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DYNAMICS OF THE FORMATION OF SPATIAL SPECIALIZATIONS OF MOUSE HIPPOCAMPAL NEURONS: STUDIES USING NEW HIGHLY SENSITIVE GENETICALLY ENCODED CALCIUM SENSORS

PhD dissertation summary

for the purpose of obtaining academic degree

Doctor of Philosophy in Cognitive Science

Academic advisor: Konstantin V. Anokhin,

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The PhD dissertation was prepared at the Institute for Advanced Brain Studies of the Federal State Budget Educational Institution of Higher Education M.V.Lomonosov Moscow State University.

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CONTENTS

GENERAL CHARACTERISTICS OF RESEARCH

Dissertation topic

The formation of stable neural representations of the environment has traditionally been the focus of neuroscience. It is known that individual neurons can be specialized in relatively complex concepts, such as a mouse nest [Lin L. et al., 2007] or movie characters in humans [Quiroga R. et al., 2005]. At the same time, cognitively specialized neurons perform complex integration of sensory information of various modalities. An important example of such integration is the spatial specialization of place neurons (place cells) in the field CA1 of the hippocampus [O'Keefe J., Dostrovsky J., 1971].

The long-term stability of representations of place cells (aka place fields) is well studied. Thus, in the work of Ziv and Schnitzer [Ziv Y. et al., 2013] it was shown that the population of neurons providing stable spatial representations underwent gradual changes in its composition in several weeks. At the same time, the place fields themselves were preserved, but they were provided by a slightly different set of place cells. In addition, it was shown [Rubin A. et al., 2015, Sheintuch L. et al., 2020] that place neurons can provide multiple cognitive maps for different environments simultaneously for a long time.

At the same time, data on how quickly the spatial specialization of place cells is formed still remains fragmentary and contradictory. Thus, in a number of electrophysiological studies on rats [Hill et al., 1978; Muller et al., 1987; Wilson, McNaughton, 1993] it was shown that place fields are formed during the first few minutes that rats spend in a new environment. In a seminal work [Ulanovsky et al., 2011], conducted on bats using echolocation to navigate in space, it was suggested that place cells form a specialization within a few milliseconds, based on data on a rapid change in the spatial information of such neurons. However, this approach cannot be directly applied in the case of continuous acquisition of information about the environment during the navigation of non-echolocating animals. In addition, a study [Hill et al., 1978] showed that most of

the registered place cells (10 out of 12) begin to show spatially selective activation from the very first visit of the animal to the corresponding place fields. On the other hand, in a recent study [Dong et al., 2021] it was shown that in the hippocampal CA1 field, the rate of place cells forming their place fields during the very first visit to the corresponding places reached 30%, while in the hippocampal CA3 field, this proportion was about 10%.

In this work, by means of miniscopic calcium imaging, the main parameters of the formation of hippocampal cognitive maps in mice were measured and characterized, starting from the very first moments of the animal's navigation in a new environment in the form of a one-dimensional circular track. For this purpose, a comparative analysis of new genetically encoded calcium indicators NCaMP7 and FGCaMP7, developed specifically for miniscopic imaging, was carried out.

It was found that a significant (25%) part of the registered place cells forms their place fields from the very first visit, while on average it takes them several minutes to form the place field. It has also been shown that with repeated navigation sessions in the same environment, cognitive maps are formed faster, regardless of how much they coincide with the cognitive maps of the first session. Similar dynamics were observed when mice navigated in a two-dimensional "open field" type arena with a variable number of obstacles. The obtained results were verified using population analysis: a consistency was observed between the average dynamic selectivity of the place cells and the mean accuracy of the reconstruction of the trajectory of animals from neural activity data performed by means of dimensionality reduction.

Purpose and objectives of research

The purpose of this work was to study and describe quantitatively the dynamics of the formation of spatial selectivity of place neurons in the CA1 field of the hippocampus of freely behaving mice exposed to a novel environment, and then in subsequent navigation sessions in this environment, as well as to compare the dynamics of the

formation of spatial specialization of neurons in different environments. To achieve this goal, the following tasks were set:

1. Analyse and compare the response characteristics of various calcium indicators at the spontaneous activity of CA1 hippocampal neurons of freely behaving mice.

2. Characterize the main parameters of the dynamics of the spatial selectivity of place cells both during the first navigation session in a new environment and during subsequent navigation sessions in the same environment.

3. Compare these parameters for navigation sessions in two environments of different types: one-dimensional and two-dimensional.

4. Verify the results obtained using population analysis.

Scientific novelty of research

In the present work, the formation of spatial specializations of CA1 hippocampal place cells in free, non-reinforced exploration task in a novel environment was described and characterized in detail for the first time both during the first navigating session in the novel environment itself and during repeated sessions in the same environment. Then, a criterion for assessing the spatial selectivity of place cells, based not only on the total spatial statistics of neuron activation but also on data on repeated activations of place neurons in their putative place fields, was applied for the first time. Also, in the course of the analysis of neural activity data, a method for identifying significant calcium events, which makes it possible to evaluate their amplitude and temporal parameters, was developed and applied for the first time. In addition, the successful use of the new genetically encoded calcium indicators NCAMP7 and FGCaMP7, designed specifically to visualize the calcium activity of neurons in awake mice using miniscopes, was demonstrated for the first time.

Theoretical and practical significance of research

In the present study, specific values of the parameters of the dynamics of the formation of place cell specialization in new and familiar environments were evaluated. In

particular, a significant number of “instant” place cells that acquire specialization immediately, from the first animal’s visit to the corresponding place field, were identified. Such results are of great theoretical significance since they actualize questions about the cellular mechanisms of the formation of the specialization of place cells, as well as about the influence of specific acts of behavior on the specialization of neurons.

The results of this work concerning the comparison of the new calcium sensors NCaMP7 and FGCaMP7 with the “traditional” GCaMP6s sensor are of significant practical importance: their successful application for miniscopic imaging of mice in the free navigation task was demonstrated for the first time.

In addition, the dynamic selectivity proposed and verified in this work as a characteristic of the spatial selectivity of neurons can be used not only to assess the selectivity of place cells but also to assess the selectivity of neurons with respect to other stimuli and animal behavioral states.

Propositions to be defended

The new genetically encoded calcium sensors NCaMP7 and FGCaMP7 exhibit parameters of the average spatially relevant calcium event comparable to the conventional calcium sensor GCaMP6s, but have potential advantages. Namely, the spatial conformation of both sensors suggests a more efficient binding of sensory subunits to calcium ions in the intracellular space, which makes it possible to reduce the cytotoxic effect of these sensors. Also, the NCaMP7 sensor contains the mNeonGreen fluorescent protein as a fluorescent part, which has a 3 times higher molecular brightness compared to the widely used EGFP fluorescent protein.

While navigating in a new one-dimensional circular track environment, hippocampal cognitive maps in mice stabilized during the first few passages of animals through the environment. At the same time, a significant part (25%) of the place fields formed at the very first visit by an animal. In repeated navigation sessions, cognitive maps stabilized faster: the latency of the specialization of place cells decreased, and the initial gain of

the dynamic selectivity of neurons in the first moments of navigation increased significantly. At the same time, this effect did not depend on the location and presence of place fields of these place cells in the first navigating session.

While navigating in a new two-dimensional environment of the “open field” type with a variable number of obstacles, the parameters of the formation of cognitive maps, similar to the case of a one-dimensional circular track, were observed. At the same time, in the case of a two-dimensional arena, a slower increase in the selectivity of place cells was observed compared to a one-dimensional circular track.

The mean place field selectivity was found consistent with the error of the animal’s trajectory reconstruction obtained from neuronal activity data by means of population analysis using Laplacian eigenmaps. Thus, both of these parameters can be interpreted as a quality metric of the neural encoding of space.

Publications and approbation of research

The content of the dissertation was published in 6 articles in peer-reviewed scientific journals indexed in the Scopus database.

