Adaptive Changes in Firing of Primary Auditory Cortical Neurons following Illumination Shift from Light to Dark in Freely Moving Guinea Pigs

Some animals are forced to rely more on non-visual signals, such as audition or olfaction, than on vision when a bright environment becomes dark. By recording from a primary-like auditory cortex (field A) in freely moving guinea pigs, possible changes in the responsiveness of single units were explored in association with illumination changes. For a subset of units, we found that robust decreases (off-decrease) or increases (off-increase) in baseline discharge (BsD) were initiated soon after room light was silently extinguished. These neuronal changes were accompanied by the initiation of explorative locomotion, possibly reflecting a changed internal brain state. Preferred acoustic stimuli evoked salient excitatory responses against the reduced BsD level in the dark for the off-decrease units, and salient inhibitory responses against the increased BsD level for the off-increase units. Histological verification indicated that the units showing such BsD changes were located predominantly in layer V or its vicinity. These results are discussed in the context of the effects of the brainstem neuromodulatory systems that are activated during behavioral adaptation to new environments.

Keywords: baseline discharge, free moving, laminar specificity, light and dark, natural sounds

Introduction

In natural situations, sensory signals are frequently associated with the initiation of context-dependent behavior (Manning and Dawkins 1998). Animals, including humans, are urged in certain situations to rely on signals of only one modality or signals devoid of some modalities. Examples are found not only in the impairment of particular sensory pathways due to accidents, but also with the abrupt onset of darkness. For experimental animals, the daily light–dark shift in home cage illumination can be one of the most common environmental changes that animals experience.

Although primary sensory cortices have been traditionally considered modality-specific, it is becoming evident that visual stimuli modify response magnitude in the auditory cortex (Laurienti et al. 2003; Ito et al. 2004; Lehmann et al. 2006). In fact, even somatosensory stimuli can modulate auditory activity (Foxe et al. 2000, 2002). In animals and humans, brightness detected by the visual system contributes to the formation of images, and is also thought to generate nonimage responses outside the visual system. Apart from the circadian rhythm (Goff and Finger 1966), a shift in ambient light intensity between light and dark appears to modulate motor activities (Borbély 1976), arousal (Borbély et al. 1975; Badia et al. 1991; Miller et al. 1998), attention (Eimer et al. 2003; Perrin et al. 2004), and fear/anxiety (Grillon et al. 1997). In experimental situations, darkness facilitates the acoustic startle reflex in humans, as well as behavioral expression of negative emotional states induced by acoustic stimuli (Grillon et al. 1997, 1998). In comparison, rats, a nocturnal animal species, demonstrate an increased acoustic startle reflex when they are placed in a bright light environment (Walker and Davis 1997a; Ison et al. 1991). These responses related to changing levels of illumination do not require the animals to be behaviorally conditioned (Walker and Davis 1997b). Furthermore, experimental results have shown that light deprivation accelerates recovery from inattention in rats that have undergone ablation of the medial agranular cortex (Crowne et al. 1983; Vargo et al. 1998). Similar effects of darkness on human patients with unilateral visual neglect have also been reported (Hjaltason and Tegnér 1992). At sufficient light intensities, acute changes in brightness have been shown to exert significant effects on rest–activity patterns in rats, primates and humans (Lisk and Sawyer 1966; Borbély et al. 1975; Borbély 1976). Thus, nonimage forming responses induced by shifts in illumination level are very likely to affect the internal brain states.

Behaviors of domestic guinea pigs are relatively stereotyped (Harper 1976) and their activity in the wild is governed by temperature and sunlight intensity, showing a crepuscular activity rhythm (Rood 1972). They are most active at low ambient light levels around dawn and dusk. However, activity–sleep rhythms in laboratory situations show polyphasicism (Escudero and Vidal 1996) which is characterized by a lack of predominance of either a diurnal or nocturnal activity period, and multiple cycles of alternating activity–sleep epochs during a day. In the food reward test, guinea pigs were shown to be able to discriminate among different levels of brightness (Miles et al. 1956). These behavioral observations may raise the possibility that guinea pigs' behavior can be artificially modulated by changes in ambient illumination level.

According to recent views of the multimodal processing potentiality of the primary auditory cortex (AI) (Falchier et al. 2002; Rockland and Ojima 2003; Werner-Reiss et al. 2003; Fu et al. 2004; Brosch et al. 2005; Budinger et al. 2006; de la Mothe et al. 2006; Bizley et al. 2007; Lakatos et al. 2007; Martuzzi et al. 2007; Kayser et al. 2008), the sequential activation of different senses following a change in illumination, starting with the visual activation and followed by motion-associated somatosensory/motor activities, might affect acoustic signal processing in the AI in a naturalistic situation. In particular, we wondered whether the responsiveness of AI neurons was changed in an altered environment where nonacoustic senses became unavailable or less helpful. By recording single unit activities from freely moving guinea pigs, we show that the baseline discharge (BsD) of a subset of AI units abruptly shifts

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to a robustly decreased or increased level soon after light offset. These neuronal changes appear to occur in synchrony with covert changes in certain internal brain state reflected by altered animal behaviors.

