Sensory and Motor Effects of Etomidate Anesthesia

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Engelmann, Jacob, Joao Bacelo, Erwin van den Burg, and Kirsty Grant. Sensory and motor effects of etomidate anesthesia. J Neurophysiol 95: 1231–1243, 2006. First published November 2, 2005; doi:10.1152/jn.00405.2005. The effects of anesthesia with etomidate on the cellular mechanisms of sensory processing and sensorimotor coordination have been studied in the active electric sense of the mormyrid fish Gnathonemus petersii. Like many anesthetics, etomidate is known to potentiate GABA\textsubscript{A} receptors, but little is known about the effects on sensory processing at the systems level. A better understanding is necessary for experimental studies of sensory processing, particularly concerning possible effects on the dynamic structure of excitatory and inhibitory receptive fields and to improve the knowledge of the mechanisms of anesthesia in general. Etomidate slowed the electromotor discharge rhythm, probably because of feedback inhibition at the premotor level, but did not alter the structure of the electromotor command. Sensory translation through primary afferents projecting to the cerebellum-like electrosensory lobe (ELL) was not changed. However, central interneurons and projection neurons were hyperpolarized under etomidate, and their spiking activity was reduced. Although the spatial extent and the center/surround organization of sensory receptive fields were not changed, initial excitatory responses were followed by prolonged inhibition. Corollary discharge input to ELL was maintained, and the temporal sequence of excitatory and inhibitory components of this descending signal remained intact. Later inhibitory corollary discharge responses were prolonged by several hundred milliseconds. The result was that excitatory reafferent sensory input was conserved with enhanced precision of timing, whereas background activity was greatly reduced. Anti-Hebbian synaptic plasticity evoked by association of sensory and corollary discharge input was still present under anesthesia, and differences compared with the nonanesthetized condition are discussed.

I N T R O D U C T I O N

The possible bias introduced by the effect of anesthetics is a general problem for systems level studies of brain processes in whole animal preparations (e.g., Edeline 1999; Gottschalk 2003; Maclver and Kendig 1989). To date, the mechanism of loss of sensory perception during anesthesia remains poorly known.

The electrosensory system of mormyrid electric fish is a particularly interesting model in which this can be studied because the pathways and physiology of corollary discharge signals that gate sensory integration are well known. The corollary discharge is driven by an efference copy arising in the electromotor command nucleus and is a descending signal that actively filters on-going sensory input, in the manner of a central prediction based on recent sensory history correlated with electromotor strategy. It may also include multimodal sensory feedback from proprioceptive, visual, or lateral line sensory systems. Here, we have examined the effects of the anesthetic etomidate on the integration of sensory and corollary discharge signals to better understand how etomidate affects both sensory processing in the initial stages of the central processing pathway.

The intrinsic network of the electrosensory lobe (ELL) is shown in Fig. 1. Corollary discharge input arrives through several pathways that terminate in different layers of this cerebellum-like network: from the juxtalobar nuclei, terminating in the granular layer, and from the nucleus preeminentialis either directly, terminating in the lower molecular layer, or indirectly through parallel fibers originating from eminentia granularis posterior that terminate in the outer molecular layer (Bell and Szabo 1986; Maler 1973). The sensory responses of interneurons and projection neurons of the ELL are strongly influenced by the descending corollary discharge input, which creates a succession of inhibition and excitation timed precisely with the arrival of reafferent sensory input generated by the fish’s own electric discharge (Bell and Grant 1989).

Etomidate potentiates the action of GABA on GABA\textsubscript{A} receptors (Belelli et al. 2003; Rudolph and Antkowiak 2004; Yang and Uchida 1996; Zhang et al. 2002), and for this reason can be expected to influence inhibitory synaptic activity in ELL, whose intrinsic network contains several different GABAergic microcircuits.

Most anesthetics block the central electromotor command, and because of this, until recently, the physiology of ELL neurons has been studied principally in curarized fish without anesthesia. Curare blocks the activation of the electric organ, but not the central motor command, making it possible to study the effects of the corollary discharge in the absence of reafferent sensory input. This is an advantage for the study of central signals alone, and artificial sensory input can be generated by external point stimulation. However, sensory images generated in this way do not have the same spatial distribution or statistical structure as those generated by the fish’s own electric discharge.

