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NEURONAL activity in the perirhinal cortex was recorded while rats performed a spatial task on a four arm radial maze. The maze was defined by proximal multisensory cues on the arm surfaces and distal complex visual cues at the surround. During each recording session, rats were run in three conditions: baseline, a condition in which proximal and distal cues were manipulated, and a second baseline. Compared with the activity of hippocampal neurons in the same paradigm, a much smaller proportion of perirhinal neurons exhibited spatial selectivity and perirhinal place fields were larger than hippocampal place fields. Although perirhinal place fields exhibited a high degree of spatial tuning and reliability within a condition, they were not stable across conditions. *NeuroReport* **9**: 3013–3018 © 1998 Lippincott Williams & Wilkins.

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Positional firing properties of perirhinal cortex neurons

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Introduction

Hippocampal complex-spike cell firing in awake behaving rats demonstrates robust spatial selectivity.[1] Spatial firing correlates have also been described for single units in the subiculum and the medial entorhinal area (MEA) of the entorhinal cortex, although subicular and entorhinal place fields are larger than hippocampal place fields in complex environments.[2–5] The perirhinal cortex is reciprocally connected with the entorhinal cortex, hippocampus and subiculum, thus providing an important interface between the neocortex and the structures of the hippocampal formation.[6–10] Because of this close connectivity, it is reasonable that spatial selectivity might also be observable in the firing patterns of perirhinal neurons. Indeed, there is some indication from experimental lesion studies that the perirhinal cortex may participate in spatial information processing in the water maze and in a spatial working memory task[11,12] (but see Refs 13,14).

The present study was undertaken to address the question of whether perirhinal neurons encode spatial information. Neuronal activity in the perirhinal cortex was examined while rats ran a four arm radial maze for lateral hypothalamic stimulation reward. The results are discussed within the framework of prior studies using the same behavioral paradigm to examine the spatial firing correlates for neurons in the hippocampus and the subiculum.[5,11,12]

Materials and Methods

Subjects were six male Long–Evans hooded rats obtained from Charles River Laboratories and weighing ~350 g at the beginning of the study. Animals were housed individually under a 12:12 h light:dark cycle with *ad lib* access to food and water. Microelectrodes were stereotaxically implanted under halothane anesthesia either in the perirhinal cortex or in CA1 as a control. Stereotaxic coordinates for perirhinal electrode placements were 5.0 mm posterior and 5.1 mm lateral to bregma, 5.6 mm ventral to the cortical surface and 15° from lateral. Coordinates for the CA1 electrode placement were 3.2 mm posterior to bregma, 2.0 mm lateral to bregma and 1.9 mm ventral to the cortical surface. An additional bipolar electrode was implanted to provide lateral hypothalamic stimulation as a reward. Coordinates for the stimulating electrode were 0.5 mm anterior to bregma, 1.5 mm lateral to bregma, and 8.6 mm ventral to the skull surface. Each subject received an implant of one or two electrodes at a single location.

Extracellular spike activity in cortical and hippocampal regions was monitored and recorded using a DataWave Technologies, Inc data collection system interfaced with a Pentium computer. Single unit neuronal recordings were obtained in freely behaving animals via one or two chronic tetrode implants plus a stereotrode to serve as a reference electrode. Tetrodes and stereotrodes were constructed from

twisted 30 μm Formvar-coated nichome wires (California Fine Wire) and mounted to a moveable microdrive.[13] The signal was pre-amplified at the head stage by three Quad J-FET chips (Active Electronics). The activity on each wire was then amplified, filtered, and transmitted to a time/amplitude window discriminator. Signals on all four wires, or channels, were digitized when a spike on any channel exceeded a predetermined threshold. The stimulation tetrode was constructed from a twisted pair of 100 μm Formvar-coated wires.

The behavioral task was controlled by a user programmed exit in the DataWave (DataWave Technologies, Inc.) software that controlled the onset and offset of all stimulation and recorded the spatial location of the rat. The position of the rat in the maze was recorded by a DataWave video tracking system that tracked two incandescent light bulbs mounted on the head stage.

