Table 1. Feeding deterrence of compounds *1 – 6* to imagines of *E. varivestis*. Each compound (dissolved in MeOH) was coated as a thin film on disks (diameter 1.6 cm) of bean leaves (*Phaseolus vulgaris*) and analyzed for feeding deterrence to imagines of *E. varivestis* in a range of doses (0.01 – 0.4 μmol/disk, corresponding to 0.34 – 13.7 μmol/g fresh weight). Leaf disks for controls were coated with MeOH only. $ED_{50}$ (doses which reduce feeding by half compared to controls) were obtained by probit analysis of the respective dose/response curves; * inactive at the doses analyzed

| Compound | $ED_{50}$ [μmol/leaf disk] |
|---|---|
| *1* | – * |
| *2* | 0.17 |
| *3* | 0.19 |
| *4* | 0.05 |
| *5* | 0.10 |
| *6* | 0.075 |

instance, in leaves of *Ageratina adenophora* (Asteraceae) (encecalin up to 20 μmol/g fresh weight) [7] or flowering heads of *Ageratum houstonianum* (Asteraceae) (precocene II up to 9 μmol/g fresh weight) [8]. Imagines present on leaf disks treated with encecalin or precocene II immediately resumed feeding when transferred to untreated bean leaves, indicating that the rejection of treated food was due to feeding deterrence and not to intoxication of the insects by the two chromenes during the bioassay. The benzofuran euparin (*1*) was again inactive in the range of doses analyzed.

The contact toxicity of the compounds studied corresponds well to their feeding deterrence. The two most toxic chromenes encecalin (*4*) and precocene II (*6*) are also the strongest feeding deterrents to *E. varivestis*, whereas the non-toxic benzofuran euparin (*1*) produces no feeding deterrence.

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1. Grainge, M., Ahmed, S.: Handbook of Plants with Pest-Control Properties. Chichester: Wiley 1988
2. Proksch, P., Rodriguez, E.: Phytochemistry *22*, 2335 (1983)
3. Isman, M. B., Yan, J.-Y., Proksch, P.: Naturwissenschaften *73*, 500 (1986)
4. Isman, M. B., Proksch, P., Yan, J.-Y.: Entomol. Exp. Appl. *43*, 87 (1987)
5. Proksch, P., Proksch, M., Towers, G. H. N., Rodriguez, E.: J. Nat. Prod. *46*, 331 (1983)
6. Srivastava, R. P., Proksch, P.: Entomol. Generalis (in press)
7. Proksch, P., Wray, V., Isman, M. B., Rahaus, I.: Phytochemistry *29*, 453 (1990)
8. Siebertz, R., Proksch, P., Witte, L.: ibid. *29*, 2135 (1990)

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# Neurons in the Midbrain of the Barn Owl Are Sensitive to the Direction of Apparent Acoustic Motion

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An animal's environment is rarely static. Stimuli from moving sources abound, and, in one sensory system, vision, the neurological basis of motion processing has been well characterized on experimental [1 – 3] and theoretical grounds (for a recent review see [4]). The acoustic environment is no less dynamic. Psychophysical studies have shown that while we are less sensitive to the movement of a sound source than that of a visual stimulus, our ability to judge direction and speed, once we detect the motion, is as accurate as that for vision [5 – 7]. Nevertheless, the neural basis of acoustic motion perception has not received the attention that has been devoted to visual motion processing or the localization of stationary sound sources. The barn owl (*Tyto alba*), whose ability to localize sound has been extensively studied [8, 9], is also known to be able to judge the direction in which prey are moving [10]. We report here the existence of mesencephalic neurons whose response depends upon the direction of apparent acoustic motion, in short, are motion-direction sensitive.

Recordings were made in three adult barn owls. Anesthesia, surgery, perfusion, histological localization of lesions, and animal care were described earlier [11]. All recordings were made in an anechoic chamber (3 × 3 × 3 m). Search stimuli consisted of tone or noise bursts having an interaural time difference and interaural level difference, important cues for azimuthal and elevational sound localization in the barn owl, respectively [8, 9]. The search stimuli were presented dichotically over stereo earphones (Sony MDR-E272). After a cell was identified we switched from dichotic stimulation to stimulation through seven 10-cm speakers arranged in a semicircular array about 10° below the owl's horizon (inset, Fig. 1). Speakers were placed at 30° intervals from +90° (owl's right, speaker #1) to −90° (owl's left, speaker #7).

