Neuronal activity in the primate globus pallidus during smooth pursuit eye movements

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ABSTRACT

Although the roles of the basal ganglia in the control of saccadic eye movements have been extensively examined, little is known about their roles in smooth pursuit. Recent anatomical data suggest that, like somatic movements, smooth pursuit may also be regulated by signals through the basal ganglia thalamocortical pathways. To understand whether the basal ganglia, especially the globus pallidus (GP), could play roles in pursuit, we examined the firing of individual GP neurons when monkeys performed smooth pursuit. We found that a subset of neurons in both the external and the internal segments of the GP modulated firing during pursuit, suggesting that pathways through the GP might play roles in the control of smooth pursuit eye movements.

Key words: Eye movements; Smooth pursuit; Globus pallidus; Basal ganglia; Monkey; Single neurons

INTRODUCTION

To track a moving object with eyes, two voluntary eye movements have been developed in primates. Smooth pursuit continuously moves eyes to stabilize the retinal images of a moving object, while saccades rapidly relocate the eye position to align the object images with the high-acuity fovea. Although many previous studies have revealed the roles of the basal ganglia in the volitional control of saccades [1–4], little is known about their roles in smooth pursuit.

Accumulating evidence suggests that the basal ganglia may also play roles in the control of smooth pursuit. Anatomically, both the saccade-related and the pursuit-related subregions in the frontal eye field send projections to the caudate nucleus [5], the putamen [6] and the subthalamic nucleus [7]. In addition, both subregions in the frontal eye field receive inputs from the thalamic nuclei that relay signals from the basal ganglia [8]. The involvement of the basal ganglia in smooth pursuit is also supported by recent imaging studies showing a significant activation of the caudate nucleus during pursuit [9], and by clinical observations showing that the gain of pursuit is reduced in subjects with Parkinson's disease [10,11].

Despite these observations, the firing of single neurons in the basal ganglia during pursuit has not been examined, with the exception of one recent study that explored signals in the substantia nigra pars reticulata (SNr) [12]. As a step toward understanding the roles of the basal ganglia in smooth pursuit, we examined neuronal activity in the globus pallidus (GP) in monkeys. The GP was chosen because it is involved in the control of saccades [13,14], and because anatomical data suggest that smooth pursuit might involve the cortico-basal ganglia loop, which consists of the frontal eye field, caudate nucleus, putamen, GP and the thalamus. [5–8]. Our study reveals that a subset of neurons in both the external and the internal segments of the GP modulate firing during pursuit, suggesting that pathways through the GP could play roles in the control of smooth pursuit.

MATERIALS AND METHODS

Animal preparation: Data were collected from two Japanese monkeys (*Macaca fuscata*, 6–14 kg). All experimental protocols were approved in advance by the Animal Care and Use Committee of the Hokkaido University School of Medicine, and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). Animals were prepared for chronic unit recording experiments using the procedures described previously [15,16]. Briefly, under general anesthesia, a pair of head holders was implanted in the skull using titanium screws and dental acrylic. A coil of stainless steel wire was also implanted under the conjunctiva to record eye movements. During training and experimental sessions, the monkey's head was secured to the primate chair, and horizontal and vertical eye position was recorded continuously using the search coil technique. After training on a variety of eye movement tasks, a recording cylinder was installed over a small craniotomy that allowed for vertical electrode penetrations aimed at the GP. Animals received analgesia after each surgery. Topical antibiotics were administered around the implant and in the cylinder as necessary. Water intake was controlled daily so that monkeys were motivated to perform behavioral tasks.

Visual stimulus and behavioral paradigms: Experiments were controlled by a Windows-based real-time data acquisition system (TEMPO; Reflective Computing, St. Louis, MO, USA). All events were updated every 5 ms, and visual stimuli were presented on a 24-inch CRT monitor (GDM-FW900; Sony, Tokyo, Japan; refresh rate: 60 Hz) that subtended 64 × 44° of visual angle. A 0.5° square spot served as a visual stimulus. Visual stimuli were presented in individual trials, and monkeys were rewarded with drops of apple juice for maintaining eye position within a 'window' that surrounded the target position at specific time intervals during each trial. A trial was aborted and followed by a newly selected trial if monkeys failed to maintain eye position within a specified window.

