Single-neuron representation of nonsymbolic and symbolic number zero in the human medial temporal lobe

Highlights

- Single-neuron recordings in the medial temporal lobe of patients
- Neurons signal both nonsymbolic and symbolic zero
- Neuronal representations of zero integrated along the number line
- Nonsymbolic empty sets distinctly encoded from other small numbers

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In brief

Kutter et al. identify and characterize single-neuron activity in the human brain correlated with the representation of both nonsymbolic (dot numerosities) and symbolic (Arabic numerals) number zero.





Report

Single-neuron representation of nonsymbolic and symbolic number zero in the human medial temporal lobe

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SUMMARY

The number zero holds a special status among numbers, indispensable for developing a comprehensive number theory.^{1–4} Despite its importance in mathematics, the neuronal foundation of zero in the human brain is unknown. We conducted single-neuron recordings in neurosurgical patients^{5–7} while they made judgments involving nonsymbolic number representations (dot numerosity), including the empty set, and symbolic numbers (Arabic numerals), including numeral zero. Neurons showed responsiveness to either the empty set or numeral zero, but not both. Neuronal activity to zero in both nonsymbolic and symbolic formats exhibited a numerical distance effect, indicating that zero representations are integrated together with countable numerosities and positive integers at the low end of the number line.^{8,9} A boundary in neuronal coding existed between the nonsymbolic empty set and small numerosities, correlating with the relative difficulty in discriminating numerosity zero behaviorally. Conversely, no such boundary was found for symbolic zero activity, suggesting that symbolic representations integrate zero with other numerals along the number line, reconciling its outlier role. The status of zero as a special nonsymbolic numerical quantity is reflected in the activity of neurons in the human brain, which seems to serve as a scaffold for more advanced representations of zero as a symbolic number.

RESULTS

The neural representation of numbers has been deciphered in some detail.^{10,11} Neuroimaging studies in humans^{12–15} and electrophysiology in nonhuman primates¹⁶ have identified cortical brain areas encoding nonsymbolic countable numerosity, serving as a developmental and evolutionary scaffold for symbolic number representations such as Arabic numerals and number words.^{10,11,17} In recent years, intracranial neuron studies in patients have revealed cells tuned to specific numerical values.^{5–7} However, the representation of "zero," pivotal for number theory, remains unclear.

Zero's emergence unfolds gradually across four stages in human history, ontogeny, evolution, and brain processing.⁹ Initially, the absence of a stimulus reflects a neural resting state lacking a specific signature. In the second stage, "nothing" is recognized as a meaningful behavioral category contrasting "something" but still lacks quantitative relevance. In ancient cultures, zero was employed as a placeholder, "nothing" devoid of its numerical identity in positional notation systems.^{1–4}

As the third stage, absence gains quantitative significance, depicted as an "empty set" at the numerical continuum's lower end. Around the age of four, children begin to integrate a nonsymbolic understanding of nothing as an empty set into their mental "number line," demonstrated by a tendency to confuse it more frequently with numerosity 1 than with the more distant numerosity 2, showcasing a "numerical distance effect."⁸ Thus, if neurons encoding empty sets represent them as the smallest quantity in the numerical continuum, they would display a neuronal numerical distance effect by exhibiting diminishing responses to increasingly higher numerosities. Without such a neuronal distance effect, neurons would simply represent a nothing category devoid of numerical meaning.

Finally, the empty set representation progresses into the number zero, a crucial part of the numerical symbol system used in mathematics. By the seventh century, zero is fully recognized as a symbolic number, leading to the adoption of the decimal positional number system in the West.^{3,4} Children typically understand zero as the smallest number in the series of positive integers around age six, long after learning to count.¹⁸ Even in adulthood, zero stands out on the mental number line, as shown by reaction time (RT) and performance variations in behavioral studies,^{19–22} highlighting its unique nature among integers. Despite zero being part of the number line, its neuronal representation may therefore be distinct compared to positive integers.



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Here, we investigated zero representations through singleneuron recordings in the medial temporal lobe (MTL) of neurosurgical patient volunteers. We revisited and analyzed the dataset that constituted the basis of our previous analysis." We presented numerical values ranging from 0 to 9 on a computer screen and asked the participants to judge the parity (even vs. odd) of numbers.^{23,24} In each trial, a number stimulus was presented after a short fixation period, followed by a brief delay during which the number stimulus was removed (Figure 1A). Afterward, participants decided whether the number had been even or odd by pressing the left or right arrow key, respectively, on the keyboard as indicated on the response screen. The keys associated with the respective response were switched block-wise to control for potential motor bias. The parity judgments were a means to ensure that the participants actively processed the numerical values.⁷ The simple parity task was suited to test a broad range of explicit number representations devoid of other cognitive factors (such as working memory) and in short time for the patients.

