Exp Brain Res (2001) 139:106–110
DOI 10.1007/s002210100721

RESEARCH NOTE

Elizabeth F. Wells · Geula M. Bernstein
Brian W. Scott · Patrick J. Bennett
Julie R. Mendelson

Critical flicker frequency responses in visual cortex

Received: 22 November 1999 / Accepted: 27 February 2001 / Published online: 12 May 2001

Abstract Critical flicker frequency (CFF) threshold is defined as the frequency at which a flickering light is indistinguishable from a steady, non-flickering light. CFF is useful for assessing the temporal characteristics of the visual system. While CFF responses are believed to reflect activity in the central visual system, little is known about how these temporal frequencies are processed in the visual cortex. The current paper estimated the CFF threshold for cells in the rat visual cortex by recording single unit responses to flickering stimuli. Results showed that: (1) there was a broad range of temporal tuning, (2) CFF threshold was lower in simple cells than in complex and hypercomplex cells, and (3) there was no significant difference in CFF threshold between areas 17 and 18.

Keywords Critical flicker frequency · Temporal processing · Visual cortex

The ability to process temporally varying visual stimuli is important for the perception of moving targets. One method of investigating the visual system's temporal processing properties is to examine the response to a flickering light stimulus. Critical flicker frequency (CFF) threshold is the lowest frequency of flickering light (measured in cycles/s or Hz) that is required to produce an appearance of steady light to an observer. When a light is flickered at rates equal to (or greater than) the CFF threshold, the individual flashes cannot be resolved and the light is indistinguishable from a steady, non-flickering light. At flicker rates below the CFF threshold, each flash can be resolved and flickering lights can be discriminated from steady lights. Behaviourally derived CFF thresholds are approximately 70 Hz in humans (Coile et al. 1989), 65–70 Hz in cats (Schwartz and Cheney 1966; Taravella and Clark 1963), and 30–39 Hz in rats (Williams et al. 1985), though it should be mentioned that CFF thresholds can be affected by such parameters as luminance of the flashing stimuli (Dodt and Wirth 1953; Loop et al. 1980) and anaesthetic (Simonson and Brozek 1952).

Although behaviourally measured CFF thresholds were originally thought to rely completely on retinal function, research has since shown the importance of the central visual system (Simonson and Brozek 1952; van de Grind et al. 1973). Physiological recordings (e.g., EEG and extracellular) have indicated that CFF thresholds are significantly higher in retinal than in cortical cells (Walker et al. 1943; van de Grind et al. 1973). Kimura (1980) showed that the CFF thresholds of extracellularly recorded cells in cat striate cortex differ with cell type such that simple cells have higher CFF thresholds than complex cells. Recently, Rager and Singer (1998) found that multiunit and local field potential recordings in cat visual cortex were responsive to a broad range of CFFs consistent with the cat's behaviour. While it is apparent that the visual cortex is involved in processing CFF, it is unclear how this is accomplished. Thus, one goal of the present study was to investigate the nature of cortical CFF processing.

A secondary goal of this study was to further our understanding of the rat's visual system. The rat is used in many types of experiments, such as aging (due to its relatively short lifespan), as well as in the development of paradigms and techniques for developmental studies, pathologies, etc. Therefore understanding how its brain and, in particular, its visual system process visual information is a worthwhile endeavour. Even though the rat is

E.F. Wells · J.R. Mendelson (✉)
Department of Speech-Language Pathology, Faculty of Medicine, University of Toronto, Tanz Neuroscience Building, 6 Queen's Park Crescent West, Toronto, Ontario M5S 3H2, Canada
e-mail: j.mendelson@utoronto.ca
Tel.: +1-416-9781574, Fax: +1-416-9781596

P.J. Bennett
Department of Psychology, University of Toronto, Toronto, Ontario, M5A 1A8, Canada

G.M. Bernstein · B.W. Scott · J.R. Mendelson
Institute of Medical Sciences, University of Toronto, Toronto, Ontario, M5A 1A8, Canada

