Behavioral/Cognitive

Enhanced Neuronal Activity in the Medial Prefrontal Cortex during Social Approach Behavior

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Although the medial prefrontal cortex (mPFC) is known to play a crucial role in rodent social behavior, little is known about mPFC neural correlates of social behavior. In the present study, we examined single-neuron activity in the mPFC of mice performing a modified version of the three-chamber test. We found that a subset of mPFC neurons elevate discharge rates when approaching a stranger mouse but not when approaching an inanimate object or an empty chamber. Our results reveal mPFC neural activity that is correlated with social approach behavior in a widely used social-interaction paradigm. These findings might be helpful for future investigations of mPFC neural processes underlying social interaction in health and disease.

Key words: in vivo recording; mPFC; object recognition; social interaction; three chamber test

Significance Statement

Although the prefrontal cortex is known to play a crucial role in rodent social behavior, little is known about prefrontal neural correlates of social behavior. This study shows that the activity of a subset of prefrontal neurons increases in association with social approach behavior during a three-chamber test—a widely used behavioral paradigm. Such responses might be a signature of prefrontal neural processes underlying social approach behavior.

Introduction

Mouse models of social dysfunction are widely used to investigate neural mechanisms underlying various neuropsychiatric diseases, such as autism spectrum disorders (ASDs), depression, and schizophrenia (Bey and Jiang, 2001; Yang et al., 2011; Ting et al., 2012; Kleijer et al., 2014; Lombardi et al., 2015; Kazdoba et al., 2016). Previous studies have found that the medial prefrontal cortex (mPFC) plays a crucial role in mouse social behavior. A disturbed excitatory and inhibitory balance in the mPFC has been shown to induce social dysfunction (Yizhar et al., 2011). Social stress causes reduced mPFC neural activity and social avoidance, effects that can be reversed by ketamine treatment, optogenetic stimulation of mPFC neurons, or optogenetic modulation of

dopaminergic projections to the mPFC (Covington et al., 2010; Li et al., 2010; Chaudhury et al., 2013; Lee et al., 2015). In addition, functional alterations of the mPFC have been observed in mouse models of ASDs that show impaired social interactions. For instance, reduced inhibitory synaptic transmission and decreased NMDA receptor function have been demonstrated in the mPFC of neuroligin-2- and Shank3-deficient mice, respectively (Duffney et al., 2015; Liang et al., 2015). Moreover, an NMDA receptor antagonist has been shown to normalize suppressed mPFC neural activity in mice lacking the excitatory postsynaptic adapter protein IRSp53 and rescue their associated social deficits (Chung et al., 2015).

Although mounting evidence implicates the mPFC in the regulation of social behavior, mPFC neural correlates of social interaction are poorly understood (Jodo et al., 2010). We addressed this issue in the present study by examining the activity of mPFC neurons in mice during a three-chamber test (Crawley, 2004), which is widely used to measure social dysfunction in animal models of ASDs (Won et al., 2012; Huang et al., 2014; Chung et al., 2015), schizophrenia (Del Pino et al., 2013; Nguyen et al., 2014), and other neurodevelopmental disorders (Grayton et al., 2013; Barnes et al., 2015). In the three-chamber test, a subject mouse is allowed to explore two opposing chambers containing another mouse (social stimulus) or an inanimate object (nonsocial stimulus), and the relative amount of time spent exploring

the social versus nonsocial target is used as an index of social preference. A caveat to the use of this index for assessing social behavior is that the extent to which the response to a salient stimulus—whether social or nonsocial—accounts for the social target-preferring behavior is unknown. Also unknown is what makes a "social" stimulus social. It could be the target animal's movement, odor, or sound, or some combination of these factors. A previous study has shown that olfactory cues are sufficient to elicit social approach behaviors in mice (Ryan et al., 2008). However, it is possible that other sensory cues, or their combinations with olfactory cues, contribute to social approach behaviors. Clearly, additional studies are necessary to clarify these issues. Nevertheless, given the widespread use of the three-chamber test in examining mouse social behavior, it would be informative to examine mPFC neural activity in the task. As a first step toward understanding mPFC neural correlates of social behavior, we examined mPFC neuronal activity in mice subjected to a modified version of the three-chamber test. We found that the discharge rates of a subset of mPFC neurons increased in the test mouse during the approach to a social target.

Materials and Methods

Animals
C57B/6J male mice (13–15 weeks old, $n = 6$) were used in all experiments. Mice were maintained with access to food and water *ad libitum* under a 12 h light/dark cycle, and their behaviors were examined during the dark phase of the cycle. All animal care and experimental procedures were performed in accordance with protocols approved by the directives of the Animal Care and Use Committee of Korea Advanced Institute of Science and Technology (approval number KA2013-04).

Linear chamber test
The conventional three-chamber apparatus (Crawley, 2004) was modified to a linear one (45 × 10 × 21 cm) without dividing walls to allow single-unit recording, to minimize exploration of unnecessary areas, and to increase the number of visits to the targets (Fig. 1A). Two removable chambers (target chambers; 10 × 10 × 20 cm) were placed at both ends of the apparatus. Each target chamber contained a stranger mouse (129/Sv, male, 12–16 weeks old; social stimulus), an inanimate object (nonsocial stimulus), or nothing (empty chamber). A nonmoving bobble-head doll (~4 × 3 × 7 cm; Fig. 1A) was used as the inanimate object throughout the experiments. The apparatus was made of translucent acrylic walls (0.5 cm thick). The lower 10 cm of the target-chamber wall facing the other end of the apparatus was barred with 1-cm-spaced thin metal wires to allow the subject mouse to interact with the target.

