

Synchronization of visual responses in the superior colliculus of awake cats

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Multi-unit responses to moving stimuli were recorded simultaneously from several sites in the superior colliculus of awake cats. Correlation analysis revealed that response synchronization was a prominent feature of visually evoked neural activity in both superficial and deep collicular layers. Responses at about half of the recordings separated by ≤ 1 mm showed significant correlations. The synchronized responses oscillated in the gamma frequency range (30–70 Hz) which contrasts to conditions in anaesthetized cats where oscillations predomi-

nantly occurred in the alpha and beta frequency range (10–20 Hz). Response synchronization was most pronounced with coherent motion stimuli and broke down with incoherent stimuli. These results agree with previous findings on cortico-tectal synchronization and support the hypothesis that synchronization in the millisecond range serves to group collicular neurons into functionally coherent assemblies. *NeuroReport* 12:43–47 © 2001 Lippincott Williams & Wilkins.

Key words: Assembly; Cat; Cross-correlation; Gamma-band; Oscillation; Superior colliculus; Synchronization; Temporal binding; Visual system

INTRODUCTION

Multielectrode recordings in visual cortex have revealed stimulus-dependent synchronization of neuronal responses in the millisecond range and it has been proposed that this might serve to define functional relations among distributed neurons [1–3]. Due to coarse coding, multiple spatially overlapping stimuli evoke overlapping population responses in visual areas and it becomes difficult for subsequent processing stages to distinguish which of the many simultaneous responses should be associated with which stimulus. Synchronization of responses evoked by the same object could contribute to the disambiguation of such overlapping population responses [3]. Neurons in the mammalian superior colliculus (SC) have large receptive fields and a broad tuning for motor responses [4,5] and, thus, it is unclear how such a distributed system can cope with the problem of stimulus superposition [6,7]. Recent evidence suggests that response synchronization can occur in the tectum of a variety of different species including pigeons [8], rats [9] and cats [10–12]. These findings suggest the possibility that synchronization might serve also in this structure to group responses evoked from the same object and to segregate them from responses to different objects. Most of these studies have been carried out in anesthetized preparations, and since there is evidence that synchronization and oscillatory patterning of responses strongly dependent on central states [13,14], recordings in awake animals are indispensable. Here we present data from multielectrode recordings of visually

evoked activity in the SC of awake cats and address the following questions: (i) Is correlated activity present in the SC of awake cats? (ii) Are the temporal correlations state-dependent, i.e. do they differ from interaction patterns observed previously in anesthetized animals [11,12]? (iii) Does collicular synchronization depend on the coherence of visual stimuli?

MATERIALS AND METHODS

We used standard surgical and recording techniques described in detail previously [11,12]. Multi-unit activity was recorded simultaneously from several sites of the SC of four head-fixed awake cats either with tungsten microelectrodes inserted through a recording cylinder implanted on the head of the animal or, alternatively, with chronically implanted platinum–iridium wires which could be advanced in the tissue by small microdrives. For implantation general anesthesia was induced by ketamine and xylazine (10 and 2 mg/kg, i.m., respectively) and maintained by ventilating the animals with a mixture of 70% N₂O, 30% O₂ and 0.8–1.2% halothane. Implants consisted of a recording cylinder or a microdrive over a small trepanation above the SC, a head fixation bolt and Ag/AgCl electrodes for DC electro-oculography.

Experimental sessions began once the animals had become accustomed to 2–3 h head fixation. In the experiments with tungsten microelectrodes (200 μ m diameter), these were advanced through the intact dura. Contact with the superficial laminae of the SC was inferred from a

sudden onset of visually evoked activity and depth below the SC surface was then estimated from micromanipulator readings. Signals were amplified $\times 10\,000$, band-pass filtered between 1 and 3 kHz and fed through a Schmitt trigger. Threshold for spike detection was set to two times the noise level. A variety of natural and computer generated visual stimuli were presented. Computer-generated visual stimuli comprised gratings, radially moving flowfields, and patterns of parallel moving random dots which were presented at a frame rate of 100 Hz on a RGB monitor. Natural stimuli included objects like rulers, or hands that were moved at variable distances and speeds through the receptive fields of the respective cells. For all neuronal responses peristimulus-time histograms as well as auto- and cross-correlation functions were computed. To assess the significance of the correlogram modulation, a generalized Gabor function was fitted to the correlograms [15]. Since our study focuses on the synchronization behavior of collicular neurons, only data from cross-correlation analysis are presented here.