Before using etomidate to explore natural electrosensory imaging, the aim of this study was to investigate how this anesthetic affects sensory processing in this low level central network that shares many of the basic design principles of the cerebellum and the dorsal cochlear nucleus of mammals.

M E T H O D S

Nineteen mormyrid fish of the species Gnathonemus petersii, measuring 10–17 cm in length, were used in electrophysiological experiments, and three fish were used for noninvasive studies of changes in the electric organ discharge rhythm under anesthesia. Fish were

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FIG. 1. A: section through the electrosensory lobe (ELL) indicating parallel fiber input to the molecular layer (white) and the mormyromast electroreceptor afferent input to the granular cell layer (black). B: enlarged schematic view of the ELL showing several (but not all) different cell types based on morphological reconstruction after labeling with biocytin. Corollary discharge input arrives through 1) parallel fiber input to the outer molecular layer, 2) nucleus preeminentialis axons ending in the inner molecular layer, and 3) juxtalobar nucleus input to the granular cell layer. Mormyromast primary afferents convey sensory information to the granular cell layer. EGp, eminentia granularis of cerebellar caudal lobe; gang, ganglionic layer; gran, granular cell; plex, plexiform layer; inter, intermediate layer; mol, molecular layer. Interneurons: MG, medium ganglionic layer cells (GABAergic); MF, medium fusiform cell (GABAergic); TSD, thick smooth dendrite cell (non-GABAergic); G, granular cells (probably GABAergic). Projection neurons (glutamatergic): LG, large ganglionic layer neuron; LF, large fusiform neuron.

Data acquisition

The triple electromotoneuron volley known as the “command signal” was recorded with an Ag-AgCl wire placed around the electric organ in the tail. The first negative peak of the command signal is defined as time 0 (Fig. 2A, t0). This served as a temporal reference for data analysis and stimulation. The command signal was amplified (Cyberamp, Axon Instruments) and fed to a trigger unit (Window Discriminator 120, WPI, Sarasota, FL) that generated a square pulse at the time of the first negative peak of the triple volley. This pulse was used to trigger data acquisition, as well as the stimulator (Anapulse, WPI), for the presentation of artificial electrosensory stimuli. In those cases where acquisition was not triggered by the command signal, data were recorded in continuous mode and cut into episodes off-line, starting at a chosen time before t0 using Ephys data acquisition software (G. Sadoc, UNIC, CNRS, Gif sur Yvette, France).

Field potential recordings were made with low-resistance micropipettes (3 M NaCl; 4–10 MΩ) recorded with an Axoclamp 2B amplifier (Axon Instruments; filter settings: DC, 3 kHz). Before data were transferred to the acquisition software, they were amplified and digitized (Cyberamp and DigiData 1323A interface, sampling rate: 10–250 kHz, Axon Instruments). For current source density (CSD) analysis, the recording electrode was advanced in steps of 25 μm. Field potentials were averaged over 20–30 episodes at each site. One-dimensional CSD was calculated as described in (Gomez et al. 2005), using arc-tangent transformed data to enhance small events. Color plots were constructed using MatLab (MathWorks).

Intracellular recordings were obtained with microelectrodes filled with 2 M potassium methyl sulfate containing 2% biocytin (Sigma-Aldrich, Lyon, France) (140–220 MΩ). To characterize individual cells, biocytin was injected intracellularly by passing a depolarizing and hyperpolarizing current of 0.1–0.6 nA for 2–10 min. Input resistance was measured at a delay of 80 ms after time 0 by passing square pulses of 10- to 20-ms duration and 0.1-nA amplitude. Acquisition of intracellular data was carried out as for field potential recordings, with filters set at DC to 10 kHz.
Stimulation

Electroreceptive stimuli were delivered to local areas of the skin through a pair of chloride silver balls, 0.5 mm in diameter, set 2.5 cm apart. The axis of the dipole was held perpendicular to the skin, with the negative electrode positioned closest to the skin. Stimulus duration was 0.1 ms, and intensities were 2–20 μA.