Numbers were presented in a nonsymbolic format as dot numerosities, as well as symbolically as Arabic numerals. Different protocols per format were used to control for non-numerical visual parameters: dot numerosities were shown in a standard (variable dot size and arrangement) and two control displays (constant total dot area and dot density across numerosities, and linear arrangement); Arabic numerals were shown in four different font types (standard and three controls) (Figures 1B and 1C). The number format and the protocols of the stimuli varied randomly and in equal proportions from trial to trial.

Behavior

Small countable numerosities from 1 to 4 were effortlessly judged with few errors (Figure S1A) and short RTs (Figure S1B), which is indicative of subitizing for small numerosities, whereas numbers 5 and higher were associated with noticeably increasing error rates and RTs as expected for number estimation.^{25–27} However, among the small numbers, judgment of the empty set stood out by showing significantly higher error rates (Figure S1A) and longer RTs (Figure S1B). For symbolic numerals, the participants' performance showed flat response curves (Figure S1A) and RT functions (Figure S1B) suggestive of a unified symbol system for representing small and large numbers. As an exception, numeral zero was judged with the lowest accuracy of all numbers (Figure S1A). These behavioral data suggest that numerosity zero and number zero have a special status on the mental number line.²⁰⁻²² The patients' behavioral performances were replicated in healthy subjects with shorter trials to gain insights into the enumeration process involved. The control subjects exhibited similar behavioral patterns (Figures S1C and S1D), indicating that the patients had a good grasp of the concept of zero and were not counting the dots symbolically.

Neuronal coding of nonsymbolic visual dot numerosities

We analyzed 801 single neurons in the MTL of 17 neurosurgical patients performing the parity judgment task with dot numerosities (for details on anatomical differences, see Table S1; for details on recordings per participant, see Table S2) (parahippocampal cortex, PHC: 109 units; entorhinal cortex, EC: 262 units; hippocampus, HIPP: 275 units; amygdala, AMY: 155 units). As previously reported,^{5,7} the firing rates of many units were systematically modulated by the numerical value of the numerosity stimulus, responding strongest to a preferred numerosity and decreasing their activity progressively with increasing numerical distance. Here, we also found neurons tuned to the empty set (Figures 1E and 1F). Tuning can not only arise due to excitation relative to some baseline activity but also as a result of suppression or inhibition to nonpreferred numbers; the latter scenario is depicted in Figures 1E and 1F. We analyzed these sideband suppression effects in our previous study on countable numerosities and found them to be specific to small numbers.⁷ The new finding here is that neurons were not only tuned to countable numerosities (1-9) but also to the empty set, despite it containing no countable item.

We combined a 2-factor ANOVA with factors "numerical value" (0-9) × "protocol" (standard vs. control) to statistically identify selective neurons, and a separate Mann-Whitney U-test with factor "parity" (even vs. odd) to exclude those neurons responsive to parity judgments (both evaluated at α = 0.01; see STAR Methods). A significant proportion of 15.1% of all units across MTL regions (121/801; p < 0.001, binomial test with $p_{chance} = 0.01$) showed a significant main effect for the factor numerical value but no main effect for factors protocol or parity and were thus identified as exclusively empty-set and numerosity-selective neurons (Figure S2). These neurons responded invariantly to the appearance of the dot patterns. The highest fraction of numerosity-selective neurons was found in the PHC (24/ 109; 22%; ρ < 0.01, binomial test with p_{chance} = 0.01, Bonferroni-corrected for multiple comparisons across brain regions, n = 4), followed by EC (41/262; 15%; p < 0.01), HIPP (42/275; 15%; *p* < 0.01), and AMY (14/155; 9%; *p* < 0.01) (Figure S2). Of those, neurons tuned to the empty set constituted the largest proportion of selective neurons (25/121; 20%) and were overrepresented by a factor of approximately two compared to all other numerosities (Figure 1G). Cross-validation analyses of numerosity tuning functions confirmed tuning to numerical values (Figures S3A and S3C). Preferred numerosities from one-half of the trials were significantly correlated with preferred numerosities from the other half for numerosity-selective neurons.

As a control, we repeated the entire sliding-window analysis, replacing the ANOVA with a linear mixed-effects (LME) regression model. Figure S4A shows the significant overlap of numerosity-selective neurons detected with the two alternative methods, indicating robust numerosity coding. The following analyses are therefore based on the population of ANOVA-selective neurons.

We analyzed the tuning characteristics of selective neurons and calculated population tuning functions of exclusively numerosity-selective units by averaging normalized activity across neurons that preferred a given numerical value. Such neurons' preferred numerosity and tuning curves are reliable and robust, as evidenced by cross-correlation analyses performed in our previous studies with numerosity-selective neurons in the MTL of humans⁵ and the prefrontal cortex of monkeys.²⁸ The resulting overlapping tuning curves showed progressively reduced discharge rates as distance from the preferred number increased (Figure 1H), also for empty-set neurons, reflecting





Figure 1. Behavioral task, example stimuli, and neural responses of single neurons tuned to dot numerosities

(A) Experimental design of the parity judgment task (sequence top to bottom).