The daily experiment consisted of three experimental sessions (Fig. 1A). The animal was first placed in the apparatus and allowed to explore the apparatus freely for 10 min with both chambers containing no object or animal (session 1: habituation or E-E session; empty chamber, E). Then, a stranger mouse (social stimulus, S) and the inanimate object (nonsocial stimulus, O) were placed in the chambers with their left/right locations randomly determined. The subject mouse was allowed to explore the targets freely for 10 min during the second experimental session (first S-O session). The positions of the two targets were then swapped, and the subject mouse was allowed to explore the targets for another 10 min in the third experimental session (second S-O session). The same stranger mouse was used for both S-O sessions. The second S-O session was designed to distinguish social target-dependent neural activities from spatial location (left/right)-dependent ones. The subject mouse was removed from the apparatus and placed on an elevated platform outside the apparatus between successive sessions (intersession intervals <1 min). Additionally, before and after the three experimental sessions, the subject mouse was placed on the platform for 5 min to check the stability of unit signals. Because social isolation is known to increase social interaction (Crawley, 2004), each animal was tested with a 4 day interval between successive experiments. Each mouse was tested in 2–10 experiments and exposed to a new stranger mouse in each experiment.

Single-unit recording
An array of microdrives (Harlan 4-drive; Neuralynx) carrying four tetrodes was implanted in the right mPFC (1.7 mm anterior, 0.3 mm lateral from bregma, and 1.8–2.5 mm ventral from the brain surface) under ketamine anesthesia. After 1 week of recovery from surgery, tetrodes were gradually lowered and unit signals were recorded from the prelimbic cortex. Unit signals were amplified 10,000×, filtered between 600 and 6000 Hz, digitized at 32 kHz, and stored on a personal computer using a Cheetah data acquisition system (Neuralynx). Tetrodes were lowered by 42.5 μm after each daily experiment so that different units were recorded across successive experiments. The anatomical location of each recorded unit was determined based on the location of a marking lesion (histological examination of 40 μm coronal brain sections under a light microscope) and the advancement history of the corresponding tetrode. A digital camera was mounted on the ceiling (100 cm above the center of the apparatus) to monitor the animal's behavior, and video images were recorded with Ethovision XT 10.1 (Noldus). Spike clustering was performed offline using MClust (A. D. Redish, University of Minnesota, Minneapolis, MN). Only well isolated unit clusters were included in the analysis (L ratio <0.1, isolation distance >25). Both putative pyramidal cells and putative interneurons were included in the analysis.

Analysis
Interaction with a target. Video images were analyzed offline with Ethovision XT 10.1. Target nose-poke, in-zone entry, and facing a target were quantified as measures of the interaction with a target. The onset of target nose poke was defined as when the subject mouse's nose (tracked by Ethovision) entered the 12 × 12 cm area in the video image covering the wired section of the target chamber wall (1 cm margin from each side of the 10 × 10 cm square). A valid nose-poke trial was defined as an incidence of target nose poke for >1 s with an interval from the previous nose poke trial >2 s. In-zone entry was scored when the center point of the subject mouse (tracked by Ethovision) entered the 10 × 10 cm area of the floor adjacent to the chamber; Fig. 1A). A valid in-zone trial was defined as a >1 s visit of the in-zone area with an interval from the previous visit >2 s (center of the body was used). Facing a target was defined as when the projection of the forward orientation vector of the mouse (determined using nose and body coordinates) passed through a chamber in the video image.

Neuronal firing rate. Trials were divided into six groups according to the session (3×) and spatial (right or left) location (2×). Trials were aligned to the onset of target nose poke or in-zone entry for all valid trials, and spike density functions (SDFs) were constructed by applying a Gaussian kernel ($\sigma = 50$ ms) to each spike. For each neuron, maximum firing rates during the first 1 s period after nose-poke onset or in-zone entry were obtained for six SDFs, and the highest of them was used to normalize each SDF. Spike data during the entire nose-poke period was included in the analysis, but similar results were obtained when spike data during the first 1 s of the nose-poke period was analyzed (data not shown). For the analysis of spatial firing rate, the center chamber was divided into nine zones (5 × 10 cm each) along the longitudinal axis, and the mean spatial firing rate (the number of spikes divided by occupancy time) was calculated for each zone for each neuron. Spatial firing rates were normalized to the highest of the nine zone firing rates for each neuron.