**Materials and Methods**

**Animals**
The care and use of animals in this study was approved (no. 0070119) by the local animal committee of the Tokyo Medical and Dental University, and conforms to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. We also made all efforts to minimize the number of animals used and their suffering.

Guinea pigs (Hartley, *Cavia porcellus*, both sexes, body weight 600–800 g) were housed in their home cages for at least 1 month before experiment. Room light was automatically turned on and off at 6 AM and 6 PM, respectively. Illuminance inside the cages during the on-period was 46 lux. Animals could gain free access to pellets and water throughout the day.

**Surgery**
Animals were anesthetized with a mixture of xylazine (5 mg/kg) and ketamine (50 mg/kg). Under aseptic conditions, the skull was exposed and cleaned of attached soft tissue. Five small holes were opened: 1 for a ground pin of an amplifier socket and 4 for small anchoring screws. Two implant electrodes (5–10 M ohm, A-M systems), 2 additional larger holes were opened on the left side. For differential amplification, one electrode, positioned at the cortical surface 2–3 mm posterior from the anterior pole of the temporal bone and 1.0–1.5 mm lateral from the pseudosylvian sulcus (field A of Redies et al. 1989 or AI of Wallace et al. 2000, see Fig. 1A), was attached firmly to a screw-driven micromanipulator (MO-09; Narishige, Japan) that had been fixed to the skull with dental resin. This electrode was used for recording. The other electrode, positioned 1 mm dorsoposterior to the recording electrode and attached to another manipulator (SM-11; Narishige), was lowered to a point where injury activities were no longer detected (i.e., within white matter), while multiple-unit discharges were continuously monitored. The second electrode was then firmly fixed to the skull with dental resin. This electrode was used as the reference. An amplifier socket with a silver-coated ground pin was mounted on the skull top. This allowed us to attach and detach a head amplifier (JH-110J; Nihon, Kohden, Japan) repeatedly. The stage was connected to the electrodes with fine cables using conductive glue (Silver Print, Toronto, Canada). Electroencephalogram (EEG) was recorded epidurally via 2 silver-coated pins positioned at the vertex and posterior temporal cortex ipsilateral to the recording site. In addition, a miniature, elettetrodenso microhphone (EMIT9T, Primo, Japan; sensitivity: –6 ± 3 dB at 1 kHz, 0 dB = 1 V/Pa; frequency range, 50–20 000 Hz) was mounted on the skull to directly monitor sounds reaching the animal. Cables from the head amplifier, microphone, and EEG pins were bundled and suspended from the ceiling with thin elastic strings. After surgery, animals were injected with antibiotics (Baytril, 5 mg/kg, Bayer HealthCare, Germany) and analgesic (Stadol, 20 mg/kg, Bristol-Myers, Lawrenceville, NJ, USA), and returned to their home cages once they had started to walk.

**Electrophysiology, Illumination Shift as Stimulus, and Acoustic Stimulation**
An arena (50 × 50 × 20 cm) was lined with acoustic carpeting to minimize sound reflections and placed on an antivibration table (Visolator; Meiritsu, Japan) in a sound proof cabin (ACO, Japan). A fluorescent lamp with an ultra-high refresh rate (50 kHz, EFA15EN/13-Zj, Toshiba, Japan) was set on the ceiling 1 m above the arena floor, with an illuminance of 60 lux as measured at the arena center. Absence of switching noise was confirmed with a calibration microphone (type 7012, ACO) placed 5 cm from the lamp surface, both when the lamp was turned on and off and also while it was kept on. Two identical dynamic speakers (NS-10MFT, 75–33 000 Hz, Yamaha, Japan) were set on the ceiling 1 m apart from each other, and with the lamp in the middle between them. Before starting recording and behavioral experiments, animals were accustomed to the arena, with gradually lengthened stay over several days. Animals were allowed to move freely within this arena, including eating pellets from a glass-made container and drinking water from a metal nozzle. They were returned to home cages after daily recording sessions. Recordings from the same animal were generally made over about 20 days.

Guinea pigs have no clear circadian rhythm (Harper 1976); they exhibit repeated wake-sleep cycles during a day (Escudero and Vidal 1996). The awaking-sleep cycle did affect the firing rate and pattern of some single units in the AI (Peña et al. 1999). Therefore, the illumination change was applied while animals were behaviorally active (in motion) or in the awakened state (as determined from the EEG waveform). Illumination of the animal’s environment was controlled by switching the light off outside the sound proof cabin. This was done only when both the animal’s behavior and neuronal firing being recorded were in a stable state for at least 1 min. In addition to this
regular pattern of illumination change, some animals were subjected to a slightly modified pattern of illumination change in which brief light and dark periods were alternated repeatedly using a solid-state relay (each 0.8 s in duration, total period was 15 s). The relay timing was precisely controlled by a pulse generator (Master-8, A.M.P.I., Israel).

