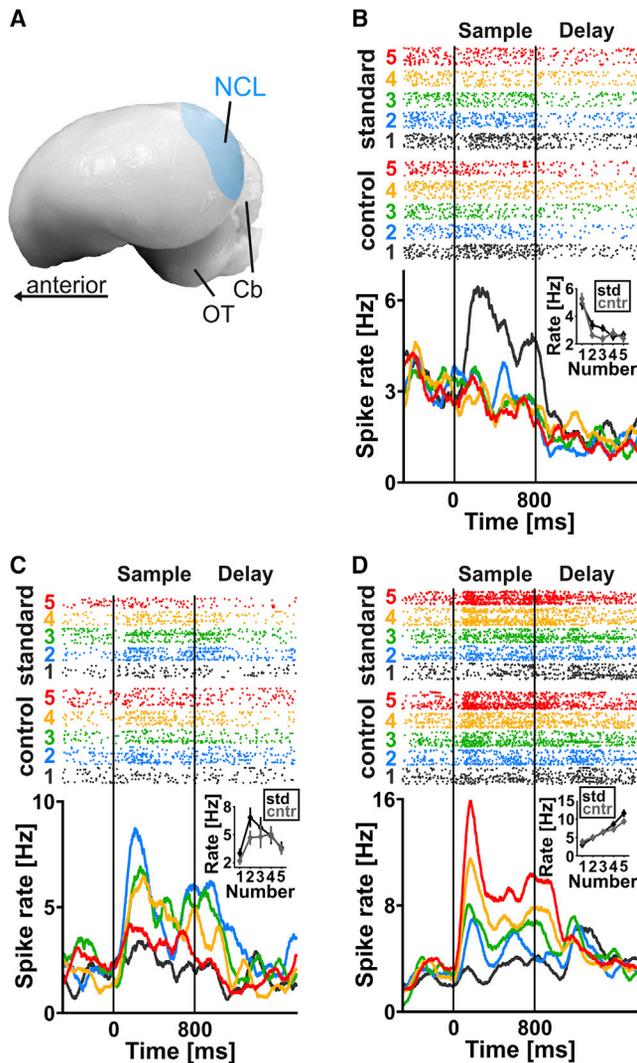
Figure 3. Brain Area and Neuronal Responses

(A) Lateral view of a crow brain with the nidopallium caudolaterale (NCL) located inside the telencephalon color coded. Cb, cerebellum; OT, optic tectum.

(B–D) Neuronal responses of exemplary neurons to the number of presented dots in the sample stimulus. The neurons were selective to numerosity 1 (A), 2 (B), and 5 (C). Top: Dot-raster histograms with each line indicating one trial and each dot representing an action potential. Activity is separated for standard and control conditions. Bottom: Corresponding spike-density functions, representing the time course of the average response to each numerosity (smoothed by a 150 ms Gauss kernel). Colors of dot-raster histograms and spike-density functions correspond to the numerosity of the sample stimulus. Vertical line at 0 ms indicates onset of the sample that was shown for 800 ms. Tuning function insets indicate the average firing rate to numerosity in the standard (std) and control (cntr) condition. Error bars represent SEM. See also Tables S1 and S2.

of all neurons that had the same preferred numerosity. This resulted in overlapping numerosity tuning curves (Figure 4B). Across the population, NCL neurons covered the entire tested range of numerosities 1–5. Finally, we plotted the average normalized activity across the population of numerosity-selective neurons as a function of the numerical distance from the

preferred numerosity (Figure 4C). On average, neuronal activity dropped as a function of the numerical distance from the preferred numerosity, a neuronal correlate of the "numerical distance effect" that has been reported for numerosity-selective NCL neurons in trained crows [15, 16].

DISCUSSION

In the current study, we tested the core idea of the "number sense" and explored, for the first time in a non-primate species, whether numerosity-selective neurons spontaneously exist in the brain of crows. To that aim, we recorded single-cell activity from the NCL, a high-level avian association brain area [17–19], of numerically naive crows. We show that a proportion of NCL neurons is selectively tuned to the number of items in a set. This demonstrates that numerosity-selective neurons are not the result of behavioral training but spontaneously exist in crows that have never been trained to discriminate numerosity.