To induce smooth pursuit, we used the step-ramp paradigm [17]. Each trial began with the onset of a red fixation point (8.1 cd/m^2). The fixation point was extinguished after a random

1200–1500 ms interval, and a white moving target (22.6 cd/m^2) appeared 4° from the location of the initial fixation. The target moved toward the fixation location at 20°/s so that it crossed the fixation location 200 ms after motion onset. After an excursion for 1000 ms, the target stopped and remained visible for a further 900–1300 ms. Monkeys were required to move their eyes within 2° of the fixation point and 4° of the tracking target except for the initial 300 ms of target motion.

Recording procedures: A tungsten microelectrode (FHC, Bowdoin, ME, USA) was lowered through a 23-gauge guide tube using a hydraulic micromanipulator (MO-97S; Narishige, Tokyo, Japan). Signals through the electrodes were amplified, filtered, and monitored using oscilloscopes and an audiomonitor. For each experiment, we were able to locate the dorsal surface of the GP rather easily by recording the characteristic tonically firing neurons with relatively short action potential duration [18]. Once task-related neuronal activity was encountered, spikes of single neurons were isolated using a real-time spike sorter with template-matching algorithms (MSD; Alpha Omega Engineering, Nazareth, Israel). The occurrence of action potentials was time-stamped, and saved in files with the data of eye movements and visual stimuli during the experiments.

Data acquisition and analysis: Horizontal and vertical eye position signals were obtained directly from the eye coil electronics (MEL-25; Enzanshi Kogyo, Chiba, Japan), and eye velocity signals were obtained using the analog differentiators (DC to 25 Hz, −12 dB/oct; System Koubou, Otaru, Japan). Data were digitized and sampled at 1 kHz, and were analyzed off-line using Matlab (Mathworks, Natick, MA, USA). For each neuron, traces of eye position were aligned on the target motion onset, and were reviewed with rasters and spike density profiles. To obtain spike densities, means of the millisecond-by-millisecond occurrence of action potentials across multiple trials were convolved using a Gaussian filter (σ = 15 ms).

All quantitative measures were performed on the basis of spike counts for specific time

intervals. The significance of firing modulation during pursuit was assessed by comparing neuronal activity during target motion (1000 ms) with that during the 1000-ms fixation period immediately before target motion (Wilcoxon rank-sum test). The firing rate during pursuit was also measured during the 800-ms interval after pursuit initiation (Figs. 1f and 2c). Pursuit onset was detected when eye velocity for trials in opposite directions showed a significant difference (Wilcoxon rank-sum test, $p<0.01$) for more than 20 ms. Directionality of pursuit-related activity was quantified by computing the modulation index (MI), which was defined as MI = (*Pref* – *Opp*) / (*Pref* + *Opp*), where *Pref* was the firing rate during target motion for trials in the preferred direction, and *Opp* was that measured for trials in the opposite direction. The MI was greater than 0.0 when the firing rate increased during pursuit, while a negative value indicated that neuronal activity was suppressed during pursuit. Directionality was also assessed by performing a statistical test (Wilcoxon rank-sum test) between trials in opposite directions.

Histological procedures: The sites of recorded neurons were reconstructed from histological sections for one monkey (monkey *E*, Fig. 3). At the end of the experiments, several electrolytic lesions were made by passing a direct current (10–20 μA) through the recording electrodes for 30–40 s. The monkey was anesthetized deeply with a lethal dose of pentobarbital (>50 mg/kg), and was perfused transcardially with 0.1 M phosphate buffer followed by 3.5% formalin. Then, the brain was removed, blocked, and fixed with the same solution overnight. Once the brain was equilibrated with 0.1 M phosphate buffer containing 30% sucrose, histological sections were cut from each hemisphere using a freezing microtome. Sections were stained with cresyl violet.