Although the animals under study were not required to fixate, DC electro-oculography revealed that they did relatively few eye movements during recording sessions. When eye movements occurred they consisted of both saccades and pursuit movements. All of the correlation patterns described here, including the broad correlation peaks shown in Fig. 2e could be observed in recording episodes free of eye movements.

RESULTS

Data were obtained from 220 multi-unit recording sites. Assuming a transition from superficial to deep collicular layers 1000 μm below the SC surface, recordings were distributed roughly equally between superficial and deep layers. Cross-correlation analysis was performed for a total of 310 recording pairs comprising 187 intercollicular pairs (electrodes located in the right and left SC, respectively) and 123 intracollicular pairs. Not all pairs of recordings were tested with the whole set of visual stimuli. As specified in Table 1, a majority of cell pairs was tested with handheld stimuli and moving dot patterns, and a smaller subset of recording pairs with flowfields or whole field gratings.

Response synchronization was abundant in the SC of awake cats and occurred with all of the applied stimuli.

Table 1. Numbers of pairs and incidence of synchrony with different types of visual stimuli.

Stimulus type	No. pairs tested	No. pairs showing synchrony
Hand-held stimuli	197	43 (22%)
Person moving through the RF	182	29 (16%)
Moving dot patterns	40	8 (20%)*
Flowfields	30	5 (17%)
Gratings	24	19 (80%)

Note that many of the 310 recordings pairs were tested with multiple visual stimuli. Handheld stimuli were usually moved at speeds around 5 deg/s and consisted of a handheld pen or a handheld ruler. Moving dot patterns, flowfields and gratings were displayed on a computer monitor which subtended $35 \times 25^\circ$ of the central visual field. Gratings had a spatial frequency of 0.1° .

*This value applies to dot displays with coherent motion only.

Figure 1a shows a typical example of synchronized multi-unit responses recorded in the superficial layers of the right SC. To quantify the correlation strength we selected for each pair of recording sites the cross-correlogram with the strongest modulation of all stimulation conditions. The grand average of the modulation amplitude (height of the center peak divided by the offset of the correlogram [15]) of these correlograms was 0.40 ± 0.23 (s.d.). As shown in Fig. 1b, significant correlations were less frequent in intercollicular pairs (12%) than in intracollicular pairs (46%; $p < 0.001$, U-test). Most intracollicular pairs were recorded with a horizontal electrode separation of ≤ 1 mm and a vertical electrode separation of < 0.5 mm. The majority of recordings were therefore intralaminar, i.e. they were restricted to either superficial or deep layers. In a subset of data we compared the probability of intracollicular synchronization as a function of horizontal electrode distance (Fig. 1c). For sites with > 1.5 mm separation the incidence of synchronization was 2-fold lower than for sites separated by < 1.5 mm ($p = 0.015$, U-test).

In a substantial fraction of cases, the neuronal interactions were characterized by an oscillatory patterning. Such oscillations appeared in 31% of the pairs exhibiting significant correlations (considering only the correlogram with the strongest modulation amplitude for each pair). As shown in Fig. 1d, the distribution of oscillation frequencies was bimodal with distinct peaks in the alpha/beta (10–20 Hz) and in the gamma range (30–70 Hz), respectively. In most of the cases, the correlation peak occurred at close to zero phase lag, the phase shifts being < 5 ms in $> 80\%$ of the correlograms with the strongest modulation (Fig. 1e). The distribution of peak widths indicates that in a substantial fraction of cases the peaks were rather narrow, the median width (at half height) being around 25 ms (Fig. 1f).

The correlation patterns depended on the type of visual stimuli presented (Table 1). Drifting whole field gratings were particularly effective in inducing synchronization and strong oscillatory patterning. They evoked synchronized responses in 80% (19 of 24) of the intracollicular recording pairs while other stimuli induced synchronized responses only in around 20% of the pairs (see Table 1, $p < 0.05$, U-test). Of the 24 pairs tested with gratings only six pairs (25%) exhibited synchronized responses when stimulated with a handheld ruler.