First-tier publications

1. Sotskov, V.P., Pospelov, N.A., Plusnin, V.V. and Anokhin, K.V., 2022. Calcium Imaging Reveals Fast Tuning Dynamics of Hippocampal Place Cells and CA1 Population Activity during Free Exploration Task in Mice. International Journal of Molecular Sciences, 23(2), p.638.
2. Sotskov, V., Plusnin, V. and Anokhin, K., 2021, May. The Rapid Place Field Tuning in Mice Exploring a Novel Environment. In FASEB Journal (Vol. 35). 111 River st, Hoboken 07030-5774, NJ USA: Wiley.
3. Subach, O.M., Sotskov, V.P., Plusnin, V.V., Gruzdeva, A.M., Barykina, N.V., Ivashkina, O.I., Anokhin, K.V., Nikolaeva, A.Y., Korzhenevskiy, D.A., Vlaskina, A.V., Lazarenko, V.A., Boyko, K.M., Rakitina, T.V., Varizhuk, A.M., Pozmogova, G.E., Podgorny, O.V., Piatkevich, K.D., Boyden, E.S. and Subach,

F.V., 2020. Novel Genetically Encoded Bright Positive Calcium Indicator NCaMP7 Based on the mNeonGreen Fluorescent Protein. International Journal of Molecular Sciences, 21, p. 1644.

4. Barykina, N.V., Sotskov, V.P., Gruzdeva, A.M., Wu, Y.K., Portugues, R., Subach, O.M., Chefanova, E.S., Plusnin, V.V., Ivashkina, O.I., Anokhin, K.V., Vlaskina, A.V., Korzhenevskiy, D.A., Nikolaeva, A.Y., Boyko, K.M., Rakitina, T.V., Varizhuk, A.M., Pozmogova, G.E. and Subach, F.V., 2020. FGCaMP7, an Improved Version of Fungi-Based Ratiometric Calcium Indicator for In Vivo Visualization of Neuronal Activity. International Journal of Molecular Sciences, 21, p. 3012.

Other publications

1. Sotskov, V., Plusnin, V., Pospelov, N. and Anokhin, K. The Rapid Formation of CA1 Hippocampal Cognitive Map in Mice Exploring a Novel Environment. In Advances in Cognitive Research, Artificial Intelligence and Neuroinformatics. Intercognsci 2020. Advances in Intelligent Systems and Computing, Velichkovsky, B., Balaban, P., Ushakov, V., Eds.; Springer: Cham, Switzerland, 2021; pp. 452–457
2. V. P. Sotskov, V. V. Plusnin, K. S. Sorokin and K. V. Anokhin, 2022. Rapid tuning dynamics of CA1 place codes in one-and two- dimensional free exploration tasks. Fourth International Conference Neurotechnologies and Neurointerfaces (CNN), Kaliningrad, Russian Federation, 2022, pp. 168-171.

Reports at conferences and seminars

Based on the materials of the work, the author made 11 reports at conferences, including 6 at international ones.

1. FENS Virtual Forum 2020 (online, United Kingdom)
2. Experimental Biology 2021 (online, USA)
3. Volga Neuroscience Meeting 2021 (Nizhniy Novgorod, Russia)

4. XXIX International Scientific Conference for Undergraduate and Graduate Students and Young Scientists "Lomonosov 2022" (Moscow, Russia)
5. FENS Forum 2022 (Paris, France)
6. Baltic Forum: Neuroscience, Artificial Intelligence and Complex Systems, 2022 (Kaliningrad, Russia)
7. XLV Final scientific session “System Organization of Physiological Function” of P.K.Anokhin Institute of Normal Physiology RAS (Moscow, Russia, 2020)
8. XIX Scietific School “Nonlinear Waves – 2020” (Bor, Russia)
9. I National Congress on Cognitive Science, Artificial Intelligence and Neuroinformatics (Moscow, Russia, 2020),
10. XXIV Scientific School-Conference of Young Scientists on the Physiology of Higher Nervous Activity and Neurophysiology (Moscow, Russia, 2021)
11. XLVI Final scientific session “System Organization of Physiological Function” of P.K.Anokhin Institute of Normal Physiology RAS (Moscow, Russia, 2022)

Personal contribution of the author

The planning and design of all the experiments described in dissertation was carried out by the author himself under the guidance of the supervisor K.V. Anokhin. The experiments, as well as the analysis of the data obtained, were carried out by the author at the Institute for Advanced Brain Studies, Lomonosov Moscow State University, in collaboration with A.A. Vorobyev, O.A. Ivleva, V.V. Plyusnin and N.A. Pospelov. The main publications based on the results of the study were written by the author himself.

Structure and scope of dissertation

Dissertation contains: introduction, literature review, materials and methods, results, discussion, conclusion, conclusions, terms list and references list, including references in Russian (8) and English (141) languages. The dissertation is presented on 108 pages and contains 4 tables and 19 figures.

MATERIALS AND METHODS

17 adult male C57Bl6 mice were used in this study. Prior to the main experiments, the animals were sequentially subjected to three operations under anesthesia: intracranial injection of viral particles encoding calcium indicators, implantation of microendoscopes, and installation of a baseplate for chronic connection of miniscopes (Fig. 1).

For three consecutive days with an interval of 24 hours, the animals with a miniscope attached to their heads were placed in a specially designed arena in the form of a circular track 50 cm in diameter with navigation keys for 15 minutes, where their calcium neural activity was imaged while animals freely explored the environment. After 8 weeks, animals 1-4 were subjected to three additional imaging sessions at 24 hour intervals in a circular 2D arena with obstacles. In the first of these sessions, three cylindrical obstacles were present in the arena, in the second, two, and in the third, one.

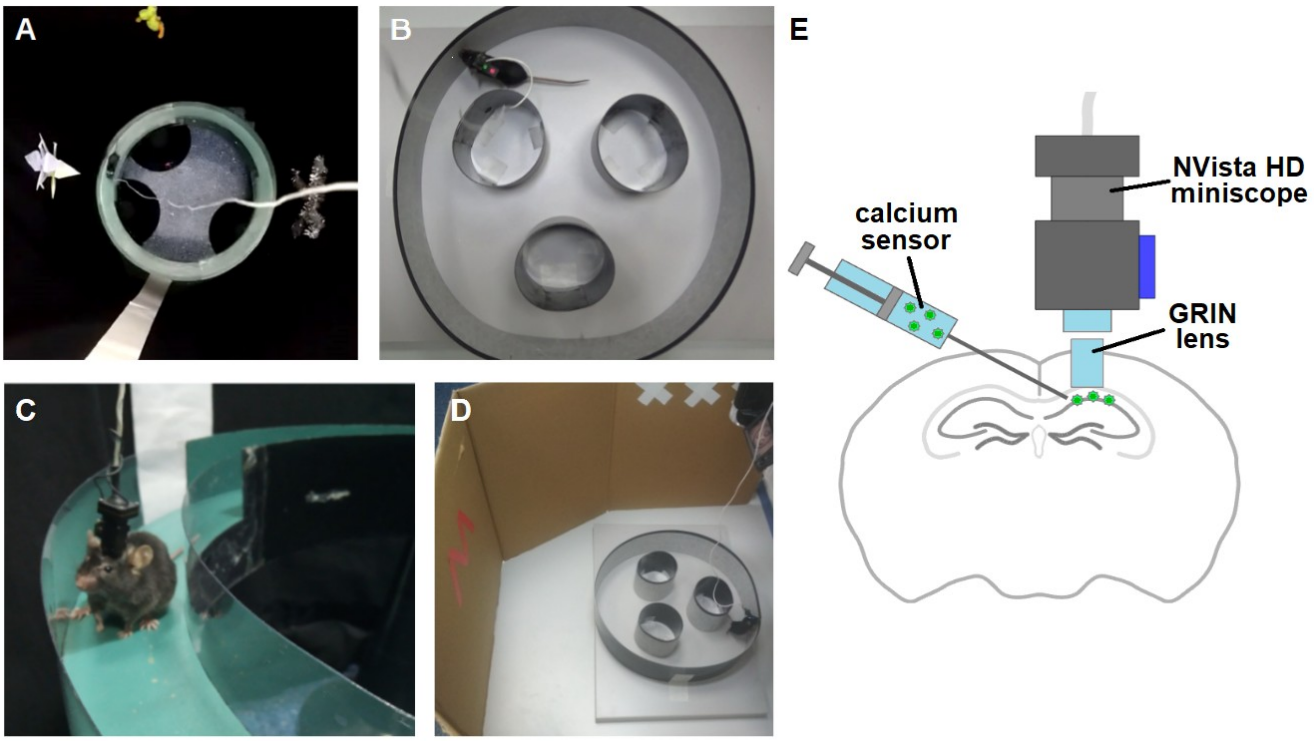

Figure 1. Animals in 1D circular track (**A**, **C**), and in 2D arena with obstacles (**B**, **D**), and a scheme of microinjection of calcium sensors, implantation of microendoscopes, and connection of a miniscope over the CA1 field of the hippocampus (**E**).