The behavioral apparatus was a four arm radial maze elevated to 70 cm from the floor. A central octagonal platform was 12 cm on each side. Each arm was 40 cm by 10 cm. The maze was surrounded on all four sides by dark blue curtains. The floor and ceiling were dark grey or black. On each curtain was a distinctive complex visual cue that served as a distal cue. Tactual, visual, and olfactory stimuli on the maze arms served as local cues. The surface of the arms were covered with either sandpaper, plastic mesh, fine wire screen, or ridged rubber. Additionally, each arm was sprayed at the beginning of each session with an aerosol odorant consisting of orange, strawberry, anise or peppermint. The maze was illuminated by four 12 V DC lights located at the four corners of the curtained area. White noise was provided by two speakers located diagonally at opposite corners of the curtained area.

Rats were trained to visit the ends of the maze arms for a lateral hypothalamic stimulation reward (0.5 trains/s of 0.5 ms pulses at 100 Hz, 10–300 μA). A trial consisted of a visit to each of the four arms. A working memory contingency was employed such that rats received reward only when visiting an arm not previously rewarded on that trial. A new trial began when each arm had been visited. All rats rapidly adopted a strategy of visiting each arm in sequence in a stereotyped manner such that errors were rarely made.

During each recording session, recordings were made in three conditions: a baseline condition, a double rotation in which distal cues were rotated counterclockwise and local cues were rotated clockwise, and a second baseline condition (Fig. 1). During each session data were collected continuously. Between sessions, the rat was placed in a covered bucket in the middle of the maze while cues were rotated. After cues were rotated, the rat was placed in one of the four arms. The start arm was randomly chosen. The experimenter entered and left the curtained area by one of three randomly chosen routes. At the end of each recording day, the tetrode was lowered either 40 μm or 80 μm.

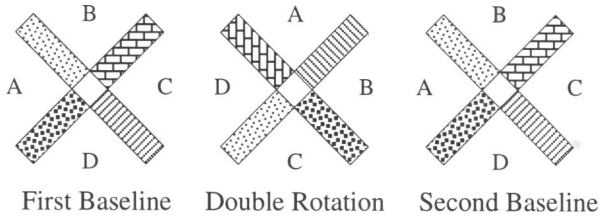

FIG. 1. Schematic of the configuration of maze cues in the three recording condition. Letters A–D represent complex visual cues at the maze surround. Patterns on the maze arms represent different olfactory, tactile, and visual cues on the surface of the maze arms. The configuration of cues is shown for the first baseline (left), the double rotation (center) in which distal cues were rotated clockwise and local cues were rotated, counterclockwise, and the second baseline (right) in which distal and local cues were returned to the original configuration.

At the end of all recording sessions, the subjects were deeply anesthetized with chloral hydrate and transcardially perfused. The brains were coronally sectioned at 30 μm, mounted, and stained for Nissl according to standard histological procedures.[8] Electrode placements were verified by microscopic analysis.

The neuronal activity of single units was statistically isolated by cluster analysis using characteristics of the signals that are recorded as variables. Examples of characteristics that may be used are peak and valley amplitude, latency to peak, and spike duration for each tetrode wire. In most cases, peak and valley amplitude for each of the tetrode leads was used. Recordings were considered stable if cluster boundaries remained stable across conditions.

The spatial distribution of firing rates was calculated by dividing the maze into 3×3 cm pixels and computing the firing rate for each pixel as the total number of spikes divided by the total time spent in that pixel. Firing rates were calculated only for periods when the rat was moving at least 2 cm/s. A grand mean was calculated as the total number of spikes/total time moving in maze. Additionally, a mean firing rate was calculated that included only the time during which firing occurred. A place field was defined as an area of at least three adjacent pixels each having a firing rate of at least three times the grand mean rate. For each isolated unit in the baseline condition, the number of place fields was calculated. For each place field, measures included the mean infield firing rate and the mean place field area. Additionally, a spatial tuning measure was calculated as the ratio of the mean infield firing rate to the

mean firing rate outside the field. A spatial certainty measure was calculated as the ratio of the mean infield firing rate to the mean place field area.

Across trial conditions, place fields were identified as unchanged, rotated, changed, appeared or disappeared, using the following criteria. A place field was considered to be unchanged if it appeared in the same arm at the same radial location (radial location defined as a distance from the center of the maze varying no more than one quarter of the total arm length). A place field was considered rotated if it appeared on an adjacent arm at the same radial location. A place field was considered to have changed if it reappeared on a different, non-adjacent arm. A place field was considered to have appeared if there was no subfield located at the same radial location on an adjacent arm in the previous trial. A place field was considered to have disappeared if there was no subfield located at the same radial location on an adjacent arm in the subsequent trial.