For isolation of single units, the recording electrode was vertically lowered by manually rotating the manipulator's screw. Advancement of the electrode by several tens of μm per day yielded only one penetration per animal in approximately 20 days. Once single units were isolated, we searched for the preferred acoustic stimuli of each unit, irrespective of whether animals were awake or asleep. All or some of the basic acoustic components such as pure tones, white noise, band-passed noises, and click (MalLab system; Krishna and Semple 2000; Ojima et al. 2005), as well as a set of digitized natural sounds (System 3, TDT) kept in a Windows platform computer, were generally applied through a dynamic speaker (1 of 2), depending on the animal’s location within the arena, or through both speakers to minimize acoustic shadow. Natural sounds including species-specific calls were collected from the environment near the animal’s home cage or in the experimental arena. Sound pressure level was calibrated at the arena center with a half-inch condenser microphone (*70*12 type, ACO). Three video images taken at different angles by in-room cameras (2 standard video cameras, WAT-204CX, Watec, Japan; 1 infrared video camera, SH-6C, WTW, Japan) and an additional image of online raster display or spike waveform (WAT-204CX) were saved together in one DVCAM video channel (DSR-45, Sony Japan) via a quad switcher (YS-Q40, Sony). Signals from the head amplifier, EEG pins, and condenser microphone were amplified (AB-610J, JB-101J, and SS-2140, respectively; Nihon) and band-passed with ranges of 500–5000 Hz, 0.5–100 Hz, and 100 to 25 000 Hz. These signals were independently saved in DVCAM audio channels; the video frame rate was 29.97 fps, and the sampling rate of each audio channel was 32 kHz at 12-bit length.

**Behavioral Observation**

Changes in behavioral pattern following ambient illumination shift were characterized in a different set of experiments using 6 guinea pigs. In each session, the animal was first transferred from its home cage to the experimental arena. After a 30-min adaptation period, the room light was alternately turned on and off, at random intervals ranging between 1 and 30 min. Observation of animal behavior was performed online by a volunteer through 3 in-room video cameras. Movements of the entire body such as walking, wobbling, and/or shaking, were considered motion-positive.

One session, consisting of approximately 15 trials of a paired offset and onset of light, was conducted per day. For most animals (5 out of 6), the behavior test was repeated 2–6 times, with a 7-day interval between the consecutive sessions (Fig. 2). To confirm light-sensitivity in some animals, their behavior was examined after fitting them with a custom-maded eyepatch that prevented light from reaching the animal's eyes. Illumination shift trials were applied both with and without attachment of the eyepatch.

**Cell Sorting**

A Spike-2 interface (Micro 1401 II, CED, UK) was used for offline analysis. Although unit discharges appeared to be well isolated, all recordings were subjected to a sorting process based on the algorithm of waveform template matching (denoted as unit discharge lane in figures) to eliminate possible contamination of mechanical artifacts generated by jaw and/or body movements.

**Changes in BsDs**

Because BsD fluctuates spontaneously and varies from unit to unit, we examined its change not only at the time of the illumination shift but also 10 s before the illumination shift for all units. Three sequential time windows, with a light offset between the second and third windows (pre-off and post-off windows, respectively; see Fig. 3), were set along a unit’s spike train. The 3 windows had an equal duration for most units (10 s, see below). Spontaneous fluctuation in BsD was evaluated as the ratio of the spike count in the pre-off window to the spike count in the preceding, first window (spontaneous index). Similarly, light-shift effect was calculated as the ratio of the spike count in the post-off window to the spike count in the pre-off window (light-evoked index). The spontaneous indices obtained from the entire population were pooled to define the range of spontaneously occurring changes in neuronal activity. Units whose light-evoked indices were outside this range were designated as robust units and their response properties were characterized in detail (Fig. 3). Some units were lost soon after light offset because of head shaking. Shorter time windows were assigned to these units, but they were never shorter than 5 s (Fig. 3B).

**Data Analysis**

The occurrence of behavioral changes following illumination shift was represented as a percentage trial and compared between the light offset and onset situations using the unpaired, 2-tailed *t*-test. To test whether the change in BsD at light-off is different from that measured...
earlier, variances of the light-evoked and spontaneous indices of the population was compared using the F-test. Changes in BsD before and after the light offset were tested using the unpaired, 2-tailed t-test. Fisher's exact probability test was used to ensure the laminar segregation of the units showing the changes in BsD. Statistics were performed using Statistical Analysis Software (SAS Institute, Cary, NC, USA).