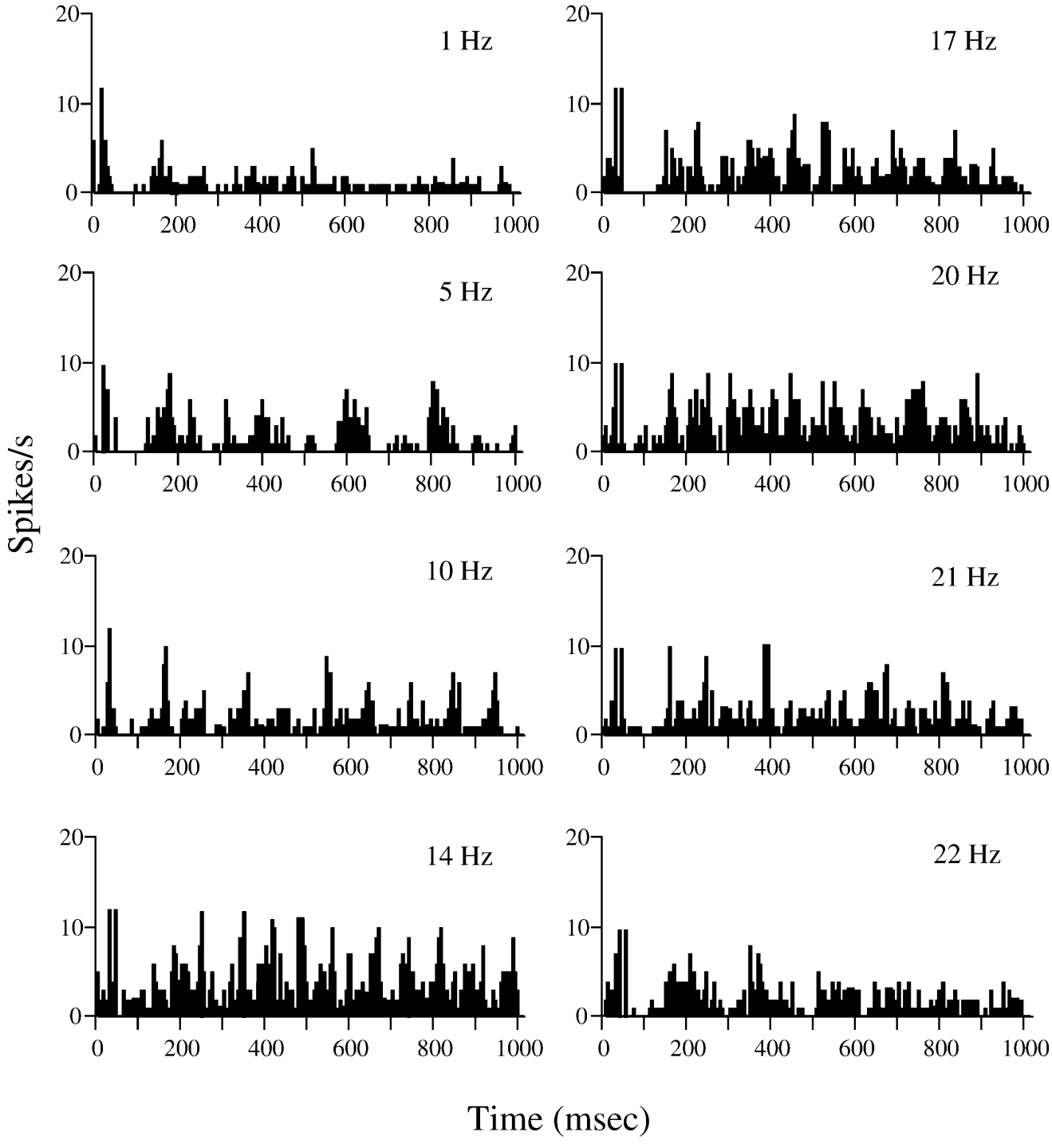

Fig. 1 A A series of peristimulus time histograms indicating the response of a complex cell to increasing rates of flicker (expressed in hertz). Time of stimulus presentation (1000 ms), divided into 200 bins of 5 ms, is displayed on the *x*-axis. *Dashes below the histograms* indicate the timing of the flashes. This cell (CFF = 21 Hz) was able to entrain to frequencies up to 20 Hz while at 22 Hz it responded primarily to the onset of stimulation. **B** The FFT of the response of the cell illustrated in **A**. Two curve-fitting procedures were applied to each transform: a linear regression (*solid line*) and an exponential fit (*dashed line*). The linear regression of the power at the stimulus fundamental frequency confirmed that the CFF threshold of this cell was 21 Hz

