Supplementary Materials and Methods

Animal preparation
In the present study we used GAD67-GFP (Δneo) mice. The previous immunohistochemical study of this species of animals reported that almost all the GFP-positive cells were GAD67-positive and there was no ectopic GFP expression in apparently GAD67-negative cells or any obvious lack of GFP expression in apparently GAD67-positive neurons (Tamamaki et al., 2003). Also we previously reported that GFP-positive neurons in this species of animals have no apparent abnormal electrophysiological properties, although the analysis was not systematic (Jiang et al., 2004).

Multicell bolus dye loading and in vivo two-photon microscopy
The pressure applied to the pipettes for dye loading was 1-10 psi, which lasted for 60-120 s (Picospritzer III, General Valve, Fairfield, NJ). After confirming that the dyes were spread in the cortex, the pipettes were withdrawn and the hole in the skull was sealed with agarose and a 0.08-0.12 mm-thick square (5 x 5 mm) cover glass (Matsunami Glass, Osaka, Japan). In the two-photon laser scanning system, laser power was constantly monitored with a power meter (Model 407A, Spectra-physics). To minimize the reduction in the two-photon excitation efficacy the group velocity dispersion of the optical materials in the 800 nm beam path was compensated by the chirp compensation (Femto Control, APE, Berlin, Germany).

Visual stimulation and data acquisition
Drifting square wave gratings (0.05 cycles/degree) were presented on a 19-inch LCD monitor in eight directions (from 0° to 315° in 45° steps) at a velocity of 9.0°/s. The gratings consisted of white and black bars, and the widths of white and black bars were 4° and 16°, respectively. The luminance of white and black bars was 6-20 and 0.01-1 cd/m², respectively. The eyes of the animals were located 26 cm from the surface of the LCD monitor. The response magnitude during the stimulus period for 5 s was obtained by averaging the intensity of fura-2 signals of 14-15 frames. This number of frames was in the same range as that during the prestimulus period, although the number was not always exactly the same because of a slight fluctuation of scanning time per frame.
Supplementary Discussion

The proportion of visually responsive neurons

In the present study, 34% of the non-GABAergic and 40% of GABAergic neurons were visually responsive. As discussed in the main body of text, such a low proportion of visually responsive cells may be ascribable to the application of visual stimulation with a fixed set of parameters (e.g., frequency and contrast of gratings, and velocity of movements) except for orientation and movement direction to a population of neurons that may have different optimal responses to various parameters of visual stimuli. There is another possibility that urethane anesthesia used in the present study reduces the number of visually responsive neurons. Regarding this possibility, we would like to point out that the previous studies using the same anesthetic at about the same dose as in the present study reported vigorous visual responses of neurons in the mouse visual cortex (Mangini and Pearlman, 1980; Metin et al., 1988). Thus, this possibility seems low, although it cannot completely be excluded.

In Figure 3A and B, some GFP-positive cells appear to be clustered. To examine the possibility that the same type of cells is clustered, a thorough and quantitative analysis of distance between the same or different group of cells is necessary. In the present study, thus, we cannot make any conclusive statement regarding such a possibility.

Possibility of saturation of peak responses in GABAergic neurons

There is the possibility that the peaks of the optimal stimulation-induced changes in the fluorescence intensity of GFP-positive cells might be saturated so that orientation selectivity is masked, because a group of GABAergic neurons (fast-spiking cells) in the visual cortex of awake rabbits and Halothane-anesthetized cats generate action potentials at high rates in response to visual stimulation, and the fluorescence intensity of Ca$^{2+}$ indicators such as fura-2 can be saturated. We believe that this possibility is unlikely on the following grounds: 1) As shown in Supplementary Figure 2A, the peak fluorescence intensity of responses of GFP-positive neurons was almost the same as that of GFP-negative neurons. If saturation had occurred only in the GFP-positive neurons, their peak values should have been larger than those of the GFP-negative neurons. 2) As seen in the right column of Supplementary Figure 1, the peaks of the maximal responses of GFP-positive cells were very sharp. If saturation had occurred, the peaks should have been dull or nearly flat.
**The slower rising slope of GABAergic neurons**