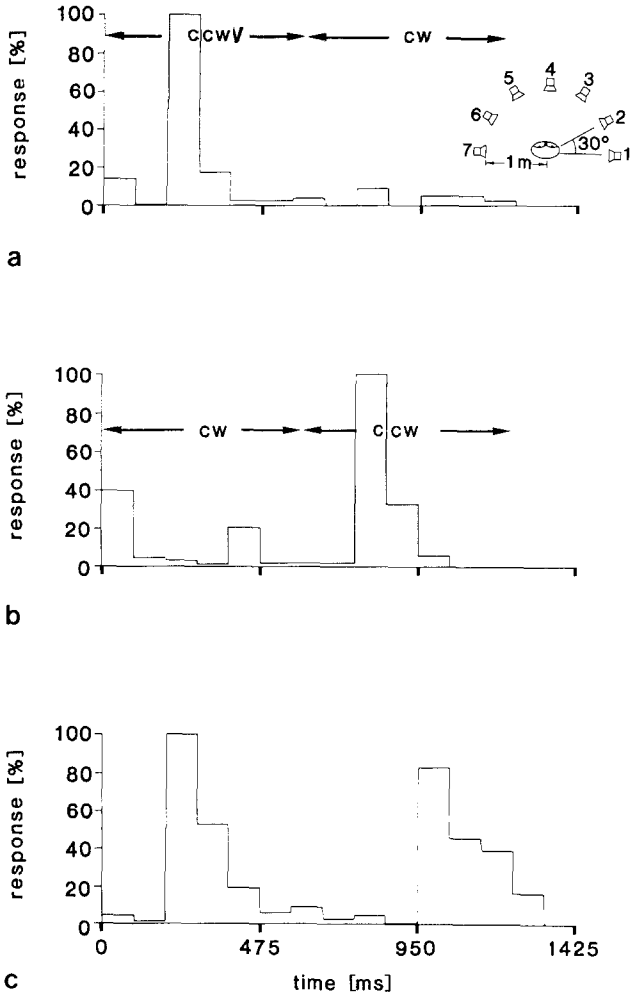


Fig. 1. Tectal cell sensitive to the direction of apparent acoustic motion. The cell responded much better to counterclockwise motion (*ccw*) than to clockwise motion (*cw*) irrespective of whether the motion started in the counterclockwise direction (a) or the clockwise direction (b). A dot-raster display demonstrating the spike discharge to 20 roundtrips is shown above each PST histogram. The maximum of the response was 130 spikes in (a) and 88 in (b). If only the most effective speaker in the motion conditions (a, b) was enabled on (c), a symmetrical PST histogram resulted, but the response maximum was still high (112 spikes). The onset response of this neuron, occurring in the first 100 ms after the beginning of the roundtrip (a, b), was excluded from the analysis. The DI values in the motion conditions are statistically significant at the $p < 0.01$ level. On-on time was 95 ms, and successive stimuli overlapped in time by 5 ms or the rise/fall time of the individual bursts. The units of analysis (bins) in the PST histograms correspond to the on-on time. In the *inset* the loudspeaker arrangement is shown

Speakers were sequentially activated in a "roundtrip" pattern, which simulated a source moving from 90° on one side to 90° on the other side, and back. Successive roundtrips were separated by 3 to 7 s of silence. Stimuli from the individual speakers consisted of noise bursts (flat between 1 and 25 kHz) having 5 ms rise/fall times and variable durations. For most of the experiments, the earphones were left in place, because control experiments revealed that a neuron's motion-direction sensitivity was unaffected by the presence of the earphones. The duration of the stimulus, the interval between the onset of successive sounds ("on-on time"), which determines the velocity of the apparent motion, and the overlap or silent interval ("gap") between successive sounds (inset, Fig. 3) were controlled by a computer (IBM AT). Note that only two of these three variables can be chosen independently.