**Relationship between Responses and Behavior**

Temporal relationships between animal behavior and stimulus/response events were determined by using video editing software (Final Cup Pro, Apple) at a temporal resolution corresponding to the video frame rate. The possibility that neuronal response changes were caused by motion-associated noises could be eliminated by monitoring sounds captured by the miniature condenser microphone (denoted as microphone input lane in figures), while synchronized video images were examined frame by frame. Spectral analysis of captured sounds was made using spectrogram software (Raven, Cornell Lab of Ornithology) when necessary. The overall frequency range of the system used for the recording and analysis was 150 Hz to 16 kHz.

**Histology**

After recording from the last unit, an electrolytic lesion (8 μA negative × 8-10 s) was made at the last recording point (Fig. 1B) and the reference site in each animal. Animals deeply anesthetized with Nembutal (50 mg/kg, Abbott) were perfused with saline followed by 0.1 M phosphate buffer solution containing 4% paraformaldehyde (Sigma). Serial coronal sections were cut at 50 μm and processed alternatively for Nissl and cytochrome oxidase staining (Wallace et al. 2000; Fig. 1B). Depth of recording points was extrapolated relative to the center of the electrolytic lesion localized on Nissl-stained sections and corrected with a shrinkage factor (factor = 0.8). The shrinkage factor was preliminarily determined in 2 brains. A pair of electrolytic lesions with a fixed distance was made in live brains, as mentioned above, and this distance was compared with the distance measured after histological processes to determine the shrinkage factor.

**Results**

**Behavioral Changes following Offset of Ambient Illumination**

In bright conditions, guinea pigs favored remaining stationary in a corner of the experimental arena irrespective of the arousal state reflected by their EEG patterns (video frames a to c in Fig. 2A). If they were awake, they occasionally oriented their head upward or laterally (see head position change in Fig. 2B). On the contrary, following the opposite direction of illumination change, they frequently became motionless (i.e., frozen) soon after light onset, and so initiated the locomotion in only 7.9% (SE, 7.5) and 9.5% (SE, 9.8) of trials during the first and second sessions, respectively, the animals elicited locomotion within 30 s following the light-off (Fig. 2B). On the contrary, following the opposite direction of illumination change, they frequently became motionless (i.e., frozen) soon after light onset, and so initiated the locomotion in only 7.9% (SE, 7.5) and 9.5% (SE, 9.8) of trials during the first and second sessions, respectively. The occurrence of locomotion was significantly different between the offset and onset situations in both the first and second sessions (P < 0.0001, unpaired, 2-tailed).

If animals were blinded with an eyepatch, they did not respond to the illumination shift at all. When they were walking, they simply continued to walk irrespective of whether the light was turned off or on; when they were motionless, they...
continued the same posture irrespective of changes in illumination status. Quantitatively, accidental occurrence of locomotion at light-off and light-on was almost equal (28% vs. 31%, respectively, \( n = 3 \)).

**Neuronal Response Changes following Light-Off and Light-On**

Single unit recordings were made from 16 unanesthetized, unrestrained guinea pigs while their behavior was continuously monitored by video cameras. Out of a total of 152 isolated units, 94 units were subject to the illumination shift. Later offline analysis of video images revealed that some units were activated by jaw movement, such as chewing or gnashing, and that the spike discharges evoked by such movements could not be distinguished from those initiated following illumination change. These units were excluded from the analysis. For the remaining population (\( n = 72 \)), distribution of their light-evoked indices was significantly different from that of their spontaneous indices (\( F \)-test, \( P < 0.0001 \), 2 tailed), suggesting that changes in BsD following the light offset were not accidental. Twenty-four out of the 72 tested units showed robust changes in BsD, with 17 units (23.6%) showing decreases in BsD (off-decrease units, Fig. 3B) and 7 units (10.0%) showing increases in BsD (off-increase units, Fig. 3B). The temporal discharge patterns of these units are summarized in Figure 3B.

**Decrease in BsD following Light-Off (Off-Decrease Units)**

Typical recording from an off-decrease unit in the middle layer of the guinea pig AI is shown in Fig. 4 (also see the movie in Supplementary Material 1). The unit retained a relatively high BsD level (7.5 spikes/s) in the light (the nonshaded part of the response waveform lane), during which the animal was awake (denoted as EEG lane) but almost motionless except for a transient head orientation (frames \( a \) to \( b \) of video image lane). This relatively high BsD level changed to a much lower level (1.6 spikes/s) immediately after the room light was turned off (highlighted by the upward arrow in the response waveform lane), almost simultaneously triggering an abrupt initiation of walking in the dark (frames \( d \) to \( e \)). In the dark (the shaded part of the response waveform lane), the animal continued to walk with occasional accesses to a glass container of pellets and/or water nozzle. Although the animal was walking in the dark, the unit's BsD level remained consistently low. This decreased level of BsD level following the light offset continued at least for 68 s in the dark. Minimum continuation of the decreased BsD level of all 17 off-decrease units ranged from 5 to 113 s (mean, 32.3 s; SE, 6.7 s, \( n = 17 \)). Examples of other units are shown in Supplementary Material 2.

