Title: Social odors drive hippocampal CA2 place cell responses to social stimuli

Running title: CA2 place cells respond to social odors

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Abstract

Hippocampal region CA2 is essential for social memory processing. Interaction with social stimuli induces changes in CA2 place cell firing during active exploration and sharp wave-ripples during rest following a social interaction. However, it is unknown whether these changes in firing patterns are caused by integration of multimodal social stimuli or by a specific sensory modality associated with a social interaction. Rodents rely heavily on chemosensory cues in the form of olfactory signals for social recognition processes. To determine the extent to which olfactory signals contribute to CA2 place cell responses to social stimuli, we recorded CA2 place cells in rats freely exploring environments containing social stimuli that included or lacked olfactory content. We found that CA2 place cell firing patterns significantly changed only when social odors were prominent. Also, place cells that increased their firing in the presence of social odors alone preferentially increased their firing during subsequent sharp wave-ripples. Our results suggest that olfactory cues are essential for changing CA2 place cell firing patterns during and after social interactions. These results support prior work suggesting CA2 performs social functions and shed light on processes underlying CA2 responses to social stimuli.

Keywords

hippocampus, place cells, CA2, social cognition, social memory, social odor

1. Introduction

Social recognition memory, or the ability to recognize and remember conspecifics, facilitates social interactions that are essential for animals to survive. Rats can discriminate between familiar conspecifics (Husted and McKenna, 1966; Thor and Holloway, 1982) based on their distinct characteristics and olfactory signatures (Gheusi et al., 1997; Popik et al., 1991; Sawyer et al., 1984). Many recent studies have implicated hippocampal area CA2 in social memory (for a review, see Oliva, 2022). Lesioning or inactivating CA2 pyramidal neurons leads to impaired social recognition memory in mice (Hitti and Siegelbaum, 2014; Stevenson and Caldwell, 2014) and optogenetic silencing of CA2 activity shows that CA2 is crucial for encoding, consolidating, and recalling social memories (Meira et al., 2018). Moreover, CA2 neurons are uniquely enriched in receptors selective for a variety of social neuropeptides, including vasopressin and oxytocin (Lee et al., 2008; Lin et al., 2018; Pagani et al., 2015; Raam et al., 2017; Wersinger et al., 2002, 2008).

All subregions of the hippocampus contain place cells, neurons that are selectively activated in a particular location in space (their “place fields”) (O’Keefe, 1976; O’Keefe and Dostrovsky, 1971). Place cells alter their firing in response to significant environmental changes in a process called “remapping” (for a review, see Colgin et al., 2008). During remapping, place fields may appear, disappear, or change location. Neurophysiological recordings of place cells have shown that place fields in neighboring hippocampal subregions CA1 and CA3 remain relatively stable in unchanging environments (Muller et al., 1987; Thompson and Best, 1990). In contrast, CA2 place cells show gradual changes in firing patterns over time (Alexander et al., 2016; Mankin et

al., 2015). CA2 place cells also appear to be highly sensitive to small updates to familiar environments (Wintzer et al., 2014). Previous work has shown that a significant proportion of CA2 place cells remap when a familiar conspecific is presented (Alexander et al., 2016). This remapping in response to social stimuli may support the ability of CA2 to encode social memories (Hitti and Siegelbaum, 2014). However, it remains unclear whether CA2 place field changes that occur in response to presentation of a conspecific rat are caused by integration of multimodal social stimuli or primarily due to a specific sensory modality associated with the social interaction.

Previous work has also shown that CA2 firing patterns during sleep are altered following a social experience (Oliva et al., 2020). During awake rest or sleep, hippocampal place cells fire during distinctive events in the hippocampal local field potential (LFP) known as sharp wave-ripples. Place cells that were active during exploratory behaviors later reactivate during sharp wave-ripples, and reactivated place cell ensembles are believed to support memory consolidation (Ego-Stengel and Wilson, 2010; Ramadan et al., 2009; Wilson and McNaughton, 1994). CA2 place cells that represent a conspecific have been shown to reactivate during sharp wave-ripples following a social experience (Oliva et al., 2020). Further, disrupting CA2 sharp wave-ripples impairs social recognition memory (Oliva et al., 2020). Therefore, the reactivation of CA2 place cells that encode a social experience may promote consolidation of social memories (Oliva et al., 2023).

In this study, we recorded CA2 place cells in dorsal hippocampus of rats during free exploration of a two-dimensional spatial environment and during subsequent rest periods. We compared changes in CA2 place cell firing patterns across sessions in which different social stimuli incorporating various sensory modalities were presented. Significant changes in CA2 place cell firing patterns were observed when social odors were presented in the absence of a rat and

when a familiar rat was presented in a soiled home cage containing social odors (as in our prior study; Alexander et al., 2016). However, no significant changes in CA2 place cell firing patterns were observed when a familiar rat was presented in a clean and relatively odorless cage. The results suggest that olfactory cues are the key sensory component of social experiences that drives CA2 place cell remapping. Furthermore, CA2 place cells that increased their firing rates in response to social odors preferentially increased their firing rates in sharp wave-ripples during subsequent rest periods. These results improve our understanding of how CA2 place cells code information related to social interactions.

2. Materials and Methods

2.1. Subjects

Six wild-type male Sprague-Dawley rats (Inotiv, USA) were used for this study. Rats were between the ages of 4 and 10 months at the time of surgery. Before surgery, rats were double or triple housed and were pre-trained to freely explore an open field enclosure. After surgery, rats were singly housed in custom-built acrylic cages (40 cm x 40 cm x 40 cm) containing enrichment material (wooden blocks, paper towel rolls, etc.) and maintained on a reverse light cycle (light: 8 p.m. – 8 a.m.). Rats were housed next to their former cage mates after recovering from surgery and throughout behavioral testing. Rats recovered from surgery for at least one week before behavioral training resumed. All behavioral experiments were performed during the dark cycle. When necessary to encourage spatial exploration, rats were placed on a food-deprivation regimen that maintained them at ~90% of their free-feeding body weight. All experiments were conducted according to the guidelines of the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals and under a protocol approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

2.2. Surgery and tetrode positioning

Drives with 14 independently movable tetrodes were implanted in five of the rats. A drive with 21 independently movable tetrodes was implanted in one of the rats. Drives were implanted above the right dorsal hippocampus (anterior-posterior -3.8 mm from bregma, medial-lateral -3.0 mm from bregma). To stabilize the recording drives, eleven bone screws were affixed to the skull and covered in dental acrylic. Two of the screws were connected to the recording drive ground. Prior to surgery, tetrodes were built from 17 µm polyimide-coated platinum-iridium (90/10%) wire (California Fine Wire, Grover Beach, California, USA). The tips of tetrodes designated for single-unit recording were plated with platinum to reduce single-channel impedances to ~150 to 300 kOhms. All tetrodes were lowered ~1 mm on the day of surgery. Thereafter, tetrodes were slowly lowered to the hippocampal pyramidal cell body layer over the course of several weeks.

One tetrode was designated for use as a reference for differential recording. All four wires of this tetrode were connected to a single channel on the electrode interface board. The differential recording reference tetrode was placed in an electrically quiet area approximately 1 mm above the hippocampus and adjusted as needed to ensure quiescence. The reference signal was duplicated using an MDR-50 breakout board (Neuralynx, Bozeman, MT, USA) and recorded continuously to ensure that unit activity or volume conducted signals of interest were not detected. Another tetrode was placed in the apical dendritic layer of CA1 to monitor LFPs and guide placement of the other tetrodes using estimated depth and electrophysiological hallmarks of the hippocampus (for example, sharp wave-ripples).

2.3. Data acquisition

Data were acquired using a Digital Lynx system and Cheetah recording software (Neuralynx, Bozeman, MT, USA). The recording setup has been described in detail previously (Hsiao et al., 2016; Zheng et al., 2016). Briefly, LFP signals from one randomly chosen channel within each

tetrode were continuously recorded at a 2000 Hz sampling rate and filtered in the 0.1–500 Hz band. Input amplitude ranges were adjusted before each recording session to maximize resolution without signal saturation. Input ranges for LFPs generally fell within ±2,000 to ±3,000 µV. To detect unit activity, all four channels within each tetrode were bandpass filtered from 600 to 6,000 Hz. Spikes were detected when the filtered continuous signal on one or more of the channels exceeded a threshold set daily by the experimenter, which ranged from 55–65 µV. Detected events were acquired with a 32,000 Hz sampling rate for 1 ms. For both LFPs and unit activity, signals were recorded differentially against a dedicated reference channel (see "Surgery and tetrode positioning" section above).

