Behavioral and neural correlates of hide-and-seek in rats

Annika Stefanie Reinhold1, Juan Ignacio Sanguinetti-Scheck2, Konstantin Hartmann1, Michael Brecht1,2†

Evolutionary, cognitive, and neural underpinnings of mammalian play are not yet fully elucidated. We played hide-and-seek, an elaborate role-play game, with rats. We did not offer food rewards but engaged in playful interactions after finding or being found. Rats quickly learned the game and learned to alternate between hiding versus seeking roles. They guided seeking by vision and memories of past hiding locations and emitted game-specific vocalizations. When hiding, rats vocalized infrequently and they preferred opaque over transparent hiding enclosures, a preference not observed during seeking.

Neuroal recordings revealed intense prefrontal cortex activity that varied with game events and trial types (“hide” versus “seek”) and might instruct role play. The elaborate cognitive capacities for hide-and-seek in rats suggest that this game might be evolutionarily old.

Neuroscience traditionally relies on strict experimental control and conditioning. However, characteristics of play behavior (1), namely that play is freedom and provides no profit, are incompatible with such experimental design. Several investigators have analyzed rough-and-tumble play (2) and ticklishness and rat vocalizations during play (3), thereby identifying neural substrates of ticklishness (4). We studied hide-and-seek, a so far poorly studied role-play game (5) known in many cultures. The complexity of hide-and-seek affords rules, another defining characteristic of play (4). We asked if rats could play a simplified two-player rat-and-human hide-and-seek game and investigated neuronal representations of such role-play in the medial prefrontal cortex, an area of the brain that in humans has been implicated in perspective taking (6) and social cognition (7).

We played hide-and-seek with adolescent male rats in a large, 30-m² room. Games began with the rat jumping into the start box (Fig. 1A). In “seek” trials (Fig. 1A, pink), closing the lid of the start box signaled that the animal was the seeker. The experimenter hid in one of three locations (fig. S1A), remotely opened the box (fig. S1B), and then the rat searched for the experimenter. An approach ≤40-cm distance) to the experimenter with a clear line of sight was scored as “find.” After “finding,” the experimenter initiated a playful interaction before returning the rat. In alternating blocks of trials, we reversed roles and assigned the rat as the hider. In “hide” trials (Fig. 1A, green), the experimenter left the start box open and crouched immobile next to it, cueing the animal to “hide.” In the following 90 s, the rat could jump out and hide. We offered seven hiding locations. Upon successful hiding, the experimenter searched for the rat, initiated a playful interaction, and returned it. All animals learned to “seek” (n = 6) within 1 or 2 weeks. Of these six animals, five also learned to “hide” and switch roles. Another experimenter trained four more animals and all became proficient hide-and-seek players (data not shown).

Rat “seek” trials are shown in movie S1. We tracked rats and show color-coded behavior classification and ultrasonic vocalizations (black dots) on the animal’s trajectory (Fig. 1B). “Seek” trials are schematized in Fig. 1C. Rats called preferentially during interaction, return, and darting. We devised different “seek” protocols to understand cues guiding rat searching (Fig. 1D). In “random seek,” the experimenter randomly picked hiding places. In “visual seek,” the experimenter left the box open and hid while the animal was watching and could initiate the search immediately. In “blocked seek,” the experimenter hid five times at the same place before changing location (movie S2). We observed longer searches in “random seek” than in “visual seek” (Fig. 1E), indicating that rats use visual cues. In “blocked seek,” search times dropped with hiding location repeats, suggesting that rats keep track of past hiding locations. Failures followed a similar pattern to that of search times (Fig. 1F).

We played “hide” with similar rules (movie S3). Compared with “seek,” rats moved around less and instead looked for cover (Fig. 2A). Rats emitted numerous calls during the game (Fig. 2, A and B). They took less time to hide than to find (Wilcoxon rank-sum test, p < 0.001; “hide”: nsession = 15, ntrial = 157; “random seek”: nsession = 14, ntrial = 182; Fig. 2C) and usually hid successfully (Fig. 2D).

Fig. 1. Hide-and-seek schematic and rat seeking behavior.

(A) Schematic of rat and human hide-and-seek. (B) Room and trajectory of a “seek” trial. The rat was placed into the start box, the experimenter hid and remotely opened the box, and then the rat searched (trajectory, behaviors color-coded). Upon finding, the rat was rewarded with a playful interaction and returned. Black dots, rat vocalizations.