Without numerosity training, we found that 12% of NCL neurons responded selectively to the number of presented dots. This proportion was significantly smaller compared to the 20% of numerosity-selective neurons from the same NCL region in crows trained to perform a numerosity-discrimination task [15] (chi-square tests, p < 0.01). However, the selectivity of the numerosity-selective responses was comparable for data from naive and trained crows. We compared the widths of the numerosity-tuning curves as measured by sigma of Gauss-fits to the (logarithmically scaled) tuning functions [20] and found no difference between numerically naive and trained crows (Mann-Whitney-U test, p = 0.86). Based on these comparisons, we conclude that numerosity training may increase the proportion of numerosity-selective cells in NCL but not their coding properties.

The only other animal species for which single-unit data about numerosity coding is available are macaque monkeys. In these primates, the ventral intraparietal area (VIP) and prefrontal cortex (PFC) have been identified as key areas for number representations [21, 22]. Interestingly, the proportion of selective neurons (12%) in the NCL of numerically naive crows is almost identical to the 13% and 14% of numerosity-selective neurons in the VIP and PFC, respectively, of numerically naive monkeys [7]. This suggests the NCL as a neuronal substrate for representing numerical information, much in the way as the VIP and PFC constitute the core number system in primates.

Our study also speaks to the question of the neuronal code for numerical quantity in the animal kingdom. Two competing hypotheses have been proposed. Numbers could either be encoded by a "summation code" as witnessed by monotonic discharges as a function of quantity [23], or by a "labeled-line code" as evidenced by neurons tuned to preferred numerosities [21]. In agreement with influential computational models of number processing [24, 25], the numerosity-selective neurons we found in the NCL of numerically naive crows were tuned to their individual preferred numerical value. The same code has been found in numerically trained crows [15, 16] and multiple times in single-cell recordings in monkeys, both trained [26–31] and numerically naive [7]. It therefore seems that the neuronal code for number information is a labeled-line code. This code seems to have
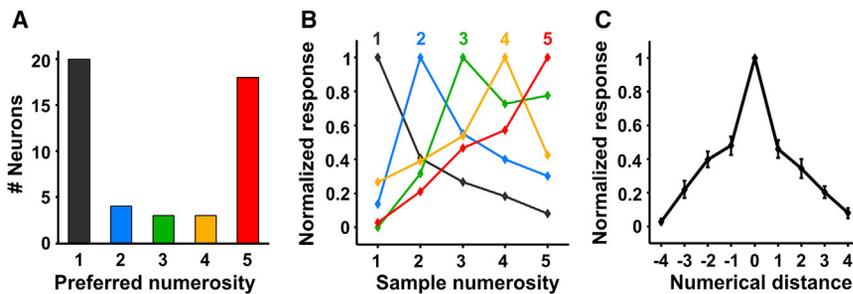
Figure 4. Average Tuning Properties

(A) Frequency distribution of the neurons' preferred numerosities.

(B) Average tuning functions for neurons preferring the same numerosity.

(C) Average normalized activity of all numerosity-selective neurons as a function of numerical distance from the preferred numerosity. Error bars indicate SEM.

evolved independently in phylogeny in birds and mammals, two distantly related vertebrate taxa [32]. The labeled-line code may be computationally superior when compared to alternative neuronal representations such as summation coding.

The ability to spontaneously assess the number of items in an approximate way is widespread across the animal kingdom, indicating that it is of adaptive value. Tests in which animals can choose between sets of food objects show that different species spontaneously "go for more" and pick the sets containing more food items [33–37]. Similarly, animals in the wild spontaneously exploit quantitative information in social interactions [2, 38, 39]. For these animals to successfully discriminate set size, numerosity-selective neurons must spontaneously be implemented in their brains. Without such neurons, they could not solve such numerical tasks in the first place.

The current data in crows together with a report about numerosity-selective neurons in the parietal and prefrontal cortex of monkeys [7] argue that the neuronal mechanisms for approximate number discrimination are readily available without number training in differently designed endbrains. This begs the question whether animals might be born with hard-wired neuronal networks that can represent numerical information. Alternatively, numerosity selectivity could emerge implicitly as a function of increased visual experience with different numbers of objects throughout development. To address this question directly, recordings in juvenile crows at the moment of eye opening would be necessary. However, even without such data, behavioral investigations suggest that numerical competence is present from early on in birds.

The young domestic chick is an extremely precocial species and has been tested for numerical competence right after hatching from the egg and thus with a minimum of visual experience. Exploiting filial imprinting few hours after hatching, chicks have been shown to discriminate numerosity and even perform rudimentary arithmetic [40, 41]. Moreover, newborn human infants at the age of 50 hr also discriminate abstract numerosity, even across sensory modality and sequential and simultaneous presentation formats [1].