RESULTS

Neuronal activity during smooth pursuit was examined for 151 single neurons recorded from three GPs of two monkeys. Of these, 78 neurons (52%; $n = 65$ from the external segment, GPe; $n = 13$ from the internal segment, GPi) exhibited significant firing modulation during pursuit compared with during fixation (Wilcoxon rank-sum test, $p<0.01$). Slightly more than half of

these neurons (n = 33/65, 51% of GPe neurons; n = 7/13, 54% of GPi neurons) reduced the firing rate during pursuit ('decrease-type' neurons), while the remaining neurons elevated the firing rate during pursuit ('increase-type' neurons). Figures 1a and b illustrate representative examples of the two types of neurons. To examine whether the firing modulation during pursuit was related to small catch up saccades, we sorted the trials according to the occurrence of saccades during the initiation of pursuit (400 ms after target motion). Because both neurons exhibited firing modulation for trials without early saccade (raster lines with left bracket), the activity during pursuit was not solely due to the occurrence of catch up saccades. Although the neuronal modulation during pursuit was observed even when catch up saccades were absent, most pursuit-related neurons in the GP also responded to saccades to a stationary target, like neurons in the SNr [12] and in the thalamus [15]. When neuronal activity during saccades of 16° was examined, 83% neurons (n = 33/38 increase-type neurons; n = 32/40 decrease-type neurons) exhibited clear firing modulation. The properties of the saccade-related activity in the GP have been reported elsewhere [16].

Figures 1c and d show the time courses of the population activity during pursuit in the preferred direction for the increase-type neurons (n = 38) and for the decrease-type neurons (n = 40), respectively. For both types of neurons, the modulations of the population activity were greater during the initiation of pursuit, and lasted even after the termination of target motion, indicating that neuronal activity did not faithfully encode eye movement parameters. The time courses of the population activity aligned on the initiation of pursuit (Fig. 1e) show that both types of neurons altered their activity slightly before pursuit, indicating that signals in the GP could play roles in pursuit initiation. As can be seen in the traces of the population activity, the firing rate of the increase-type neurons during central fixation before target motion was statistically less than that of the decrease-type neurons (31.0 ± 17.0 vs. 56.4 ± 25.5 spikes/s, Wilcoxon rank-sum test, $p<10^{-5}$). Figure 1f compares the firing rate during the 800-ms interval following pursuit initiation with that during fixation before target motion onset for individual

neurons. For both types of neurons, the magnitude of the firing modulation during pursuit tended to be greater for neurons showing higher baseline activity during fixation. The rank correlation coefficients computed between the baseline activity and the firing modulation during pursuit were 0.63 and –0.77 for the increase-type neurons and the decrease-type neurons, respectively ($p<10^{-4}$).

Many neurons in the GP exhibited firing modulation (either increase or decrease) during pursuit in multiple directions. Polar plots in Fig. 2a compare the magnitudes of neuronal activity for three representative neurons during (data connected by solid lines) and before (dotted lines) pursuit in four different directions. For each neuron, the response during pursuit was measured during the 1000-ms period of target motion, and that before pursuit was measured during the 1000-ms fixation period immediately before target motion. Both the decrease-type neuron plotted in the left panel and the increase-type neuron in the right panel exhibited directional firing modulation that was maximal during upward pursuit, whereas the size of firing modulation for the decrease-type neuron in the middle panel was comparable between trials in all different directions.

To quantify the directionality of neuronal activity during pursuit, we computed the modulation index (MI) for each neuron. The sign of the MI indicates the direction of firing modulation (i.e., decrease-type or increase-type), and the magnitude of the absolute value indicates the strength of the directionality (See Methods). For example, the MIs for the neurons in Fig. 2a were –0.35, –0.03, and 0.20, respectively. Figure 2b summarizes the distribution of the MIs for 78 pursuit-related neurons. The MIs averaged –0.12 ± 0.09 (SD) and 0.07 ± 0.08 for the decrease-type neurons and the increase-type neurons, respectively. The firing rates during pursuit in opposite directions were statistically different for 32% (n = 25) of those neurons (black bars in Fig. 2b; Wilcoxon rank-sum test, $p<0.01$). We found a slight but significant correlation between the absolute values of the MIs and the magnitude of firing modulation during pursuit (Spearman's $r_s = 0.32$, $n = 78$, $p<0.01$), indicating that neurons with greater firing

modulation tend to be directional.

Many neurons exhibited firing modulation even after the offset of target motion (Figs. 1c and d). To examine whether the activity during the second fixation in the periphery was related to eye position in the orbit, Fig. 2c compares the means of firing rate before, during and after pursuit for trials in the opposite directions. Although the activity during pursuit depended on the direction of pursuit (compare data connected with solid versus dashed lines), that during the two fixation intervals did not. This suggests that the firing modulation during the second fixation interval may not be related to the changes in eye position.