The off-decrease in BsD could be repeatedly initiated in the same unit (although only in units that were not affected by head shaking that led to cell loss). As shown in Figure 5, the second trial carried out approximately 6 min later resulted in a similar but slightly delayed onset of the BsD decrease. The animal's initial motion, such as neck extension (frames \( b \) to \( c \)) or head orientation (frames \( c \) to \( d \)), was elicited soon after the light-off (the upward arrow in the response waveform lane) but definitely occurred before the robust decrease in BsD, implying that the initial action itself may not be responsible for initiating the decrease in BsD. The initiation of the BsD decrease was almost coincident with the animal's first forward step, leading to an explorative locomotion (frame \( e \)).

Further analysis of video images in the third trial for the same unit (Fig. 6) showed that a relatively long pause (10 s, arrowheads with a horizontal line) interposed during the locomotion continuing from the light offset did not affect the already decreased BsD level at all, making it less likely that the overt body movements were responsible for the decrease in BsD during the dark period.

If averaged discharge rates from these trials were compared during the periods before and after the light-off (pre-off vs. post-off periods, each 10 s), the pre-off period had a significantly higher

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**Figure 4.** A composite display of neuronal and behavioral response changes following light offset (upward arrow in the response waveform lane). The recording is made from a single neuron in the field A1. From top to bottom: EEG waveform, waveform of acoustic stimulus generated by a synthesizer (no stimulus in this trial), notes on animal behavior together with video images of interest, waveform of microphone input of the sounds that reach the animal, and unit activity (upper, unit discharge; lower, waveform response). Video frames \( a \)–\( c \) are images captured by a standard video camera, with insets of images taken simultaneously by an infrared video camera from a different angle. Video frames \( d \)–\( h \) are images taken by an infrared video camera. Ordinates for EEG, acoustic stimulus, microphone input, and response waveform have arbitrary units. The shaded portion of the response waveform lane indicates the light-off period, whereas the nonshaded portion indicates the light-on period. The sharp (#) and asterisk (*) in the EEG lane depict artifacts generated by a short switching difficulty and a weak wobbling motion of the animal, respectively. The abbreviations h.o. and n.e. in the behavior lane indicate head orientation and neck extension, respectively. The vertical arrow in response waveform lane indicates light-off moment. These symbols are applicable to other figures.
discharge rate than the post-off period (6.91 spikes/s, SE, 0.88 vs. 1.50 spikes/s, SE, 0.83; \( t \)-test, \( P < 0.0001 \), unpaired, 2 tailed).

To exclude the possibility that shifts in arousal level might explain the change in BsD, we applied the light offset while the animal was continuously active in the light, as shown in Figure 7 (see the behavior and microphone input lanes). Before the light-off, the animal was swinging its body back and forth to approach pellets with its forelimb attaching to and detaching from the floor (frames a to f in the video image lane). Turning the light off during the animal's engagement in this motion (the upward arrow in the response waveform lane) elicited an abrupt and robust decrease in BsD, comparable to that elicited while animals were virtually motionless in the light (see Figs 4-6). A major difference between the pre-off and post-off behaviors was whether or not the animal initiated locomotion to a water nozzle (frame k) following light offset. This suggests that the BsD change following the light offset is not simply linked to the elevated arousal level, but is rather likely to be linked to covert changes in internal brain state that encourage the animal to initiate explorative locomotion activity. A similar result was shown in another example (see the movie in Supplementary Material 3).

Because illumination offset frequently accompanied each animal's locomotion, it was necessary to clarify which overt body movement (such as walking) or covert change in brain state (such as intention or motivation) would be required for changes in BsD. To segregate the physical walking activities from light-triggered changes in internal brain state, we modified the regular experimental paradigm. The modification relied on the behavioral observation that animals were driven to initiate locomotion...
The behavior of the animal when a glass pellet container was hit elicited robust, sound responses that were on and again when the light was off. One preferred sound, which had been previously recorded in the dark, was used to drive the neuron and was played back through speakers when the light was on. The preferred sound was generated when the experimenter scratched the container. In this particular unit, 4 sound stimulation trials (5.2 s in duration) during the light-on period displayed no robust increase in the acoustic response rate relative to the prestimulus period (1.30 vs. 4.81 spikes/s, respectively). The S/N ratios of the 2 lighting conditions were significantly different (1.05, SE, 0.17 for light-on vs. 0.30 vs. 2.01, SE, 0.69; P < 0.01, unpaired, 2 tailed). Two additional units were subjected to the same test. Similar improvements in the S/N ratios of acoustic responses in the darkness relative to the light were also observed and are shown in Supplementary Material 6. Altogether, the S/N ratios of these 3 units in the light and the dark were significantly different (0.95, SE, 0.30 vs. 2.01, SE, 0.69; P < 0.01, unpaired, 2 tailed).