(B) Standard and control protocols for nonsymbolic numerosity format, exemplary for numerical values "3" and "7."

(C) Standard and control protocols for symbolic numeral format.

(D) Empty set stimulus and numeral 0 stimuli.

(E) Exemplary neuron tuned to numerosity 0. Large top panels show dot-raster histograms with each row indicating one trial and each dot representing one action potential. Below are corresponding mean instantaneous firing rates as spike-density histograms obtained by averaging responses to each numerosity (smoothed by a 150 ms Gaussian kernel). Colors correspond to the numerical values of the sample stimulus. Gray shaded areas represent significant number-discrimination periods (colorcoded p values above each panel). Small bottom left panels depict a density plot of the recorded action potentials, color darkness indicating the number of overlapping wave forms according to the color scale below. Bottom right panels show the number-tuning functions in the significant trial interval.

(F) Second exemplary neuron tuned to numerosity 0. Layout as in (E).

(G) Proportion of exclusively number-selective neurons tuned to different preferred numerosities.

(H) Normalized average tuning curves of neurons tuned to the ten numerosities.

(I) Average empty set tuning curve (red), compared to random tuning of artificial units (gray). Asterisks represent significant differences between responses to adjacent numerical distances (*p < 0.05, **p < 0.01, ***p < 0.001). Error bars denote SEM. See also Figures S1–S4 and Tables S1 and S2.

significance, likely due to the few neurons

tuned to numerosity 2 resulting in a lack of statistical power). A numerical distance ef-

the well-known numerical distance effect.¹⁶ This finding was confirmed when detecting numerosity-selective neurons via an LME regression model (Figure S4B).

To explore if empty-set neurons encoded the empty set as a quantity adjacent to-and in line with-the smallest countable numerosities, we investigated how empty-set neurons represented countable numerosities 1 and 2. If empty-set neurons represent empty sets as the smallest quantity of the numerical continuum (or number line), such neurons should exhibit a neuronal numerical distance effect by responding systematically less for increasingly higher numerosities. The lack of a neuronal distance effect would be evidence that such neurons simply encode the absence of items as a non-numerical category.⁹ Indeed, empty-set selective neurons showed a significantly higher discharge to numerosity 1 than numerosity 2 (p < 0.05, Wilcoxon signed-rank test) and thus responded to the numerical value adjacent to zero (i.e., 1) more strongly than more remote values (Figure 1I). (For neurons tuned to numerosity 2, a distance effect from numerosity 1 to 0 did not reach statistical fect evidenced by a progressive decay of firing rates with distance from numerosity zero was also observed for empty-set neurons detected via an LME regression model (Figure S4C). The presence of a neuronal numerical distance effect in neurons tuned to sets without elements suggests that the empty set was not encoded as the category nothing as opposed to something, but as quantity zero that is continuous with progressively higher numerical values on the number line.

To analyze the coding dynamics of the population of numerosity-selective neurons, we conducted a multi-dimensional statespace analysis. Each dimension within the neural state space represents the activity of a single neuron (n = 121). Trajectories that traverse this space then reflect the time-varying activity of the neuronal population in response to different number conditions (Figure 2A).

Calculating the Euclidean distance between pairs of trajectories revealed that numbers were represented with increasing spatial gaps according to ordinal numerical distances during the sample and delay phases (Figures 2B and 2C). This trend

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Figure 2. Population dynamics for numerosity-selective neurons based on state-space analysis and k-means clustering

(A) Averaged state-space trajectories of numerosity-selective neurons (n = 121) for all number conditions, reduced to the three principal dimensions for visualization. Each trajectory depicts the temporal evolution in the time window -300 to 1,200 ms (stimulus onset to 100 ms after delay offset). Circles indicate boundaries between experimental periods (Fix., fixation; Smpl., sample; Del., delay; Resp., response).

(B) Intertrajectory distances between countable numbers 1–9, averaged across pairs of trajectories with the same numerical distance.

(C) Intertrajectory distances relative to the empty set.

(D) Neural states, reduced to the two principal dimensions, after averaging firing rates per trial across the sample period. Different colors correspond to different number conditions. Each dot represents one trial; squares and ellipses indicate condition mean and covariance ellipse per condition, respectively. The lower panel depicts neural states only for small numbers 0–4 subjected to a k-means clustering analysis. The colors of the dot outlines (black for empty set or white for countable number) indicate the class label assigned by the k-means classifier. The black and white crosses show the centroids of each class.

(E) Evaluation of different numbers of clusters for the k-means clustering using the Caliński-Harabasz criterion (blue) and the gap criterion (orange), which is also defined for clustering solutions containing only one cluster. Data are presented as mean values; error bars denote SEM of cross-validations. Asterisks indicate the optimal number of clusters.