Videos of rats' behavior were recorded through the Neuralynx system with a resolution of 720 × 480 pixels and a frame rate of 29.97 frames/s. Rat position and head direction were tracked via an array of red and green or red and blue light-emitting diodes (LEDs) on a HS-54 or HS-27 headstage (Neuralynx, Bozeman, MT, USA), respectively.

2.4. Behavioral task

Rats were familiarized to an open field arena (1 m x 1 m with 0.5 m wall height) for a minimum of three days before recording started. Rats freely explored the open field arena for four 20-minute sessions per day, with 10-min rest sessions preceding and following each active exploration session. During active exploration, small pieces of sweetened cereal or cookies were randomly scattered to encourage rats to explore the entirety of the arena. Rats had to cover at least 60% of the arena across each of the four sessions for a day to be included for further analysis. During each rest session, rats rested in a towel-lined, elevated flowerpot outside of the arena. A plexiglass standard rat housing cage was placed in one corner of the arena for all recording sessions. In the first and fourth sessions (A and A'), this cage contained only clean bedding. In the middle two sessions (B and B') of the different experimental

conditions, the cage contained various types of social stimuli (Figure 1). In the Odor condition, the soiled bedding from the former home cage of familiar rats (or a familiar rat) was used. In the Visual condition, a familiar rat was placed in the stimulus cage with clean bedding and a filter-top lid to minimize social odors. In the Visual + Odor condition, a familiar rat was presented in its home cage containing soiled bedding. In the Mirror condition, a familiar rat was placed in the stimulus cage with clean bedding and a filter-top lid, but the cage was lined with a one-way mirror attachment. This one-way mirror attachment prevented the stimulus rat from seeing the implanted rat, limiting visually driven reciprocal interactions between the two rats. In the Control condition, a cage containing only clean bedding was presented in all four sessions.

2.5. Histology and tetrode localization

Following recording, rats were perfused with 4% paraformaldehyde solution in phosphate-buffered saline. Brains were cut coronally in 30 µm sections using a cryostat. For two rats, brains were stained with cresyl violet. For four rats, brains were immunostained for the CA2 marker Purkinje Cell Protein 4 (PCP4) (Figure 2). For the immunostaining protocol, sections were washed three times for 15 minutes in tris-buffered saline (TBS) followed by a 10-minute water wash. Sections were then permeabilized and washed for 15 minutes in TBS containing 0.3% Triton-X followed by three 10-minute washes in TBS. Sections were blocked for 30 minutes in 10% normal goat serum in TBS. Sections were incubated overnight with rabbit anti-PCP4 (1:200, Sigma-Aldrich Cat# HPA005792) diluted in TBS containing 0.05% Tween. The next day, sections were washed twice for 10 minutes in TBS and incubated overnight with secondary fluorescent antibody (in one rat: Alexa Flour™-555 anti-rabbit, Thermo Fisher Scientific; in three rats: Alexa Flour™-555 anti-rabbit, Thermo Fisher Scientific). All washes and incubations were performed at room temperature. Slides were mounted using DAPI Fluoromount-G (Fisher Scientific). Tetrode recording sites were identified by comparing locations across adjacent sections.

2.6. Spike sorting and unit selection

Spike sorting was performed manually using graphical cluster-cutting software (MClust, A.D. Redish, University of Minnesota, Minneapolis, Minnesota) run in MATLAB (Mathworks). Spikes were sorted using two-dimensional representations of waveform properties (i.e., energy, peak, and peak-to-valley difference) from four channels. A single unit was accepted for further analysis if the associated cluster was well isolated from, and did not share spikes with, other clusters on the same tetrode. Units were also required to have a minimum 1 ms refractory period. Units with mean firing rates above 5 Hz were considered putative interneurons and excluded from further analysis. In order to be considered active in the arena, a unit had to reach a peak firing rate of at least 1 Hz. In order to be included in the sharp wave-ripple firing analysis, a unit had to have valid clusters in both the active exploration and rest sessions. CA2 cell yields for each condition are reported in Tables 1 and 2.

2.7. Place cell remapping analyses

Methods used to create firing rate maps for each single unit were based on methods used in our prior study (Alexander et al., 2016; example rate maps for the present study shown in Figure 3). First, the arena was divided into 4 cm^2 bins. The number of spikes that occurred within each bin was divided by the time spent in that bin to determine the firing rate. Only spikes that occurred while the rat was traveling 5 cm/s or faster were included. The rate map was smoothed with a two-dimensional Gaussian kernel (standard deviation = 6 cm). To determine if a place cell remapped during presentation of a social stimulus, a Pearson correlation coefficient *R* was calculated for each unit between pairs of rate maps from control and social stimuli sessions (i.e., A-B, B-B', B'-A', A-B', and A-A' session pairs, see Figure 1). To determine if spatial correlation coefficients differed across session pairs and conditions (i.e., Odor, Visual + Odor, Visual, Mirror, Control), we used a generalized linear mixed model statistical analysis (IBM SPSS Statistics,

version 29.0.2.0). Condition and session pair were fixed factors, session pairs were repeated measures within rats, and multiple place cells were nested within rats. A condition by session pair interaction effect was also included in the model to determine whether differences between session pairs varied across conditions. When a significant effect was observed, post-hoc pairwise comparisons were performed with Bonferroni correction for multiple comparisons. The estimated mean spatial correlations for each condition and session pair from the generalized linear mixed model are shown in Figure 4A. The estimated mean spatial correlations for each condition, collapsed across session pairs, are shown in Figure 4B. Individual spatial correlation values for each cell are shown for each session pair and each condition in Figure 4C-G.

To determine if positions of place fields coherently shifted during remapping, we first identified cells that remapped and sorted them into “turn on”, “turn off”, and “field shift” categories (Figure 5). Because place cells were classified as active if they reached a peak firing rate of at least 1 Hz, “turn on” cells were defined as cells that had a peak firing rate less than 1 Hz in Session A and greater than 1 Hz in Session B. We then calculated the distance between the position of the peak firing rate of the cell in Session B and the stimulus cage for “turn on” cells. “Turn off” cells were defined as cells that had a peak firing rate above 1 Hz in Session A and below 1 Hz in Session B. The distance between the position of the peak firing rate of the cell in Session A and the stimulus cage was calculated for “turn off” cells. Cells were identified as “field shift” cells if they reached a peak firing rate above 1 Hz in Sessions A and B but showed unstable place field locations. We assessed place field stability as follows, using criteria defined in a prior study (Widloski and Foster 2022). Cell IDs were randomly shuffled 1000 times within each condition and rat. Spatial correlation coefficients were then calculated for a given unit from session A and each shuffled rate map from session B. A place field was considered stable between sessions A and B if the spatial correlation coefficient exceeded the 95th percentile of the shuffled distribution. For “field shift” cells, we calculated the distances between the stimulus cage and

the positions of the peak firing rates of the cell in Sessions A and B. To determine if a cell's place field moved closer to the stimulus cage when social stimuli were presented, we calculated the difference between the distance estimates from Sessions A and B for "field shift" cells.

To quantify how selective a unit was for session A vs session B, we calculated a selectivity index for each unit, as in Hwaun and Colgin (2019). The selectivity index was defined as $(\mu_B - \mu_A)/(\mu_B + \mu_A)$, where μ_A was the mean firing rate in session A and μ_B was the mean firing rate in session B. A value of -1 would indicate that a unit was exclusively active in session A, while a value of 1 would indicate that a unit was exclusively active in session B.

2.8. Exploration time

To investigate exploration of social stimuli, the arena was divided into 4 cm^2 bins, as in a prior study (Zhu et al., 2023). The time spent in each bin during the first two minutes of every session was determined for each day. These exploration maps were then smoothed with a two-dimensional Gaussian kernel (standard deviation = 4 cm). Maps were averaged within and then across rats and plotted as a heat map for each condition (Figure 6A). The time spent within 12 cm of the cage was then calculated. A generalized linear mixed model analysis (IBM SPSS Statistics, V 29.0.2.0) was used to compare time spent investigating the stimulus cage across different sessions and conditions, with conditions and sessions as fixed factors. Conditions were repeated on different days within rats, with different days included as random factors within rats. Sessions were repeated measures within conditions. Post-hoc tests were performed to compare exploration times across conditions for the session of interest (i.e., Session B), using the Bonferroni correction for multiple comparisons.