(C) Behavior in “seek” trials: ultrasonic vocalization (black lines). S, success; F, failure. (D) “Seek” protocols: “random seek,” “visual seek,” “random seek,” “visual seek,” start box open and the animal seeing the experimenter. “Blocked seek,” experimenter hid five times at the same location before changing to a new one. (E) Search times for seek protocols (Wilcoxon rank-sum test; “random seek”: nsession = 14, ntrial = 182, “visual seek”: nsession = 12, ntrial = 147; Quade test for “blocked seek”; nsession = 12, ntrial = 176) (F) Failures [conventions as in (E)].
We compared the repeated targeting locations in “hide” and “seek” trials. When hiding, rats targeted the last hiding location less frequently than during “seek” (fig. S2). We investigated the rats’ preferences for different hiding locations. Of three different types of hiding locations offered (Fig. 2E), rats showed a significant preference for opaque and cardboard boxes over transparent boxes (Fig. 2F). We compared the rats’ box-entering behavior in “hide” and “seek” trials to understand if the opaque box preference reflected an a priori bias of rats or if rats consider visibility when choosing hiding locations. Consistent with the role in the game, rats entered boxes more rarely in “seek” than in “hide” trials (Fig. 2G). Notably, whereas rats entered transparent and opaque boxes indiscriminately during “seek,” they preferred opaque boxes during “hide” (Fig. 2H). After being found in “hide” trials, and more rarely also after finding in “seek” trials, rats often showed re-hide and run-away behaviors (fig. S3 and movie S4). These behaviors are noteworthy, as they delayed the interaction reward and prolonged the game.

Fig. 2. Rat hiding behavior. (A) Room and trajectory of a “hide” trial. The rat was placed into the start box while the experimenter crouched next to it, jumped out, and hid. (B) Behavior in “hide” trials: ultrasonic vocalization (black lines). (C) Time to hide (black line, median). (D) Successes, visible failures (rats staying visible), and other failures (see materials and methods). (E) Hiding locations. (F) Hiding location choices (Wilcoxon rank-sum, Bonferroni-corrected; \( n_{\text{session}} = 15, n_{\text{trial}} = 157, n_{\text{animal}} = 5 \)). (G) Box entering in “hide” and “seek” trials (\( t \) test; \( n_{\text{session}} = 14; \) “hide”: \( n_{\text{session}} = 14, n_{\text{animal}} = 4 \)). (H) Opaque box preference in “hide” but not “seek” trials (Fisher’s exact test; \( n_{\text{session}} = 25, n_{\text{animal}} = 4 \)).

Fig. 3. Hide-and-seek vocalization behavior. (A) Spectrograms of vocalizations (flat, left; modulated, middle) and relative incidence of rat vocalization types (right). (B) Event-triggered averages of vocalization rates in “hide” and “seek.” \( p \)-values (Student’s \( t \) test) compare “hide” (green) and “seek” (pink) trials 1 s around events (“seek”: \( n_{\text{session}} = 14; \) “hide”: \( n_{\text{session}} = 14, n_{\text{animal}} = 4 \)). (C) Vocalization rates for phases of “seek” and “hide” trials (Wilcoxon rank-sum, Bonferroni-corrected; “seek”: \( n_{\text{session}} = 14; \) “hide”: \( n_{\text{session}} = 14, n_{\text{animal}} = 4 \)).
Rats emitted ultrasonic calls dominated by flat and modulated vocalizations (Fig. 3A). Because rat calls are inaudible to the experimenter, they are not directly shaped by conditioning. We computed vocalization-rate averages for game events across all hide-and-seek sessions (Fig. 3B). Vocalization rates increased while rats were jumping into and out of the start box and were higher for “hide” than for “seek” trials. Call rates at interaction onset were similar in “seek” and “hide” trials. There was, however, a difference for the “find” event in “seek,” during which animals called, versus the being “found” event, during which animals went silent. Vocalization rates varied between phases of the game (Fig. 3C). In “hide,” call rates were low during hiding.

To address neural correlates underlying play behavior, we performed tetrode recordings of single neurons in rat medial prefrontal cortex (n = 5 rats), a brain area associated with encoding social proximity (8) and rules (9). Neuronal discharges in medial prefrontal cortex varied sharply during game events (Fig. 4). A neuron in infralimbic cortex (Fig. 4A, left) discharged for a single game event exclusively during “seek” trials (Fig. 4A, middle). The response peaks of this cell coincided with closing of the start box (Fig. 4A, right), and such responses were not observed when the animal entered a dark hiding place. Box closure–related responses were seen in ~30% of neurons. Such responses are noteworthy because box closure was the assignment cue for rats being a seeker. A neuron in prelimbic cortex responded to multiple game events (Fig. 4B).

To assess population activity, we time-warped neuronal activity according to the median length across trials, conserving the alignment of four events: jump out of the box, interaction, return, and jump into the box. This procedure allowed for visualization and comparison of the activity of all neurons recorded. Each row of Fig. 4C shows single-neuron mean firing rates (n = 177) during entire “seek” trials normalized to their maximum rates. Neurons are ordered according to the optimal order of a hierarchical clustering (left: classification tree) based on a dissimilarity matrix using pairwise correlation distance between cells and linked by Ward’s method. We clustered neurons using their “seek” activity into five groups (Fig. 4C). The average activity of “seeking clusters” was related to different phases of the game (Fig. 4D). For example, the black cluster neurons were active in the start box, whereas the salmon-colored cluster was active during interaction. The same analysis also revealed structured population activity (Fig. 4E) and clusters active in different phases (Fig. 4F) for entire “hide” trials.