Figure 3 illustrates the sites of neurons recorded from monkey *E*. We explored neurons exhaustively within the areas of approximately 5 mm square, but the locations of the pursuit-related neurons only are plotted. Most neurons were located in the anterior portion of the GP, within a few millimeters posterior to the anterior commissure. No difference in the distributions was found between the types of neurons both along the anterior-posterior and the medial-lateral coordinates of electrode penetrations, except for the left hemisphere of monkey *E* (Fig. 3) where the decrease-type neurons were located more lateral part of the GPe than the increase-type neurons (Wilcoxon rank-sum test, $p<0.05$). Locations of the pursuit-related neurons largely overlapped with those of the saccade-related neurons reported previously [16], although pursuit neurons were distributed more sparsely within the GP than were saccade neurons.

DISCUSSION

The present study showed that a subset of neurons in both the external and the internal segments of the GP modulate activity during smooth pursuit eye movements. About half of these neurons elevated the firing rate during pursuit, while the others reduced the firing rate, just like GP neurons that are related to somatic movements [19–21]. The firing rate of many of these neurons was modulated during pursuit in opposite directions, and neuronal activity often persisted even

after target motion, suggesting that the neuronal activity was strictly related neither to the direction nor to the speed of pursuit. Similar to neurons in the SNr [12] and in the thalamus [15], most pursuit-related neurons in the GP also modulated firing during saccades to a stationary target. Importantly, however, the firing modulation during pursuit was present even in the absence of catch up saccades.

There are several possible ways by which the signals in the GP regulate pursuit. First, considering the inhibitory projections from the GPi to the thalamus and from the GPe to the SNr/GPi via the subthalamic nucleus, the increase-type neurons in the GPe and the decrease-type neurons in the GPi could provide "permissive disinhibition" of pursuit [12,22,23]. As suggested in the saccade system, transient removal of persistent suppression might gate signal processing through the target structure [24]. For example, signals from the GP might boost pursuit signals within the reciprocal excitatory pathways between the cortex and the thalamus [16]. Another possibility is that the cortico-basal ganglia thalamocortical loop transmits pursuit drive signals, composing the internal feedback circuitry that allows for the high-gain pursuit in the absence of retinal image motion during the maintenance of pursuit [12,15]. Finally, because signals in the frontal eye field regulate the gain of sensorimotor transformation for pursuit [25], the signals through the basal ganglia thalamocortical pathways might play roles in setting the gain higher during the initiation and the maintenance of pursuit [12]. All these possible mechanisms could explain the previous observations that the gain of pursuit decreased in subjects with damage to the basal ganglia [10,11].

In summary, the present study was the first to describe the neuronal modulation in the GP during smooth pursuit. Some neurons exhibited directional firing modulation, and many responded to large (16°) saccades, like neurons in the SNr [12] and the thalamus [15]. To understand the roles of the pursuit-related signals in the GP reported here, further stimulation and inactivation experiments will be needed in future studies.

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CAPTIONS

Figure 1.

Neuronal activity during smooth pursuit eye movements. (**a**) An increase-type neuron recorded from the GPe. (**b**) A decrease-type neuron recorded from the GPi. Vertical solid lines indicate the onset and offset of target motion. The bracket on the left side of the raster lines indicate trials in which no catch up saccade was observed during 400 ms after target motion onset (vertical dashed line). *he* and *ve* denote horizontal and vertical eye position, respectively. (**c**) and (**d**) The mean population activity during pursuit in the preferred direction. (**e**) The mean population activity aligned on the initiation of pursuit. The dashed traces indicate the mean ± SE. (**f**) The firing rates during pursuit as a function of those during fixation for the increase-type neurons (open circles) and for the decrease-type neurons (filled triangles). The arrows indicate the data for neurons shown in **a** and **b**.

Figure 2.

Directionality of the pursuit-related activity. (**a**) Three representative neurons recorded from the left hemisphere of monkey *E*. Each polar plot indicates the mean firing rate measured during pursuit (data connected with solid lines) and during central fixation before target motion (dashed lines) for trials in four different directions. (**b**) Distribution of the modulation indices (MIs). Neurons with positive MIs increased the firing rate during pursuit, while those with negative values decreased the firing rate. For 25 neurons, the firing modulation during pursuit in opposite directions was statistically different (filled bars; Wilcoxon rank-sum test, $p<0.01$). (**c**) Means of activity before, during and after pursuit in the preferred (data connected with solid lines) and in the opposite direction (dashed lines) for the increase-type neurons (open circles) and the decrease-type neurons (filled triangles). Error bars indicate 95% confidence intervals.