2.9. Sharp wave-ripple detection and analysis

Sharp wave-ripples were detected from recordings obtained while rats rested in an elevated, towel-lined flowerpot outside of the arena. Detection and analysis methods were similar to previously published methods (Hwaun and Colgin, 2019). The LFP recorded on all tetrodes that had CA2 place cells was band-pass filtered between 150 and 250 Hz. A Hilbert transform was performed on the filtered LFP, and the absolute value of this signal was smoothed with a Gaussian kernel (standard deviation = 25 ms). Potential sharp wave-ripple events were detected when the signal exceeded at least 5 standard deviations above the mean and bounded by crossings of the mean. Overlapping events were combined across tetrodes, so the time interval of identified events could extend beyond sharp wave-ripples detected on a single tetrode (as in Hwaun and Colgin, 2019; Karlsson and Frank, 2009). Potential sharp wave-ripple events were kept for further analysis if they were between 50 and 500 ms in duration.

We then obtained the firing rate for each CA2 place cell around detected sharp wave-ripple events by constructing a spike raster with a bin size of 1 ms and smoothing the raster with a Gaussian kernel (standard deviation = 5 ms). To account for baseline firing rate differences among individual cells, the firing rate was normalized by the average firing rate 400-100 ms before ripple onset (Hwaun and Colgin, 2019). The peak normalized firing rate was obtained for each unit by taking the maximum normalized firing rate after sharp wave-ripple onset. Because a broad range of peak normalized firing rates was observed, we rescaled the distribution using a $\log_{10}$ transformation of the peak normalized firing rates. To determine the extent to which sharp wave-ripple-associated firing rates of individual cells were altered by presentation of a social stimulus, we computed the difference between the peak normalized firing rates during the rest periods after sessions A and B (i.e., peak normalized firing rate after session B - peak normalized firing rate after session A) for the Control and Odor conditions. A positive value indicates that a cell had higher sharp wave-ripple-associated firing in the rest session following session B, and a negative value indicates that a cell had higher sharp wave-ripple-associated

firing in the rest session following session A. We used multiple linear regression (IBM SPSS Statistics, V29.0.2.0) to assess the effects of session selectivity (as measured using the selectivity index, see *Place cell remapping analyses* section) and experimental condition on the difference in sharp wave-ripple-associated firing between rest sessions (as in Hwaun and Colgin, 2019). The regression analysis was only performed for conditions that had more than 100 active cells during both run and rest sessions (Control condition: n = 130 cells from 5 rats, Odor condition: n = 132 cells from 5 rats).

2.10. Data and Code Availability

MATLAB (Mathworks) scripts were custom written for the analyses in this study, based on algorithms that have been used in prior studies, as described above. Scripts and data are available upon request.

3. Results

In our prior study of CA2 place cells (Alexander et al., 2016), we compared CA2 place cell responses to a rat in a soiled home cage and responses to the presentation of an object that resembled a rat, namely a stuffed toy rat. However, the stuffed toy lacked some visual components that occur during interactions with a live rat (e.g., motion content). Also, the toy rat was presented in a clean cage and thus lacked the olfactory components of a social experience. The major goal of the present study was to determine the extent to which different sensory modalities associated with a social experience drive CA2 place cell remapping to social stimuli. We employed an open field exploration behavior paradigm (see "*Behavioral task"* section of Materials and Methods) in which different types of social stimuli were presented across sessions for various experimental conditions (i.e., Odor, Visual, Visual + Odor, Mirror) and compared to a Control condition in which no social stimuli were presented (Figure 1).

3.1. CA2 place cells remap to social odor stimuli

The olfactory system is highly important for social recognition and social behaviors in rodents (Oettl and Kelsch, 2018). Therefore, we hypothesized that olfactory cues would be a key sensory component of CA2 place cell responses to social stimuli. In the Odor condition, rats explored an environment in which social odors were presented, but another rat was not present. This condition isolated the olfactory content of a social interaction and preserved the ethological relevance of the presented stimuli. Example CA2 place cell firing rate maps during presentation of social odors (Figure 3B) show that a subset of cells changed their firing patterns when social odors were presented. To quantify CA2 place cell remapping, we compared firing rate changes in cells recorded in the Odor condition (145 cells in 6 rats, Table 1) to firing rate changes in cells recorded in the other conditions (see Figure 3 for example rate maps for all conditions and Table 1 for cell yields). We calculated the spatial correlation between rate maps from pairs of sessions for each cell (Figure 4). We found that spatial correlation values for the Odor condition were significantly lower than the Control, Visual, and Mirror conditions (generalized linear mixed model, no significant interaction between session pair and condition, $F(16, 3025) = 0.971$, $p = 0.486$ (Figure 4A); significant main effect of condition, $F(4, 3025) = 15.4$, $p < 0.001$ (Figure 4B); significant differences in post-hoc tests for Odor vs. Control ($t(3025) = 6.3$, $p < 0.001$), Odor vs. Visual ($t(3025) = 4.0$, $p = 0.001$), and Odor vs. Mirror ($t(3025) = 3.6$, $p = 0.001$)). In agreement with our prior findings, CA2 place cells also showed significant remapping when a familiar conspecific rat was presented in a soiled home cage containing social odors (Visual + Odor condition, Figures 3C and 4, significant differences in post-hoc tests for Visual + Odor vs. Control ($t(3025) = 6.4$, $p < 0.001$), Visual + Odor vs. Visual ($t(3025) = 4.5$, $p < 0.001$), and Visual + Odor vs. Mirror ($t(3025) = 4.1$, $p = 0.001$). In contrast, significant changes in CA2 place cell firing patterns were not observed when a stimulus rat was presented in a clean cage with a filter top, a condition that maintained all visual components of social interactions and minimized social odors (Visual condition, Figures 3D and 4, no significant differences in post-hoc test for

312 Visual vs. Control (t(3025) = 1.8, p = 0.2)). Similarly, CA2 place cell firing patterns did not
313 change significantly when a stimulus rat was presented in a clean cage with a filter top and a
314 one-way mirror to prevent visually mediated reciprocal interactions between rats (Mirror
315 condition, Figures 3E and 4, no significant differences in post-hoc test for Mirror vs. Control
316 (t(3025) = 2.3, p = 0.1)). Together, these results show that CA2 place cells remap when social
317 odors are presented but not during interactions with another rat when social odors are
318 minimized.
319
320 *3.2. No coherent movement of place fields during remapping*
321 CA1 place fields have been reported to shift with salient stimuli (Fenton et al., 2000; O'Keefe
322 and Conway, 1978) or move toward locations of salient rewarding stimuli (Breese et al., 1989;
323 Fenton et al., 2000). However, our previous work showed that fields of CA2 place cells do not
324 move closer to a social stimulus during social remapping (Alexander et al., 2016). To test if CA2
325 place cells moved coherently towards or away from the social stimuli in our experiment, we first
326 identified place cells that remapped between sessions A and B. These cells were identified as
327 cells that either gained a place field ("turn on" cells), lost their place field ("turn off" cells), or
328 shifted their place field ("field shift" cells). For "turn on" cells, we calculated the distance between
329 the cell's peak firing rate position in the session in which social stimuli were presented (Session
330 B) and the position of the stimulus cage (Figure 5A). Similarly, we calculated the distance
331 between the peak firing rate position and the position of the stimulus cage in Session A for the
332 "turn off" cells (Figure 5B). If place cells preferentially gained or lost fields close to the stimulus
333 cage, we would have expected a skewed distribution towards low values. Instead, we observed
334 a broad distribution of distances from the stimulus cage. For "field shift" cells, we estimated the
335 distance between the stimulus cage and cells' peak firing rate positions in Sessions A and B and
336 then calculated the difference between these two distance estimates (Figure 5C). If place fields
337 shifted closer to the cage, we would have expected values to skew negatively. However,

differences between place field distances from the cage between Sessions A and B covered a wide range of positive and negative values. Therefore, consistent with our previous findings (Alexander et al., 2016), no coherent movement of place fields toward or away from the stimulus cage was observed. This may be due to the nature of odor stimuli, as airborne odorants diffuse across widespread locations within an environment. This may allow the rat to perceive odors regardless of the exact location where odor stimuli are presented in the arena. It is also possible that the introduction of social odors to an environment was sufficient to change the context as a whole. This generalizability may help rats to associate conspecifics with larger areas of an environment.