Results

Placement of electrodes was verified by reconstruction of the electrode track from coronal sections stained for Nissl material. Figure 2 shows a representative electrode track location in the perirhinal cortex and the track location for a single dorsal hippocampus electrode implant from which control data were collected.

All subjects learned to collect lateral hypothalamic stimulation rewards at the end of maze arms within a few sessions. Each subject adopted a stereotyped strategy for performing the task of running to the end of each arm in sequence such that very few errors were made and the processing of distal and local cues was not necessary to obtain rewards.

Seventy neurons were isolated from recordings of cellular activity in the perirhinal cortex of three rats. Figure 3 shows waveforms on the four tetrode leads across three trials for a representative perirhinal unit. Twenty of 70 cells, or about 29% of the units, exhibited place fields as defined by the criteria set forth in Materials and Methods (Table 1, column labeled All Units). Of those, eight units exhibited two subfields and the remaining 12 units exhibited one place field. The mean firing rate of perirhinal single units with one or two place fields was 0.32 Hz. The mean infield firing rate was 3.68 Hz. The mean area of place fields was 6.36 pixels or 57.24 cm^2. Indices of spatial tuning and spatial certainty indicated that the place fields were well defined and reliable.

Further analysis was conducted for a subset of cells for which data from baseline and double rotation conditions were available ($n = 43$). The properties of this subset of single units were similar to those

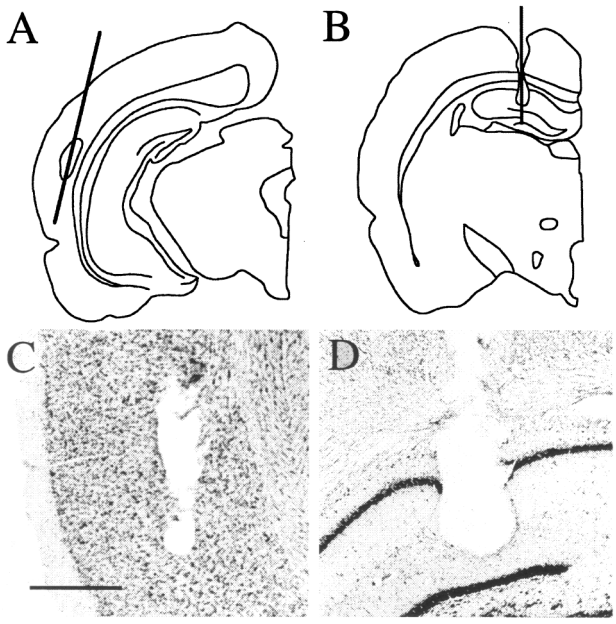

FIG. 2. (**A**) Reconstruction of electrode track for a representative perirhinal tetrode implant. (**B**) Reconstruction of electrode track for the control hippocampal tetrode implant. (**C**) Photomicrograph of perirhinal electrode track. (**D**) Photomicrograph of hippocampal electrode track. Bar = 150 μm.

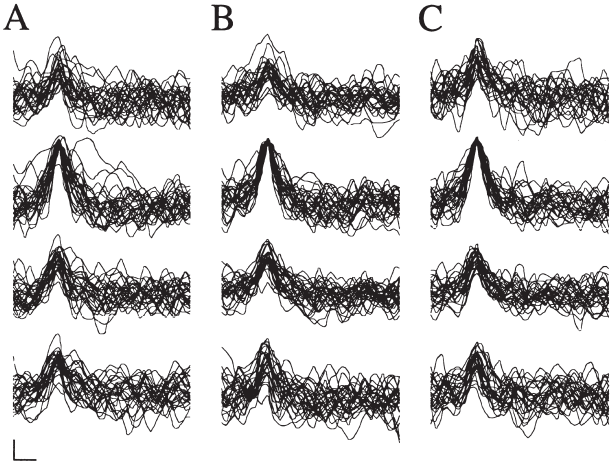

FIG. 3. Tracings of tetrode recordings in the perirhinal cortex. This is an example of the stability of single unit activity across three conditions, the first baseline (**A**), the double rotation condition (**B**) and the second baseline (**C**). Bar = 150 μV × 150 μs.

of the entire group of 70 (Table 1, column labeled Subset). Roughly one-third exhibited place fields (Table 2). Of these single units, 10 exhibited one place field and four exhibited two place fields. In the single units in which there were two subfields, the disposition of the two place fields was always the same across conditions, e.g. when one of the place fields disappeared with the double rotation of cues, so did the other. Six perirhinal units exhibited place fields that appeared with double rotation of the distal and

Table 1. Properties of perirhinal single units.