For cells that increased their discharge rate when the receptive field center was stimulated the normalized increase in discharge rate (NI) was defined as follows: NI = (discharge rate at center of RF - spontaneous discharge rate)/discharge rate at center of RF.

For cells that were inhibited by stimulation of the center of the receptive field the normalized increase was defined as follows: NI = 1 - (discharge rate at center of RF - spontaneous discharge rate)/discharge rate at center of RF.

Histology

At the end of experiments, fish were deeply anesthetized with MS222 (0.5g/l) and perfused with PBS followed by 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M PBS (pH 7.2). After overnight fixation, brains were cut at 50–80 μm thickness in the transverse plane on a Leica VT 1000 vibratome (Wetzlar, Germany) and reacted with the ABC system (Vector Laboratories, Burlingame, CA). Labeled cells were reconstructed using either a drawing tube attached to a microscope (Leitz Orthoplan) or using the Neurolucida system (MicroBrightField).

Statistics

Statistical comparisons are based on t-test for paired data (StatView, SAS Institute, Cary NC). If not noted otherwise, values concerning population data are given as means with SD, with sample sizes indicated by a lowercase n and the number of fish by a capital N.

RESULTS

Effects of etomidate on the electric organ discharge

To compare the timing of activity in different nuclei of the electromotor command chain and the electroreceptive processing network, a zero time reference \( t_0 \) (see Fig. 2A) was defined as the first negative peak of the electromotoneuron triple volley (the command signal) recorded at the skin above the electric organ (Bell and Grant 1992). The exact timing of the electric organ discharge (EOD) relative to \( t_0 \) is of importance because the processing of reafferent sensory information in the ELL is gated by means of the electric organ corollary discharge input (EOCD) arising from the electromotor command (Bell 1986, 1989, 1990; Bell and Grant 1992).

Figure 2A shows that etomidate did not alter the relative timing of \( t_0 \) and EOD. The latency of the positive peak of the EOD relative to \( t_0 \) was averaged over 126 EODs before each change in the concentration of etomidate and compared with the latency observed in the absence of the anesthetic. In
the example shown in Fig. 2, the mean latencies of the EOD with 1.6, 2.8, and 4.1 μM of etomidate were 3.58 ± 0.03, 3.55 ± 0.04, and 3.59 ± 0.04 ms, respectively. After returning to respiration without etomidate, the EOD had a mean latency of 3.59 ± 0.05 ms. For the three fish studied, etomidate did not change the timing of motor events compared with the preparation without anesthetic (Wilcoxon matched-pairs test: \( P > 0.15, n = 126, N = 3 \)).

The shape and the amplitude of the EOD were also unaffected by etomidate, as evaluated by comparing the integrals computed for the time window 1.76 ms on either side of the zero crossing separating the positive and negative peaks of the bi-phasic signal (Wilcoxon matched pairs test: \( P > 0.22, n = 126, N = 3 \)).

However, etomidate did affect the rhythm of the EOD, which is normally instantaneously variable in unanesthetized fish, but became slow and regular under etomidate in a dose-dependent manner (Fig. 2B). Averaging data from three fish, the mean inter-EOD interval increased from 90.8 ± 20.1 ms in the absence of etomidate to 1,035.2 ± 61.8 ms in 1.6 μM etomidate, which was the working concentration used for intracellular recording. At concentrations >4.1 μM, the EOD sometimes split into two components, similar in form to the normal EOD but of different amplitudes; bursting of small, desynchronized EODs also occurred. After return to ordinary water, the EOD returned to an irregular pattern within a few minutes.

In the unanesthetized state, sensory stimuli could lead to a transient increase in the motor command rate known as a “novelty response” (Post and von der Emde 1999). Such novelty responses were usually not observed at the anesthetic concentration used in the physiological experiments (1.6 μM).