3.3. Rats explore all modalities of social stimuli

It is possible that the lack of remapping to non-olfactory social stimuli may be due to inadequate exploration of these stimuli. To test this possibility, we examined rats' stimuli exploration time during the first 2 minutes of each exploration session for each experimental condition (Figure 6). We found that stimulus cage exploration time during session B of most social conditions (i.e., Odor, Visual, and Mirror) significantly increased compared to session B of the Control condition (generalized linear mixed model, interaction between session and condition, $F(12,260) = 2.589$, $p = 0.003$; post-hoc tests, session B: significant difference between Control vs. Odor: $t(260) = 2.8$, $p = 0.03$; Control vs. Visual: $t(260) = 3.3$, $p = 0.009$; and Control vs. Mirror: $t(260) = 3.3$, $p = 0.01$; No significant difference between Control vs. Visual + Odor: $t(260) = 2.1$, $p = 0.2$). It is important to note that stimulus cage exploration times significantly increased in the social conditions in which CA2 place cells did not show significant remapping (i.e., Visual and Mirror conditions), suggesting that the lack of place cell remapping in these conditions was not due to reduced exploration of social stimuli. Note also that exploration times did not significantly differ between Visual + Odor and Control conditions, although significant remapping was observed in the Visual + Odor condition. However, the insignificant difference in exploration times may be

364 due to the lower number of rats in the Visual + Odor condition (i.e., 4 rats in Visual + Odor
365 condition compared to 5-6 rats in other conditions, see Table 1).
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367 ### *3.4. Place cells that respond to social odors are preferentially recruited into sharp wave-*
368 ### *ripples*
369 CA2 place cells that encode a social experience with a novel conspecific have been shown to
370 reactivate during sharp wave-ripples in mice (Oliva et al., 2020). Therefore, we aimed to
371 determine whether presentation of social odors alone was sufficient to drive preferential sharp
372 wave-ripple-associated reactivation of CA2 place cells that responded to social odors. We used
373 a selectivity index (see Materials and Methods) to classify cells according to their firing
374 preferences during the Control and Odor conditions. The selectivity index defined the extent to
375 which CA2 place cells fired during session A (i.e., the session in which an empty cage was
376 presented) compared to session B (i.e., the session in which a cage containing social odors was
377 presented in the Odor condition). We then estimated the normalized average peak firing rate of
378 each cell during sharp wave-ripples in rest sessions after session A and session B (see
379 Materials and Methods). Next, we used the difference between a cell's peak normalized firing
380 rate during the rest session after session B and the rest session after session A as a measure of
381 the extent to which a cell changed its sharp wave-ripple-associated firing after presentation of
382 social odors. CA2 place cells show unstable firing patterns in unchanged environments over
383 time (Mankin et al., 2015). Thus, we performed the same analysis for CA2 place cells recorded
384 in the Control condition to ensure that changes in sharp wave-ripple-associated firing were not
385 explained by the effect of time. We found that a cell's selectivity for social stimuli in active
386 exploratory sessions correlated with sharp wave-ripple-associated peak firing rate changes in
387 the Odor condition but not the Control condition (Figure 7; multiple linear regression, $F(3,243) =$
388 2.661, $p = 0.049$; interaction between selectivity index and condition, $t = 2.448$, $p = 0.015$,
389 Pearson correlation between selectivity index and firing rate changes for the Control condition R

390 = -0.102, p = 0.266, Pearson correlation between selectivity index and firing rate changes for
391 the Odor condition R = 0.227, p = 0.010). Specifically, cells that increased their firing rates
392 during exploration of a social odor preferentially increased their sharp wave-ripple-associated
393 firing rates during rest. These results suggest that the presentation of a social odor alone is
394 sufficient to preferentially reactivate CA2 place cells that code social stimuli.
395
396 **4. Discussion**
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398 An increasing number of lesion, genetic, optogenetic, and pharmacogenetic manipulation
399 studies have implicated CA2 in social memory processing (Hitti and Siegelbaum, 2014; Meira et
400 al., 2018; Oliva et al., 2020; Smith et al., 2016; Stevenson and Caldwell, 2014). In this study, we
401 expanded on prior results showing that CA2 place cells remap during a social interaction
402 (Alexander et al., 2016) by investigating how various sensory modalities of social stimuli
403 contribute to this response. Here, we show that CA2 place cell remapping is primarily driven by
404 the olfactory component of social stimuli. Exposure to social odors in the absence of a rat was
405 sufficient to induce CA2 place cell remapping. CA2 place cells did not respond to social
406 interactions with another rat when social odors were reduced. The lack of remapping when
407 social odors were reduced was not explained by inadequate exploration of social stimuli. Lastly,
408 CA2 place cells that showed increased firing during exploration of social odors were
409 preferentially reactivated during sharp wave-ripples in subsequent rest. Our findings support the
410 notion that CA2 place cells integrate social olfactory information with contextual information to
411 encode and consolidate social episodic memories (Oliva, 2022; Oliva et al., 2023). While social
412 experiences did not induce significant CA2 place cell remapping when social odors were
413 lacking, the possibility remains that other sensory modalities of a social experience are
414 processed via different pathways and brain structures and are still able to support social
415 recognition and social memory processes.

The sensory modalities that drive social recognition vary across species. While humans depend predominantly on auditory and visual cues for social recognition, rodents and other mammals rely heavily on chemosensory cues in the form of olfactory or pheromonal signals (Popik and van Ree, 1998). Indeed, olfactory bulb lesions or chemically induced anosmia impairs individual recognition in rats (Dantzer et al., 1990; Popik et al., 1991). The complex mix of chemosensory signals embedded in rat urine convey information about a rat's social status and identity (Hurst, 2005). CA2 neurons differentially respond to urine from different conspecifics in mice (Hassan et al., 2023). The ability of CA2 to respond to social odors in the absence of conspecifics, as seen in our study, may allow rats to discern territory and identity information, which may drive social behavior even when other rats are not present. The precise mechanisms underlying social odor detection in CA2 neurons are unknown but may be related to the distinctive neurochemical and structural features of CA2 neurons.

CA2 neurons contain an abundance of neuropeptide receptors, including oxytocin receptors (OXTR) and vasopressin receptors (AVP1-b) (Mitre et al., 2016; Vaccari et al., 1998; Young and Song, 2020). Genetic or pharmacological ablation of CA2 OXTRs impairs the persistence of long-term social recognition memory and the ability to discriminate between social stimuli in mice (Lee et al., 2008; Lin et al., 2018; Raam et al., 2017). AVP1-b activation in CA2 has been associated with social aggression (Pagani et al., 2015) and the enhancement of social memory (Smith et al., 2016). Long-range axonal projections from the paraventricular nucleus of the hypothalamus (PVN) may be the source of neuropeptide release onto CA2 neurons during social interactions. The PVN receives input from the olfactory system (Guevara-Aguilar et al., 1988) and directly innervates CA2 (Cui et al., 2013; Zhang and Hernández, 2013).

Several other brain regions that are involved in social processing send direct inputs to CA2, including the hypothalamic supramammillary nucleus (SuM) and the lateral entorhinal cortex (LEC). SuM projections to CA2 are preferentially activated by novel social encounters (Chen et al., 2020), making SuM inputs an unlikely candidate to induce CA2 place cell responses to a familiar social odor. Conversely, neuronal activity in the LEC showed similar increases during social exploration for both novel and familiar conspecifics (Lopez-Rojas et al., 2022). Recent studies have suggested that the direct projection from LEC to CA2 is essential for social memory (Dang et al., 2022; Lopez-Rojas et al., 2022). Whether LEC input to CA2 is selectively enhanced during exploration of a social odor alone remains to be tested, but a more general involvement of the LEC in odor processing is well documented (Igarashi et al., 2012; Kerr et al., 2007).

CA2 also receives inputs from the dentate gyrus (DG). The DG directly projects to CA2 via mossy fibers that form excitatory synapses onto CA2 neurons (Kohara et al., 2014). The DG is predicted to have a role in pattern separation, a process that reduces overlap between ensembles of neurons to minimize interference (McClelland and Goddard, 1996). The DG is important for associating odors with larger contexts in discrimination tests (Morris et al. 2012). However, whether the DG responds selectively to social odors is unknown and may warrant further investigation.