To test whether response synchronization depended on the coherence of the visual stimuli we presented random dots patterns whose motion coherence was parametrically varied (Fig. 2). In eight of 40 recording pairs tested in this way, coherently moving dots (the same spatial displacement applied to all dots from frame to frame) evoked synchronized responses that oscillated in the gamma frequency range. This oscillatory patterning broke down, however, as soon as the patterns were made incoherent by adding noise, i.e. by spatially displacing a fraction of dots at random between frames. Addition of 10% noise was sufficient to abolish gamma oscillations ($p < 0.01$, U-test). The modulation amplitude of correlograms was not significantly different for coherent motion stimuli (0.31 ± 0.21) and noise (100%) stimuli (0.25 ± 0.27). As shown in Fig. 2, this dependence of high-frequency oscillations on stimulus coherence could be observed both in the superficial and deep collicular layers. While precise synchronization and

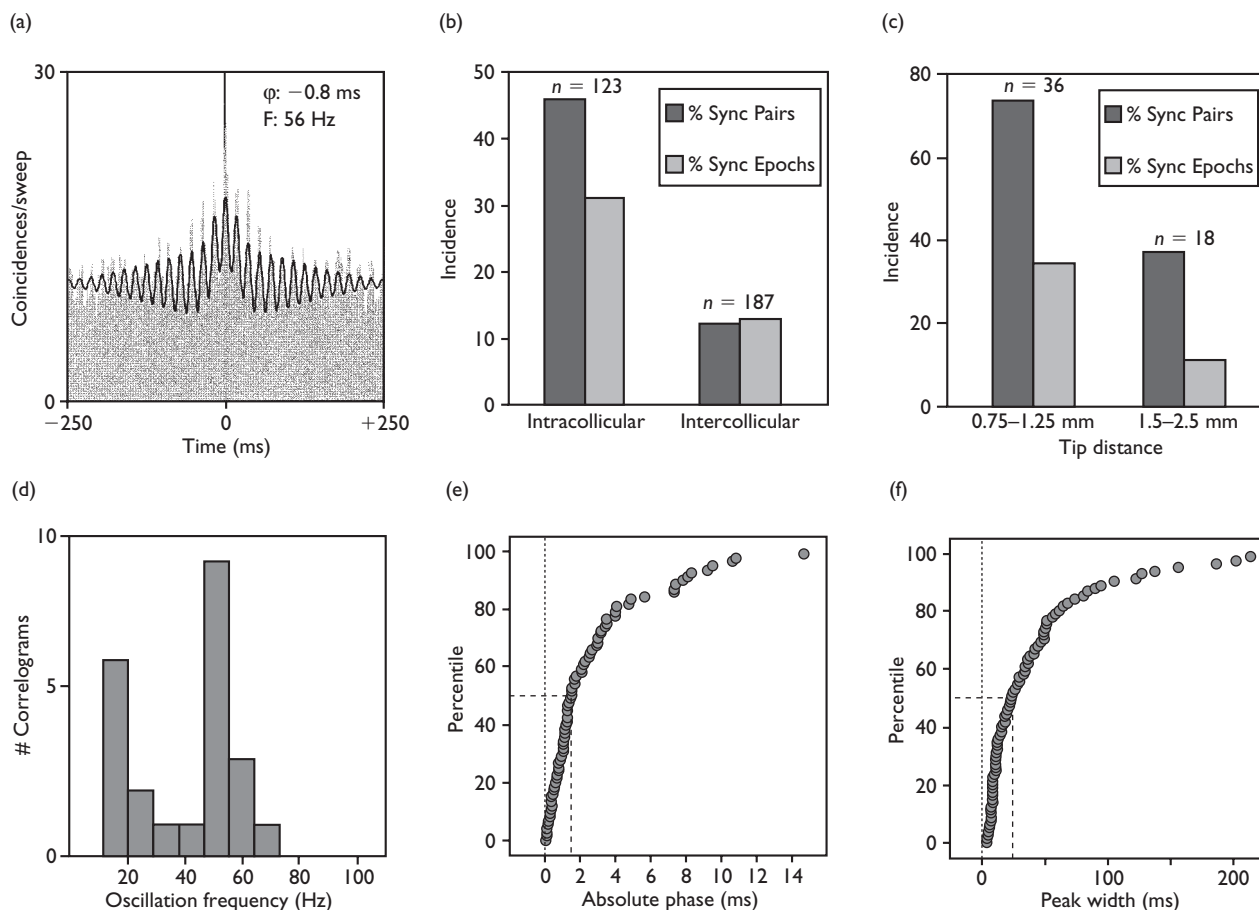


Fig. 1. Parameters of the correlation patterns recorded in awake cats. **(a)** Example of a cross-correlogram with strong oscillatory modulation. Cells were activated with a coherently moving dot pattern. Recordings were made from superficial SC layers. The black continuous line superimposed to the correlogram represents the generalized Gabor function that was fitted to the data. ϕ , phase shift of the center peak; F , frequency of the modulation. **(b)** Comparison of the incidence of neuronal synchronization for intra- and intercollicular pairs. The light grey columns (% Sync Epochs) refer to the percentage of stimulation epochs (ten stimulus repetitions each) in which synchronization was observed. Dark columns (% Sync Pairs) refer to incidence of pairs where synchronization was observed in at least one stimulation epoch. **(c)** Dependence of the probability of intracollicular synchronization on electrode distance. Data are from 18 penetrations in which we recorded with three evenly spaced electrodes. Conventions for dark and light grey columns as in **(b)**. **(d)** Distribution of oscillation