All of these data together argue that numerosity selectivity may indeed be inborn, not only in primates but also in other vertebrates. This suggests that hard-wired (but, of course, modifiable) neuronal connections extracting numerical information are not a special property of the cerebral cortex but are also implemented in the anatomically distinct endbrain circuitries of birds that evolved based on convergent evolution. How these distinct endbrain designs give rise to the same type of numerosity code needs to be addressed in the future.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes two tables and can be found with this article online at https://doi.org/10.1016/j.cub.2018.02.023.

AUTHOR CONTRIBUTIONS

L.W., H.M.D., and A.N. designed the experiment. L.W. and M.L. conducted the experiments. L.W. analyzed the data. L.W. and A.N. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing financial interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Corvus corone	University of Tübingen, Institute of Neurobiology	Crow T, crow V
Software and Algorithms		
NIMH Cortex	National Institute of Mental Health	c598; https://www.nimh.nih.gov/labs-at-nimh/research-areas/clinics-and-labs/ln/shn/software-projects.shtml
MAP Data Acquisition System	Plexon	https://plexon.com/
R2013b	MathWorks	https://www.mathworks.com
Other		
Dental Cement	Heraeus	Paladur, ISO 20795, CE 0197
Microdrives	Animal Physiology Unit	Custom fabrication
Electrodes	Alpha Omega LTD	Cat.#: 366-130620-00; www.alphaomega-eng.com

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Andreas Nieder (andreas.nieder@uni-tuebingen.de).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Carrion crows

Two hand-raised male carrion crows (*Corvus corone corone*, 6 and 2 years old) were used in this experiment. The birds were housed in social groups in indoor aviaries. They were on a controlled feeding protocol during the training and recording period. Body weight was measured daily. The daily amount of food was given as reward during, or if necessary after, the sessions. Water was *ad libitum* available in the aviaries and during the experiments. All procedures were carried out according to the guidelines for animal experimentation and approved by the responsible national authorities, the Regierungspräsidium Tübingen, Germany.

METHOD DETAILS

Apparatus

The birds were placed on a wooden perch in front of a touchscreen monitor (ART development PS-150, 15″, 60 Hz refresh rate) in a darkened operant conditioning chamber. The CORTEX system (National Institute of Mental Health, MD, USA) was used to control the stimulus display on the screen and to store the behavioral data. An infrared light barrier ensured, controlled by a reflector foil attached to the bird's head, a stable head position in front of the screen throughout the trial and was used as the response instrument by the bird. A custom-built automated feeder below the screen delivered either mealworms (*Tenebrio molitor* larvae) or bird seed pellets as reward upon correctly completed trials. Additionally, the birds received specific auditory feedback sounds for correct and error trials.

Stimuli

The visual stimuli were generated using a custom-written MATLAB software. They consisted of a colored dot array presented on a gray background circle. Each combination of five colors (red, blue, green, yellow, purple) and five numerosities (1, 2, 3, 4, 5) was used (Figure 1B). For the generalization test, black dot arrays were used. To prevent the crows from memorizing the visual patterns of the dot arrays, a new stimulus set with four different images for each color-numerosity combination was generated for each session.

For the standard stimuli, the diameter of each dot varied randomly within a given range. In addition, control stimuli controlling for total dot area (the total area of all dots in a display was equal for all stimuli within a trial) and dot density (mean distances between centers of the dots in a display was equal for all stimuli within a trial) were used in each session. Trials containing standard or control stimuli were pseudo-randomly shuffled and equally likely to occur.

Behavioral protocol

The crows performed a delayed match-to-sample (DMS) task in which they discriminated the color of dot arrays (Figure 1A). A trial was initiated by positioning the head facing the monitor whenever a go-stimulus (small white cross) was shown, thus closing an infrared light barrier, and maintaining this position throughout the trial. To indicate that the light barrier had been entered, the bird

heard a click sound and the go-stimulus turned into a small white circle for 60 ms. Whenever a crow made premature head movements and thereby left the light barrier during an ongoing trial, this trial was terminated and discarded. In the 600 ms pre-sample phase, a plain gray background circle was shown in the center of the screen. Then the sample dot array was presented within the background circle for 800 ms. The color and numerosity of the dot array were pseudo-randomly selected. During the subsequent 1000 ms delay, only the plain background circle remained on the screen. In the following test phase, another dot array, the test1 stimulus, was presented for 900 ms. It was a 'match' in 50% of the cases, i.e., the dot array had the same color and numerosity as the sample, however it was never exactly the same image. The crow had to respond by moving its head out of the light barrier to receive a reward. In the other half of the cases, the test1 stimulus was a 'nonmatch' showing a dot array of another color and numerosity as the sample. Here, the crow had to refrain from responding and wait until the test2 stimulus, which was always a 'match', appeared. Responses to the 'nonmatch' stimulus and no response to either of the two test stimuli were considered as error trials and therefore not rewarded.