Figure 7. Decrease in the BsD of another AI unit after light offset. All video frames are infrared video images. An abrupt decrease in the BsD is initiated soon after light offset (upward arrow in the response waveform lane) even while the animal is continuously engaged in active motion (continuous back and forth movement of the body, see frames a-f in the video image lane). This includes transient stepping of the right forelimb onto the floor (frames b and d) and continuous jaw movements (small and large dots as shown in the behavior lane). After the light-off, the animal's behavior shifts from pellet consumption to exploratory locomotion (frames g-k). Jaw movements such as chewing (small dots in the behavior lane) and grasping (large dots in the behavior lane) are detected clearly and reliably by a head microphone (microphone input lane) and represented as deflections of the microphone input waveform. The shaded portion of the response waveform lane indicates the light-off period, whereas the nonshaded portion indicates the light-on period. The abbreviation h.o. in the behavior lane indicates head orientation. The vertical arrow in the response waveform lane indicates the light-off moment.

Although the number of units examined in this paradigm was limited (n = 3), they all consistently showed decreases in BsD without accompanying overt locomotion during the stimulus period (4.00 spikes/s, SE, 0.78 in pre-off window vs. 2.06 spikes/s, SE, 0.85 in post-off window; t-test, P < 0.05, paired, 2 tailed). In one unit, we were able to repeat the alternating paradigm 3 times, and confirmed that the changes were reproducible in a similar behavioral context (4.24 spikes/s, SE, 0.76 and 2.76 spikes/s, SE, 0.70; t-test, P < 0.05, paired, 1 tailed).

Responses of Off-Decrease Units to Preferred Sounds in the Light and the Dark

To the neuron shown in Figure 4, we applied a preferred acoustic stimulus in both illumination and darkness, and compared the responsiveness under these different lighting conditions (Fig. 9). Preferred sounds that had been predetermined as effective in driving the neuron were saved in a computer and played back through speakers when the light was on, and again when the light was off. One preferred sound, generated when a glass pellet container was hit, elicited robust, time-locked discharges against the already decreased level of BsD in the dark (Fig. 9B), resulting in a high signal-to-noise (S/N) ratio in the responses. In the light, however, the acoustically induced response was not salient or slightly suppressed when the same digitized sound was applied, because the BsD level had already been high (Fig. 9A). This stimulus sound resembled a sound emitted when an animal's teeth touched the pellet container. In this particular unit, 4 sound stimulation trials (5.2 s in duration) during the light-on period displayed no robust change in average discharge rate during the stimulus period relative to the prestimulus period (6.97 vs. 6.82 spikes/s, respectively), whereas the trials during the light-off period showed a relative increase in the discharge rate during the stimulus period compared with the prestimulus period (1.30 vs. 4.81 spikes/s, respectively). The S/N ratios of the 2 lighting conditions were significantly different (1.05, SE, 0.17 for light-on vs. 0.30 vs. 2.01, SE, 0.69; P < 0.05, unpaired, 2 tailed). Two additional units were subjected to the same test. Similar improvements in the S/N ratios of acoustic responses in the darkness relative to the light were also observed and are shown in Supplementary Material 6. Altogether, the S/N ratios of these 3 units in the light and the dark were significantly different (0.95, SE, 0.30 vs. 2.01, SE, 0.69; P < 0.01, unpaired, 2 tailed).

Increase in BsD following Light Offset (Off-Increase Units)

We also observed robust and abrupt increases in BsD following light offset in 7 units (see Fig. 3). As shown in Figure 10A, an increase in BsD was induced soon after light-off (the upward arrow in the response waveform lane), in apparent association with a brief stepping of the right hind limb (note the small white arrows in frames b and c) followed by a head orientation (frames c and d). Similar neuronal and behavioral associations were found for the remaining off-increase units (Fig. 3B). The preferred sound was generated when the experimenter scratched the
arena wall. This sound did not affect BsD when applied to the animal in the light (Fig. 10B, upper panel); when applied in the dark, the sound suppressed the prevailing high BsD only during the sound stimulus periods (Fig. 10B, lower panel).

**Laminar Location of Recordings**

Histological verification of the laminar position of recording points was made using hemispheres from 13 out of 16 animals (Fig. 11). Although recordings could not be made homogeneously throughout the cortical depth, 20 out of 21 units that showed the robust changes in BsD (both off-decrease and off-increase) were located at the border of layers IV and V or within layer V; only 1 off-decrease unit was located in layer 3. The units that did not show robust BsD changes (n = 49) were distributed from layers 2 to 6. The localization of off-changed units in the specific cortical sites was statistically significant (Fisher's exact probability test, P < 0.05, 2 tailed).