After all imaging sessions, histological analysis of brain sections of all experimental animals was carried out. According to the results of the analysis, the compliance of the actual coordinates of the site of expression of the calcium sensor, as well as the trace from implantation of the microendoscope with the declared coordinates, was checked.

An approach based on constrained non-negative matrix factorization (CNMF) was used to localize active neurons and extract time series of their activity [Pnevmatikakis et al., 2016]. Then, significant (exceeding the threshold of 4 median absolute deviations) calcium events were identified from the obtained time series using custom MATLAB scripts developed by the author.

To determine the location and posture of the animals, a pipeline was used in the Bonsai visual programming environment, which consisted of successively applied brightness thresholds in red and green channels and allowed determining the trajectory of the animal's center of mass, as well as the trajectories of colored markers attached to the miniscope.

A conservative two-stage approach was chosen to assess the spatial selectivity of place cells. First, putative place fields were determined for each of the neurons based on spatial activation statistics. Then, for each putative place field, a check was made for the frequency of activation of the corresponding neuron. To achieve this, each time when animal visited a given place field, dynamic selectivity score was calculated as the ratio of the number of calcium events in a given neuron during the time period of visiting the place field to the total number of calcium events for the above period and for adjacent time periods in which the animal was not in any of the place fields of this neuron (Fig. 2). Place fields for which the selectivity score did not reach the 50% threshold during at least three consecutive visits were excluded from further analysis.

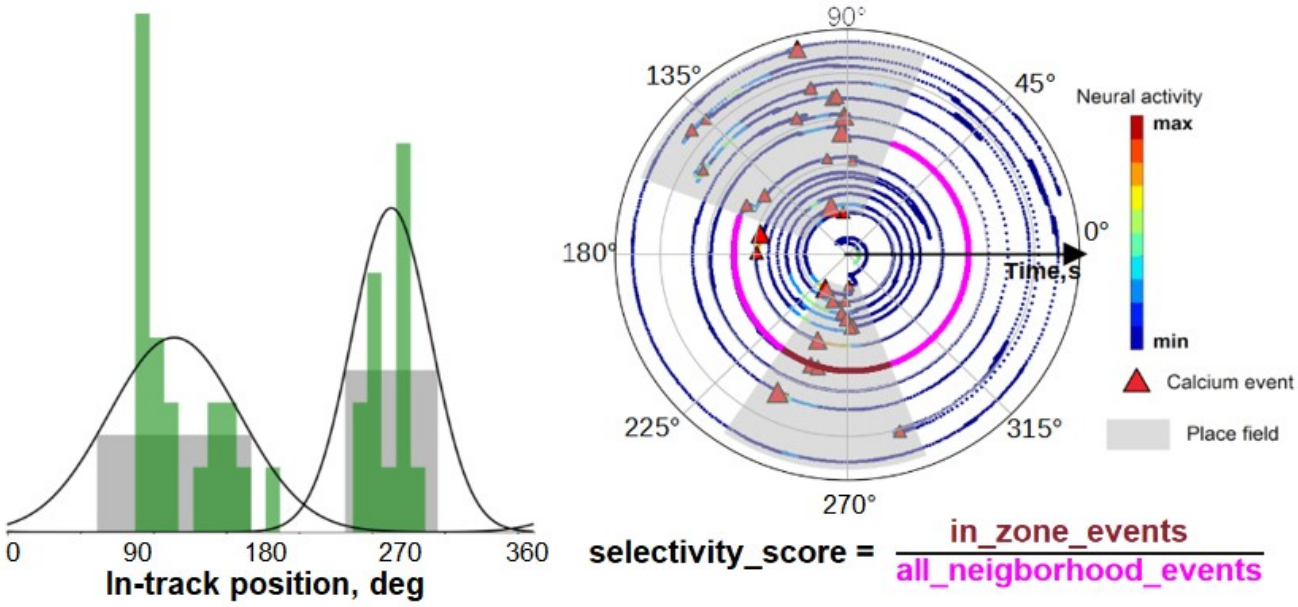

Figure 2. Isolation of spatially selective neurons (place cells). Left: distribution of activations of one of the neurons. Right: the trajectory of the animal in the track, unfolded in time in the radial direction.

To carry out population analysis in this work, the method of Laplacian eigenmaps (LEM) was used, which allows projecting population vectors into a latent space of reduced dimension, determined naturally from the geometry of the original manifold [Belkin M., Niyogi P., 2003].

Residual variance [Tenenbaum J. et al., 2000] between real mouse coordinates and points in the space of reduced dimension was used as a metric for the accuracy of track geometry reconstruction. Then, for similar time points, by linear interpolation, the average selectivity score for each of the animals was calculated. For comparison with the reconstruction error, a value opposite to the selectivity score was taken, namely, the difference between one and the selectivity score. Then, for each of the animals, a cosine similarity measure was calculated between these time series.

KEY RESULTS

1. Comparative analysis of various calcium sensors

The disadvantages of widely used genetically encoded calcium sensors of the GCaMP family of various modifications include high cytotoxicity at high levels of sensor expression, caused by nonspecific interaction of the sensor with the intracellular environment [Qian et al., 2018]. In this study two new sensors are considered, NCaMP7 and FGCaMP7, designed to reduce the cytotoxic effect in various ways.

The N and C terminals of the NCaMP7 sensor molecules are located in the fluorescent part of the sensor, and possible bonds with them do not prevent the capture of calcium ions by the sensor part, while in the GCaMP6s sensor, the open N and C ends fall on the sensor part, which can adversely affect the dynamic range of the sensor and its affinity for calcium. In addition, the mNeonGreen fluorescent protein used in the NCaMP7 sensor has a molecular brightness that is 3 times higher than that of the EGFP protein used in GCaMP [Piatkevich et al., 2019], which makes the design of the NCaMP7 sensor promising in this regard.

The new FGCaMP7 sensor, in turn, solves the problem of nonspecific interaction with the intracellular space in another aspect. It uses calmodulin from the fungi *Aspergillus niger* and *Aspergillus fumigatus* as a sensory part, which excludes the non-specific binding of such calmodulin to the intracellular environment. In a recent work [Barykina N.V. et al., 2017] it was shown that the previous version of the FGCaMP sensor demonstrated free diffusion in the intracellular space, while up to 30% of the expressed GCaMP6s protein was incapable of intracellular diffusion, which can be explained by nonspecific binding of the sensor to other proteins in the intracellular space.

However, the actual performance of the two new calcium sensors described above needed to be verified under conditions as close to naturalistic as possible, that is, in the brains of freely moving animals. At the same time, successful confirmation of the performance of these sensors makes it possible to use them in the following parts of this work.

In this work, the main parameters of the response kinetics of these sensors versus that of one of the most popular calcium sensor GCaMP6s were compared in awake, freely moving animals. To do this, a series of experiments were carried out with miniscope recordings of hippocampal field CA1 neurons of awake mice freely exploring a new environment in the form of a circular track 50 cm diameter with keys for navigation.

The both spontaneous and space-specific activity of neurons was analysed. Since the dynamics of the membrane potential during the spontaneous activity of neurons in free behavior, and, consequently, the frequency of action potentials, can vary greatly and depend on uncontrolled factors, spatially selective activations of neurons were chosen to reliably compare individual calcium events between animals with different calcium sensors.