In the present study we also found that the rising slope of the optimal visual responses of GFP-positive neurons was significantly slower than that of GFP-negative neurons. This may reflect the slower kinetics of Ca\(^{2+}\) transients in inhibitory neurons than excitatory neurons, as reported in cultured hippocampal neurons (Lee et al., 2000). There is another possibility that the expression of GFP *per se* may change kinetics of Ca\(^{2+}\) indicator responses so as to slow the rising slope of responses. Although we cannot completely exclude this possibility in the present study, such a possibility seems unlikely for the following reason: In a previous study (Jiang et al., 2004), we found that electrophysiological properties of GFP-positive GABAergic neurons were not obviously different from the normal properties. Also stated in previous studies using the same species of transgenic mice as in the present study (e.g., Uusisaari et al., 2007), there is no reason to think that neurons expressing GFP have abnormal cellular properties.

**Sources of non-oriented responses of GABAergic neurons**

We have found that most of GABAergic neurons are not orientation-sensitive. There are two possible explanations for such an orientation-insensitive response; 1) As reported previously, most neurons in layer IV of the visual cortex of the mouse are not orientation-sensitive (Mangini and Pearlman, 1980; Metin et al., 1988). So, if GABAergic neurons receive main inputs from these layer IV neurons, visual responses of GABAergic neurons become orientation-insensitive. 2) If GABAergic neurons receive diverse inputs from neighboring neurons which have different preferred orientations, GABAergic neurons become orientation-insensitive. It was reported that visual cortical neurons with the same or similar orientation preference are clustered in the cat, but not so in the rat (Ohki et al., 2005). So, the second explanation may be applicable only to the visual cortex of rodents. In the present study we cannot conclusively distinguish these two possibilities.

**References**


properties between excitatory and inhibitory hippocampal neurons from the rat. J Physiol 525:405-418.


**Legends for Supplementary Figures**

Supplementary Figure 1. Examples of visual responses of GFP-negative (left column) and GFP-positive (right column) neurons to eight different patterns of visual stimuli, as shown on top. Each trace represents averages of 5 sweeps. Calibration bars at bottom, right indicate 10% (-ΔF/F₀) and 10 s, and apply to all of the traces. The value of orientation selectivity index (OSI) is given at the right of each trace. Cells 1-4 were obtained from the focal plane of 160 µm depth of the cortex of a mouse, cells 5-8 were from the plane of 140 µm depth of the cortex of another mouse, and cells 9, 10, 11 and 12 were from the planes of 150, 150, 130 and 140 µm depth, respectively, of the cortex of a third mouse.

Supplementary Figure 2. Distributions of peak fluorescence intensity (A) and rising slope (B) of optimal visual responses obtained from the averaged traces of GFP-negative and -positive neurons, respectively. The rising slope was calculated using the value for 10-90% of the initial rising phase of the averaged traces of the optimal responses, using Mini Analysis Program (Synaptosoft Inc., Fort Lee, NJ). The mean values of the peak fluorescence intensity for 181 GFP-negative neurons and 28 GFP-positive neurons are 14.0 ± 6.3 (SD) and 14.2 ± 7.0%, respectively. The mean values of the rising slope for the GFP-negative and -positive neurons are 9.2 ± 7.3 and 5.8 ± 3.7 %/s, respectively. At bottom are shown cumulative distributions of the peak fluorescence intensity (A) and the rising slope (B) of the optimal responses of the GFP-negative (black) and -positive (green) neurons. The distributions of the rising slope of the two types of neuron were significantly different (Kolmogorov-Smirnov test, P < 0.01), while those of the peak fluorescence intensity were not (P = 0.57).