Discrimination index. The discrimination index (d'; Macmillan and Creelman, 2004; Latimer et al., 2015) was used to compare levels of neuronal responses for two targets in a given session. d' was defined as follows:

$$d' = \frac{\mu_1 - \mu_2}{\sqrt{\frac{1}{2}(\sigma_1^2 + \sigma_2^2)}}$$

where μ and σ represent mean and SDs, respectively, of neuronal firing rate during valid nose-poke or in-zone trials. For the S-O sessions, μ_1 and μ_2 represent mean firing rates for social and object targets, respectively, and positive and negative d' values indicate preferential firing toward the social and objet target, respectively. For the E-E session, μ_1 and μ_2 represent mean firing rates for left and right targets, respectively. Three d' values were calculated for all three experimental sessions (E-E, first S-O, and second S-O) for each neuron.
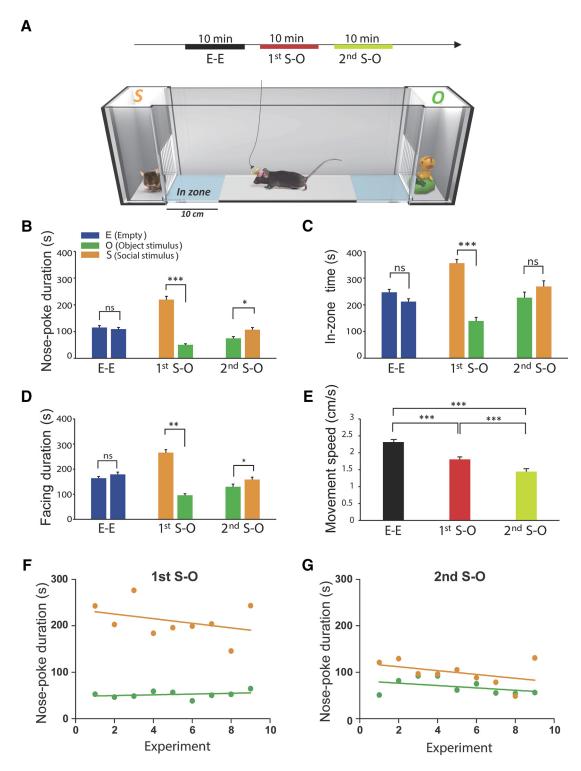
Figure 1. Preferential interaction with a social target in the linear chamber apparatus. **A**, Experimental scheme. Mice were subjected to three 10-min exploratory sessions in the linear chamber apparatus. E-E, Habituation session (both chambers were empty); first S-O, the first social interaction session [a stranger mouse-containing chamber (S) versus an inanimate object-containing chamber (O)]; and second S-O, the second social interaction session (the stranger mouse and the inanimate object swapped their positions). **B–D**, Preferential interaction with the social target, as measured by nose-poke (**B**), in-zone (**C**), and facing (**D**) durations (44 experiments, 6 mice; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns, not significant; two-way ANOVA with Tukey's *post hoc* test). **E**, Decreased movement speed (total distance/recording time) across sessions (***$p < 0.001$). **F, G**, Total nose-poke duration per session across successive experiments. Nose-poke durations for the social (orange) and object (green) stimulus are shown separately for the first (**F**) and second (**G**) S-O sessions. The lines were determined with linear regression. None of them showed a significant relationship (*p* values of the slopes >0.05).

Statistical tests

For the analysis of behavioral data, two-way repeated-measures ANOVA was used to examine effects of stimulus type (social vs nonsocial) and session (first vs second S-O) on animal's approach behaviors (durations of nose poke, in-zone stay, and facing a target), and the repetitive measurement one-way ANOVA was used to compare animal's movement speeds across the three experimental sessions. Student's *t* test was used to compare the indices of approach behaviors for the left and right targets

during the habituation (E-E) session. For the analysis of neural data, two-way ANOVA was used to examine the effects of stimulus type (social, object, and empty) and location (left vs right) on neural activities, and one-way ANOVA was used to compare neuronal discrimination index (d') across the three experimental sessions. Three-way repeated-measures ANOVA was also used to examine effects of stimulus type, stimulus location, and session sequence on neural activities. Student's t test was used to test whether neuronal discrimination index (d') was significantly different from zero. Tukey's test was used in all *post hoc* analyses after ANOVA. All statistical tests were two-tailed tests. A p value <0.05 was used as the criterion for a significant statistical difference, and all data were expressed as mean ± SEM unless noted otherwise.