For analysis, single calcium events were selected across calcium activity traces of all registered neurons, including spatially specific ones (place cells), the onset of which occurred when the animals visited the place fields corresponding to these place cells. Each of these events was fitted by a curve characteristic of calcium events with fast rise and slow decay, and the rise and decay constants themselves, as well as the peak amplitude, were free parameters of this fit. As a result of the approximation, the values of the peak amplitude (in units of $\Delta F/F0$) were obtained, as well as the rise and decay constants of the peak (in seconds), showing the time it takes to rise and decay from half the amplitude to the peak value and vice versa. Individual and averaged forms of calcium events, the values of the above parameters, and a comparison of the amplitudes of spatially selective calcium events are shown in Fig. 3.

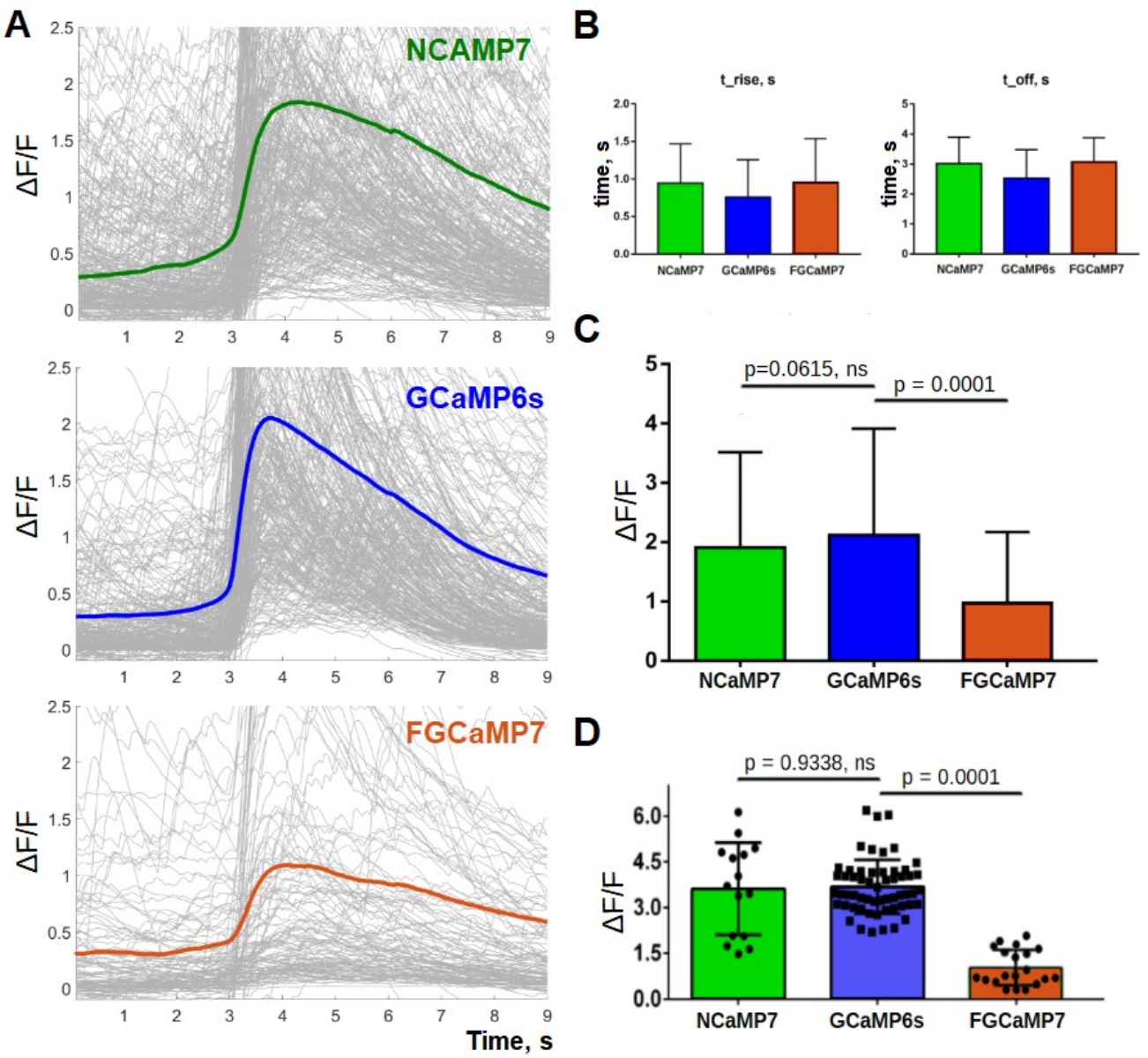

Figure 3. A. Individual (gray) and averaged (color) forms of calcium events for calcium sensors NCaMP7, GCaMP6s, and FGCaMP7. **B.** Comparison of the mean rise (left) and decay (right) constants for all calcium events for all sensors. **C-D.** Comparison of the average values of the peak amplitude ΔF/F0 for all calcium events (**C**) and spatially selective calcium events (**D**) for all sensors.

According to the results of the comparison, calcium events, including spatially selective ones, recorded with the NCaMP7 sensor had a comparable amplitude with events recorded with the GCaMP6s sensor, demonstrating a slower decay and rise of fluorescence. Events recorded using the FGCaMP7 sensor had, on average, a

significantly (by a factor of 2.1, and in the case of spatially selective events, by a factor of 3.7) lower amplitude compared to the analogous parameter for GCaMP6s. At the same time, the dynamics of fluorescence growth and decay for calcium events recorded using the FGCaMP7 sensor turned out to be comparable with those for the NCaMP7 sensor, while remaining slower compared to GCaMP6s.

Nevertheless, taking into account the high dispersion of all observed parameters, the new sensors NCaMP7 and FGCaMP7 in terms of visualization of spatially selective calcium events in neurons were almost as good as the common calcium sensor GCaMP6s, which made it possible to include data obtained on animals with these types of calcium sensors in further analysis of the dynamics of spatial specializations of hippocampal neurons.

2. The main parameters of the dynamics of spatial selectivity of neurons during navigation in a new environment

In this work, a series of experiments was carried out to assess the rate of formation of spatial specializations of place neurons. Using NVista HD miniscopes, the activity of neurons in the field CA1 of the hippocampus of freely moving mice was imaged during three sequential, 24h apart, 15-minute sessions of free exploration of a new environment (a circular track with navigation cues).

For each of the registered neurons with preferred areas of activation in the track space (place fields), the selectivity score was calculated, reflecting the rate of relevant activations of such a neuron at each visit by the animal to the corresponding area, as well as the time point (and number of the visit), starting from which the neuron can be considered selective (specialization latency).

Selectivity score and specialization latency were averaged over all place cells in all animals and analyzed between sessions (Figure 4). During each of the imaging sessions, the average selectivity of place neurons increased significantly; at the same time, the initial levels of average selectivity for all neurons in all animals did not differ significantly (Fig. 4F), i.e., there was no accumulation of dynamic selectivity between

sessions. However, the average latency of specialization significantly decreased in the second session relative to the first, as the novelty factor of the environment decreased (Fig. 4C). At the same time, the initial (for the first 5 visits to the site field) increase in dynamic selectivity in the second session significantly exceeded the same value for the first session (Fig. 4D). This may be associated with the incremental improvements in space coding described in [Karlsson, Frank 2008].

Also, a significant (25.1% for the first session, on average for all animals) proportion of place neurons had a specialization latency equal to one visit to the place field, i.e. turned out to be specialized from the very first visit to the corresponding place field. This proportion did not change significantly in the second session relative to the first (Fig. 4E). This fact agrees with the results of classical works [Hill et al., 1978; Muller et al., 1987; Wilson, Mcnaughton, 1993], where it was shown that a significant part of place neurons (“immediate” neurons) are selectively activated starting from the very first visit to the corresponding place fields by the animal. However, Hill's original work demonstrated that 10 out of 12 recorded place neurons are, in fact, "immediate", which is not entirely consistent with the proportion of such neurons (from 4% to 42% depending on the animal) obtained in the present study. Such a discrepancy may be due to the geometry of the T-shaped arena used, in which the animals could see the entire environment at once in the process of being placed in it. At the same time, the distributions of specialization latencies obtained in the work are consistent with the data obtained earlier in the one-dimensional virtual navigation task [Dong et al., 2021].