frequencies. **(e)** Cumulative distributions of the absolute phase values, i.e. the lateral displacement of the correlogram center peaks as inferred from the fitted function. The dashed line indicates the median absolute phase value. **(f)** Cumulative distribution of peak width. The dashed line indicates the median. Peak width was inferred from the fit with a generalized Gabor function and computed at half height of the center peak. Note that in **(d-f)**, only the respective correlogram with the strongest modulation amplitude for each pair with significant interactions has been included.

oscillatory patterning of responses were abolished by noise stimuli, these stimuli could evoke broad correlation peaks with a width of up to 70–100 ms at half height. As illustrated by the example in Fig. 2e, increasing noise levels could lead to a progressive increase in the width of these broad peaks. The effect of motion noise on the offset of the correlograms was variable.

DISCUSSION

Our data show that response synchronization and oscillatory modulation of synchronized responses occur frequently and for a variety of stimulation protocols in the SC of awake cats. The synchronization patterns observed in the SC resemble those described previously in the visual cortex with respect to their strength, phase relationships,

temporal precision and oscillatory patterning (for review see [1–3]). This is not surprising given the massive cortical input to the SC [16] and the evidence that collicular responses get synchronized with cortical activity [11]. The probability, strength and the temporal patterning of response synchronization in the colliculus depend on a number of factors such as the animal's state of arousal (see below), the distance between cells, and the coherence of applied stimuli. These dependencies, too, resemble those identified previously for cortical responses [17–21].

When compared with previous data obtained in the SC of anesthetized cats [12], the present results provide clear evidence that collicular correlation patterns are state dependent, in a way that is similar to the state dependence of cortical synchronization phenomena [13,14]. Oscillation