**Discussion**

This study revealed that the baseline activity of a subset of neurons in AI or AI-like field was consistently modulated in the
naturalistic behavioral context in which animals set off sequential movements suggestive of explorative behavior upon exposure to a shift of ambient illumination from light to dark (see Fig. 3 for summary). The different BsD levels of AI neurons were apparently in close association with the 2 modes of overt behavioral states (i.e., stationary rest vs. active motion such as locomotion) (Figs 4–6). However, close examination of guinea pig behaviors demonstrated that the initiation and continuation of changes in BsD level were not necessarily concurrent with the animal’s motion (Fig. 6). BsD activity was also modulated by the application of light-off stimulation when animals were actively engaged in continuous consumption (Fig. 7). In the experimental situation where animals abstained from locomotive activity in spite of their intention to initiate it, decreases in BsD were still triggered by the stimulation of repeated light-on and light-off (Fig. 8). These results imply that simple somatosensory/motor activities resulting from vigorous body movements such as locomotion may not be responsible for changes in neuronal activity. The differences in behavior might reflect different internal brain states of the animal, and the shifts in internal brain state may trigger or involve changed perceptual and cognitive functions, requiring coordinated activation of multiple cortical sensory areas. Flexible adaptation of animals to an abruptly changed environment (e.g., from lightness to darkness) would require immediate operational adjustments of neuronal networks in various cortical areas. For some neurons in AI or AI-like field in the dark environment, adjustments such as the improved S/N ratio of sensory responses or enhanced contrast in stimulus-driven vs. spontaneous activities (Fig. 9) are effective strategies.

Modulation of sensory processing in cortical neurons has been reported in relation to the actions of various neuro-modulatory transmitters including norepinephrine (NE), acetylcholine, serotonin, and dopamine (Foote et al. 1983; Jacobs 1986; Metherate and Weinberger 1989; Metherate et al. 1990; Nicoll et al. 1990; Gu 2002; Berridge and Waterhouse 2003; Aston-Jones and Cohen 2005; Ma and Suga 2005; Briand et al. 2007). Stimulation of NE-containing neurons in the locus coeruleus (LC) or NE injection in target cortical regions are known to reduce BsD tonically, apparently comparable to the sustained decrease in BsD triggered by light offset as described in the present experiments. Multimodal processing may also provide a means to change the responsiveness of AI neurons via the activation of visual and/or somatosensory-related pathways (Brosch et al. 2005; Lakatos et al. 2007). Therefore, our results will be discussed in comparison with known multimodal AI processing and in line with the possible involvement of the LC-NE diffuse projection system.

Comparison with AI Multimodal Processing

Recent electrophysiological studies have revealed that neurons in the AI respond to nonacoustic stimuli. The responses require either highly repeated training of animals with nonacoustic signals (Brosch et al. 2005) or peripheral nerve stimulation, which does not require animals to initiate any behavioral response (Lakatos et al. 2007). It was noted in these studies that the responses to these nonacoustic stimuli were generally transient, indicating the phasic modulation in the responses of AI neurons, which is typical of responses to sensory stimuli. Our findings were also obtained from the AI or AI-like field, but were different from those mentioned above in a few respects. First, the behavioral changes following the ambient illumination shift occurred without any training, suggesting that it was innate or instinctive to the animal. Because the animals had constant access to food and water at any time in their home cages and the experimental arena, they did not have to learn this visual environmental change as a cue signal. Second, animals consistently initiated a sequence of active movements following light-off. However, neither the initial action itself, such as head orientation or neck extension evoked soon after the single illumination change (see an example in Fig. 5), nor a similar action occurring spontaneously before light offset, triggered changes in BsD. In addition, the decreased BsD level triggered by light-off was retained during the time when the locomotive activity was paused in the darkness (Fig. 6). However, in the situation in which animals abstained from locomotive activity in spite of their intention to initiate it, the BsD of AI or AI-like neurons was also strongly modulated (Fig. 8). These findings imply that motion itself, and therefore the somatosensory/motor activities resulting from motion, may not be responsible for the BsD modulation of AI or AI-like neurons or underlie its continuation. Rather, the light-off-associated BsD changes in AI or AI-like neurons could be linked to changed internal brain states, such as decision making, motivation, or augmented expectation, reflected overtly by behaviors such as active stepping forward. However, which brain state(s) is responsible for the changes in BsD level remains to be
Off-Decrease in BsD Linked to Adaptive Activation of NE