(F) Proportions of trials per small number condition labeled as belonging to class "empty set" (black) or class "countable numbers" (white). Data are presented as mean values; error bars denote SEM of cross-validations.

was observed not only for countable numbers 1–9 (Figure 2B) but also for the empty set (Figure 2C), again suggesting that the zero quantity was represented as the smallest element on the number line. As this analysis involves smoothing of firing rates, the time course of the dynamics is blurred (Figure 2B). Note that visual response latencies of MTL neurons are generally in the range of 400 ms,²⁹ suggesting that the divergence of number trajectories relates to number representations during the sample period.

We had previously demonstrated that small numbers up to 4 within the subitizing range and large numbers 5 to 9 were spatially segregated from each other within this state space.⁷ As a new discovery, the current analysis shows an additional

significant gap between the empty set and countable numbers 1 to 4. We statistically quantified this graphical grouping effect between the empty set and small numerosities 1 to 4 by performing an unsupervised clustering analysis using the average firing rates of numerosity 0 to 4 trials during the sample period. Figure 2D depicts the two most meaningful dimensions of this neural state space, obtained via principal component analysis. We determined the optimal number of clusters that divide our data best into distinct categories using the Caliński-Harabasz (or variance ratio) criterion defined for clustering solutions containing two or more clusters, as well as the gap criterion that is defined for one or more clusters. Both measures indicated two clusters as the optimal cluster number (Figure 2E). Based on



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Figure 3. Neural responses of single neurons tuned to symbolic numerals

(A) Exemplary neuron tuned to Arabic numeral 0. Large panels show dot-raster histograms and corresponding mean instantaneous firing rates. Gray shaded areas represent significant number-discrimination periods (color-coded *p* values above each panel). Small bottom left panels depict a density plot of the recorded action potentials. Bottom right panels show the number-tuning functions. Same conventions as in Figures 1E and 1F.

(B) Second exemplary neuron tuned to numeral 0. Layout as in (A).

(C) Proportion of neurons tuned to different preferred numerals.

(D) Normalized average tuning curves of units tuned to the ten numerals.

(E) Average "zero" numeral tuning curve (red), compared with random tuning (gray). Asterisks represent significant differences between responses to adjacent numerical distances (* $\rho < 0.05$, ** $\rho < 0.01$, *** $\rho < 0.001$). Error bars denote SEM.

See also Figures S1, S3, S5, and S6.

this, the algorithm partitioned the state space into one cluster comprising the empty set trials and a second one containing trials of countable numbers 1 to 4. This objectively determined boundary in neuronal population activity argues for distinct representation of the empty set (or numerosity zero) among small numerosities.

Neuronal coding of symbolic visual Arabic numerals

We repeated the same neuronal analyses using ANOVAs when participants judged the parity of symbolic Arabic numerals. Significantly more MTL neurons than expected by chance were selectively responsive to symbolic numerical values of the sample stimulus (15/801; 1.8%; p < 0.05, binomial test with $p_{chance} = 0.01$; Figure S3), including neurons that were tuned to numeral 0 (Figures 3A and 3B). These neurons responded invariantly to the font type of the numerals. Indeed, the highest fraction of units was tuned to the numeral 0 (6/15; 40%) (Figure 3C). Cross-validation analyses of numeral tuning functions confirmed that preferred numbers from one-half of the trials were significantly correlated with preferred numerosities from the other half across individual numeral-selective neurons (Figures S3B and S3D).

Similar counts of numeral-selective neurons were obtained when using an LME regression model, with overlaps between ANOVA- and LME-determined selective neurons (Figure S6A). Figure 3D displays the average normalized tuning functions of numeral 0-selective neurons in comparison to other numeral-selective neurons. Similar population tuning functions were obtained when detecting numerosity-selective neurons via an LME regression model (Figure S6B). The following analyses are based on the population of ANOVA-selective neurons.

When we analyzed the tuning functions of neurons tuned to numeral 0 (in the same way as for the empty-set neurons before), we again observed a gradual decay of normalized activity with increasing numerical distances toward numeral 1 and 2 (p < 0.05, Wilcoxon signed-rank test; Figure 3E). A comparable decay of activity from zero to higher numerals was seen when using the LME model but without significant differences due to the low number of zero neurons (Figure S6C). This reflects a mild neuronal numerical distance effect in neurons tuned to numeral 0 and shows that symbolic representation of zero is represented as a number value at the low end of the symbolic number line. This aligns with human discrimination behavior, where a distance

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Figure 4. Population dynamics for numeral-selective neurons based on state-space analysis and k-means clustering (A) Averaged state-space trajectories of numeral-selective neurons (*n* = 15) for all number conditions, reduced to the three principal dimensions for visualization. Same conventions as in Figure 2A.

(B) Intertrajectory distances between countable numbers 1–9, averaged across pairs of trajectories with the same numerical distance.

(C) Intertrajectory distances relative to the zero numeral.

(D) Neural states, reduced to the two principal dimensions, after averaging firing rates per trial across the sample period. Same conventions as in Figure 2D.