Field potentials and CSD analysis of corollary discharge responses

Field potentials and current sinks and sources evoked by the EOCD input are compared for an exemplary data set in anesthetized and unanesthetized fish in Fig. 3. In the absence of etomidate, the earliest EOCD response recorded throughout all layers of the ELL is a ramp-like positive wave (Fig. 3A, p1) (Bell 1990; Bell and Grant 1992). After p1, n2 peaks as a sharp negative wave 5.5 ms after \( t_0 \) in the intermediate and granular layers (Fig. 3A). n2 is explained by a transient activation of cells in the granular layer of ELL by juxtalobar input (Bell and Grant 1992; von der Emde et al. 2000). A second brief positive wave (p2) is recorded from the plexiform to the molecular layer simultaneously with n2. It has been suggested that p2 represents the activity of the axon terminals of the granule cells, i.e., their terminals act as passive current sources, and the presence of p2 up into the molecular layer can be explained by volume conduction (Bell and Grant 1992). In the plexiform layer, n2 fuses with a third negative going n3 (Fig. 3A), which has a latency of ~7 ms. After n3, a broad positive wave (p3, arrow; Fig. 3A and C) is recorded throughout the deep layers of the ELL. This wave peaks with a latency of ~15.7–17.3 ms in the ganglionic layer.

The CSD analysis (Fig. 3) reveals a first sink at the level of the intermediate and granular layers that reaches up to the plexiform layer, corresponding to field potential n2. The corresponding source extends from the plexiform to the molecular layer with a shortest latency of ~6 ms. A source corresponding to n3 can be seen in the plexiform layer with the corresponding sink reaching up to the ganglionic layer (Fig. 3A, *). A second and later sink/source constellation is characterized by the
propagation of the sink (Fig. 3A, ***) from the ganglionic layer into the molecular layer (~200–380 μm).

In summary, it seems that early juxtalobar input excites cells in the granular layer, and this activation invades the upper granular and plexiform layer, leading to the activation of different cell types (~6–10 ms after \( t_0 \)), resulting in the n2–n3 complex. Action potentials propagate into the proximal molecular layer (Gomez et al. 2005).

Thirty minutes after the application of etomidate, the early components of the field potentials and CSD pattern in response to the EOCD input were, in general, quite similar to those observed in the absence of etomidate (cf. Fig. 3, A and B). However, the time-course of the later components of the field potentials differed. The n3–p3 complex became sharper (Fig. 3C), and the duration of p3 (Fig. 3C arrow) was prolonged (measured as the width at 50% amplitude in the plexiform layer; Wilcoxon matched-pairs test: \( P = 0.018, z = -2.36, n = 7 \)). This resulted in a prolongation of the late sink in the molecular layer and the corresponding source in the granular layer.

In summary, both field potentials and CSD profiles showed that etomidate did not disrupt the temporal sequence of synaptic events corresponding to the initial stages of sensory information transfer in the ELL. However, the later processes after the initial EOCD input within the ELL are affected by anesthesia, suggesting that etomidate does alter the balance of activity in the inhibitory circuits intrinsic to the ELL.

Field potentials and CSD analysis of sensory inputs

Primary afferent fibers are coupled electrotonically with postsynaptic granular layer neurons in the ELL. Thus in intracellular records from receptor primary afferents, it is possible to see both the centrally conducting action potential of the afferent fiber itself, as well as synaptic potentials actually occurring in the postsynaptic granule cell (Bell et al. 1989; Slesinger and Bell 1985), generated by central corollary discharge input or by sensory input from convergent primary afferent fibers. The mean corollary discharge excitatory postsynaptic potential (EPSP) latency (measured to the peak) in six primary afferents recorded in presence of etomidate was 4.9 ± 0.72 ms. This is comparable to the corollary discharge EPSP latency of 5.4 ± 0.5 ms reported in the presence of curare alone (Mohr et al. 2003a). Two of these primary afferents were recorded both before and during the application of etomidate (Fig. 4A), and no change was observed in the latency or amplitude of the corollary discharge EPSP or the sensory EPSPs and spikes evoked in response to an artificial stimulus given at a longer delay, beyond the corollary discharge window. Field potentials and CSD analysis (Fig. 4, B and C) showed that the initial part of sensory responses in the upper granular layer to artificial stimulation at a delay of 80 ms were not changed under etomidate. This was assessed by measuring the half-width and peak latency of the field potential in the granular layer (Wilcoxon matched-pairs test: \( P = 0.34, z = -0.94, n = 7 \)). However, the later part of the negative sensory field potential was prolonged in two cases in the presence of etomidate. This increased the length of the sink centered at the granular/plexiform layer border and the corresponding source in the molecular layer. One reason for this might be that because etomidate prolonged the p3 component of the corollary discharge activity, the response to a late sensory stimulus occurring even at a delay of 80 ms may be superimposed on this.