The time of arrival of action potentials, relative to the onset of the roundtrip, was recorded in computer memory and analyzed as peristimulus time (PST) histograms. To quantify the motion-direction sensitivity of a neuron, we derived a directionality index DI:

DI = 1 − (lower spike count/higher spike count).

"Lower spike count" and "higher spike count" refer to the halves of the PST histogram with the lower and higher numbers of spikes, respectively (Fig. 1). To judge the statistical significance of the DI, we compared, using a chi-square test, the halves of the PST histogram from trials in which all speakers were enabled (motion condition), with those from trials in which only the most effective speaker produced a sound (stationary condition). The stationary condition represents the null hypothesis for the chi-square test, because a symmetrical PST histogram is expected if only the best speaker is activated twice each roundtrip. When a cell was lost before the stationary test was performed, the spike counts to clockwise and counterclockwise stimulation were averaged for the chi-square test. This was always done to test for a bias in the one-speaker test. One cell out of 50 tested with the stationary condition was biased and was excluded from the further analysis.

The results are based on 68 recordings from the optic tectum (17 recordings), the external nucleus of the inferior colliculus (ICx) (9), and the lateral shell of the central inferior collicular nucleus (ICc-ls) (30). The remainder of the recordings (12) lay also in the vicinity of these nuclei, but the association of a recording with a specific nucleus was not possible in these cases. Anatomical studies have shown that ICc-ls projects to the ICx, which, in turn, innervates the optic tectum [11, 12]. About two-thirds of the recordings were isolated as single units. No difference in the response to moving stimuli was seen between single-unit recordings and multi-unit recordings. The general response characteristics of these neurons to stationary sound bursts are well known

 

[11, 13–15]. All neurons that responded to stationary stimuli responded also to the apparent motion stimulus. In about 70% of the recordings, maximal spike rate in the stationary condition equaled the maximal spike rate in the motion condition. In 15%, the response was better to motion, and in 15% the response was better to stationary stimuli.

The PST histograms in Fig. 1 demonstrate a neuron with high motion-direction sensitivity. The speaker array was first activated in the counterclockwise direction (ccw), from speaker #1 to #7, then, in the clockwise direction (cw), from #7 to #1 (Fig. 1a). A strong discharge could be associated with the activation of speaker #3 when the array was activated in the counterclockwise direction, but not when the array was activated in the clockwise direction. This resulted in a PST histogram with a DI of 0.82 (Fig. 1a). To exclude the possibility that this was simply an onset effect, we reversed the activation sequence, beginning with clockwise motion followed by counterclockwise motion (Fig. 1b). Under these circumstances as well, the neuron discharged heavily during counterclockwise motion, confirming that it was direction and not stimulus onset that was responsible for the asymmetry of the PST histogram (DI = 0.77). When only the most effective speaker, #3, was enabled, the neuron responded twice per roundtrip or each time speaker #3 emitted a sound. This resulted in a symmetrical PST histogram and a low DI of 0.07 (Fig. 1c). The PST histogram of Fig. 1c further demonstrates that the single burst, caused by the first activation of speaker #3, did not fatigue the neuron. Finally, comparison of the top two histograms with the bottom histogram revealed that the number of spikes in the nonpreferred, or "null", direction was less than that evoked by the activation of speaker #3 alone, and that the maximum number of spikes per bin in the preferred direction was similar to the number of spikes in the stationary test. Therefore, this neuron's motion-direction sensitivity was due to an inhibition in the null direction.

The distribution of all DI values is shown in Fig. 2. Of the total number of 68 cells, some 34% had a statistically significant DI. These DI values are

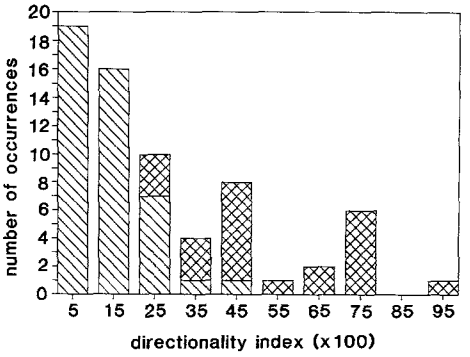


Fig. 2. Distribution of directionality indices. Of the 68 recordings, the directionality indices of the motion-direction insensitive cases are plotted by the *lightly hatched bars,* those of the motion-direction sensitive cases (chi-square test, $p < 0.05$) by the *cross-hatched bars.* Stimulus conditions for all recordings were as noted in Fig. 1. If more than one test was performed with a cell, all spikes registered in response to clockwise and all to counterclockwise stimulation were summed for the calculation of the DI value and the chi-square test. Some cells, like that shown in Fig. 1, showed onset responses. Such responses were excluded from the analysis. Bin width is 0.1