Attributes	All units[1]	Subset[2]
Total no. cells	70	43
No. cells with place fields	20	14
Mean firing rate (Hz)	0.32 ± 0.06	0.32 ± 0.08
Mean no. place fields	0.94 ± 0.12	0.70 ± 0.08
Mean place field area (pixels)	6.36 ± 0.37	6.59 ± 0.47
Mean infield firing rate (Hz)	3.68 ± 1.72	4.50 ± 2.46
Mean spatial certainty	1.69 ± 1.19	2.28 ± 1.69
Mean spatial tuning	8.40 ± 0.63	8.88 ± 0.79

[1]All units are all isolated by statistical analyses. [2]A subset of units for which data were collected in at least two conditions were further analysed.

Table 2. Spatial properties of perirhinal neurons.

Spatial characteristics	Number of units	Percent total ($n = 43$)
Cells without place fields	29	67.4
Cells with place fields	14	32.6
Unchanged	0	0.0
Appeared with DR	6	14.0
Disappeared with DR	6	14.0
Changed unrelated to cues	1	2.3
Rotated with distal cues	1*	2.3
Rotated with local cues	0	0.0

*For one single unit, the place field appeared with the double rotation (DR) and then rotated with the distal cues at the second baseline.

local cues, and six units exhibited place fields that disappeared in the double rotation condition. Although place fields exhibited a high degree of spatial certainty and spatial tuning within a condition, none of the place fields observed in the first baseline or the double rotation condition reappeared in the second baseline. One place field changed as a result of the double rotation, but was independent of either local or distal cues, i.e. it did not change to an adjacent arm, but rather to the opposite arm.

The spatial activity of only one of the recorded perirhinal units was determined by the controlled cues. For that neuron, the place field appeared with the double rotation and then rotated with the distal cues in the second baseline condition (Fig. 4A). Thus, only one cell provided any evidence that the activity of perirhinal neurons might be controlled by distal, spatial cues. As a control, neuronal activity was also recorded from the dorsal hippocampus in a fourth rat. Although only a small amount of data was collected from that subject, the data were consistent with findings of other studies using the same task in that hippocampal neurons tended to exhibit localized firing controlled by maze cues.[5,11,12] Figure 4B shows a hippocampal place cell from the control subject in which the place field was controlled by distal, spatial cues.

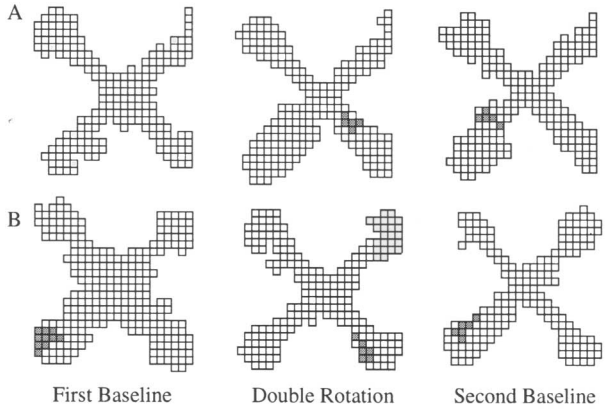

FIG. 4. Place fields across the three conditions for a perirhinal single unit (**A**) and a hippocampal single unit (**B**). Only one perirhinal unit of 43 showed characteristics similar to those of a hippocampal place field that is controlled by distal cues. Prior studies have documented that the majority of hippocampal single units show spatial selectivity that is controlled by local or distal cues.