Effects of etomidate at the cellular level

Twenty-three of the 24 cells analyzed in the presence and absence of etomidate were stable enough to investigate alterations in their passive properties. On average, etomidate reduced spiking activity and produced slight membrane hyperpolarization (control: \(-71.8 ± 7.5 \text{ mV}, >10\) min after etomidate application: \(-74.6 ± 8.7 \text{ mV}\); t-test for paired data: \( P = 0.003; df = 22, 23 \) cells; Fig. 5B). Figure 5A shows the change in membrane potential distribution for different cell types that showed an overall hyperpolarizing shift. In the LF, LG, and MG cells, secondary peaks appeared in the membrane potential distribution. These peaks were caused by the development of a large inhibitory postsynaptic potential (IPSP) in response to EOCD input.

Input resistance (\( R_{in} \)) decreased in the presence of etomidate in 16 of the 18 cells where this was studied (Fig. 5B; before etomidate: \(42.9 ± 22.4 \text{ MΩ}\); with etomidate: \(29.4 ± 22.3 \text{ MΩ}\); t-test for paired data: \( P = 0.007; df = 19, 16 \) cells) and slightly increased in one MG cell and one LF cell. After washout, the mean membrane potential and the input resistance were indis-
distinguishable from the control levels (\( t \)-test for paired data: \( P > 0.35 \); df = 19, 8 cells).

These changes were accompanied by a reduced membrane potential variability (Fig. 6, A–C) in all neurons (\( t \)-test for paired data: \( P < 0.014 \), df = 2499, 18 cells) except medium fusiform cells (4 of 4) and 1 of the 11 MG cells tested (\( t \)-test for paired data: \( P > 0.14 \), df = 2499, 5 cells). This was determined by comparing the time-locked inverse of the SD of the membrane potential during the first 100 ms after time 0 for both states in the individual cells. This method can reveal “clamping” of the membrane potential because of underlying shunting inhibition even though no hyperpolarizing IPSP is visible (Monier et al. 2003).

Etomidate normally did not alter the general sequence of EOCD-evoked EPSPs and/or IPSPs, but the timing of the different excitatory and inhibitory components of the EOCD response could be shifted. In curarized, nonanesthetized fish, the earliest EOCD response was an EPSP in 20 of the 23 cells (Fig. 6). However, in one MG cell (Fig. 6B) and two LF cells (Fig. 6, C and D), a weak IPSP preceded the EPSP. These IPSPs were enhanced and prolonged under etomidate in the LF cells while it was reduced in the MG cell. In the LF cells, this delayed the EOCD-evoked EPSP by \( \sim 4 \) ms, whereas in the MG cell, the EPSP peaked 3 ms earlier. These latency shifts and changes in the IPSPs were not caused by membrane potential hyperpolarization, i.e., to changes in the EPSP driving force. This is shown for one LF cell (Fig. 6D, inset); when the cell was hyperpolarized by passing current through the recording microelectrode in the absence of etomidate (thick black trace), the early IPSP was not different from that seen at the resting membrane potential (thin black trace). Under etomidate (gray trace), both the initial and later IPSPs were potentiated and the latency of the interposed EPSP was shifted.