Results

Behavior
Six mice were tested in 44 experiments, with each consisting of three sessions. Social preference was assessed by comparing the amount of time spent investigating a social versus a nonsocial object stimulus, determined by monitoring nose poking and facing the stimulus and occupation of its in-zone area (Fig. 1A). During the habituation session (session 1), in which both chambers were empty (E-E), mice showed no tendency to preferentially explore the left or right chamber, measured as nose-poke duration ($p = 0.518$), facing duration ($p = 0.200$), and in-zone duration ($p = 0.058$; $n = 44$; paired t test). However, mice showed a strong preference for the social stimulus during the first S-O session (session 2), which was substantially reduced during the second S-O session (session 3; $n = 44$; two-way repeated-measures ANOVA). The results of ANOVAs (expressed as F values) and *post hoc* Tukey's test (first vs second session) for each of the three measured parameters can be summarized as follows (Fig. 1B–D): (1) nose-poke duration: main effect of stimulus type, $F_{(1,86)} = 48.58$ ($p < 0.001$); main effect of session, $F_{(1,86)} = 40.17$ ($p < 0.001$); stimulus × session interaction, $F_{(1,86)} = 204.0$ ($p < 0.001$); S versus O during the first S-O session, $p < 0.001$; S versus O during the second S-O session, $p = 0.011$; (2) facing duration: main effect of stimulus type, $F_{(1,86)} = 34.91$ ($p < 0.001$); main effect of session, $F_{(1,86)} = 30.40$ ($p < 0.001$); stimulus × session interaction, $F_{(1,86)} = 184.4$ ($p < 0.001$); S versus O during the first S-O session, $p < 0.001$; S versus O during the second S-O session, $p = 0.048$; and (3) in-zone duration: main effect of stimulus type, $F_{(1,86)} = 17.67$ ($p = 0.004$); main effect of session, $F_{(1,86)} = 0.011$ ($p = 0.751$); stimulus × session interaction, $F_{(1,86)} = 62.80$ ($p < 0.001$); S versus O during the first S-O session, $p < 0.001$; S versus O during the second S-O session, $p = 0.387$. As shown in Figure 1E, the movement speed of mice decreased progressively across the three sessions ($n = 44$), as determined by repeated measures one-way ANOVA ($F_{(2,86)} = 62.54$, $p < 0.001$) and Tukey's *post hoc* test (E-E vs first S-O, $p < 0.001$; E-E vs second S-O, $p < 0.001$; first S-O vs second S-O, $p < 0.001$), suggesting that the reduced social interaction during the second relative to the first S-O session is likely attributable to habituation. We found no significant change in nose-poke duration across successive experiments (linear regression, $p > 0.05$), likely because we used a new stranger mouse for each experiment (Fig. 1F, G).

Neuronal activity during nose poking
We recorded 248 well isolated units from the prelimbic and infralimbic cortices, with the majority recorded from the prelimbic cortex. Of these, neurons that emitted spikes during all three experimental sessions (E-E, first S-O, and second S-O) with overall mean discharge rates >0.5 Hz during the nose-spoke period were included in the analysis (176 of 248 units; Table 1). Figure 2,

Table 1. Acquired cell information

Mouse identification number	No. of experiments	Recorded units	Analyzed units	Social-selective units	Object-selective units	Empty-selective units
Nose poking						
1	9	32	15	2	0	1
2	2	10	9	0	0	3
3	10	43	31	3	3	7
4	11	68	48	2	1	6
5	11	69	51	11	2	7
6	2	26	22	1	0	4
Total	45	248	176	18	6	28
In zone						
1	9	32	15	2	3	1
2	2	10	9	2	1	0
3	10	43	31	4	9	4
4	11	68	48	2	9	5
5	11	69	51	14	12	2
6	2	26	22	0	7	2
Total	45	248	176	24	41	14

A and B, depicts an example of elevated neuronal activity after nose poking directed toward the social target but not toward other (object or empty) targets. An analysis of nose-poke-associated neural activity of all neurons ($n = 176$) indicated elevated responses to the social relative to the nonsocial target during the first S-O session ($d' = 0.312 \pm 0.078$; t test, $p < 0.001$) but not during the second S-O session ($d' = -0.095 \pm 0.066$; t test, $p = 0.149$; Fig. 2C,D). The discrimination index (d') of the first S-O session was also significantly greater than those of E-E and second S-O sessions, as determined by one-way ANOVA ($F_{(2,175)} = 13.84$, $p < 0.001$) and Tukey's *post hoc* test (E-E vs first S-O, $p < 0.001$; E-E vs second S-O, $p = 0.918$; first S-O vs second S-O, $p < 0.001$; Fig. 2D). As expected, neural discrimination was higher for both first and second S-O pairs compared with E-E pairs (greater absolute d' values), as determined by one-way ANOVA ($F_{(2,175)} = 15.49$, $p < 0.001$) and Tukey's *post hoc* test (E-E vs First S-O, $p < 0.001$; E-E vs second S-O, $p = 0.011$; first S-O vs second S-O, $p = 0.021$; Fig. 2E).

We categorized the 176 neurons according to their responses to different stimulus types (social, object, and empty targets). To control for neuronal responses to spatial location (left vs right), we performed a two-way ANOVA (main factors, stimulus type, and spatial location) and selected those neurons that fired discriminately across stimulus types (Tukey's *post hoc* test). The results are summarized as a Venn diagram (Fig. 3A). As shown, diverse target-dependent responses were observed. Some discriminated only between two different targets (object versus empty, $n = 10$; social vs object, $n = 6$; and social vs empty, $n = 18$), some discriminated one type of stimulus from the other two (object vs others, $n = 10$; social vs others, $n = 22$; empty vs others, $n = 31$), some discriminated all three types of stimulus from each other ($n = 5$), and others showed no significantly different firing pattern between any two types of stimulus ($n = 74$).