Then, the hypothesis was tested that if the cognitive map is retains in the next session, the average selectivity will be higher, and the latency of specialization will be lower, compared with cases of remapping. To do this, I compared these parameters, as well as the initial increase in dynamic selectivity and the proportion of “immediate” place neurons between groups of animals that differ in terms of the preservation or rebuilding of the cognitive map. Such a comparison did not reveal statistically significant differences between the groups of animals in which the cognitive map in the second session was preserved or rebuilt relative to the cognitive map of the first session.

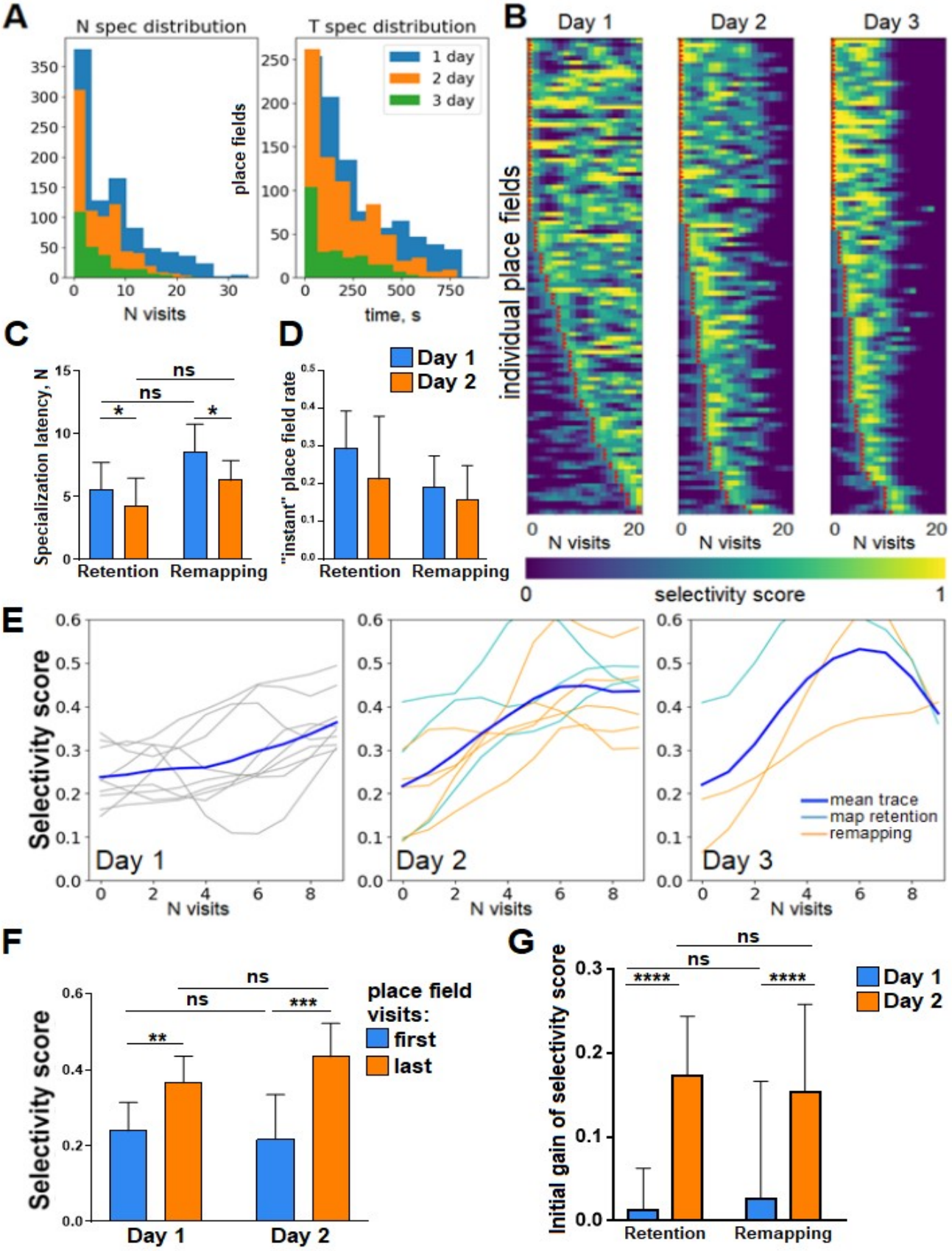

Figure 4. A. Distribution of specialization latency in all place fields of all place cells of

all animals in all sessions. On the left, the latency is measured in the number of visits to the place field, on the right, in natural time scale (in seconds) since the start of the session. **B.** An example of the evolution of the selectivity of individual place fields (separate lines on heat maps). Markers denote visits to place fields, starting from which they were considered stable (stabilization latencies). Place fields were sorted independently in each of the sessions. **C-D.** Comparison of stabilization latency expressed in visits (**C**), as well as the proportion of "immediate" place fields stable from the very first visit **(D)**, averaged in the first and second sessions for animals that had a remapping or preservation of a cognitive map in the second session relative to the first. Two-way ANOVA, *p = 0.0488 for the day factor, ns, p > 0.05. **E.** Evolution of selectivity score in each session, averaged along each animal (thin lines) and over all animals (thick blue lines). **F.** Comparison of the mean values of selectivity score at the beginning and at the end of the first and second sessions, averaged over all animals. Two-way ANOVA, Bonferroni post-hoc test, ** p = 0.0261, *** p = 0.0006, ns, p > 0.05. **G.** Comparison of the initial (for the first 5 visits) increase in selectivity score, averaged in the first and second sessions for animals that had a remapping or preservation of a cognitive map in the second session relative to the first. Two-way ANOVA, **** p = 0.0026 for session factor, ns, p > 0.05.

Thus, it was shown that as the factor of novelty decreased, cognitive maps stabilized faster: the latency of place cell specialization decreased, and the average selectivity of neurons did not change at the initial moments of the session, but its initial increase significantly increased at the first moments of navigation. This effect did not depend on whether these place cells participated in the first navigation session.

3. Comparison of the dynamics of spatial selectivity of neurons in different environments

It is well known [Muller et al., 1994] that in one-dimensional environments, in contrast to two-dimensional ones, place cells can have different place fields depending on movement direction. Since, in the model of free navigation in a circular track, animals

could arbitrarily change the direction of movement, it was difficult to take into account the direction specificity of place fields, and only bidirectional place fields were considered. Therefore, an additional experiment was set up, in which animals 1-4 explored a two-dimensional environment of the "open field" type for three sessions with a different number of obstacles (from 3 to 1) for 15 minutes 24 hours apart. Thus, the factor of the dependence of the fields of the place on the direction was excluded, while maintaining the complete arbitrariness of the movement of animals.

During the analysis of the obtained data, 1231 place fields were identified in 894 place cells in 4 animals in 3 imaging sessions; examples of overlaying the activity of individual place cells on the trajectory of animals are shown in Fig. 5.