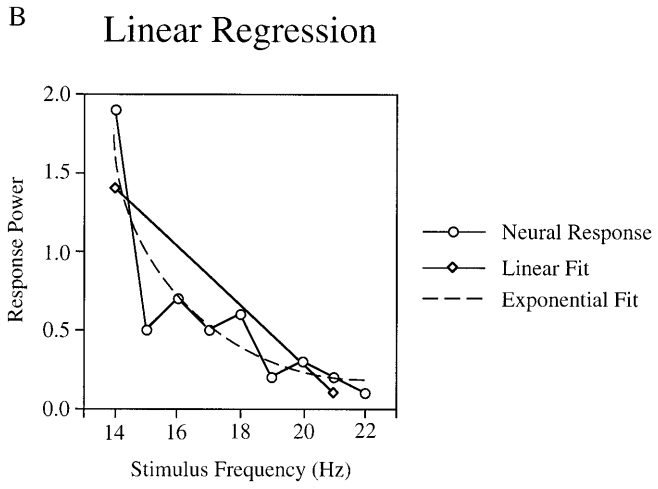

Fig. 1 B

a nocturnal animal, it still relies on visual information. Yet, despite its usefulness, relatively little is known about its visual cortex. Studies have shown that functional properties of cortical cells are tuned for orientation and velocity, respond to sine wave gratings (Burne et al. 1984; Girman et al. 1999) and can be grouped into simple, complex and hypercomplex categories (Parnavales et al. 1981). However, relatively little is known about temporal processing in the rat visual cortex, especially how CFF is processed. Further, while much is known about the relationship between function and anatomy in the rat retina and lateral geniculate nucleus (LGN), little is known about this relationship in its visual cortex (Perry 1979; Lennie and Perry 1981). Thus, it is of interest to determine whether CFFs are processed differently by primary and secondary visual cortex and/or by cell type, i.e., simple, complex, and hypercomplex (Hubel and Wiesel 1962, 1968). To this end, we examined the responses of neurons in rat visual cortical areas 17 and 18 to flicker stimuli in order to gain further insights into CFF processing as well as that of the rat visual system.

Twenty-eight male Long Evans hooded rats weighing between 180 and 400 g were used. The surgical procedures employed were approved by the Canadian Council for Animal Care (CCAC) and comply with the stipulations regarding the care and use of experimental animals set out by the American Physiological Association and Experimental Brain Research. Subjects were anaesthetized with Equithesan (3 mg/kg, i.p.) and administered 0.1 ml atropine sulphate subcutaneously to prevent respiratory distress. Glycerin-based eye drops (Isoptotears) were administered periodically to prevent the corneas from drying out. A constant infusion of Equithesan (1 mg/kg/h i.p.) in lactated Ringer's solution was administered throughout the course of the experiment to ensure a proper level of anaesthesia and hydration. All wound margins and pressure points were infiltrated with the long-lived local anaesthetic bupivacaine hydrochloride (2.5%). A craniotomy was performed over the left occipital cortex using the stereotaxic coordinates of Paxinos and Watson (1986).

Whole-field CFF stimulation was provided by light pulses (10 μs) delivered by a Grass PS33 PLUS stimulator lamp. The lamp was located outside a window of an electrically shielded, sound-attenuating chamber (IAC), and positioned 40 cm from the animal's eye. The intensity of the flash corresponded to a luminance value of 17 cd/m^2.

A Macintosh Quadra 950 computer with A/Dvance software was used to generate bars of light that could be varied in size, direction, orientation and speed. Whole-field stimuli were projected onto a dark screen placed at a distance of 30 cm from the animal's contralateral eye which maximizes the rat's optical viewing ability (Weisenfeld and Kornel 1975; Parnavales et al. 1983). All recordings were made in a completely darkened room.

CFF threshold was assessed by pseudo-random presentation of flicker frequencies between 1 and 60 Hz. Data were collected during 20 presentations of each frequency with an interstimulus interval of 2–4 s, depending on the response rate of the cell. The cell was said to entrain to the stimulus when it was able to respond to each individual flash.