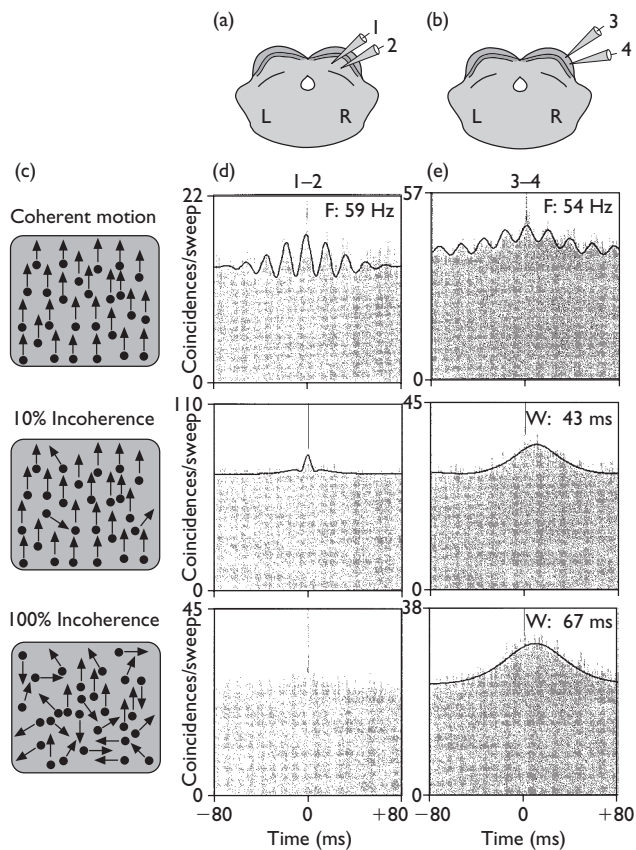


Fig. 2. Effect of stimulus coherence on neuronal interactions in the SC. The figure illustrates one case in which simultaneous recordings (1, 2) were made in the deep layers of the SC (**a,d**) as well as one example for simultaneous recordings (3, 4) from the superficial SC layers (**b,e**). (**c**) Three types of whole-field random dot stimuli were employed: Either coherently upward moving dots (top), upward moving dots intermixed with 10% dots displaced randomly from each frame to the next (middle), or with 100% dots showing random spatial displacement in every frame (bottom). (**d**) Cross-correlograms computed for the responses at sites 1 and 2 under the three different stimulation conditions. Note that the coherent motion stimulus induces pronounced synchronous gamma-oscillations (top correlogram) which disappear with the less coherent visual stimuli (middle and bottom correlograms). F, oscillation frequency. (**e**) Similar effects are visible in the cross-correlograms computed for sites 3 and 4. The gamma-oscillation occurs only in response to the coherent motion stimulus (top correlogram). In this case, a broad correlation peak is observed in the responses to the more incoherent stimuli (middle and bottom correlogram). Note that the peak width (W, computed as width at half height) increases with increasing noise level. Where present, the black continuous line superimposed to the correlograms represents the generalized Gabor function that was fitted to the data.

frequencies are higher and average phase lags smaller in the awake than in the anesthetized animal. In the latter, oscillations in the SC occurred predominantly in the alpha/beta frequency range, and only 60% of the pairs showed cross-correlograms with phase lags of < 5 ms [12]. However, it needs to be emphasized that visual stimulation conditions in the awake animals differed from those applied in the anesthetized preparation where we had used mainly light bars as stimuli [12]. Moreover, the awake

animals examined in the present study were not trained to visual fixation and, hence, eye movements might have affected our results. However, DC electro-oculography (see Materials and Methods) revealed that, with the stimuli used here, the animals made either no or only low-gain pursuit movements during the stimulation episodes sampled for correlation analysis.

The dependence of the temporal patterning on stimulus coherence also suggests close similarities between collicular and cortical synchronization. As shown previously [12], temporal correlations in the SC depend on whether the cells are responding to the same or to different objects, synchrony being observed only in the former case, but not the latter. The same effect has been documented for a variety of visual cortical areas in cats and monkeys (for review see [1–3]). Along similar lines, our measurements with random-dot stimuli demonstrate a dependence of collicular synchrony on motion coherence. We have recently applied the same stimulation paradigm to cortical neurons in the cat and obtained very similar results [22]. Thus, we observed a loss of gamma oscillations with noise in the patterns and, furthermore, a decrease in the precision of synchrony (as measured by correlogram peak widths) with a decrease in the coherence of visible motion (i.e. increase in the noise level). The differences between correlations evoked by computer generated stimuli such as gratings and those correlations evoked by handheld stimuli could be related to a variety of factors such as stimulus size, velocity, stability etc.

As we have suggested previously, stimulus- and state-dependent synchronization among collicular neurons might serve the disambiguation of collicular activity patterns in cases where the animal has to select between multiple targets for an orienting response [11,12]. The present finding of fast oscillations and precise synchrony in the awake cat SC is in line with this hypothesis. Supportive evidence for a possible functional role of collicular synchrony comes from recent microstimulation experiments that we have performed in awake cats [23]. In these measurements, we investigated eye movements evoked by synchronous or asynchronous electrical pulse trains applied to multiple sites in the deep SC layers. These experiments revealed a critical influence of the relative timing of the microstimulation pulses on the saccadic eye movements. When the trains of stimulation pulses applied to different sites were synchronized (phase shifts < 5 ms), averaging of the individual saccade vectors was observed. However, asynchronous (phase shifts > 5 ms) delivery of the pulses led to summation of the individual saccade vectors, as if the stimulation trains had been applied in succession [23]. Thus, the relative timing of neuronal signals on a millisecond scale appears to be critical for the motor output of the system. As shown here, the temporal precision of naturally occurring synchrony is in good agreement with the coincidence window defined by these microstimulation experiments and, together, these studies open up the possibility that spike timing might be involved in collicular target selection.

CONCLUSION

Our results show that response synchronization is a prominent feature of visually evoked neural activity in

superficial and deep collicular layers of the awake cat. In the awake state, synchronized responses often exhibit an oscillatory patterning in the gamma frequency range (30–70 Hz), whereas lower oscillation frequencies predominate in the colliculus under anesthesia. While coherent motion stimuli evoke temporally precise synchronization, such synchronization patterns are not induced by incoherent motion patterns. These results are consistent with the hypothesis that synchronization in the millisecond range serves to group collicular neurons into functionally coherent assemblies.

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