Generalization test

To confirm that the crows discriminated the stimuli based on color and not on the irrelevant parameter numerosity, we tested them with pure numerosity stimuli (black dot arrays, numerosity 1 to 5). These trials contained no color information (sample and test stimuli black) and were randomly inserted during the ongoing color discrimination task. The ratio of generalization trials was between 12.3% and 17.1% of the total number of trials. Reward was given for correctly solved numerosity trials (i.e., responding to the test stimulus which showed the same numerosity as the sample), however the birds were not forced to solve these trials correctly. Three generalization test sessions without neural recording were done for each bird: before, during and after the recording period.

Surgery and neuronal recordings

The surgery was performed while the animal was under general anesthesia with a mixture of ketamine (50 mg/kg) and Rompun (5 mg/kg xylazine). The head was placed in a stereotactic holder. To locate the target region, stereotaxic coordinates (center of craniotomy: AP 5 mm, ML 13 mm) were used. Neurons were sampled a few millimeters around these coordinates. Two custom-built microdrives with four glass-coated tungsten microelectrodes (2 MΩ impedance, Alpha Omega LTD, Israel) each and a connector for the head stage were chronically implanted. The eight electrodes were located in the NCL of the left hemisphere of crow T and the right hemisphere of crow V. No clustering of numerosity selectivity was detected across electrodes or recordings depths. A small head post for the reflector of the light barrier was already implanted in the course of previous experiments. After the surgery, the birds were provided with postoperative analgesics (Morphasol, 1 mg/kg butorphanol).

Each recording session started with adjusting the electrodes until a proper neuronal signal was detected on at least one channel. The neurons were never pre-selected for any involvement in the task. Single-cell separation was done offline (Plexon Offline Sorter, version 2.6.2).

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavioral analysis

Data analysis was performed using MATLAB (MathWorks, R2013b). The behavioral performance, which quantifies the ratio of correct answers, was calculated as the number of correct trials divided by the total number of trials. For the color discrimination task, the performance was calculated for each sample color in each session, statistically verified using a binomial test, and averaged across all sessions. To exclude numerosity discrimination, the performance for each numerosity in trials with black dot arrays during the generalization sessions (trials of the three sessions added together) was calculated and tested using a binomial test.

Neuronal analysis

The analyzed neuronal data included all cells that were recorded for at least 20 correct trials of each sample color and numerosity and had an average firing rate higher than 1 Hz during the entire trial. Neuronal responses to the sample stimulus were analyzed in an 800 ms window shifted by 100 ms from stimulus onset to account for response latency.

To identify numerosity-selectivity, defined as a difference in firing rate as a function of the number of presented dots, a three-factor ANOVA with main factors sample numerosity (1, 2, 3, 4, 5), sample color (red, blue, green, yellow, purple) and protocol (standard or control) was performed. A neuron was classified as numerosity-selective if it showed either a significant main effect for numerosity ($p < 0.01$) or for numerosity and color, but no significant effect for protocol and interactions. The preferred numerosity was defined as the numerosity which elicited the highest firing rate. We compared the proportion of selective neurons found in the real data with shuffled firing rate data as a measure of chance selectivity. Data were shuffled a thousand times per neuron and each time tested with the three-factor ANOVA.

To derive average tuning functions of the numerosity-selective neurons, the individual tuning functions were normalized by setting the highest firing rate to the preferred numerosity as 100% and the lowest firing rate as 0%. These were then averaged across all neurons which preferred the same numerosity and as a function of the numerical distance from the preferred numerosity, respectively.

To evaluate potential changes in the selectivity of numerosity tuning in naive versus numerically trained crows, we compared the width of the tuning functions in naive crows (this dataset) with a previously recorded dataset in numerically trained crows [15]. To that

aim, Gauss-functions were fit to the neuronal tuning functions of each numerosity-selective neuron. The Gaussian was chosen because it represents the standard symmetric distribution and, thus, provided a means to compare the tuning functions. Data were plotted on a logarithmic scale because this provides symmetric tuning functions [15]. The derived width (sigma) of the Gauss fits was then compared between data in naive and trained crows.

DATA AND SOFTWARE AVAILABILITY

Analysis-specific code and data are available by request to the Lead Contact.