Consistent with previous work, our results show that CA2 place cells that remap in response to a familiar social odor do not return to their original activity, despite the removal of the social odor in the last session (Alexander et al., 2016). It is possible that the persistent change in CA2 activity is a result of lingering social odors in the arena. Recording CA2 place cells for longer than 20 minutes after the removal of the odor would address if CA2 place cell activity eventually returns to baseline, although this would be complicated by the effect of time on CA2 place cells

(Mankin et al., 2015). However, this persistent change in CA2 place cell activity may also suggest the involvement of synaptic plasticity. Interestingly, CA2 synapses are resistant to standard long-term potentiation (LTP) compared to other hippocampal subfields (Chang et al., 2007; Zhao et al., 2007). How, then, are long-lasting changes occurring in CA2 place cells? One hypothesis is that the release of social neuropeptides, such as vasopressin and oxytocin, can promote potentiation of CA2 neurons (Lin et al., 2018; Pagani et al., 2015; Tirko et al., 2018). Selective activation of OXTRs or AVP1-b *in vitro* robustly depolarizes CA2 pyramidal neurons and lowers the threshold for LTP at excitatory synapses (Dang et al., 2022; Tirko et al., 2018). As a result, neuropeptide receptor activation may refine the responsiveness of CA2 pyramidal neurons to synaptic input from upstream structures, thus creating appropriate conditions for synaptic plasticity.

A question remains of how social information coded by CA2 is transmitted to downstream regions that control behavior. The main output of dorsal CA2 is dorsal CA1. However, at the single cell level, dorsal CA1 place cell activity was unaffected by social interactions (Alexander et al., 2016). Dorsal CA2 neurons also project to ventral CA1, a region that is essential for social memory (Okuyama et al., 2016). Recent work has shown that ventral CA1 place cells did not remap to a social stimulus (Wu et al, 2023). However, ventral CA1 place cells became significantly more spatially selective in an environment that contained a social stimulus compared to an empty environment (Wu et al. 2023). Further, the activity of a subset of ventral CA1 neurons was modulated by the presence of a conspecific (Rao et al. 2019; Wu et al. 2023). Inhibition of dorsal CA2 projections to ventral CA1 impairs social memory (Meira et al., 2018; Tsai et al., 2022), raising the possibility that social remapping in dorsal CA2 induces the alterations in neuronal firing in ventral CA1 in response to social stimuli.

Interestingly, recent work has shown that sharp wave-ripples that originate in dorsal CA2 are important for social memory consolidation (Oliva et al., 2016, 2020). While a portion of the sharp wave-ripples initiated in dorsal CA2 propagate to other subregions of the dorsal hippocampus, some do not propagate to dorsal CA1 or dorsal CA3 (Oliva et al., 2016). Given that individual place cells in dorsal CA1 did not remap to a social stimulus (Alexander et al., 2016), it is possible that the reactivation of CA2 place cells that respond to a social odor may be limited to sharp wave-ripples that do not propagate to dorsal CA1. Instead, the function of these sharp wave-ripples may be to propagate information to ventral CA1 in order to consolidate memories of a social experience (Meira et al., 2018). Consistent with this idea, the rate of sharp wave-ripples increased in ventral CA1 when conspecifics were present (Rao et al., 2019). A future study examining the reactivation of dorsal CA2 place cells that respond to social stimuli while simultaneously recording place cells in dorsal and ventral CA1 will be essential for our understanding of how reactivation of CA2 place cells during sharp wave-ripples supports social memory consolidation.

An important question left unanswered is the extent to which CA2 is specialized for social memory. While the present results show that a social odor is sufficient to induce CA2 place cell remapping, it remains unknown whether other odors of similar salience or ethological relevance have similar effects. Future studies examining the activity of CA2 place cells in response to other relevant salient nonsocial odors, such as predator urine, will help determine whether CA2 integrates many salient odors into representations of space or whether the role of CA2 is specific to social information.

The current study shows how presentation of a social odor alone provides a well-controlled social stimulus to induce changes in place cell activity in dorsal CA2. Use of a social odor can eliminate confounds that could be introduced by the presence of a conspecific, such as

518 variations in locomotor behavior, social interactions, and ultrasonic vocalizations. Presentation
519 of a social odor thereby provides a valuable paradigm for assessing CA2 physiology in rodent
520 models of diseases and disorders involving aberrant social behaviors, such as autism spectrum
521 disorders, to better understand how impairments in social behavior may arise.

522

523 Table 1. Number of CA2 place cells recorded in each rat and each condition during active
524 exploration sessions.

Animal ID	**Control**	**Odor**	**Visual**	**Visual + odor**	**Mirror**
Rat 117 (“Lauren”)	7	5	7	0	6
Rat 122 (“Chase”)	51	32	38	0	49
Rat 165 (“Gus”)	34	56	26	26	35
Rat 256 (“Viggo”)	28	13	13	13	21
Rat 391 (“Daffodil”)	15	4	6	5	5
Rat 418 (“Hugo”)	20	35	18	41	0
Total	**155**	**145**	**109**	**85**	**116**

525

526 Table 2. Number of CA2 place cells recorded in each rat and each condition during rest periods.

Animal ID	**Control**	**Odor**	**Visual**	**Visual + odor**	**Mirror**
Rat 117 (“Lauren”)	0	0	1	0	0
Rat 122 (“Chase”)	52	31	2	0	18
Rat 165 (“Gus”)	26	58	31	23	38
Rat 256 (“Viggo”)	18	10	13	8	0
Rat 391 (“Daffodil”)	14	4	7	5	5

Rat 418 (“Hugo”)	20	29	14	35	0
Total	**130**	**132**	**68**	**71**	**61**

CRediT authorship contribution statement

Emma Robson: Formal analysis, investigation, writing – original draft, visualization. **Margaret M. Donahue:** Formal analysis, investigation, writing – original draft, visualization, funding acquisition. **Alexandra J. Mably:** Conceptualization, methodology, formal analysis, investigation. **Peyton G. Demetrovich:** Investigation. **Lauren T. Hewitt:** Investigation. **Laura Lee Colgin:** Conceptualization, methodology, resources, writing – review and editing, supervision, project administration, funding acquisition.

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Data availability

Data will be made available on request.

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Figure 1. Behavioral task. Rats freely explored a familiar open field arena for four 20-minute sessions per day. A stimulus cage, a standard rat housing cage, was placed in a corner of the arena. In the first and final sessions of experimental conditions (i.e., Odor, Visual + Odor, Visual, Mirror), and in all four sessions of the Control condition, the cage contained only clean bedding. In the middle two sessions of experimental conditions, a cage containing social stimuli was presented. In the Odor condition, a stimulus cage containing soiled bedding of a familiar rat or rats was presented. In the Visual + Odor condition, a cage containing a familiar rat and the rat's soiled bedding was presented. In the Visual condition, a familiar rat was placed in the stimulus cage with clean bedding and a filter-top lid to minimize social odors. In the Mirror condition, a familiar rat was placed in the stimulus cage with clean bedding, and the cage was lined with a one-way mirror attachment. This prevented the stimulus rat from seeing the implanted rat, thereby limiting reciprocal social interactions.

Figure 2. Histology. An example hippocampal section showing immunohistological identification of a tetrode track in CA2. To identify CA2, hippocampal sections were immunostained with a CA2 marker, Purkinje cell protein 4 (PCP4, red). DAPI nuclear staining is shown in blue.

Figure 3. Example CA2 place cell firing rate maps across the four recording sessions for all conditions. Color-coded firing rate maps are shown for all place cells recorded on a single example tetrode in each condition for one example rat (Rat 165). Rate maps are shown scaled to the maximal firing rate (shown inset) of each cell across all sessions. White pixels indicate places that were not visited by the rat during a session.

Figure 4. Changes in CA2 place cell firing patterns in response to social stimuli. A. The estimated means of spatial correlation coefficients from our generalized linear mixed model are shown across each condition and session pair. Error bars represent 95% confidence intervals.