To probe whether prefrontal activity encodes “hide” and “seek” in the same way, we looked at the activity during “seek” (Fig. S4, A and B) and “hide” (Fig. S4, C and D) ordered by clustering the activity of neurons in the opposite role of the game. Doing so did not fully recover the ordered activity seen in Fig. 4, C and E; i.e., some cells were differentially active for the same events in “hide” or “seek.”

Rats were strategic players. Seeking strategies included systematic searches, use of visual cues, and targeting of past hiding locations. Hiding strategies included preferences for opaque enclosures, being silent when hiding, and changing hiding locations. Rats behaved differently when assigned as seeker or hider. Neither seeking nor hiding fulfilled a function beyond the game.

**Fig. 4. Neuronal correlates of hide-and-seek in rat medial prefrontal cortex.** (A) Left: Drawing of coronal section with tetrode track (red) and recording site (red “X”) in rat infralimbic cortex. Middle: Neuronal firing rate trace throughout a hide-and-seek session. Tick marks indicate jump in events. Right: Peristimulus time histogram of responses aligned to start-box closure (the cue that a “seek” trial follows). Cg1, cingulate area 1; PrL, prelimbic cortex; IL, infralimbic cortex. (B) Upper left: Drawing of recording site in rat prelimbic cortex [conventions as in (A)]. Upper right: Firing rate trace reflecting responses to multiple events. Bottom: Peristimulus time histogram of responses to events in “seek” trials. (C) Population activity of medial prefrontal cortex neurons in “seek” trials. Cells were selectively active in different phases of “seek.” Time-aligned normalized average firing rates of single neurons (rows) were sorted according to hierarchical clustering (dendrogram, left). (D) Average firing rates (+SEM) of clusters show different population responses during “seek” (colors as in dendrogram). (E and F) Population and cluster activity in “hide” trials [conventions as in (C) and (D)].
But why did rats play hide-and-seek? They might have been shaped to play by the social interaction reward. This shaped-to-play hypothesis implies that rats behave in a reward-oriented manner and hide to be found (and then be rewarded). Alternatively, rats might adopt the game just to play. This play-to-play hypothesis invokes complex mental representations (fun, game understanding, playing), but aligns with various observations. First, the animals looked like they are having fun. Behaviors that signify eagerness to observers include the following: quick, directed locomotion; frantic search; teasing of the experimenter; and execution of freudensprung (“joy jumps”). Rats were highly vocal and eager to engage in the game but appeared tired toward the end of a 20-trial session. Thus, motivational aspects differ from operant food-reward conditioning, where there is little evidence for vocalizations and where rats tirelessly do hundreds of trials. Second, the rats’ behavior seemed purposeful. They preferred to hide in opaque over transparent boxes as if they were hiding to hide rather than hiding to be found. Third, hiding rats tended to be silent when being found, contrary to the excitement about being found predicted by the shaped-to-play hypothesis. Fourth, when found, rats often ran away and re-hide. Contrary to the shaped-to-play hypothesis, rats prolonged the game by re-hiding. By succeeding at hiding, they inherently delayed the reward. Collectively, our observations tentatively, but not conclusively, favor the play-to-play hypothesis. However that because rats’ internal representations are inaccessible, our evidence is indirect and further work is needed to understand rat play behavior.

Rats emitted complex vocalizations during hide-and-seek. Vocalization rates peaked at game events and might coordinate play (10). All rats learned hide-and-seek quickly and we wonder if role-play games are an evolutionarily old play capacity. Hide-and-seek might be a promising paradigm for investigating perspective taking and theory-of-mind-related skills, which have been intensely investigated in non-human primates (11, 12) and children (13), but not in rodents.

Human ventromedial prefrontal cortex is involved in mind perception and social attention (14). Prefrontal cortex seems to be involved in social behavior and play (15, 16), which is important for social skill development (17). Recordings in medial prefrontal cortex revealed neuronal activity specific to phases and events of hide-and-seek. Such correlates support earlier findings linking prefrontal cortex to play (18, 19) and encoding of rules (9, 20, 21).

We observed neural correlates of hide-and-seek and sharp tuning of medial prefrontal cortex neurons to specific game events despite the relatively unrestricted experimental conditions. Nevertheless, these neuronal responses resemble previous findings in very short (3 to 5 s), overtrained, and controlled paradigms (9, 22).

Rat hide-and-seek allows important aspects of neurobiology (i.e., decision-making, navigation, motivation, and role) to be studied in the context of rich, unrestricted behavior. Such agency-affording paradigms might offer insights about the brain that go beyond what can be understood in rodents.

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SUPPLEMENTARY MATERIALS
science.sciencemag.org/content/365/6458/1180/suppl/DC1
Materials and Methods
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Role play between rats and humans

There is controversy regarding how widespread animal play behavior is and what its evolutionary function might be. Reinhold et al. demonstrated that rats can play hide-and-seek with a human. In the “seek” condition, rats learned to look for the hidden humans and kept seeking until they found them. In the “hide” condition, they learned to hide in one of several locations and waited there until being found. In both cases, the rats were rewarded by social interaction with the human. Rats vocalized when seeking and finding and were silent when hiding. Recordings in the medial prefrontal cortex detected neurons that were sensitive to the game structure.

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