(E) Evaluation of different numbers of clusters for the k-means clustering. Same conventions as in Figure 2E.

(F) Proportions of trials per small number condition labeled as belonging to class "zero" (black) or class "countable number" (white). Same conventions as in Figure 2F.

effect persists for number symbols, albeit to a lesser extent compared with nonsymbolic numerosities.^{30,31}

Since both the nonsymbolic and numeral protocols were tested in the same recording sessions, we could compare neurons tuned to both formats. The product of the proportions of neurons selective to each format (121/801 numerosity-selective neurons and 15/801 numeral-selective neurons, $p_{chance} = 121/$ $801 \times 15/801 = 0.0028$) provides the probability that a given neuron responds selectively to both formats. The number of neurons selective to both formats (i.e., 5 out of 801 numeral-selective neurons were also numerosity selective) was not significantly greater than chance (p = 0.0795, binomial test with $p_{chance} = 0.0028$). Importantly, only two out of these five neurons showed identical preferred numerical values for both formats. We repeated this analysis with all number-selective neurons, irrespective of additional main effects for protocol or parity in the ANOVA (129/801 numerosity-selective and 21/801 numeral-selective neurons), and found that 10 neurons responded to both formats, more than expected by chance (p = 0.0025, binomial test with $p_{chance} = 0.0042$); however, only two out of these ten neurons showed identical preferred numerical values for both formats. Overall, it seems unlikely that format-independent number detectors exist in MTL.

In line with our investigations for the nonsymbolic format, we conducted a multi-dimensional state-space analysis to delve into the coding dynamics of the population of numeral-selective neurons (n = 15). During fixation, when numerical information was absent, trajectories were intermingled, but they notably diverged during the sample period when numerals were presented (Figure 4A). For positive integers, trajectory distances systematically increased with numerical distance, indicating a clear numerical distance effect (Figure 4B). Remnants of this effect were also observed for distances relative to the numeral 0 (Figure 4C).

The ordinal representation of symbolic numerals is also discernible in the neural state space during the sample period



(Figure 4D). Although a notable gap between zero and countable numbers suggests distinct categories, akin to what was observed for small numerosities, the optimal cluster number calculated for the clustering analysis was one (Figure 4E). Consequently, trial labels vary unsystematically for all numbers when the algorithm is forced to partition the data into two clusters (Figure 4F). This suggests that symbolic numeral zero, as the smallest element on the symbolic numerals. This corresponds with the behavioral finding that judgments of symbolic numeral zero and other symbolic integers are exact and rarely mixed up.³²

DISCUSSION

Our single-neuron recordings in the MTL of human participants resulted in three main findings. First, we observed neurons tuned to both nonsymbolic and symbolic zero. This extends our previous findings that neurons in this brain region respond to nonsymbolic countable numerosities $1-9^7$ and symbolic positive numerals 1-5.⁵ In fact, the highest proportion of selective neurons responded to zero values, both in the nonsymbolic and in the symbolic format.

Second, these neuronal representations of zero number values were integrated at the low-quantity end of the number line. Selective neurons encoded the empty set and symbolic numeral zero not as a distinct nothing category (i.e., the absence of items) but as a quantity adjacent to countable positive integers along the number line.^{8,9} This is evidenced by both empty-set neurons and numeral-zero neurons exhibiting a neuronal numerical distance effect, which is reflected both in single-neuron-tuning curves as well as in neuronal population decoding analyses. Empty-set neurons in the human brain mirror findings observed in the prefrontal cortex and intraparietal sulcus of nonhuman primates³³⁻³⁵ as well as in the telencephalic nidopallium caudolaterale of crows³⁶ and deep learning neural networks.³⁷ These findings suggest an evolutionary predisposition of various brain networks to represent nothingness as a numerical quantity, serving as a potential evolutionary precursor for symbolic zero representations unique to humans.⁹

Third, despite this clear integration of zero on the number line, nonsymbolic empty-set representations remain distinctly encoded from other small numbers. This conclusion is based on coding boundaries between numerosity zero and other small numbers that we discovered when performing neuron population decoding analyses. This representational boundary correlated with the behavioral dichotomy between numerosity zero and higher numerosities seen in the participants' judgments.⁷ This latter finding suggests that symbolic number representations can reconcile the outlier role of number zero on the number line.

While the MTL, where we discovered numerosity- and numeral-selective neurons, is classically not regarded as part of the core number network, neuroimaging studies have increasingly implicated the MTL in numerical information processing.^{38,39} Additionally, a recent fMRI study identified map-like representations for numerosities and numerals in the temporal-occipital cortex.⁴⁰ A region in the posterior inferior temporal gyri, known as the "number form area," has shown selectivity

ber representations in the temporal lobe are influenced by, or rely on, their functional connectivity with classical number-related

to number symbols over letters and false fonts.^{41,42} These num-

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areas in the parietal and frontal regions, ^{43,44} even in the absence of visual input, ⁴⁵ seemingly inheriting semantic number information. Suggestive evidence of numerosity tuning also exists in the macaque HIPP.⁴⁶ Our study adds to mounting evidence of the critical role of temporal regions in representing numerical information, including concepts of zero.