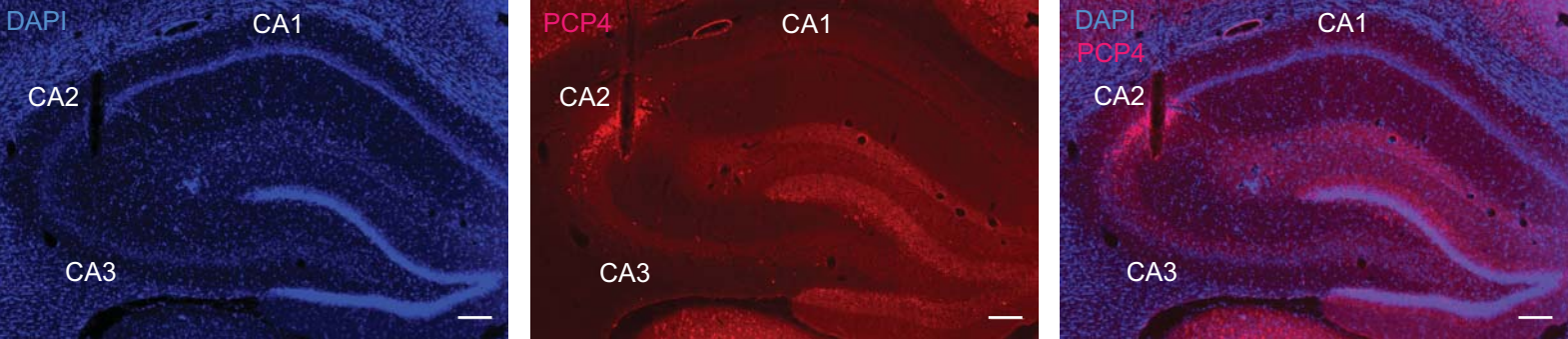
B. The estimated means of spatial correlation coefficients from our generalized linear mixed model are shown across each condition for all session pairs combined. C-G. Spatial correlation measures are shown for the entire sample of CA2 place cells for all pairs of sessions across all conditions. Each marker represents a spatial correlation value for an individual place cell. Different symbols are used for CA2 place cells recorded from different rats.

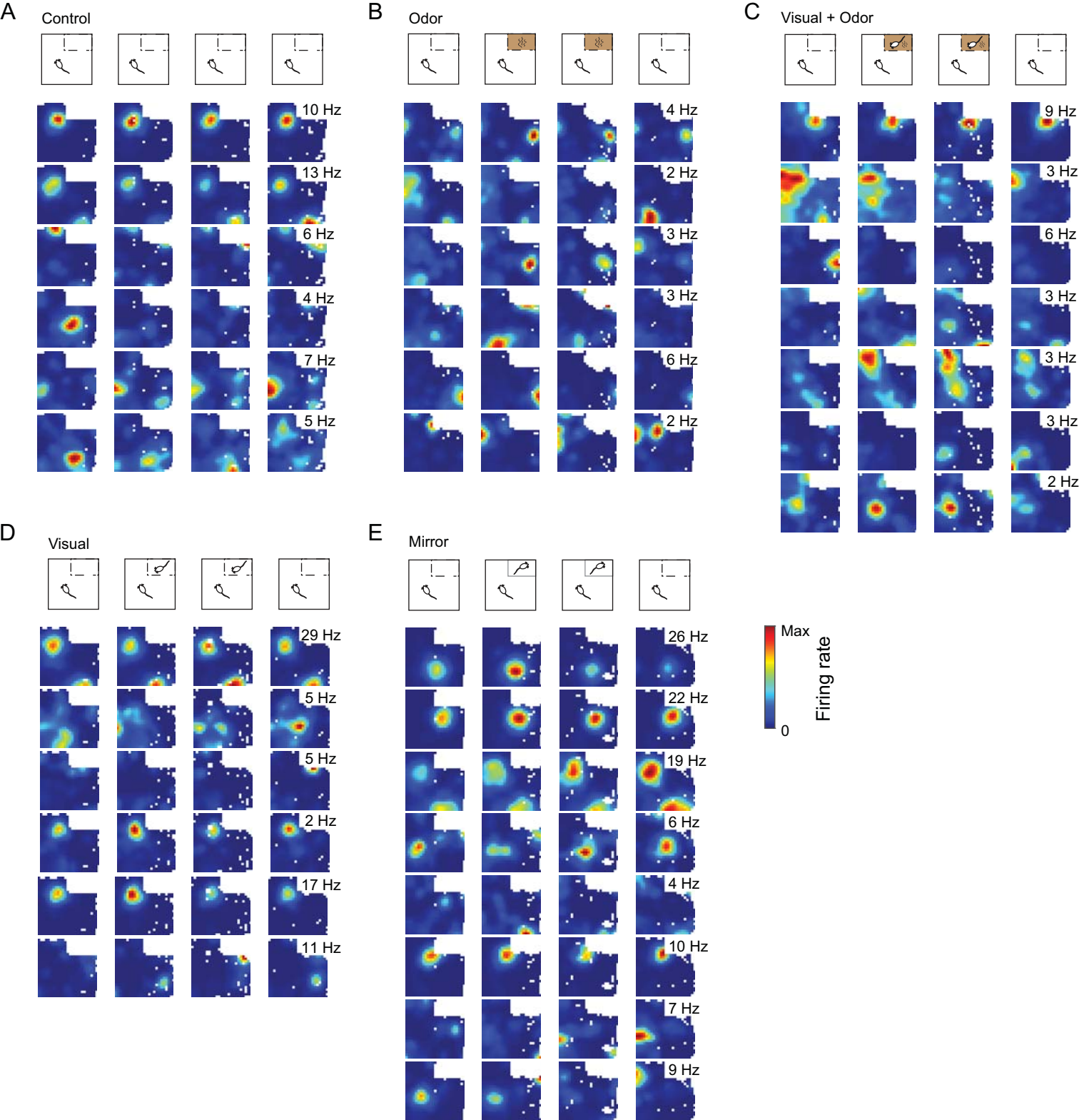
Figure 5. CA2 place cells did not show a coherent place field shift related to the location of the stimulus cage. A. Shown are the distances measured between the positions of peak firing in Session B and the stimulus cage for CA2 place cells that “turn on”, or gain a field, in Session B. B. Shown are the distances between the peak firing rate position in Session A and the location of the stimulus cage for CA2 place cells that “turn off”, or lose a field, in Session B. C. Shown are the changes in distance from the stimulus cage of the peak firing rate positions in Sessions A and B for CA2 place cells that were active in both Sessions A and B but shifted their place field locations. Individual markers represent measurements from individual place cells. Different symbols represent distance measurements for CA2 place cells recorded from different rats.