Cells were identified as either simple, complex, or hypercomplex based on criteria previously established by Hubel and Wiesel (1962, 1968) and Parnavales et al. (1983). CFF threshold was determined by performing fast Fourier transforms (FFTs) on each histogram and computing the power at the fundamental frequency for each stimulus. This procedure has been widely used in analysing these types of data (Eggermont 1991; Gur and Snodderly 1996; Rager and Singer 1998). For comparative purposes, and to account for any possible changes in CFF threshold with the increase in luminance associated with higher flicker rates, the normalized power (power at the fundamental frequency divided by the average response rate) was computed. Two curve-fitting procedures were applied to each transform. With linear regression, CFF threshold was defined as the frequency at which the corresponding regression line crossed the frequency axis. For the exponential curve, CFF threshold was defined as the frequency yielding a response that was 20% of the peak response. Because many of the units studied exhibited an exponential decay (see Fig. 1b), fitting a linear function to the responses may underestimate the true CFF threshold for a single unit. However, when alternative curve-fitting procedures were applied to the data, the results were similar due to the fact that the different procedures were highly correlated. Thus, by using linear interpolation, CFF threshold is reported as the frequency at which the power of the fundamental went to zero.

Reference lesions (6 μA for 6 s) were made at each recording site for histological verification. At the end of each experiment, subjects were deeply anaesthetized, perfused transcardially, and the brains processed for cresyl violet staining.

Extracellular recordings made with platinum-iridium microelectrodes were obtained from 68 neurons in rat visual cortex, with 30 recorded in area 17 and 38 in area

18. The range of CFF thresholds recorded was between 10 and 35 Hz, with a mean of 21.54±6.0 Hz.

Figure 1a illustrates a response of a complex cell. Visual inspection of the histogram revealed that the cell was able to entrain to frequencies up to 20 Hz. FFT analysis established the CFF as 21 Hz (Fig. 1b).

Stimuli presented at or above the CFF threshold caused neurons to respond in one of four ways: (1) a response to the onset of the first stimulus flash only (n=46); (2) a response to the onset of the first flash and the offset of the last flash (n=2); (3) a response to the offset of the last flash only (n=3); and (4) randomly throughout the entire stimulus presentation (n=17). Chi-square analysis indicated that the type of response was not equally distributed (χ^2 3 $d.f.$=74.235, P<0.001).

The mean CFF threshold for simple cells (15±1.15) was significantly lower than that for either complex (24.68±4.59; t=8.214, P<0.001) or hypercomplex cells (29±4.24; t=4.583, P<0.05). The difference in mean CFF threshold between complex and hypercomplex cells was not significant. In addition, no significant difference in the mean CFF threshold was observed between area 17 (20.42±4.84 Hz) and area 18 (21.33±6.32 Hz). However, the differences in the mean CFF threshold found between simple, complex and hypercomplex units were maintained in both cortical areas.

The results of the present study showed that CFF thresholds for individual cells in the rat visual cortex vary across a broad range of frequencies. While the mean cortical CFF threshold is somewhat lower than that derived behaviourally, the range of CFFs observed physiologically appears to be in accordance with those obtained behaviourally (Williams et al. 1985). This is consistent with what Kimura (1980) and Rager and Singer (1998) have reported in the rat.

The results also showed that responsiveness of cells in rat visual cortex to CFFs is a function of cell type. The present study found that simple cells had lower CFF thresholds than either complex or hypercomplex cells. This difference is consistent with previous studies that measured velocity tuning properties in cortical cells. Given that high temporal frequencies are an inherent feature of rapidly moving stimuli, and given that it is well established in both rat and cat that complex and hypercomplex cells prefer faster velocities than simple cells (Parnavales et al. 1981), one would expect lower CFFs for simple cells and higher ones for complex and hypercomplex cells. In contrast, as mentioned above, Kimura (1980) found that in cat striate cortex, simple cells responded to higher CFFs than did complex cells. It is unclear what may account for the apparent discrepancy between the present results and those of Kimura (1980).

The issue of how CFFs are encoded in the rat visual cortex remains unclear. Fukada and Saito (1971) have shown in the cat optic nerve that X- and Y-type cells appear to respond differently to flashing light stimuli. Rager and Singer (1998) have suggested that in cat visual cortex these LGN afferents may play a role in encoding cortical responses to flicker stimuli. While Perry and others have shown that X-, Y-, and W-like cells exist in the rat retina and LGN (e.g., Perry 1979; Lennie and Perry 1981), to our knowledge, their cortical afferents have not been reported. Unfortunately, our study did not allow for a quantitative analysis of how the retinogeniculocortical afferents may encode CFFs.