Recently, neural correlates of zero in the human brain have been measured using magnetoencephalography (MEG),⁴⁷ which detects the collective magnetic fields generated by the synchronized electrical activity of large groups of neurons. Similar to our findings from single-neuron recordings, the MEG representations of zero were positioned along a graded neural number line shared with other countable numbers. These representations of zero, which are format-independent and generalize from empty sets to symbolic zero, were localized across the posterior association cortex. This suggests that neuronal representations of zero are widely distributed within the broader number network of the human brain.⁴⁸

RESOURCE AVAILABILITY

Lead contact

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

Materials availability

This study did not generate new, unique reagents.

Data and code availability

Data and analysis software for this paper are available from GitHub: https://github.com/EstherKutter/Single-Neuron-Representation-Of-Nonsymbolic-And-Symbolic-Number-Zero-In-The-Human-MTL.

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AUTHOR CONTRIBUTIONS

A.N., E.F.K., and F.M. designed the study; F.M. and R.S. recruited patients; V.B. and F.M. implanted the electrodes; E.F.K. and G.D. collected the data; E.F.K. and A.N. analyzed the data with contributions from F.M.; A.N., E.F.K., and F.M. wrote the paper. All authors discussed the results and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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 can be found online at https://doi.org/10.1016/j.cub.2024.08.041.

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STAR*METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Cheetah software	Neuralynx Inc.	https://neuralynx.com/software/cheetah
Pegasus software	Neuralynx Inc.	https://neuralynx.com/clinical-overview/clinical-products/ pegasus-software/
Combinato spike sorting software	Niediek et al. ⁴⁹	https://github.com/jniediek/combinato
MATLAB R2017a	MathWorks	https://de.mathworks.com/
Psychtoolbox	Psychophysics Toolbox Version 3	http://psychtoolbox.org/
Data and Code	github.com	https://github.com/EstherKutter/Single-Neuron-Representation- Of-Nonsymbolic-And-Symbolic-Number-Zero-In-The-Human-MTL
Other		
Behnke-Fried depth electrodes	AD-TECH Medical Instrument Corp.	https://adtechmedical.com/depth-electrodes
ATLAS neurophysiology system	Neuralynx Inc.	https://neuralynx.com/news/techtips/atlas-neurophysiology- system-for-cogneuro-applications

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Seventeen human participants (five male, mean age 37.6 years) with medically refractory focal epilepsy undergoing invasive pre-surgical seizure monitoring participated in the main study. A control experiment was performed with nineteen healthy volunteers (three male, mean age 30.2 years). Informed written consent was obtained from each participant. All studies conformed to the guidelines of the Medical Institutional Review Board at the University of Bonn, Germany. The study was not preregistered and the analysis plan was exploratory.

METHOD DETAILS

Experimental task and stimuli

Participants sat in bed and performed a parity judgment task on a laptop (display diagonal 11.7 in, resolution 1366x768 pixels) on which stimuli were presented at a distance of approximately 50 cm.

Prior to the experiment, the task instruction was verbally explained by the experimenter and displayed on the screen, specifying which numbers were 'even' and which ones 'odd'. Additional training trials (excluded from all analyses) during which the experimenter emphasized, once more, that an empty gray circle represented the 'number zero' and that zero was an even number ensured that participants were familiar with all stimuli. To exclude any bias, they were not informed about hypotheses or purposes of the experiment.

Each trial started with a fixation period of 300 ms, followed by a number stimulus that was presented for 500 ms. After a delay period displayed for 600 ms, participants had to decide whether the number had been even or odd by pressing the left or right arrow key on the keyboard, respectively, as indicated on the response screen ('gerade' [even] or 'ungerade' [odd]). The keys associated with the respective response were balanced and switched across blocks to control for potential motor bias. Participants responded in a self-paced manner, but were asked to respond as fast and accurately as possible. The next trial started automatically after a 200 ms feedback display.

Three factors were varied systematically in this task: 'numerical value' (0–9), 'format' (nonsymbolic and symbolic numerals), and 'protocol' (standard and controls). A session was divided into four blocks, comprising all conditions in pseudo-random order. With each number stimulus per format being presented 16 times, each session comprised 320 trials.

Stimuli were presented within a filled gray circle (diameter approximately 6° of visual angle) on a black background. A white fixation spot was presented in the center of the gray area during fixation and delay phase. During stimulus presentation, the fixation spot was removed to avoid confusion with nonsymbolic stimuli.