The most frequent change under etomidate was a reduction of the EPSP duration caused by a potentiation of a late IPSP. This late IPSP was not always visible in the absence of etomidate (Fig. 6, A and B) but became prominent under anesthesia. Its presence changed the \( V_m \) distribution to a two-peaked distribution (Fig. 5A). The time-course of this late IPSP was further studied using an analysis of membrane potential variation, calculated as the inverse of the SD of time-locked membrane potential for the period 30–100 ms after \( t_0 \). In 2 of 3 LF cells, 2 of 2 LG cells, and 9 of 11 MG cells, the variability of the membrane potential was less than that in the control situation (\( t \)-test for paired data: \( P < 0.033 \), df = 1699, 13 cells). However, in all medium fusiform cells, one of the three LF cells, two MG cells, and the single TSD cell, there was no significant difference in membrane potential variability in the two situations (\( t \)-test for paired data: \( P > 0.281 \), df = 1699, 7 cells). The TSD cell and medium fusiform cells were the only cell types where the late IPSP was never observed in presence of etomidate. We conclude that in efferent and MG cells, an EOCD-driven IPSP was enhanced under etomidate that was less prominent in the absence of etomidate.

A consequence of the enhancement of the early and late IPSPs of the EOCD response was that, in most cells, the spiking probability was constrained to a short time after EOCD input. The cross-correlation (bin width of 2 ms) between the timing of action potential firing and the motor command signal was normalized by the number of motor command cycles. The resulting distribution gives the probability that, at a given time, an action potential will occur after the motor command, and the peak of this distribution was defined as the correlation coefficient. The probability that a given action potential is driven by the EOCD input was obtained by normalizing the cross-correlation distribution by the number of action potentials. The peak of this distribution gives the contribution index (Derjean et al. 2003; USrey 2002). Three examples that show the major effects of anesthesia on the spiking probability are shown in Fig. 7. In 8 of 11 cells, the EOCD-coupled spikes occurred with a higher probability during anesthesia, whereas the probability of later spikes was reduced (Fig. 7A). This resulted in an increase of the correlation coefficient and the contribution index (Fig. 7A, inset). In these cells, the overall effect of etomidate was thus to increase the contrast between EOCD-evoked firing and background activity.

In two of seven MG cells analyzed in this manner (Fig. 7B), spiking probability was reduced during the EOCD window. These cells differed from all other cells as spiking activity was markedly increased in the period after the long-lasting IPSP; thus the contribution index decreased in these cells. No change...
in probability of EOCD-coupled spikes (Fig. 7C) was encountered in only one LF cell.

Because the tested population showed considerable variability and because for each cell type the sample size remains small, no significant global changes (paired t-test: $P > 0.16$, df = 10,11 cells) could be identified in the contribution index, the correlation index, or the latency of the EOCD coupled spikes (Fig. 7D).

Receptive fields

Electrosensory receptive fields of 11 cells ($n = 7$) were explored in the presence and absence of etomidate and characterized in terms of subthreshold properties and the spiking behavior in response to artificial stimulation given at the reafferent sensory input timing. We compared the discharge rates, the normalized increase of the discharge rate in the center of a given receptive field, and the width of the receptive field in anesthetized and nonanesthetized conditions.

Figure 8 shows the raster plots of the spiking behavior and also the subthreshold responses to an artificial local stimulus for a medium fusiform cell (Fig. 8A), an LF cell (Fig. 8B), and an MG cell (Fig. 8C) in the absence (left) and presence (right) of etomidate. The stimulating electrode was moved from rostral to caudal along a straight line extending roughly from the tip of the Schnauzenorgan to the operculum of the fish (see fish in Fig. 8A) and passing in front of the center of the receptive field of the recorded cell. In the control situation, in all cells except LG cells, stimulation in the center of the receptive field caused an increase in action potential firing and decreased spike latency compared with the response to the EOCD input alone. In the line-plots shown in Fig. 8, the average discharge rate measured at the different positions along the body surface, relative to the maximal rate measured in the center of the receptive field, is shown for the three cells.