While CFF processing appears to be related to receptive field type (i.e., simple, complex, hypercomplex), it does not seem to map onto anatomical subdivisions within the rat visual cortex. These results appear to be consistent with those of Rager and Singer (1998), who compared the response of clusters of cells in areas 17 and 18 in the cat visual cortex and found no difference in the ability of the cells to synchronize their firing with temporally structured stimuli.

In conclusion, this study provides additional information on information processing in the rat's visual system. In particular, it showed that CFF appears to be treated in a similar way in both areas 17 and 18.

Acknowledgements This research was supported by NSERC Grant WFA0123096. We thank R. English for his assistance with this project.

References

Burne RA, Parnavelas JG, Lin C-S (1984) Response properties of neurons in the visual cortex of the rat. Exp Brain Res 53: 374–383

Coile DC, Pollitz CH, Smith JC (1989) Behavioral determination of critical flicker fusion in dogs. Physiol Behav 45:1087–1092

Creel D, Beck EC (1974) Intensity of flash illumination and the visually evoked potential of rats, guinea pigs and cats. Vis Res 14:725–729

Dodt E, Waldenstein L (1954) The use of flicker electroretinography in the human eye. Acta Ophthalmol 32:165–180

Dodt E, Wirth A (1953) Differentiation between rods and cones by flicker electroencephalography in pigeon and guinea pig. Acta Physiol Scand 30:80–89

Eggermont JJ (1991) Rate and synchronization measures of periodicity coding in cat primary auditory cortex. Hear Res 56:153–167

Fukada Y, Saito H-A (1971) The relationship between response characteristics to flicker stimulation and receptive field organization in the cat's optic nerve fibers. Vis Res 11:227–240

Girman SV, Sauve Y, Lund RD (1999) Receptive field properties of single neurons in rat primary visual cortex. J Neurophysiol 82:301–311

Gur M, Snodderly DM (1997) A dissociation between brain activity and perception: Chromatically opponent cortical neurons signal chromatic flicker that is not perceived. Vision Res 37: 377–382

Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J Physiol 160:106–154

Hubel DH, Wiesel TN (1968) Receptive fields and functional architecture of monkey striate cortex. J Physiol 195:215–243

Kimura M (1980) Neuronal responses of cat's striate cortex to flicker light stimulation. Brain Res 192:560–563

Lennie P, Perry VH (1981) Spatial contrast sensitivity of cells in the lateral geniculate nucleus of the rat. J Physiol (Lond) 315:69–79

Loop MS, Petuchowski S, Smith DC (1980) Critical flicker fusion in normal and binocularly deprived cats. Vision Res 20:49–57

Parnavelas JG, Burne RA, Lin C (1981) Receptive field properties on neurons in the visual cortex of the rat. Neurosci Lett 27:291–296

Parnavelas JG, Burne RA, Lin C (1983) Distribution and morphology of functionally identified neurons in the visual cortex of the rat. Brain Res 261:21–29

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, San Diego

Perry VH (1979) The ganglion cell layer of the rat: a Golgi study. Proc R Soc Lond [Biol] 204:363–375

Rager G, Singer W (1998) The response of cat visual cortex to flicker stimuli of variable frequency. Eur J Neurosci 10:1856–1877

Schwartz AS, Cheney C (1966) Neural mechanisms involved in the critical flicker frequency of the cat. Brain Res 1:369–380

Simonson E, Brozek J (1952) Flicker fusion frequency: background and applications. Physiol Rev 32:349–378

Taravella CL, Clark G (1963) Discrimination of intermittent photic stimulation in normal and brain-damaged cats. Exp Neurol 7:282–293

van de Grind WA, Grusser OJ, Lunkenheimer H-U (1973) Temporal transfer properties of the afferent visual system. Psychological, neurophysiological and theoretical investigations. In: Jung R (ed) Handbook of sensory physiology, vol VII/3. Springer-Verlag, Berlin, pp 432–573

Walker AE, Woolf JI, Halstead WC, Case TJ (1943) Mechanism of temporal fusion effect of photic stimulation on electrical activity of visual structures. J Neurophysiol 6:213–219

Wiesenfeld Z, Kornel EE (1975) Receptive fields of single cells in the visual cortex of the hooded rat. Brain Res 94:401–412

Williams RA, Pollitz CH, Smith JC, Williams TP (1985) Flicker detection in the albino rat following light-induced retinal damage. Physiol Behav 34:259–266