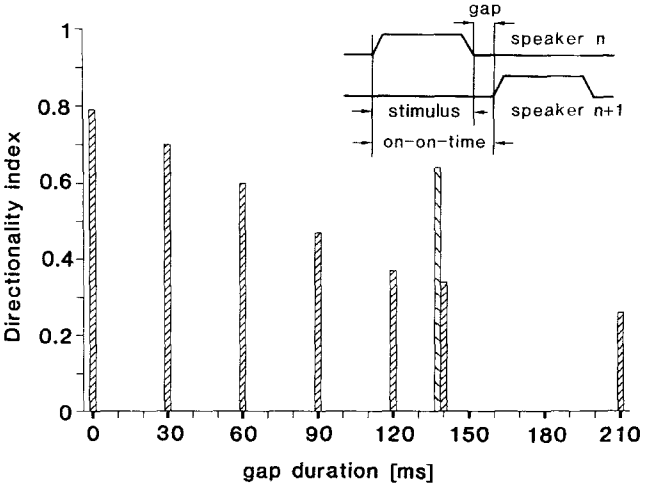


Fig. 3. Dependence of the DI value on gap duration. In the *inset* the relation between on-on time, stimulus duration and gap duration is shown. As the on-on time of 240 ms, equivalent to an apparent velocity of 125°/s, was kept constant, the DI value decreased with increasing gap duration in this neuron (*heavily hatched bars*). The *lightly hatched bar* shows the DI value for an on-on time of 95 ms and a stimulus duration of 100 ms, a parameter combination that reduced the gap to zero. Note that this bar is plotted next to the bar with the same stimulus duration but 140 ms gap duration under the 240 ms on-on time condition

scattered in the upper half of the distribution and are responsible for its long tail. To judge whether motion-direction sensitivity was due to an inhibition in the null direction, wherein the response in the null direction is less than the stationary response, or to a facilitation in the preferred direction, wherein the response in the preferred direction is greater than the stationary response, we compared the responses in the motion condition with that in the stationary condition. Inhibition in the null direction was observed in 89% of the cases. Facilitation in the preferred direction occurred in 22% of the neurons, and 11% of the neurons showed both inhibition in the null direction and facilitation in the preferred direction.

Further evidence for inhibition as a major factor in generating motion-direction sensitivity under our stimulus conditions came from experiments in which a gap was introduced between the activation of a speaker and the activation of the next speaker. To in-

troduce the gap, apparent velocity was kept constant while stimulus duration was decreased. As the gap increased, motion-direction sensitivity decreased (Fig. 3). In contrast, the response level was independent of gap duration (not shown). The smooth decrease of DI with gap duration seen in Fig. 3 could not be explained by a decrease in the sound energy that resulted from the decrease of stimulus duration, because for a stimulus duration of 100 ms, a much higher DI value resulted for a zero gap than for a gap of 140 ms (Fig. 3, lightly hatched bar). Seventeen of the 23 motion-direction sensitive cells were stimulated with more than one combination of on-on time and gap duration. For zero gap duration and upon variation of the apparent velocity, the DI varied only slightly. The mean DI changed from 0.50 for 125°/s ($N=11$) and 0.53 for 310°/s ($N=23$) to 0.41 for 1000°/s ($N=10$). If, however, in the case of 125°/s a gap was introduced so as to generate the same stimulus duration as in the 1000°/s case, the mean DI was only 0.25 ($N=11$).

Our results demonstrate that a large proportion of neurons in the midbrain of the barn owl is sensitive to the direction of apparent acoustic motion in the horizontal plane. Our sample may underestimate the actual number of motion-direction sensitive neurons because we only assessed motion along the horizontal axis, and, because our dichotic search stimulus simulated a stationary source.