Of the 176 mPFC neurons, 27 (15.3%) showed significant differences in social-versus-object and social-versus-empty responses (Fig. 3A,B). Of these, those that showed consistently higher or lower firing rates to the social target compared with object and empty targets, constituting 13.6% (24 of 176) of all neurons tested, were determined to be social target-selective neurons (Fig. 3B, neurons in the first and third quadrants). The vast majority (23 of 24; 95.8%) of social target-selective neurons ex-
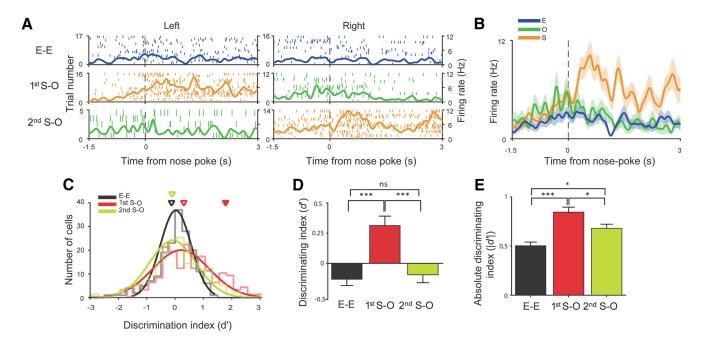
Figure 2. Social target-selective neuronal responses during nose poke. ***A***, ***B***, An example of a social target-selective neuron. ***A***, Spike raster plots and SDFs are shown overlaid for all valid nose-poke trials. ***B***, Nose-poke responses (SDFs) to each target averaged across sessions. Shading indicates ±SEM. ***C–E***, Population data for all analyzed neurons (*n* = 176). ***C***, Distributions of discriminability (*d′*) between two targets (curves, Gaussian fitting). Positive and negative *d′* values of S-O sessions indicate preferential responses to the social and object target, respectively. Empty triangles, mean *d′* values; filled triangle, *d′* value of the example neuron shown in ***A***,***B***. ***D***, The *d′* values for the three sessions, presented as means ± SEM. ***E***, Absolute *d′* values (discriminability without consideration of response direction; **p* < 0.05, ****p* < 0.001; ns, not significant; one-way ANOVA with Tukey's *post hoc* test).

hibited enhanced responses to the social target such that their averaged response to the social target, including all session data in the analysis, was greater than that to the other targets (Fig. 3 *B*,*C*). A separate examination of data from each session revealed that their responses to the social target were more prominent during the first than the second S-O session (Fig. 3*D*).

To further examine relative contributions of stimulus type, spatial location, and session (first vs second S-O) to discharges of mPFC neurons, we performed a three-way ANOVA, plotting *F* values of all mPFC neurons in two-dimensional space as stimulus-versus-spatial and stimulus-versus-session factor plots (the spatial-versus-session factor plot is not shown). Some neurons showed stronger dependence on stimulus type than spatial (or session) factor, but others showed the opposite pattern (Fig. 3 *E*,*F*), consistent with the results shown in Figure 3*A*. In the case of the 24 social target-selective neurons, neuronal activity was primarily accounted for by the stimulus type rather than spatial location or session (Fig. 3 *E*,*F*). These results confirm that the activity of social target-selective neurons was most strongly influenced by the stimulus type rather than the spatial or session factor. However, 15 of the 24 neurons (62.5%) exhibited a significant reduction in their firing rates during the second session compared with the first (main effect of session, *p* < 0.05). Nine of the social-selective neurons (37.5%) showed significant spatial location-dependent firing as well (main effect of spatial location, *p* < 0.05). Thus, social target-selective responses were reduced across the first and second S-O sessions, similar to the reduced social approach behaviors across the two S-O sessions (Fig. 1*B–E*).

The remaining neurons (i.e., not social target-selective) showed diverse response patterns (Figs. 3*A*, 4). To compare these neurons with social target-selective neurons, we examined responses of object target-selective neurons, determined in the same manner as for social target-selective neurons. Of the 176

mPFC neurons, 15 (8.5%) showed significant differences in social-versus-object and object-versus-empty responses (Figs. 3*A*, 5*A*), of which 13 (7.4%) showed consistently higher or lower firing rates to the object compared with social and empty targets; these were determined to be object target-selective neurons (Fig. 5*A*, neurons in the first and third quadrants). Nine object target-selective neurons showed higher rates in response to the object compared with the other targets (Fig. 5*C*), and four exhibited lower rates (Fig. 5*G*; no significant deviation from equal distribution; *p* = 0.473, Fisher's exact test). Unlike the social target-selective neurons, the mean normalized firing rates of these latter neurons was not elevated above the baseline level (before nose poke) on exposure to the object stimulus (Fig. 5*B*). Diverse response patterns were observed for the remaining neurons that were not object target selective (Fig. 5*D–F*,*H–J*).