Numerical values of the stimuli ranged from 0 to 9, and were presented in two different 'formats', each using different 'protocols' to control for low-level visual features. First, 'nonsymbolic' stimuli consisted of black dot arrays with the number of dots corresponding to the respective numerical value ('numerosities'). For the standard protocol, diameter and location of each dot varied randomly within a given range (diameter 0.3° to 0.8° of visual angle). In the control displays, the total dot area and dot density (mean distances between centers of the dots) across numerosities was equated. Additionally, in half of the control trials, the dots were linearly arranged.



We incorporated control stimuli to maintain constant total area and dot density across different numerosities. This ensured that the neurons' responses were not driven by features that would otherwise systematically change with an increase in the number of dots. However, controlling for total area results in smaller dots with increasing numerosities. To address this, we implemented the standard protocol where the average dot size remained consistent across numerosities. Furthermore, we introduced a control condition where all dots were arranged linearly to rule out the possibility that neurons were responding to shape configurations (e.g., three dots forming a triangle, four dots forming a quadrilateral, etc.). Standard and control protocols for the nonsymbolic stimuli were shown with equal probability of 50 %.

Second, 'numerals' were presented as black Arabic digits in the center of the gray area. For standard and control protocols, four different font types were used (Arial, DS-Digital, Times New Roman and Courier New; all 45 pt), each protocol type being equally likely to occur (25 %). For single digit numerals, including the numeral 0, there is no systematic variation in luminance, area, or density because each numeral comprises a single shape. Hence, there is no requirement for incorporating control stimuli to address low-level feature variations. However, we introduced different font types to ensure that neurons generalized their responses from specific numeral shapes to their associated numerical values.

To ensure that subjects had a good grasp of the concept of zero, we performed a control experiment on 19 healthy volunteers, recording behavioral measures in a simplified version of the parity judgment task. Trials started with a fixation period of 300 ms, followed by a number stimulus presented for 500 ms.

To prevent counting and covert decisions, the delay period was excluded from the control experiment. Instead, subjects were asked to indicate the parity of the stimulus as fast and accurately as possible after stimulus offset and onset of the response screen, by pressing the left or right arrow key on the keyboard, respectively. As in the main experiment, key presses made before the presentation of the response cues were not registered. RTs were measured relative to the onset of the response cues. Verbal instructions and familiarization trials prior to the experiment, as well as all stimuli and factor variations (numerical value, format, and protocol) were analogous to the main study.

Neurophysiological recording

To localize the seizure-onset zone for possible neurosurgical resection, participants were implanted bilaterally with chronic intracerebral depth electrodes in the MTL. The implantation site and number of the electrodes was determined exclusively by clinical criteria and varied across participants. Neuronal signals were recorded using 9–10 clinical Behnke-Fried depth electrodes (AD-Tech Medical Instrument Corp., Racine, WI). Each of these electrodes contained a bundle of platinum-iridium micro-wires; eight high-impedance active recording channels, and one low-impedance reference wire that protruded from the tip of the electrode by approximately 4 mm. We used a 256-channel ATLAS neurophysiology system (Neuralynx Inc., Bozeman, MT) for recording differential neuronal signals (recording range $\pm 3200 \ \mu$ V), filtering (bandwidth 0.1–9,000 Hz), amplification and digitization (sampling rate 32768 Hz). The Cheetah and Pegasus software (Neuralynx Inc., Bozeman, MT) was used to synchronize recorded spikes and behavioral data via 8-bit timestamps.

After band-pass filtering the signals (bandwidth 300–3,000 Hz), action potentials were automatically detected and pre-sorted using our Combinato software.⁴⁹ We manually corrected the automated clustering and classified resulting units as artifact, multi- or single unit, based on spike shape and its variance, inter-spike-interval distribution per cluster and the presence of a plausible refractory period. Only units that responded with an average firing rate of > 1 Hz during stimulus presentation for any stimulus format were included in the analyses. Across 28 recording sessions from all 17 participants, a total of 801 single units were identified in the parahippocampal cortex (PHC; 109 units), entorhinal cortex (EC; 262 units), hippocampus (HIPP; 275 units) and amygdala (AMY; 155 units).

QUANTIFICATION AND STATISTICAL ANALYSIS

In a previous study, we showed that different stimulus formats are represented by distinct groups of neurons.⁵ Thus, all analyses were carried out separately for trials of each format. Overall behavioral performance was high across all participants (mean \pm standard deviation: 86.4 % \pm 3.1 %). We therefore decided to include both correct and incorrect trials into the analyses.