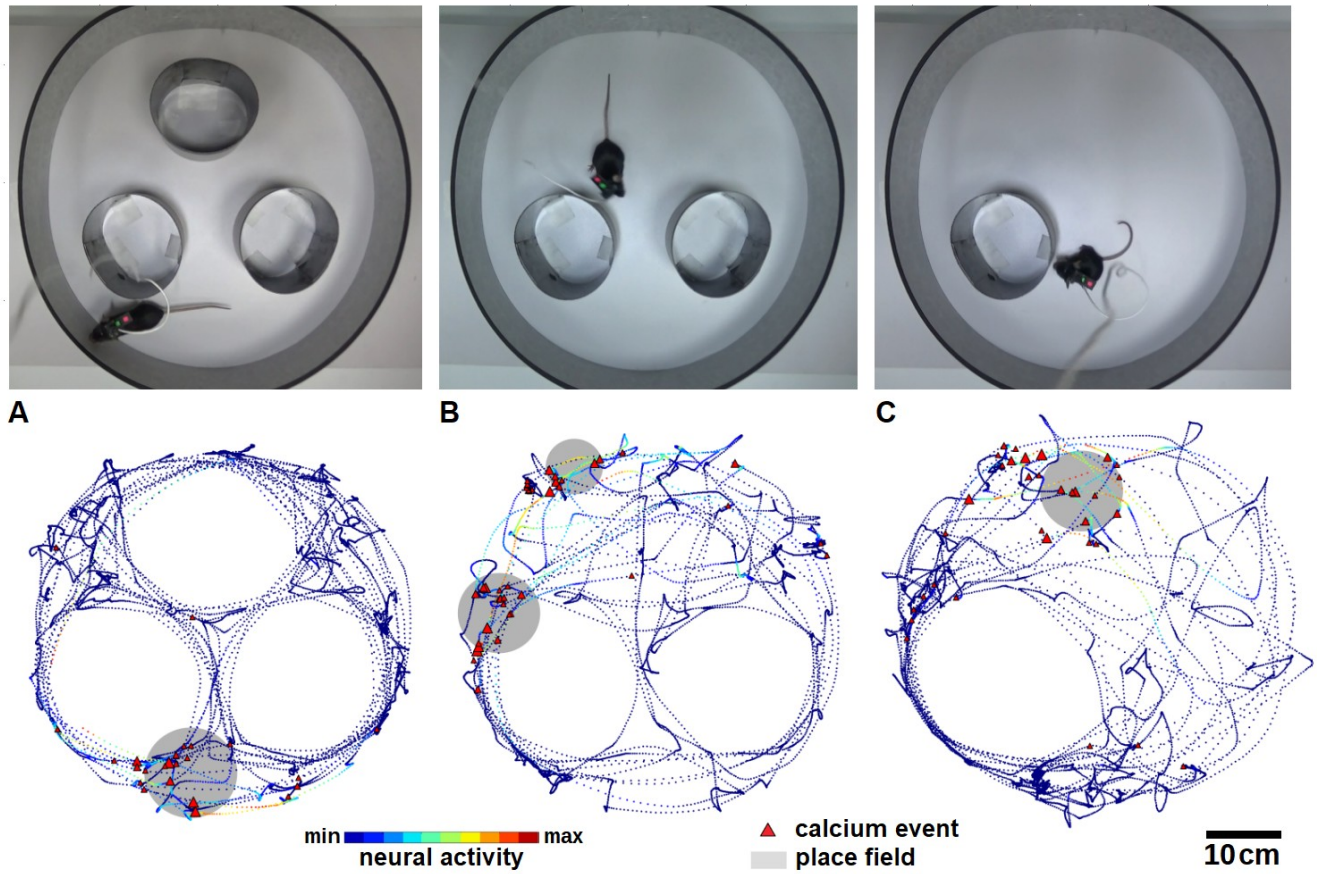

Figure 5. Examples of calcium activity of individual place cells plotted on the trajectory of animals in a two-dimensional arena with obstacles, the number of which varies from three (**A**) in the first session of the experiment, to two (**B**) and one (**C**) in the second and third imaging sessions, respectively.

Average selectivity in this 2D environment showed dynamics similar to that in the circular track, with a sharp initial increase and then a plateau (Fig. 6A). At the same time, the starting levels of selectivity score in the second and third sessions did not show a rollback to the starting value of the first session, in contrast to the situation in the 1D circular track.

The distribution of specialization latency between sessions turned out to be similar to that in the case of a one-dimensional circular track (Fig. 6B), with an average value for the first session of 199.6 s, which corresponded to the 8th visit to the place field. In the second session, the latency of specialization was significantly less than in the first one; however, no statistically significant differences were found between the second and third sessions (Fig. 6C).

In addition, a comparison was made of the proportion of "immediate" place fields (Fig. 6D), as well as the initial increase in selectivity (Fig. 6E) in both environments, one-dimensional (circular track) and two-dimensional (arena with obstacles). The initial increase in selectivity in the circular track showed a sharper rise between sessions compared to the 2D arena with obstacles, while the rate of "immediate" place fields turned out to be comparable in all considered sessions, with the exception of the first session in the 2D arena with obstacles (11.4%). Such a deviation can be explained by the more difficult-to-navigate geometry of the environment (a two-dimensional arena with three obstacles).

Thus, these results confirm the conclusions about the acceleration of the formation of cognitive maps as the environment gets more familiar to animals due to the initial increase in the selectivity of place cells. At the same time, the slower dynamics of the starting growth in sessions in the 2D arena with obstacles may be due to the maintained level of novelty by reducing the number of obstacles in each of the sessions (from 3 to 1). At the same time, the absence of significant differences in the average latency of specialization between the second and third sessions (Fig. 7) does not allow us to speak

of the critical importance of the number of obstacles in the arena as a factor in the animals' perception of novelty.

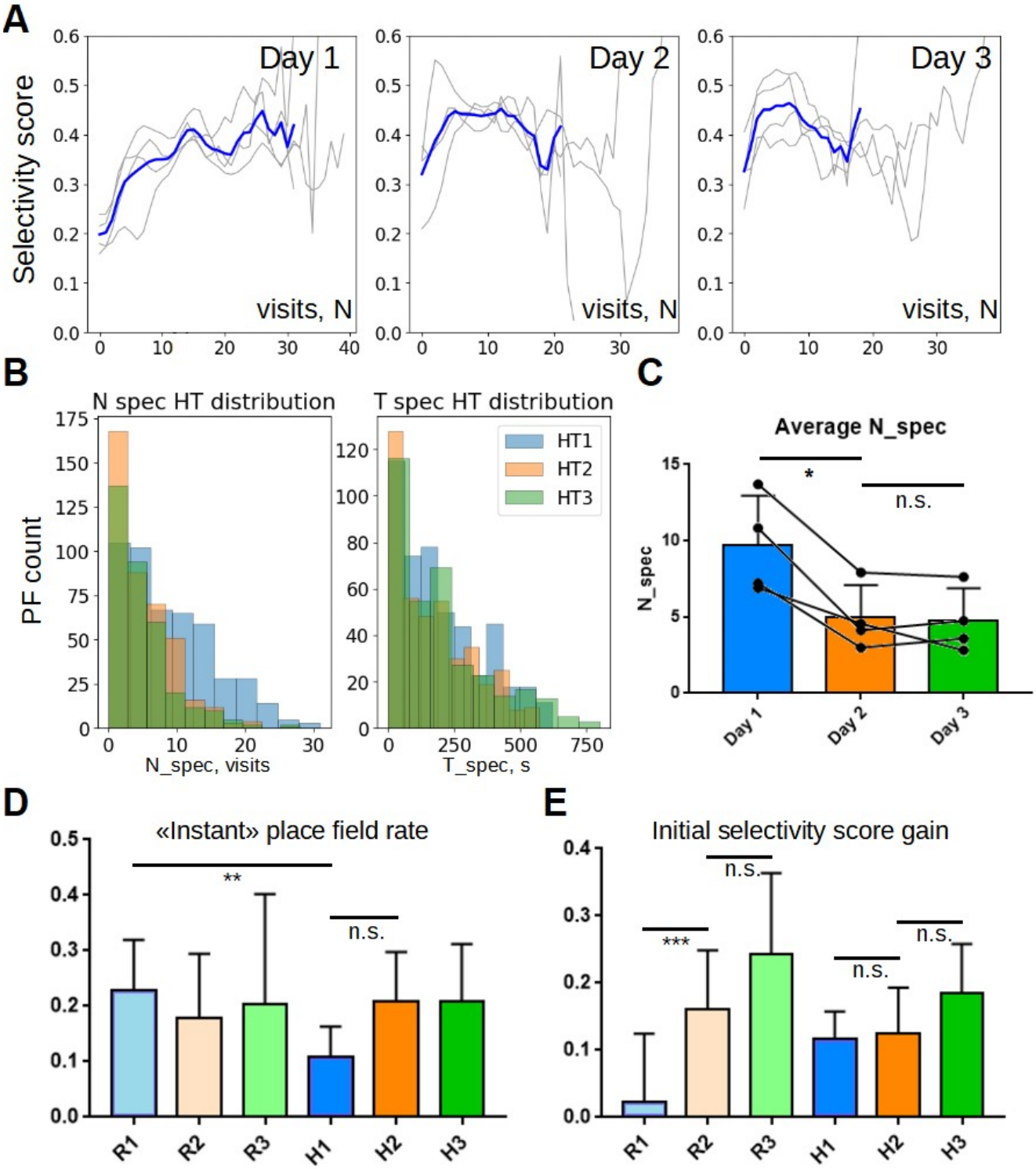

Figure 6. A. Evolution of dynamic selectivity in each session in a 2D arena with obstacles, averaged over each animal (gray lines) and across all animals (blue lines). **B.** Distribution of latency of specialization in all place fields of all place cells of all

animals in all sessions. **C.** Comparison of the average stabilization latency expressed in discrete visits between all imaging sessions in the arena with obstacles. *p = 0.0156, paired Student t-test. ns, p > 0.05. **D.** Proportion of "immediate" place fields among all place fields, averaged over all animals for each of the sessions in the circular track (R1-R3) and in the arena with obstacles (H1-H3). **p = 0.0025, paired Student t-test. ns, p > 0.05**. E.** Initial increase (over the first 5 visits) in the selectivity score, averaged over all animals for each of the sessions in the circular track (R1-R3) and in the arena with obstacles (H1-H3). ***p = 0.0012, paired Student t-test. ns, p > 0.05.