Earlier neurophysiological studies of mammals have also demonstrated motion-direction sensitivity in auditory neurons by a variety of techniques [16 – 19]. The earlier studies relied on the inspection of neuronal spike trains and PST histograms rather than on a statistical analysis to judge the motion-direction sensitivities. In these studies motion-direction sensitive neurons were not as common as in the owl's midbrain, independent of stimuli used, e.g., dichotic clicks [16], binaural beats [17] or apparent motion [18].

Although most of our motion-direction sensitive cells were found in the optic tectum, it is of interest to note that some of the cells in the ICc-1s were motion-direction sensitive. Unlike the optic tectum or the ICx, the ICc-1s is composed of cells that are sharply tuned in frequency and have multiple spatial receptive fields [11, 13]. Cells in the tectum and ICx have a single spatial receptive field, which is a consequence of having a broad frequency selectivity [11, 13 – 15]. Not only does this suggest that motion-direction sensitivity can precede the formation of space-specific cells that have unique spatial receptive fields, it also implies that the motion-direction sensitive cells have the ability to preserve the spectrum of the moving sound. Such capability, we speculate, may signal to higher centers that a particular group of frequencies moved in unison, and can therefore be treated as having a common origin.

Many neurons responded to stationary as well as to moving stimuli. Therefore, these neurons cannot signal whether or not a source is moving without further neural computation. We have shown that inhibition is a major factor in generating the acoustic motion-direction sensitivity in the barn owl. In some 22 % of the cells facilitation in the preferred direction was also found. These data are in accord with models of motion detectors that require a nonlinear, notably inhibitory interaction between two inputs as proposed for visual motion detectors [1]. They do not, however, exclude the possibility of excitatory interactions in detection of acoustic motion. More experiments, with various sound levels, different acoustic characteristics (e.g., tones, clicks), and various spatial arrangements of the sound sources are necessary to analyze the mechanisms underlying the detection of acoustic motion and to determine whether they closely resemble visual motion detection mechanisms [1 – 4] in their implementation, or, whether acoustic-motion detection mechanisms are different.

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1. Barlow, H. B., Levick, W. R.: J. Physiol. Lond. *178,* 477 (1965)
2. Dubner, R., Zeki, S.: Brain Res. *35,* 528 (1971)
3. Mikami, A., Newsome, W. T., Wurtz, R. H.: J. Neurophysiol. *55,* 1308 (1986)
4. Borst, A., Egelhaaf, M.: TINS *12,* 297 (1989)
5. Perrott, D. R., Musicant, A. D.: J. Acoust. Soc. Am. *62,* 1463 (1977)
6. Grantham, D. W.: ibid. *79,* 1939 (1986)
7. Waugh, W., Strybel, T. Z., Perrott, D. R.: J. Audit. Res. *19,* 103 (1979)
8. Konishi, M., Takahashi, T. T., Wagner, H., Sullivan, W. E., Carr, C. E., in: Auditory Function: Neurobiological Bases of Hearing, p. 721 (eds. Edelman, G. E., Gall, W. E., Cowan, W. M.). New York: Wiley 1988
9. Takahashi, T. T.: J. Exp. Biol. *146,* 307 (1989)
10. Payne, R.: ibid. *54,* 535 (1971)
11. Wagner, H., Takahashi, T. T., Konishi, M.: J. Neurosci. *7,* 3105 (1987)
12. Knudsen, E. I., Knudsen, P.: J. Comp. Neurol. *218,* 187 (1983)
13. Knudsen, E. I., Konishi, M.: J. Neurophysiol. *41,* 870 (1978)
14. Knudsen, E. I.: ibid. *52,* 709 (1984)
15. Takahashi, T. T., Konishi, M.: J. Neurosci. *6,* 3413 (1986)
16. Bechterev, N. N., Syka, J., Altman, J. A.: Experientia *31,* 819 (1975)
17. Yin, T. C. T., Kuwada, S.: J. Neurophysiol. *50,* 1000 (1983)
18. Rauschecker, J. P., Harris, L. R.: Brain Res. *490,* 56 (1989)
19. Schlegel, P. A., in: Animal Sonar Systems, p. 973 (eds. Busnel, G., Fish, J. J.). New York: Plenum Press 1980

 