Figure 3.

Sites of the pursuit-related neurons in monkey *E*. Circles and triangles indicate the increase-type

neurons and the decrease-type neurons, respectively. Levels of frontal sections are shown as millimeters from the anterior commissure (AC). GPe and GPi, external and internal segments of the globus pallidus, respectively.

Figure1

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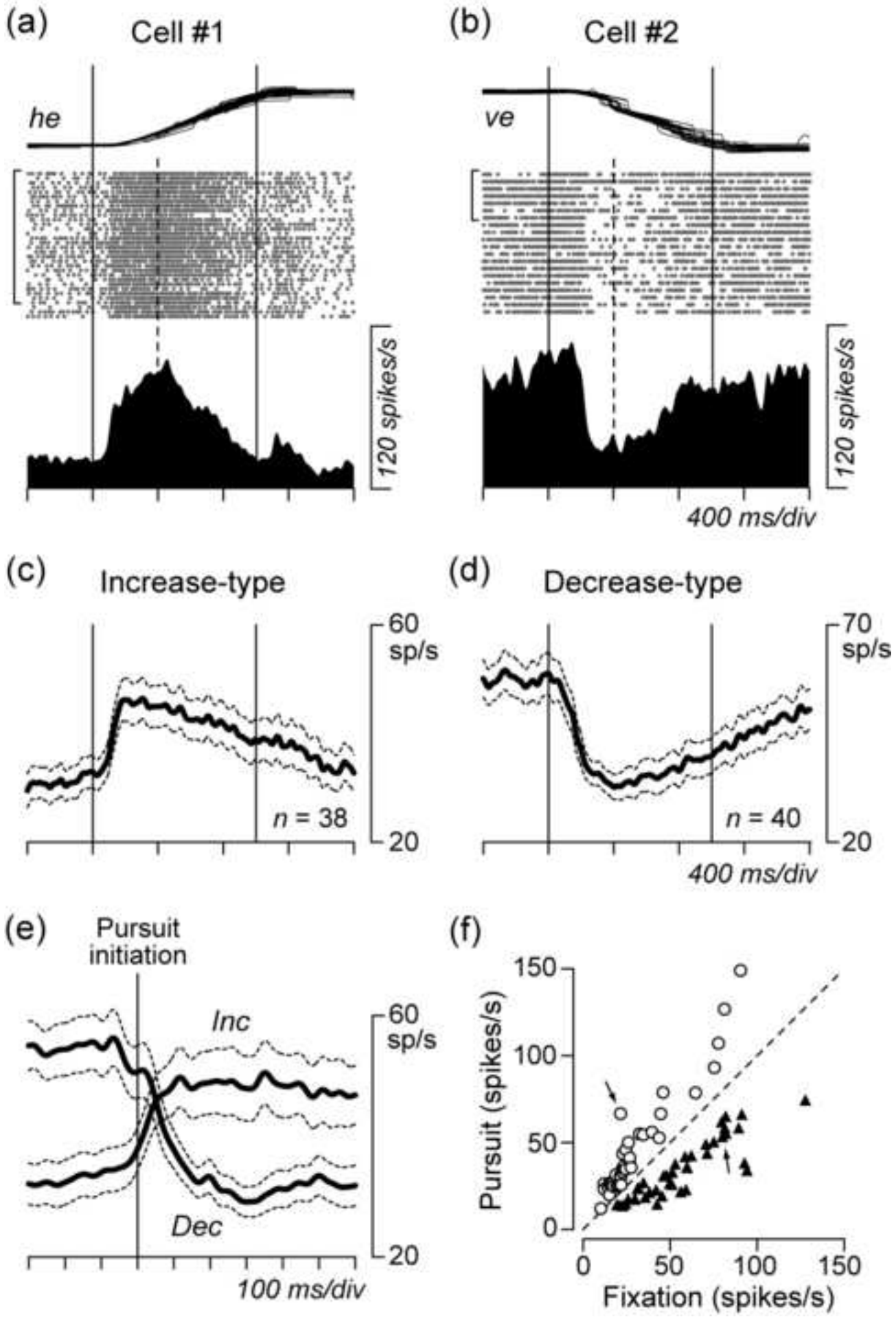