In a naturalistic context, sensory signals can be perceived as a cue on which animals initiate appropriate behavior, and this behavioral change is directed toward increasing the probability of survival (Manning and Dawkins 1998). This must require not only a cortical area necessary to process a particular sense but also cortical areas involved in adapting animals to new situations. Neural substrates mediating the requirement of animals to behave differently in changing environments have been ascribed to the brainstem neuromodulatory systems (Luppi et al. 1995; Gu 2002; Robbins 2005; Briand et al. 2007). Of these, the LC–NE system (Aston-Jones et al. 2000) would be of special relevance for interpreting the present findings because the modulation pattern generated by NE application, and its long-term effect, resemble the changes in BsD shown here. It is known that cortical neurons generally reduce their spontaneous activity when NE is directly applied to various cortical areas, including the auditory (Foote et al. 1975), barrel (Waterhouse and Woodward 1980) and visual (Waterhouse et al. 1990) cortices of restrained or anesthetized animals. A recent hypothesis in which LC–NE activity was involved in the decision-making process, namely in overt behavioral shift from disengagement from the ongoing behavior to engagement in an alternative behavior in a context-dependent manner (Bouret and Sara 2004; Devilbiss and Waterhouse 2004; Aston-Jones and Cohen 2005; Bouret and Sara 2005). Thus, the present findings that the response changes in Al or Al-like units and the associated behavioral and psychological shift toward the adaptive action are each likely to include a decision-making process may support the involvement of the LC–NE system and its interaction with prefrontal areas (Sesack et al. 1989; Jodo et al. 1998; Lee et al. 2005). In this scenario, sudden darkness in the visual environment may serve as a trigger-like signal to activate the retinal area that is dedicated to peripheral rather than central vision, and this retinal activation must be transferred to the cortex (likely via the superior colliculus-to-pulvinar extrastriate pathway), as pointed out previously (Dean and Redgrave 1984). Indeed, it is proposed for rats and hamsters that the interaction between the prefrontal cortex and the LC–NE system is involved in the association of novel signals with a relevant behavior (Redgrave et al. 1993). Such a behavioral response necessarily needs to accompany modulations of sensory systems; for example, improved gain or accuracy in sensory detection by neurons in the AI, and probably in other cortical areas as well. This would be advantageous in a new environment in which visual information is less helpful. Selective impairment of the LC–NE system (e.g., pharmacological impairment using N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine [DSP-4], which exerts a selective degenerative effect on LC–NE axons) could illuminate this possibility (Ross 1976; Jonsson 1980).

Functional Implication of a Subset of Cortical Neurons Showing Alterations in BsD

We hypothesize that covert changes in an animals’ internal brain state that are reflected by overt changes in its behavior would be responsible for or involved in changes in the BsD of neurons in Al or Al-like fields. The present anatomical finding demonstrating that the units showing the robust BsD changes are predominantly confined to layer V or its vicinity (see Fig. 11) raises the possibility that modulation of the activity of a pervasive cortical network contributing to the behavioral changes could be mediated, at least in part, by pyramidal neurons in layer V.

How can changes in internal brain state be linked to changed activity levels in other brain areas? Specific intra-area and interarea connections of layer V pyramidal neurons may provide some clues. Two major types of pyramidal neurons have been identified in layer V; namely, one having a thick apical dendrite ending with a dendritic tuft of dense arborization in layers 1–2, and the other having a slender dendrite ending with poor arborization in the upper layers (Larkman 1991; Ojima et al. 1992). Assuming that BsD
modulation reflects a change in internal brain state, this change should be made possible via top-down feedback projections. As anatomy shows, the top-down projections terminate densely in layer 1 (Rockland and Pandya 1979; Felleman and Van Essen 1991; Rouiller et al. 1991), and therefore it is likely that the pyramidal neurons with a thick apical dendrite are more susceptible to this top-down influence than the other type. Activity in layer V is known to be transferred to superficial cortical layers (Silva et al. 1991). Pyramidal neurons with a thick apical dendrite in layer V are proposed to play a major role in the initiation of local network excitability, namely cortical synchronization (Connors and Amitai 1995). Interestingly, feedback input to the rich dendritic tuft of this neuronal type is regulated by the spontaneous excitability of the neuron itself (Rhodes and Llinás 2001; Larkum et al. 2004). Therefore, responsiveness of this layer V pyramid is dependent upon its own BsD level. Furthermore, a recent model proposing the transthalamic activation of cortical areas (Sherman and Guillery 2006) via the axonal projection of this type of layer V neuron to the thalamus (Ojima 1994) suggests that the cortical synchronization could be transferred indirectly from a primary (first-order) cortex to higher-order cortical areas. Furthermore, this type of pyramidal neurons project corticofugal axons to brainstem stations such as the inferior colliculus (Diamond et al. 1969; Ojima 1994). Indeed, it is known that this feedback system exerts modulatory effects on the processing of basic acoustic parameters by inferior collicular neurons (Yan and Suga 1996; Zhang et al. 1997). Considering these connections with higher cortical and subcortical stages, together with indirect projections from the perirhinal and parahippocampal areas (Lavenex and Amaral 2000), AI or AI-like field might participate in cognitive functions through the strategic dendritic and axonal morphology of a particular type of layer V pyramidal neurons by means of the changes in their baseline activities.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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