4. Population analysis of hippocampal neural activity

It is known that not only place neurons can contribute to space coding [Rubin et al., 2015; Meshulam et al., 2017; Chang et al., 2021]. In this regard, a population analysis of all registered neurons in the first session of each of the experiments, not necessarily spatially selective was carried out. In the course of the analysis, using the Laplace eigenmaps, the trajectories of animals in the circular track and in the two-dimensional arena with obstacles were reconstructed frame by frame from neural activity data. The possibility of such a reconstruction was shown earlier in a number of papers [Wilson, McNaugnton 1993; Zhang et al., 1998; Guger et al., 2011; Rubin et al., 2015]. Next, the average reconstruction error (i.e., the distance between the actual animal coordinates and the representation of the trajectory obtained during the reconstruction) and the average dynamic "anti-selectivity" (i.e., the difference between unity and selectivity) were compared for each of the animals in the dynamics of the navigation session.

As a result, a correspondence was found between these values: the similarity of the curves was estimated using the Ochiai measure (cosine similarity), which averaged 0.97 ± 0.04 for all curves for the first session of the experiment in the circular track (Fig. 7). For the first session in the arena with obstacles, the analogous value was 0.99 ± 0.02 (Fig. 8).

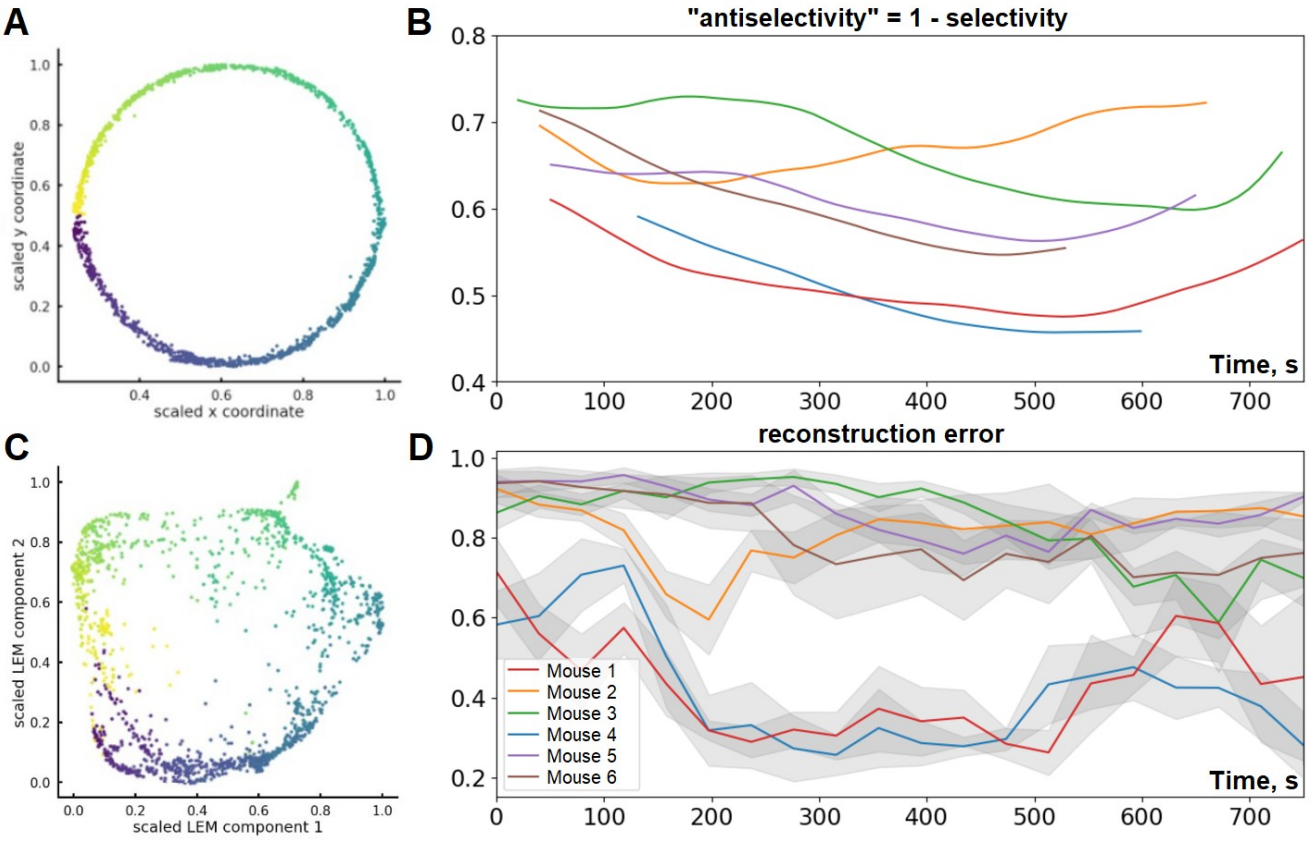

Figure 7. A, B. The actual trajectory of the animal in the circular track (**A**), each point corresponds to one video frame. The color indicates the position of the points in the track and the representation of the trajectory constructed from the data of neuronal activity (**B**). **C, D.** Average "anti-selectivity" of all place fields of all animals in the first session in the circular track (**C**) and the average error of the reconstruction of the animal trajectory in the circular track, smoothed by averaging over a time window of 250 s (**D**).