Sliding-window ANOVA analysis

For each unit, spike trains were smoothed trial-wise (Gaussian kernel with $\sigma = 150$ ms) within the trial window -300–1200 ms (fixation onset to 100 ms after delay offset). To detect tuning to numerical values, instantaneous firing rates were subjected to a sliding-window (window size 300 ms; step size 20 ms) 2-factor analysis of variance (ANOVA) with factors 'numerical value' (0–9) and 'protocol' (standard vs. control), and a separate Mann-Whitney U-test with factor 'parity' (even vs. odd). This resulted in a temporal sequence of *p*-values for each of the three factors. Time intervals of significant number encoding were then identified using a cluster-permutation test ($\alpha_{clus} = 0.01$; $p_{rank} = 0.01$; $n_{perm} = 100$).⁵⁰ A neuron was then termed 'exclusively number-selective' (NUM-ONLY) if a significant time window for the factor 'numerical value' was observed between 0–1000 ms (stimulus onset to 100 ms before delay offset) and if there were no overlapping significant intervals for the factors 'protocol' or 'parity'. Proportions of these number units were determined separately for each MTL region. To evaluate whether the observed proportions were higher than expected by chance, we applied a binomial test ($\rho_{chance} = 0.01$), Bonferroni-corrected for multiple comparisons across brain regions (n = 4).



In single-cell neurophysiology, the firing of a single neuron is typically regarded as independent from other neurons, thanks to the concept of neuronal autonomy. This principle suggests that an individual neuron's activity is mainly governed by its intrinsic properties and the inputs it receives, rather than being influenced by the activity of other neurons in the network. In addition, single trial recordings are treated as statistically independent observations in single-neuron studies because each trial represents a distinct instance of neuronal activity, influenced by stochastic processes and variability in the system. Due to the concept of neuronal autonomy, many studies, including ours, have assessed the selectivity of single neurons based on their individual firing rates in response to parametric variations of numerosity. ANOVA analyses have emerged as a common and widely accepted method for determining whether a neuron selectively responds to specific stimulus features.

As a control, we repeated the entire sliding-window analysis replacing the ANOVA with a linear mixed-effects (LME) regression model of the form $FRs \sim NUM + PROT + PAR$ (Wilkinson notation), thus testing for the main effects of numerical value, protocol and parity, including a fixed effect for the intercept. Time intervals of significant number encoding were then identified based on a cluster-permutation test analogous to the one described above. Proportions of exclusively number-selective neurons were then compared with the ones obtained via the ANOVA using Venn diagrams.

Tuning characteristics

Tuning functions were calculated for each number neuron by averaging the firing rates across trials for different numerical values during the significant time window. The numerical value eliciting the maximum response was defined as 'preferred number'. Functions were then normalized by setting the lowest response rate to 0 %, and the highest response rate (to the preferred number) to 100 %. Population tuning functions were then obtained by averaging across all units that preferred the same number.

To estimate the reliability of the preferred number assessment, we split the data of each selective neuron into two halves (odd and even trials), calculated the preferred number for the two datasets and quantified the relationship calculating Pearson's linear correlation coefficient.

Activity of all neurons preferring the numerical value '0' was considered as a function of numerical distance from the preferred number. Pairs of response activity to adjacent numerical values were then separately compared using Wilcoxon signed-rank tests. Moreover, we used a permutation test ($n_{perm} = 1000$) to generate response patterns to be expected in case of random tuning (repeating the analysis with shuffled labels) and tested whether the true response obtained for a specific numerical distance differed significantly from chance.

Population state-space analysis

To analyze activity of a neuronal population, we calculated the trajectories for the ten different number conditions that traverse the neuronal state-space over time. In this *n*-dimensional space (in our case, 121-dimensional for the subpopulation of numerosity-selective neurons, and 15-dimensional for the subpopulation of numeral-selective cells) each axis represents the instantaneous firing rate of one neuron. These were obtained by averaging, normalizing (z-scoring) and smoothing spike trains per condition (Gaussian kernel with $\sigma = 150$ ms) for each unit. Solely for visualization, trajectories were reduced to the top 3 dimensions (in terms of covariance explained) using a Gaussian-process factor analysis.⁵¹

To evaluate population tuning in terms of numerical distances, we calculated the Euclidean distances between all adjacent pairs of trajectories. For countable numbers, we then averaged all trajectory pairs with the same numerical distance.

Next, we calculated the neuronal population state for all trials by averaging firing rates across the sample period (window size 500 ms; shifted by 200 ms to account for response latency). The state space was then orthonormalized using principal component analysis. Solely for visualization, only the top 2 dimensions were depicted.

To investigate the status of the 'zero' quantity within the range of small numbers, we subjected all trials for small numbers 0-4 to an unsupervised *k*-means clustering algorithm.⁵² In a first step, we determined the optimal number of clusters that would partition the data best into *k* non-overlapping, distinct clusters applying two different criteria. First, the Caliński-Harabasz criterion (or variance ratio criterion, VRC) according to which the optimal number of clusters corresponds to the solution with the highest ratio between overall between-cluster variance and overall within-cluster variance.⁵³ As a second and even more important criterion, as it is also defined for solutions containing only one cluster, we calculated the gap criterion which estimates the heuristic 'elbow' location at the most dramatic decrease in error measurement that indicates the optimal number of cluster and every cluster's centroid) as a distance metric, and repeating the algorithm 50 times with new randomly chosen initial cluster centroid positions. For cross-validation, the analysis was repeated 50 times, each time using only 75 % randomly selected trials per condition.