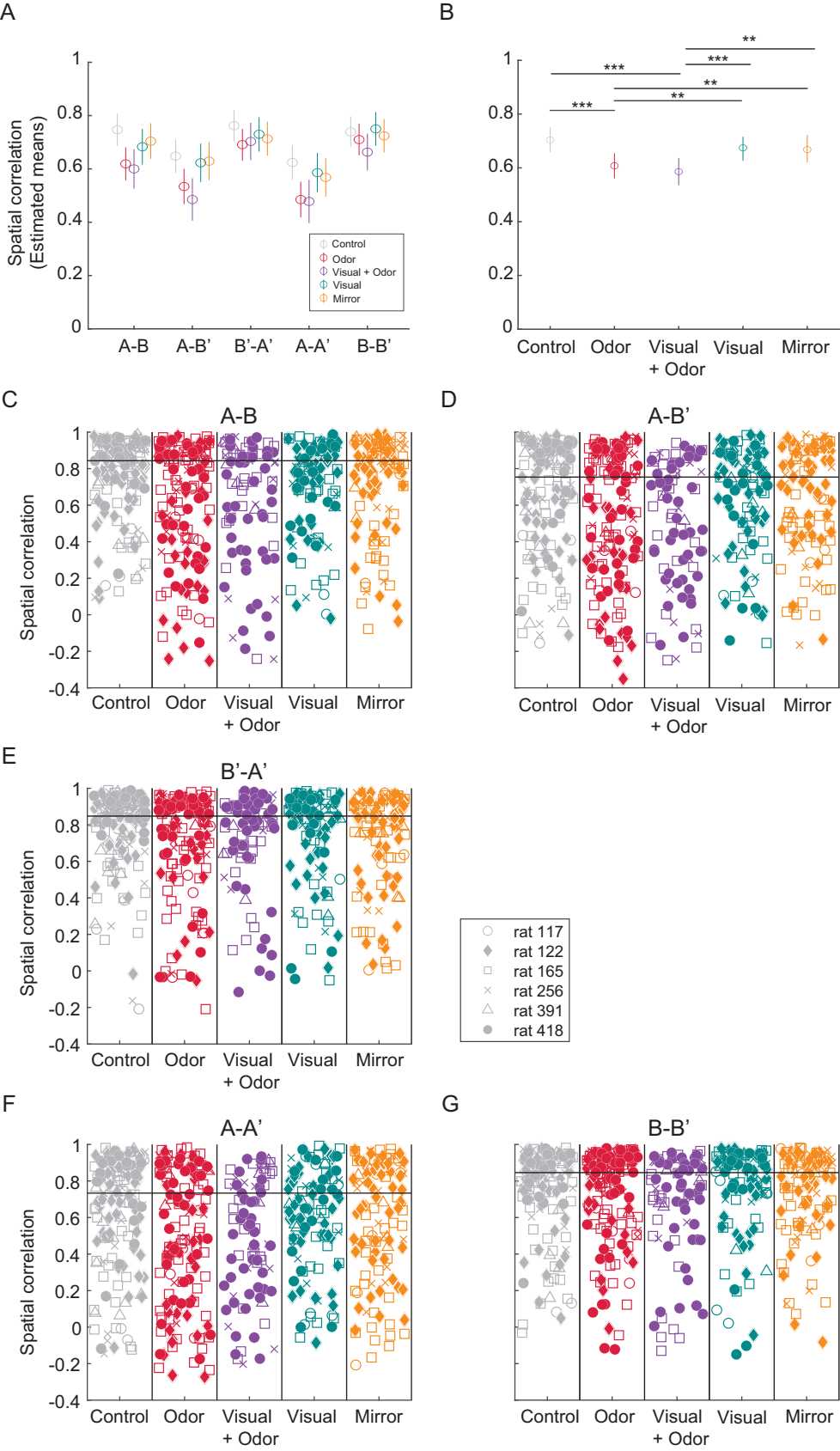
Figure 6. Exploration of social stimuli. A. Heat maps show mean exploration times of different locations in the arena, with the stimulus cage shown in the top right corner of the arena. Time spent was calculated for the first 2 minutes of each session individually and then averaged within each rat. Heat maps shown are averaged across rats. B. Time spent exploring locations close to the cage (within 12 cm) was calculated for each session and then averaged within a rat. Individual dots represent the mean exploration time for each rat, and error bars represent 95% confidence intervals across all sessions within a rat. Rats increased their exploration time of the stimulus cage in session B for the Odor, Visual, and Mirror conditions compared to the Control condition.

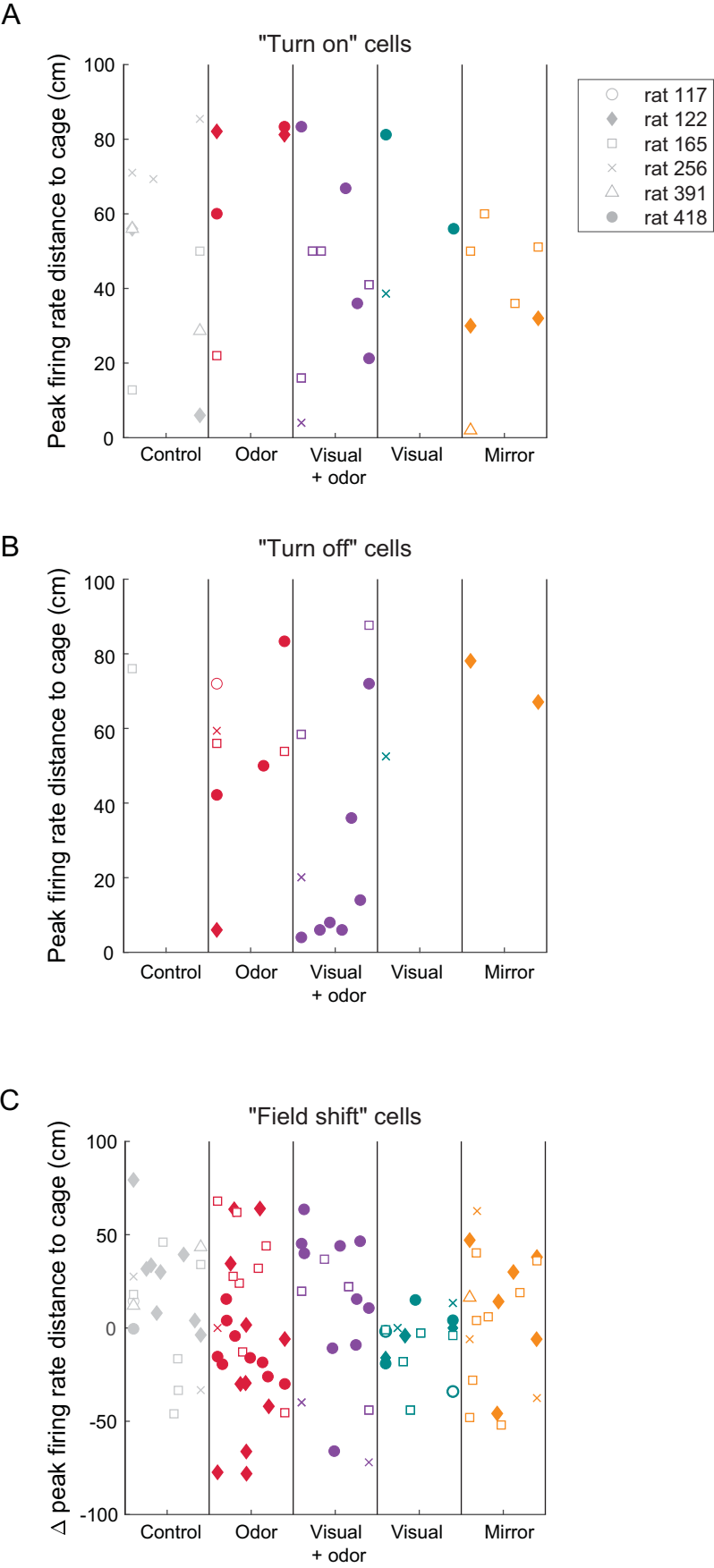
Figure 7. CA2 place cells that responded to a social odor preferentially fired during sharp wave-ripples. A. The difference between peak normalized firing rates of CA2 place cells during sharp wave-ripples in the rest period following session B compared to peak normalized firing rates of CA2 place cells during sharp wave-ripples in the rest period following session A was positively correlated to the selectivity index for the Odor condition (B) but not the Control condition (A). The selectivity index indicates a cell's preference for session A (value of -1 for maximum selectivity in session A) or session B (value of 1 for maximum selectivity in session B).