Neuronal activity in the in-zone area

Units that emitted spikes in all three experimental sessions with overall mean discharge rates >0.5 Hz during the in-zone period were included in the analysis (178 of 248 units; Table 1). As indicated in Figure 6, which presents some of the main findings, the results were similar to those obtained for the nose-poke period analysis (Fig. 6). Almost all (23 of 24; 95.8%) of the social target-selective neurons displayed enhanced responses to the social target, with greater averaged responses relative to the other targets (Fig. 6*A–C*). An analysis of in-zone period activity of all neurons (*n* = 178) revealed elevated responses to the social stimulus relative to the object stimulus during the first S-O session, as demonstrated by one-way ANOVA ($F_{(2,177)}$ = 9.7, *p* < 0.001) and Tukey's *post hoc* test (E-E vs first S-O, *p* < 0.001; E-E vs second S-O, *p* = 0.756; first S-O vs second S-O, *p* = 0.002; Fig. 6*D*).
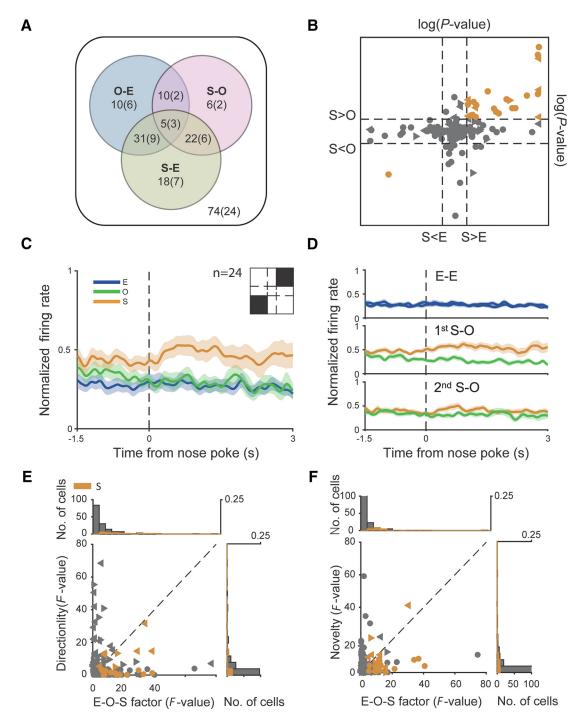
Figure 3. Discharge characteristics of social target-selective neurons. Social target-selective neurons were determined based on neural activity during the nose-poke period. ***A***, Types of neurons that discriminated between different types of stimulus (social, object, and empty targets) were determined using a two-way ANOVA (main factors, stimulus type, and spatial location). The Venn diagram shows the numbers of neurons whose firing rates were different between two stimulus types (Tukey's *post hoc* test), and the numbers in parentheses indicate those neurons that also showed spatial (left vs right) selectivity. The numbers outside of the circles indicate those neurons that showed no target specificity (total, $n = 74$; those with spatial selectivity, $n = 24$). ***B***, p values for social-versus-object and social-versus-empty discriminations (two-way ANOVA with Tukey's *post hoc* test) for all neurons ($n = 176$), presented in log scale (dashed lines, $p = 0.05$). Twenty-four neurons (orange) were determined to be social target-selective neurons (circles within the first and third quadrants). ***C, D***, Averaged responses (shading, $\pm$SEM) to each stimulus (***C***, across sessions; ***D***, within sessions). ***E, F***, The distribution of F values for each factor (3-way ANOVA) was compared between stimulus type (E-O-S factor) and spatial location (left-right directionality factor; ***E***) and between stimulus type and session (novelty factor; ***F***). Orange symbols denote the 24 total social target-selective neurons during nose poke, and gray symbols represent the remaining neurons. Triangles indicate those neurons with significant spatial selectivity. Frequency histograms of F values are shown for the social target-selective and remaining neurons in orange and gray colors, respectively, on top and right for the corresponding factor. Neural data from all three sessions were analyzed together except in ***D***.

Spatial firing

We also examined the relationship between neuronal activity and the distance from a chamber. Analyses of both nose-poke period and in-zone area identified 24 neurons as social target selective.

Of these, 20 (83.3%) were social target-selective neurons in both analyses. Thus, 28 neurons were determined to be social target-selective neurons in at least one analysis. An examination of spatial firing patterns of these neurons showed that their discharge
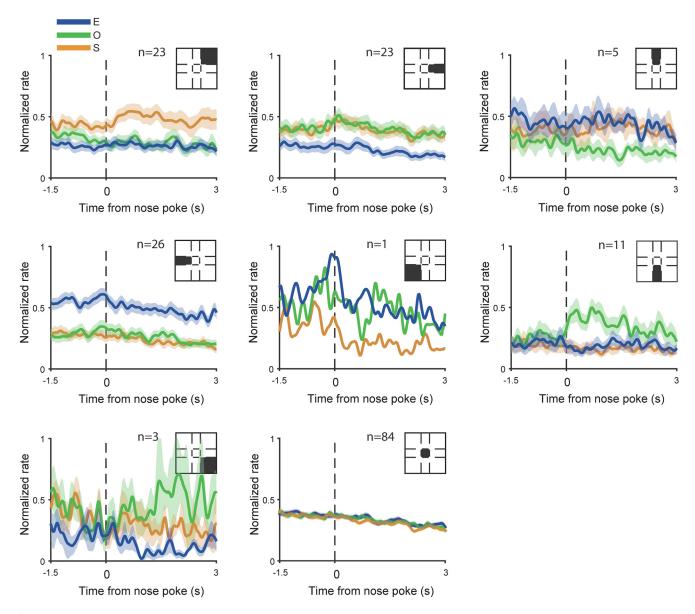
Figure 4. Diverse responses of mPFC neurons. Mean normalized firing rates during nose poke are shown for different types of neurons. Inset squares correspond to the log(p value) square in Figure 3*B*. The same format as in Figure 3*C*.

rates gradually increased as the subject mouse approached the social target during the first S-O session ($p < 0.001$) but not during the E-E ($p = 0.854$) or second S-O ($p = 0.082$) session (Fig. 7, linear regression analysis).