Figure2

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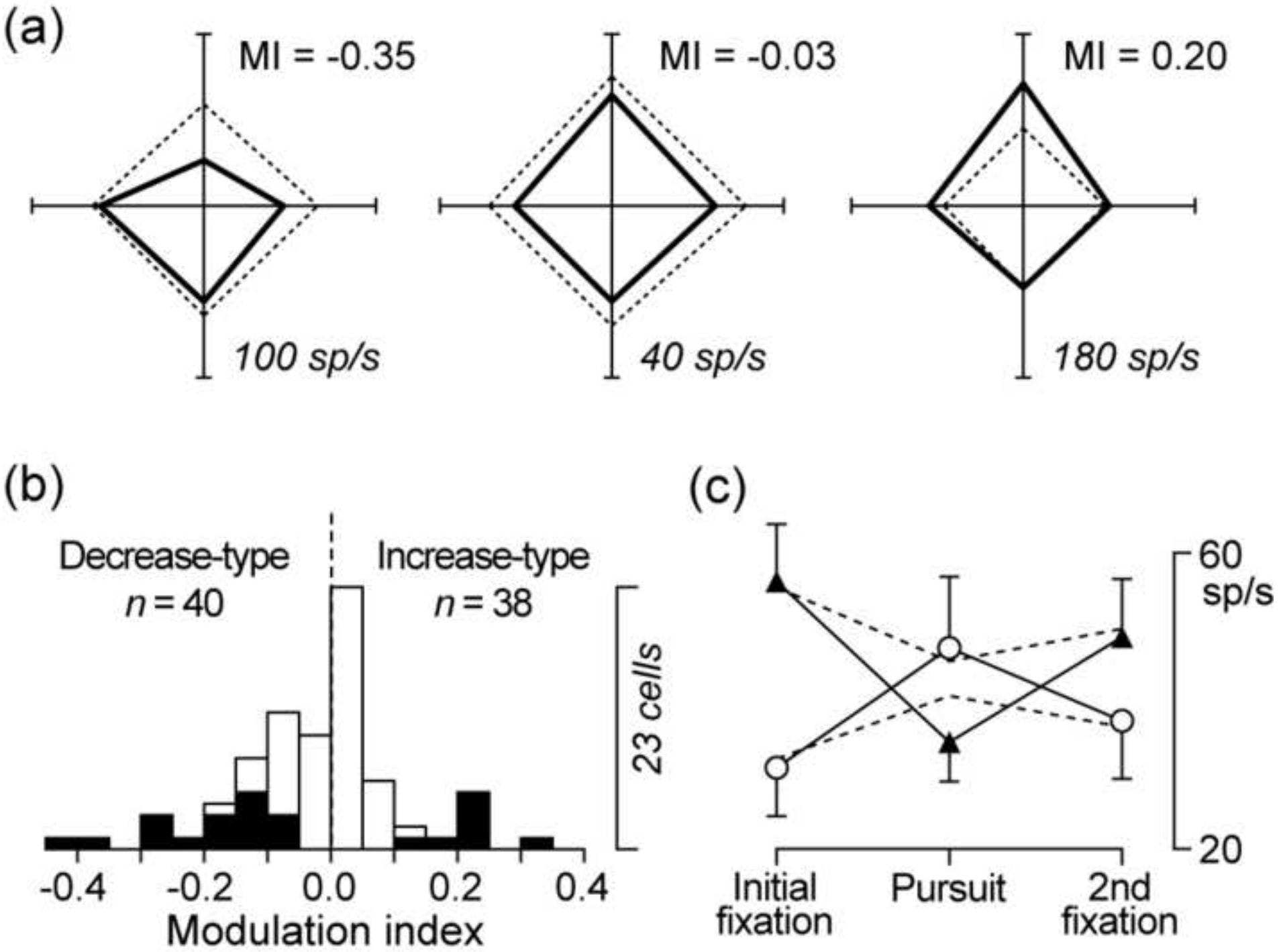

Figure3

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