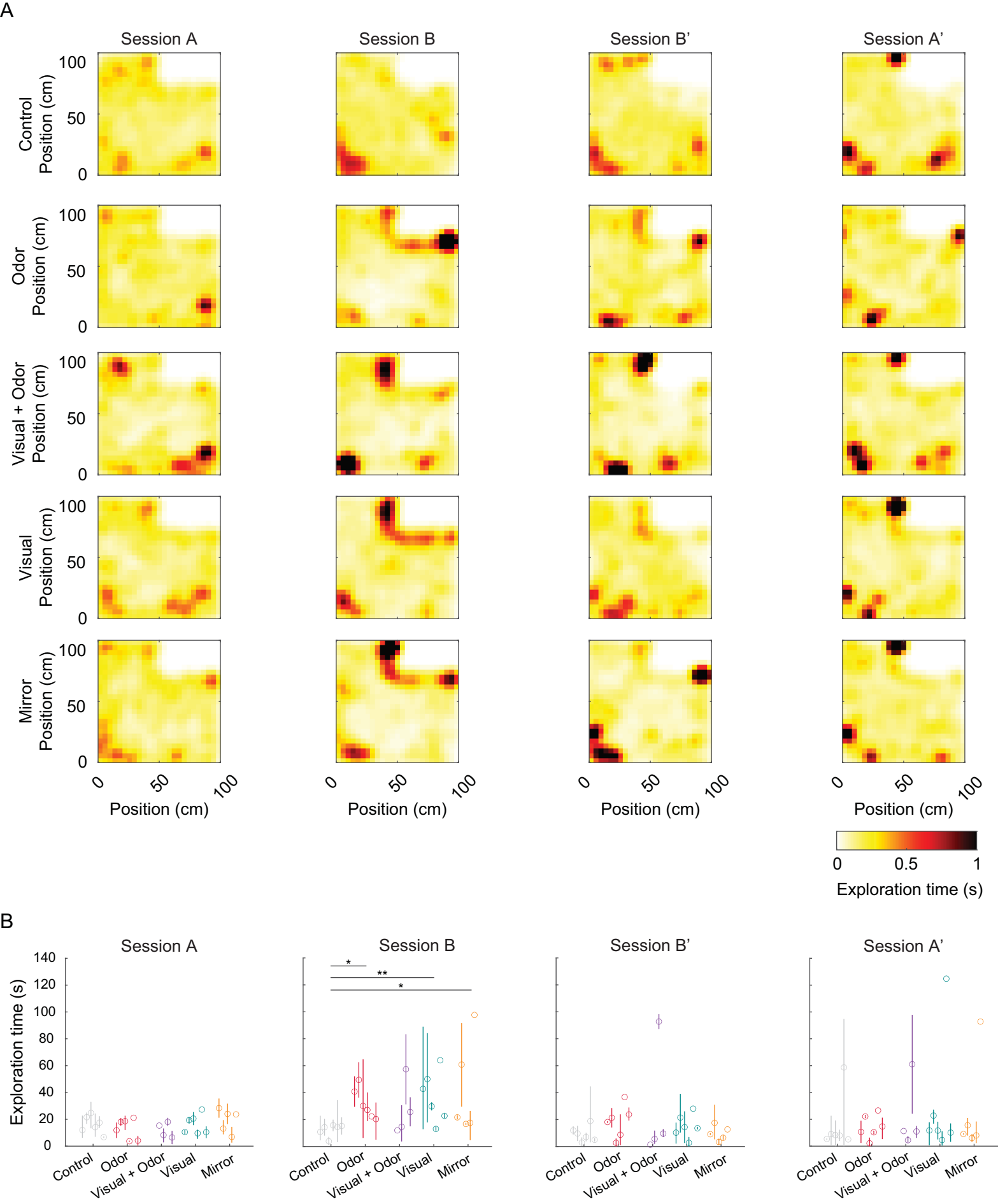
Mirror
Visual
Visual + Odor
Odor
Control
A
B
B'
A'
1 m
1 m

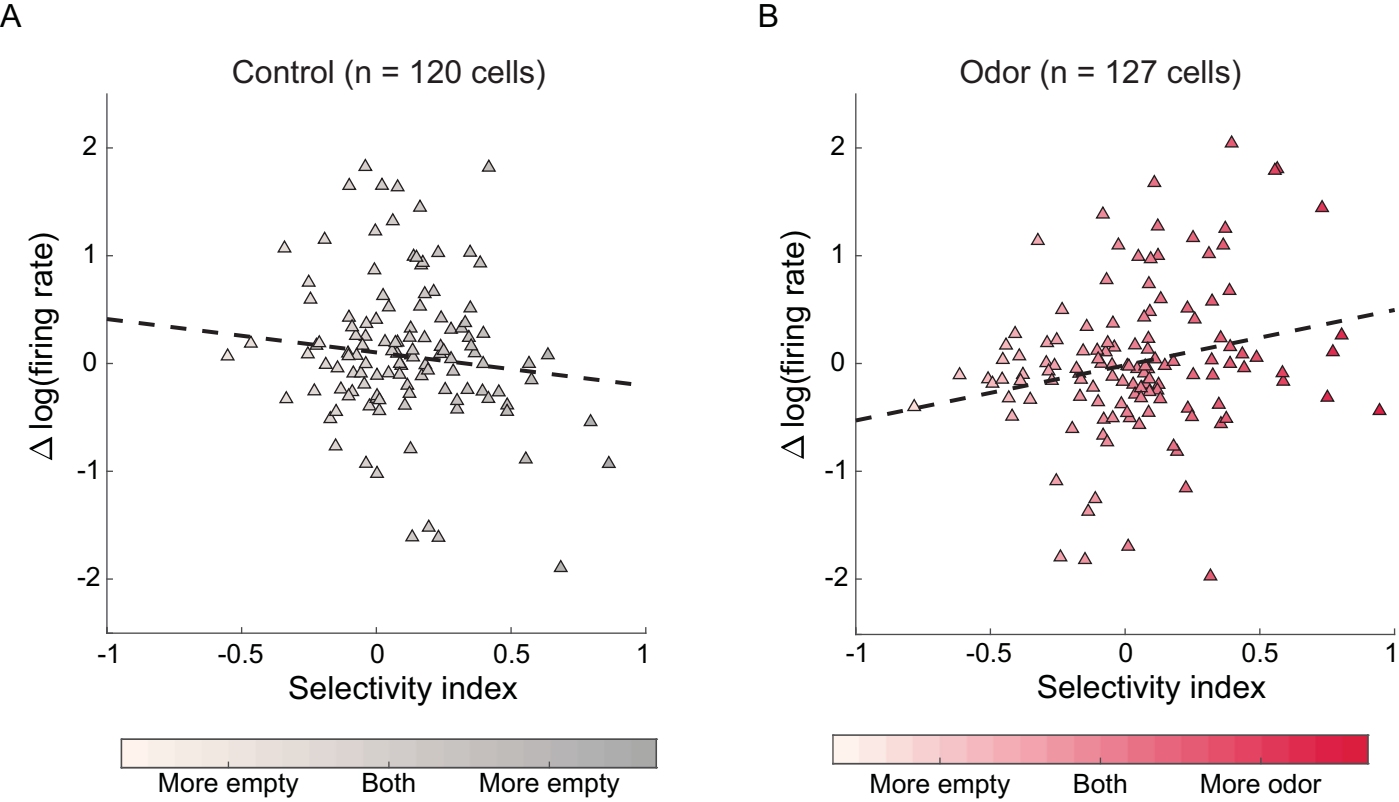

DAPI
CA1
CA2
CA3
PCP4
CA1
CA2
CA3
DAPI
PCP4
CA1
CA2
CA3


A
Control
10 Hz
13 Hz
6 Hz
4 Hz
7 Hz
5 Hz
B
Odor
4 Hz
2 Hz
3 Hz
3 Hz
6 Hz
2 Hz
C
Visual + Odor
9 Hz
3 Hz
6 Hz
3 Hz
3 Hz
3 Hz
2 Hz
D
Visual
29 Hz
5 Hz
5 Hz
2 Hz
17 Hz
11 Hz
E
Mirror
26 Hz
22 Hz
19 Hz
6 Hz
4 Hz
10 Hz
7 Hz
9 Hz
Max
0
Firing rate


A
Spatial correlation (Estimated means)
Control
Odor
Visual + Odor
Visual
Mirror
A-B
A-B'
B'-A'
A-A'
B-B'
B
***
***
***
**
**
**
Control
Odor
Visual + Odor
Visual
Mirror
C
A-B
Spatial correlation
D
A-B'
E
B'-A'
rat 117
rat 122
rat 165
rat 256
rat 391
rat 418
F
A-A'
G
B-B'


A
"Turn on" cells
Peak firing rate distance to cage (cm)
100
80
60
40
20
0
Control
Odor
Visual + odor
Visual
Mirror
rat 117
rat 122
rat 165
rat 256
rat 391
rat 418
B
"Turn off" cells
Peak firing rate distance to cage (cm)
100
80
60
40
20
0
Control
Odor
Visual + odor
Visual
Mirror
C
"Field shift" cells
Δ peak firing rate distance to cage (cm)
100
50
0
-50
-100
Control
Odor
Visual + odor
Visual
Mirror


A
Session A
Session B
Session B'
Session A'
Control
Odor
Visual + Odor
Visual
Mirror
Position (cm)
0
50
100
Exploration time (s)
0
0.5
1
B
Session A
Session B
Session B'
Session A'
Exploration time (s)
0
20
40
60
80
100
120
140
*
**
*
Control
Odor
Visual + Odor
Visual
Mirror


A
Control (n = 120 cells)
Δ log(firing rate)
2
1
0
-1
-2
-1
-0.5
0
0.5
1
Selectivity index
More empty
Both
More empty
B
Odor (n = 127 cells)
Δ log(firing rate)
2
1
0
-1
-2
-1
-0.5
0
0.5
1
Selectivity index
More empty
Both
More odor