Responses of different cell types to the social target

We classified the recorded neurons into wide-spike neurons (putative pyramidal cells) and narrow-spike neurons (putative interneurons) based on widths and peak-to-valley ratios of filtered spike waveforms (Fig. 8*A*). Wide-spike neurons tended to fire at lower rates ($n = 183$, 0.933 ± 0.088 Hz, mean $\pm$ SD) than narrow-spike neurons ($n = 65$, 1.179 ± 0.243 Hz). To test whether the two cell types show different social target-related responses, we compared their responses during the nose-poke period. The discrimination index (d') between social and object targets, obtained by analyzing both the first and second S-O sessions, was similar for the two cell groups (Fig. 8*B*). Of the 24 social target-selective neurons, 20 were wide-spike neurons and four were narrow-spike neu-

rons. Both groups of cells exhibited enhanced responses during nose poking of the social target (Fig. 8*C,D*). Similar results were obtained for the in-zone area analysis (data not shown). These results indicate that putative pyramidal cells and interneurons tend to show similar responses to the social target.

Discussion

As a first step toward elucidating mPFC neural correlates of mouse social behavior, we examined mPFC neuronal activity in a modified version of the three-chamber test. We found that a subset of mPFC neurons exhibited elevated discharge rates during exploration of a social object relative to an inanimate object or an empty target, a result that could not be accounted for by direction-selective firing. In addition, social target-selective responses were more prominent during the first than the second S-O session, as was also the case for social approach behaviors.
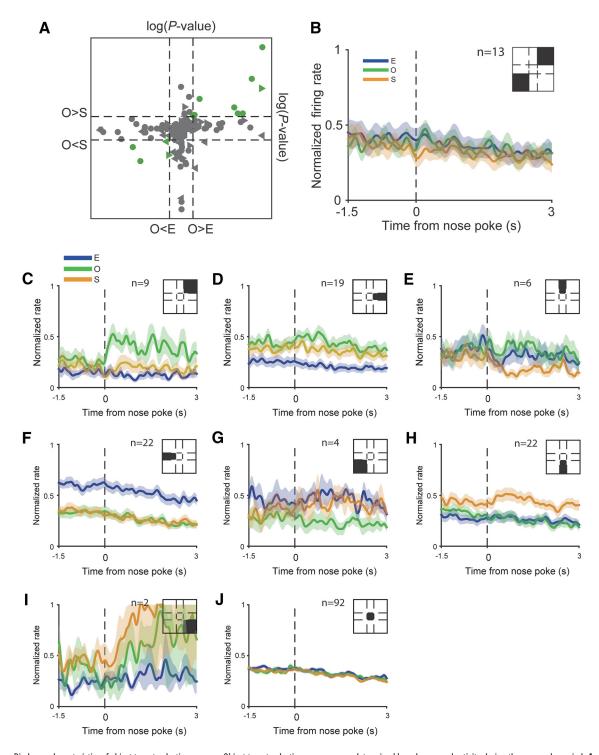
Figure 5. Discharge characteristics of object target-selective neurons. Object target-selective neurons were determined based on neural activity during the nose-poke period. *A*, p values for object-versus-social and object-versus-empty discriminations (two-way ANOVA with Tukey's *post hoc* test) for all neurons (*n* = 176), drawn in log scale (dashed lines, *p* = 0.05). Thirteen neurons (green) were determined to be object target-selective neurons (circles within the first and third quadrants). *B*, Averaged responses (shading, ±SEM) to each stimulus. The same format as in Figure 3C. *C–J*, Mean normalized firing rates during nose poke are shown for different types of neurons. Inset squares correspond to the log(*p* value) square in *A*. The same format as in Figure 3B.

In the three-chamber test, the time spent in two side chambers is known to be the most important parameter (Yang et al., 2011). However, in the present study, we were compelled to remove the chamber walls for the purpose of *in vivo* recording; thus, there were no side chambers with dividing walls. As a potential alternative measure of chamber time, we used in-zone time, which provided data in addition to sniffing time for assessing the social interaction of mice. We found that the activity of some mPFC neurons increased during interaction with the social target, regardless of which dataset was analyzed. Of 28 neurons that were determined to be social target selective based on either nose-poke period or in-zone area analysis, 20 showed increased discharge rates in both nose-poke and in-zone areas of the social target. Thus, the two groups of neurons mostly overlapped. A spatial firing analysis showed that the discharge rates of social target-selective neurons also gradually increased as the subject mouse
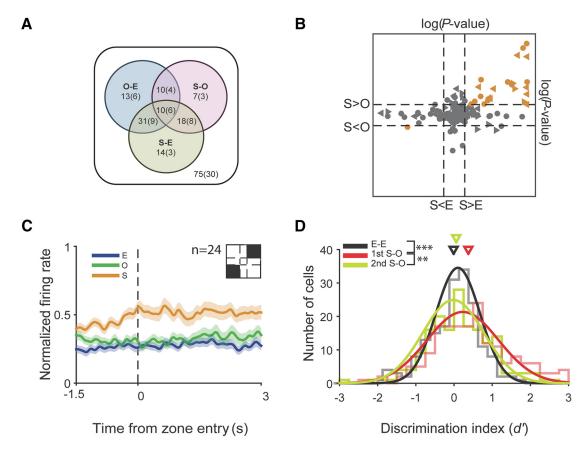
A

B

C

D

Figure 6. Social target-selective neuronal responses in the in-zone area. Analysis of in-zone neural data yielded similar results as the analysis of nose-poke neural data. *A–C*, Follows format of Figure 3*A–C*. *D*, Follows format of Figure 2*C*.
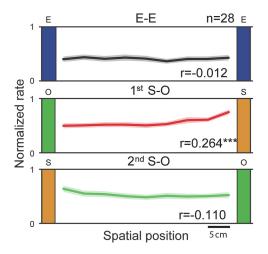

Figure 7. Spatial firing of social target-selective neurons. Spatial firing rates of the 28 neurons determined to be social-selective neurons in nose-poke or in-zone area analyses, presented as means ± SEM. *r*, Slope of linear regression. ***$p < 0.001$.

approached the social target. These results indicate that social target-selective responses of individual mPFC neurons are robust.