The observation that positional firing properties of the perirhinal neurons were not controlled by cue manipulation across conditions, as is true for many hippocampal place cells, raised the issue that spatial firing in the perirhinal cortex represents only a temporary firing pattern that may not even be persistent within a single session/condition. To address this issue, the firing patterns of five randomly chosen perirhinal place fields were visually examined to compare positional firing during the first half of the recording session *vs* the second half. For all five place fields, firing occurred within the place field in both halves of the session suggesting that positional firing properties were stable within a session.

Discussion

The present study provided evidence that position is a relatively poor correlate of perirhinal neuronal activity in the four arm radial maze task. Two-thirds of single units isolated in the perirhinal cortex did not exhibit activity that correlated with position. The remaining one-third of the neurons recorded in the perirhinal cortex did exhibit spatial selectivity during performance in the task. This proportion, however, is substantially lower than the 85–100% of hippocampal neurons observed to exhibit spatial selectivity in awake, behaving rats.[5,12] For perirhinal cells that did exhibit positional firing correlates, there was little evidence that neuronal activity was experimentally controlled either by conjunctions of maze cues or by single, simple cues. Whereas perirhinal place fields were stable within a condition, every perirhinal place field can be said to have remapped as a consequence of cue manipulation; place fields either appeared, disappeared, or changed in the double rotation condition (one place field appeared

with the double rotation and then followed distal cues with the second baseline). Moreover, in no case did a place field observed in the first baseline reappear in the second baseline. In contrast, in a large sample of hippocampal complex-spike cells observed in the same task, virtually all units exhibited spatial selectivity, but only 58% of the cells rotated with proximal or distal cues or remained unchanged.[11] The remaining place cells remapped as a consequence of the double rotation (i.e. appeared, disappeared or changed). This is similar to the behavior of perirhinal place cells except that with the return of all cues to their baseline position most hippocampal place cells exhibited the original place field. Only 10% of the hippocampal neurons exhibited place fields that behaved like perirhinal place fields across all conditions, i.e. remapped with the double rotation and did not reappear in the second baseline.

The proportion of perirhinal neurons exhibiting firing patterns correlated with position is also lower than that observed for neurons in the ventral subiculum during the same task. One half of subicular cells exhibited spatial selectivity (e.g. 5). Ten of 14 either appeared or disappeared (remapped) and four of 14 rotated with proximal or distal maze cues. In a different spatial task, all entorhinal neurons that were tested for spatial selectivity exhibited place fields.[2] Roughly one-third remapped and two-thirds followed manipulation of a distal cue. To summarize, fewer perirhinal neurons showed spatial selectivity as compared to structures of the hippocampal formation, and the entire complement of spatially selective perirhinal neurons exhibited stability within, but not across conditions, i.e. remapped as a consequence of cue manipulation and were not predictably controlled by cues. Although one-third of perirhinal neurons demonstrated place fields as defined by criteria set forth in this and prior studies using the same behavioral paradigm,[5,11] there was little evidence that perirhinal neurons exhibited the single property most closely associated with place fields in earlier studies, i.e. control by manipulation of distal cues.[1–4]

With the present task and experimental design, firing correlates could only be defined by position. Nevertheless, the finding that neuronal activity in the perirhinal cortex was largely independent of available cues is in striking contrast to the robust perirhinal neuronal activity observed to correlate with cues in other behavioral protocols, for example an odor-guided delayed non-matching to sample task[14] or the presentation of familiar *vs* novel stimuli.[15,16] Because there were robust olfactory and tactile cues on the maze and the distal cues could be considered analogous to visual patterns or objects, it is surprising that clear, reproducible firing correlates were not discernible for perirhinal neurons during the radial arm maze task. Our interpretation is that perirhinal neuronal activity did not correlate with aspects of this task because the cues were not foreground cues relevant to task performance. All subjects adopted a stereotyped performance strategy of running to the end of each arm in sequence. Once this strategy was in place, subjects made very few errors and the processing of distal and local cues was not necessary to obtain rewards. In subjects exhibiting a similar behavioral strategy in performance on the same task, a large proportion of hippocampal neurons encode place using local and/or distal cues even though the processing of these cues is not necessary to obtain rewards. Thus, the hippocampus appears to be automatically encoding background or contextual cues.