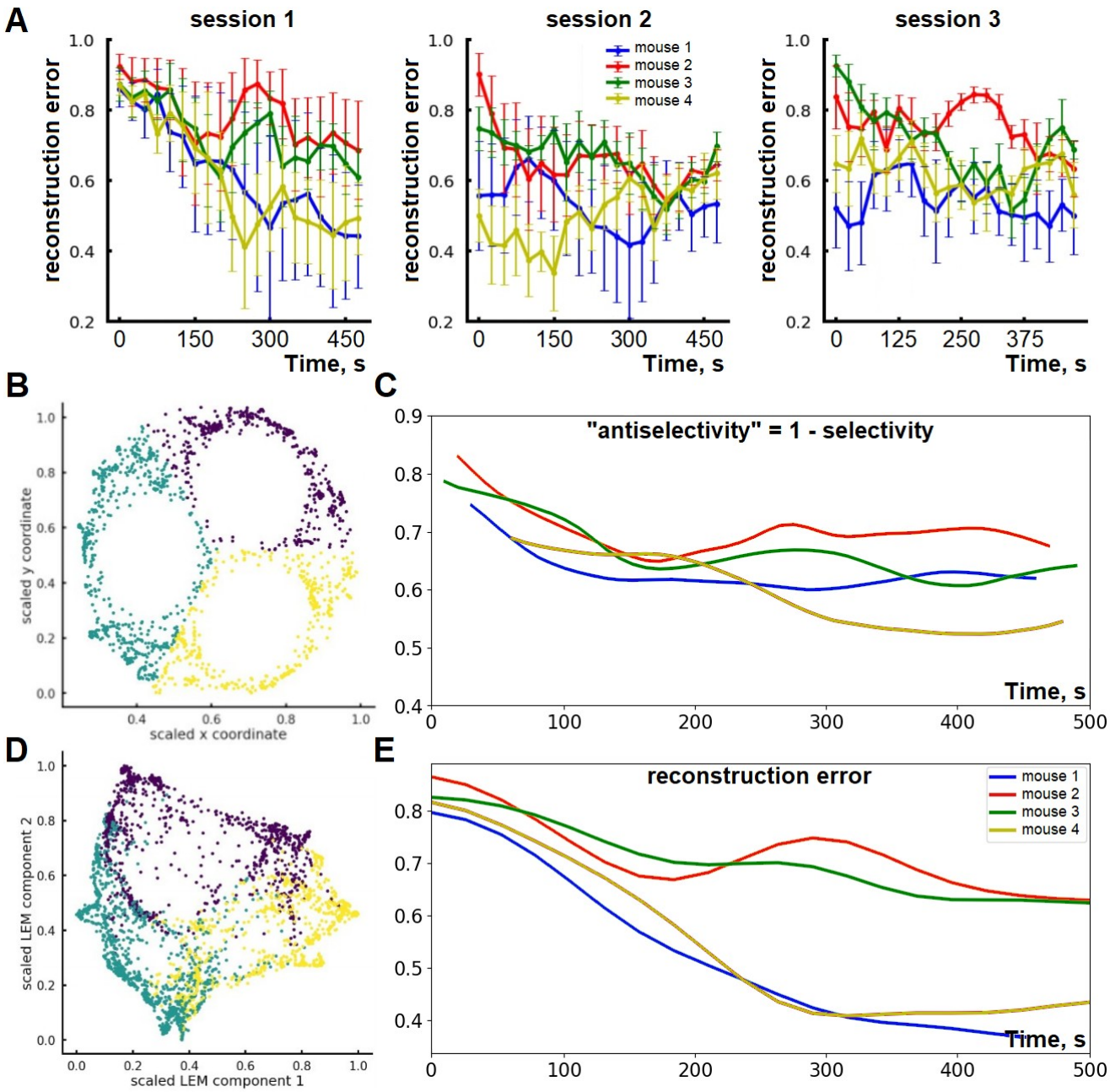

Figure 8. A. Animal trajectory reconstruction error in the arena with obstacles in each of the sessions of the experiment in the arena with obstacles. **B, D.** The real trajectory of an animal in the arena with obstacles **(B),** each point corresponds to one video frame. The color indicates the position of the points in the track and the representation of the trajectory constructed from the data of neuronal activity **(D). C, E.** Average "antis-electivity" of all place fields of all animals in the first session in the arena with obstacles **(C)** and the average reconstruction error of the animal trajectory in the arena with obstacles smoothed by averaging over a time window of 250 s **(E).**

Thus, it was shown that the quality of this reconstruction is consistent with the average dynamic selectivity, which additionally verifies both of these parameters as metrics of the quality of space encoding by a given population of neurons. In addition, such an approach can serve as a basis for assessing the exact contribution of place neurons and all other neurons to space coding, which is the subject of further research.

CONCLUSION

In this work, the new genetically encoded calcium sensors NCaMP7 and FGCaMP7 were compared with the conventional calcium sensor GCaMP6s. With the potential advantage of reduced non-specific binding of sensory subunits, these new sensors demonstrated comparable parameters of the mean space-selective calcium event to GCaMP6s in awake, moving animals in the free-navigation task in a circular track.

When mice navigated in a new environment in the form of a one-dimensional circular track, the spatial representations of neurons in the CA1 region of the hippocampus stabilized during the first few passages of the animals through the environment. At the same time, a significant part (25%) of the receptive fields of these neurons was formed during the very first visits to the corresponding areas of the environment. In repeated navigation sessions, spatial representations stabilized faster: the latency of the specialization of spatially selective neurons decreased, and the average selectivity of neurons did not change at the initial moments of the session, but its initial increase significantly increased at the first moments of navigation. At the same time, it did not matter what the place fields of these place neurons were in the first navigation session.

When navigating in a new environment in the form of a two-dimensional arena of the “open field” type with a variable number of obstacles, the parameters for the formation of spatial representations were obtained, similar to the situation with a one-dimensional circular track. At the same time, in the case of a two-dimensional arena, slower dynamics of the increase in the spatial selectivity of neurons was observed compared to a one-dimensional circular track.

According to the results of a population analysis of the activity of all registered neurons in a part of the animals of the first session in the circular track, it was shown that the error in the reconstruction of the animal trajectory based on the neural activity data is consistent with the average selectivity of the selected place neurons. Thus, the use of selectivity as a measure of the quality of neural coding of space was verified.

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References

1. Barykina N.V. et al. Green fluorescent genetically encoded calcium indicator based on calmodulin/M13-peptide from fungi // PLoS ONE. 2017. Vol. 12. P. 0183757.

2. Belkin M., Niyogi P. Laplacian eigenmaps for dimensionality reduction and data representation // Neural Comput. 2003. Vol. 15. P. 1373–1396.

3. Chang C. et al. Behavioral clusters revealed by end-to-end decoding from microendoscopic imaging // bioRxiv e-prints. 2021.

4. Dong C., Madar A.D., Sheffield M.E.J. Distinct place cell dynamics in CA1 and CA3 encode experience in new environments // Nat Commun. 2021. Vol. 12. P. 2977.

5. Guger C. et al. Real-time position reconstruction with hippocampal place cells // Front Neurosci. 2011. Vol. 5, № 85.

6. Hill A.J. First occurrence of hippocampal spatial firing in a new environment // Experimental neurology. 1978. Vol. 62, № 2. P. 282–297.

7. Karlsson M., Frank L. Network dynamics underlying the formation of sparse, informative representations in the hippocampus // J. Neurosci. 2008. Vol. 28. P. 14271–14281.

8. Lin L. et al. Neural encoding of the concept of nest in the mouse brain // Proc. Natl. Acad. Sci. USA. 2007. Vol. V. 104. P. 6066–6071.

9. Meshulam L. et al. Collective Behavior of Place and Non-place Neurons in the Hippocampal Network // Neuron. 2017. Vol. 96. P. 1178–1191.

10. Muller R. et al. On the directional firing properties of hippocampal place cells // J. Neurosci. 1994. Vol. 14. P. 7235–7251.

11. Muller R., Kubie J., Ranck J. Spatial firing patterns of hippocampal complex-spike cells in a fixed environment // J. Neurosci. 1987. Vol. 7. P. 1935–1950.

12. O'Keefe J., Dostrovsky J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat // Brain Research. 1971. Vol. 34, № 1. P. 171–175.

13. Piatkevich K.D., Murdock M.H., Subach F.V. Advances in engineering and application of optogenetic indicators for neuroscience // Appl. Sci. 2019. Vol. 9. P. 562.

14. Pnevmatikakis E.A. et al. Simultaneous Denoising, Deconvolution, and Demixing of Calcium Imaging Data // Neuron. 2016. Vol. 89, № 2. P. 285–299.

15. Qian Y. et al. A bioluminescent Ca(2+) indicator based on a topological variant of GCaMP6s // Chembiochem Eur. J. Chem. Biol. 2018. Vol. 20. P. 516–520.

16. Quian Quiroga R. et al. Invariant visual representation by single neurons in the human brain // Nature. 2005. Vol. 435. P. 1102–1107.

17. Rubin A. et al. Hippocampal ensemble dynamics timestamp events in long-term memory // eLife. 2015. Vol. 4. P. 12247.

18. Sheintuch L. et al. Multiple Maps of the Same Spatial Context Can Stably Coexist in the Mouse Hippocampus // Curr. Biol. 2020. Vol. 30. P. 1467–1476.

19. Tenenbaum J., De Silva V., Langford J. A global geometric framework for nonlinear dimensionality reduction // Science. 2000. Vol. 290. P. 2319–2323.

20. Ulanovsky N., Moss C.F. Dynamics of hippocampal spatial representation in echolocating bats // Hippocampus. 2011. Vol. 21, № 2. P. 150–161.

21. Wilson M.A., McNaughton B.L. Dynamics of the hippocampal ensemble code for space // Science. 1993. Vol. 261, № 5124. P. 1055–1058.

22. Zhang K. et al. Interpreting neuronal population activity by reconstruction: unified framework with application to hippocampal place cells // Journal of neurophysiology. 1998. Vol. 79, № 2. P. 1017–1044.

23. Ziv Y. et al. Long-term dynamics of CA1 hippocampal place codes // Nat. Neurosci. 2013. Vol. 16. P. 264–266.