It is unclear which sensory cues (e.g., movement, sound, smell) emitted by a stranger mouse played the major role in eliciting mPFC neuronal responses and approach behaviors. The social target-selective responses may be heavily contributed by smell-dependent firing (Ryan et al., 2008). However, enhanced responses to the social target were observed, albeit weakly, even

during the second S-O session. If odor traces from fur, urine, and/or feces primarily affected neuronal responses during the first S-O session, it would be difficult for a social target to elicit stronger neuronal responses than the object target after swapping their positions (i.e., second S-O session). In addition, olfactory habituation happens quickly (Yang and Crawley, 2009), and our chamber was relatively small. These considerations cast doubt on the possibility that social target-selective responses represent purely sensory responses to the smell of the target mouse. Unfortunately, odor gradients were not measured in the present study. In future studies, examining social target-selective mPFC neuronal responses together with temporal dynamics of spatial odor gradients will help resolve this issue.

All three analyses—nose poke, in-zone, and spatial firing—converged to show robust, enhanced social target-selective responses in some mPFC neurons, arguing against the possibility that social target-selective responses represent simple responses to a single sensory cue. In addition, social target-selective neuronal responses decreased substantially across the first and second S-O sessions in parallel with social approach behaviors. Because we analyzed neural activity during the same behaviors (nose poke and in-zone area duration) across sessions, these results further suggest that social target-related mPFC neuronal responses do not merely represent simple sensory responses. It is likely that social approach behaviors depend not only on immediate sensory information but also on the memory of a previously encountered social target. Likewise, social target-selective mPFC responses may depend on sensory information and memory. The rodent mPFC is known to play an important role in working memory (Vertes, 2006; Kesner and Churchwell, 2011). Sensory cues may
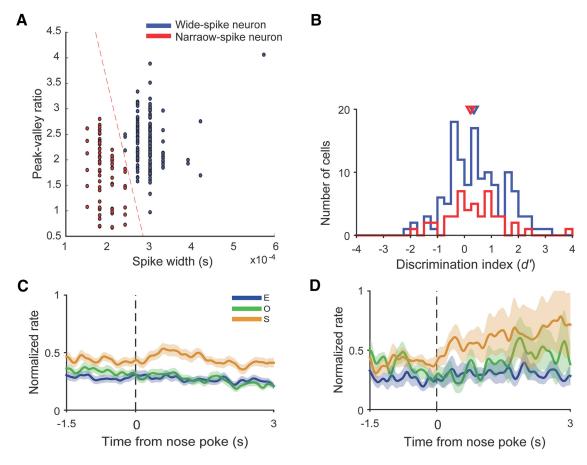
Figure 8. Responses of different cell types to the social target. **A**, Unit classification. The recorded mPFC units (n = 248) were classified into wide-spike neurons (putative pyramidal cells; blue) and narrow-spike neurons (putative interneurons; red) based on the width and peak-to-valley ratio of the averaged spike waveform. Of the 248 mPFC units, 183 (73.8%) were classified as wide-spike neurons and 65 (26.2%) as narrow-spike neurons. **B**, Comparison of the discrimination index (d') during the nose-poke period for wide-spike and narrow-spike neurons showed no significant difference (p = 0.439, t test). **C**, **D**, SDFs of social target-selective neurons during the nose-poke period are shown separately for wide-spike neurons (n = 20; **C**) and narrow-spike neurons (n = 4; **D**).

be important for the initial recognition of a stranger mouse as a social target, but their importance for eliciting social approach behaviors and social target-selective mPFC responses may diminish in the late phase of a session.

It remains to be determined whether elevated mPFC responses to the social target are related to stimulus salience or "social" stimulus. It is also unknown to which aspects of social approach behaviors the observed mPFC neuronal activity is related. Social approach behaviors are likely to consist of multiple processes, including sensory integration, social perception, and sensory–motor transformation. Memory is also likely to play a role in social approach behaviors. Future studies should address which aspect of these processes the enhanced mPFC neuronal activity represents.

In conclusion, we found that the activity of some mPFC neurons in a test mouse increased during the approach to a stranger mouse but not during the approach to an inanimate object or an empty chamber. It is currently unclear which sensory stimuli elicit the responses. Moreover, whether the responses represent genuine social responses, and if so, to which aspects of social behaviors they are related, are similarly unclear. Nevertheless, our results reveal social target-selective neuronal activity in the mPFC in a widely used social-interaction paradigm. These findings may prove helpful for future studies of neural correlates of social interaction in health and disease and neurobiological studies of animals with impaired social interaction.

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