The viewpoint that the hippocampus automatically encodes environmental stimuli in the absence of relevance to a task, whereas the perirhinal cortex does not, is consistent with observations of the role of the hippocampus *vs* the perirhinal cortex in fear conditioning to context. The balance of the evidence indicates that the hippocampus is necessary for fear conditioning to background or contextual cues.[17–20] In contrast, damage to the perirhinal cortex impairs fear conditioning to a conditioned stimulus,[21,22] but perhaps not to the training context.[21] Thus it appears that the perirhinal cortex is specialized for encoding task-relevant (foreground) cues and does not participate in the automatic processing of incoming sensory information for its spatial or contextual content.

Conclusion

Our data suggest that neurons in the perirhinal cortex, a region that receives substantial unimodal and polymodal associational input and is reciprocally interconnected with structures of the hippocampal formation, do not process this incoming sensory input to encode spatial or contextual information. Despite the presence of both distal spatial cues and local sensory cues, location was an unreliable descriptor of perirhinal cell discharge in the four arm radial maze task. In contrast, entorhinal, hippocampal, and subicular neurons demonstrate more robust spatial selectivity in a variety of spatial tasks. These findings suggest that the substantial input from sensory associational regions to the perirhinal cortex supports functions other than the further processing of this sensory input to derive spatial information. Our suggestion is that the perirhinal cortex may be specialized for encoding stimuli that are best described as foreground stimuli and that it is not involved in the further processing of sensory stimuli in the background. Rather, sensory information received via the perirhinal cortex and, most likely, other routes is further processed for its

spatial or contextual content in the hippocampal formation at a higher level of information processing, probably beginning with the entorhinal cortex.

References

1. O'Keefe J and Dostrovsky J. *Brain Res* **34**, 171–175 (1971).
2. Quirk GJ, Muller RU, Kubie JL and Ranck JBJ. *J. Neuroscience* **12**, 1945–1963 (1992).
3. Barnes CA, McNaughton BL, Mizumori SJY *et al. Prog Brain Res* **83**, 287–299 (1990).
4. Sharp PE and Green C. *J Neurosci* **14**, 2339–2356 (1994).
5. Phillips RG and Eichenbaum H. *Behav Neurosci* (in press).
6. Deacon TW, Eichenbaum H, Rosenberg P and Eckmann KW. *J Comp Neurol* **220**, 168–190 (1983).
7. Burwell RD and Amaral DG. *J Comp Neurol* (in press).
8. Burwell RD and Amaral DG. *J Comp Neurol* **391**, 293–321 (1998).
9. Naber PA, Caballero-Bleda M, Jorritsma-Byham B and Witter MP. *NeuroReport* **8**, 2617–2621 (1997).
10. Kosel KC, Van Hoesen GW and Rosene D. *Brain Res* **269**, 347–351 (1983).
11. Wiig KA and Bilkey DK. *Behav Brain Res* **63**, 101–109 (1994).
12. Wiig KA and Burwell RD. *Behav Neurosci* (in press).
13. Herzog C and Otto T. *J Neurosci* **111**, 1265–1272 (1997).
14. Ennaceur A, Neave N and Aggleton JP. *Behav Brain Res* **80**, 9–25 (1996).
15. Tanila H, Shapiro M, Gallagher M and Eichenbaum H. *J Neurosci* **17**, 5155–5166 (1997).
16. Shapiro M, Tanila H and Eichenbaum H. *Hippocampus* **7**, 624–642 (1997).
17. Hetherington PA and Shapiro ML. *Behav Neurosci* **111**, 20–34 (1997).
18. Young BJ, Otto T, Fox GD and Eichenbaum H. *J Neurosci* **17**, 5183–5195 (1997).
19. Zhu XO, McCabe BJ, Aggleton JP and Brown MW. *Neurosci Lett* **229**, 141–143 (1997).
20. Zhu XO and Brown MW. *Brain Res* **689**, 101–110 (1995).
21. Penick S and Solomon PR. *Behav Neurosci* **105**, 611–617 (1991).
22. Honey RC and Good M. *Behav Neurosci* **107**, 23–33 (1993).
23. Phillips RG and LeDoux JE. *Learn Mem* **1**, 34–44 (1994).
24. Winocur G, Rawlins JNP and Gray JA. *Behav Neurosci* **101**, 617–625 (1987).
25. Rosen JB, Hitchcock JM, Miserendino MJD *et al. J Neurosci* **12**, 4624–4633 (1992).

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