Medium fusiform cells only show a subthreshold EPSP in response to EOCD input but fired a single spike in response to reafferent sensory stimulation. There was thus a sudden jump in the discharge rate (Fig. 8, A and B) as the stimulus passed through the center of the receptive field. In contrast, the receptive fields of both efferent cells (Fig. 8, C and D) and MG cells (Fig. 8, E and F) were characterized by rather smooth changes in discharge rates and spike latencies. Action potentials elicited from skin areas bordering the receptive field center occurred with longer latencies than those elicited from...
the center. Stimulation in the region surrounding the receptive field center could reduce the number of action potentials and increase their latency, indicating the presence of an inhibitory surround. Such complex receptive fields were found in two of three LF cells and in two of four MG cells.

Anesthesia did not change the receptive fields of the three medium fusiform cells that were studied (Fig. 8A): the discharge rate, latency, and width of the receptive field were unchanged. The receptive fields of all three LF cells, the LG cell, and two of the four MG cells that were tested were also changed very little under etomidate, although the LF cell shown in Fig. 8C showed a slight caudal shifting of the receptive field center under anesthesia. The most notable effect of etomidate on receptive field organization, observed only in MG cells (2 of 4), was a marked increase in contrast between the center and the inhibitory surround. The example shown for this group (Fig. 8, E and F) showed an opposite shift of the center of the receptive field than the LF cell shown in Fig. 8, D and E.

To what extent the shifts in the center of the receptive fields can be attributed to the anesthesia or to plasticity of sensory responses caused by repetitive association of the sensory stimulus with the EOCD input is difficult to evaluate. Similar shifts in the position of the receptive field center were found in four cells for which we repeated the receptive field measurements in quick succession in the absence of anesthesia. This indicated that plasticity rather than anesthesia may be the probable explanation, and indeed, previous in vivo experiments (Bell et al. 1997b) have shown that repetitive association of a given sensory input with the corollary discharge produces plastic changes in the response to the latter after <10 associations.

For all 10 cells studied, Fig. 9A summarizes the effect of anesthesia on the width of the receptive fields. The receptive field width was not altered by etomidate (Wilcoxon test, \( z = -0.76, P = 0.43 \), 10 cells). However, the stimulus-driven response at the center of the receptive field was increased by anesthesia (Wilcoxon test, \( z = -2.36, P = 0.018 \), 10 cells), although this apparent effect is mainly caused by the overall reduction in background firing activity.

In two LF cells and one LG cell, we were able to repeat the receptive field measurements at a higher dose of etomidate (4.1 \( \mu M \)), corresponding to very deep anesthesia. Under these conditions, the width of the receptive field was considerably reduced, and the normalized increase in discharge rate at the center of the receptive field was even higher (data not shown).

Plasticity

Repetitive association of the same sensory input with descending EOCD input leads to plastic modulation of the effect of the EOCD (Bell et al. 1997b). It has been postulated that the EOCD input to ELL generates an internal “expectation” of sensory future in the form of a negative image of recent reafferent responses and that this acts as an adaptive filter of current reafferent sensory responses (Bell 1981; Bell et al. 1992, 1997a; Roberts and Bell 2000). The effect of this filter would be to reduce the effects of sensory input that can be predicted from recent association. We studied plasticity under etomidate by pairing the EOCD response with an artificial sensory stimulus evoked at the normal reafferent signal delay for 11 cells, of which 5 (1 LF, 1 TSD, 2 MG, and 1 LG cell) were also studied in the absence of the anesthetic.

Examples of the plasticity obtained in the presence of etomidate are shown for two MG cells and an LF cell in Fig. 10. MG cells fire two sorts of action potentials: small spikelets that are probably axon spikes that do not invade the soma and large, broad action potentials that backpropagate into the apical dendritic tree. The latter have been implicated in the expression of plastic changes in responses to molecular layer EOCD input in vitro. An example of pairing a sensory stimulus with the EOCD input in an MG cell is shown in Fig. 10A, where the sensory stimulus inhibited the first spikelet evoked by the EOCD input and at the same time increased the latency of a second spikelet. At the end of pairing, the inhibition of the first spikelet was becoming less complete, and the latency of the

FIG. 7. A–C: post-tg spike probability for 2 LF cells (A and C) and 1 MG cell (B). Each probability is based on 100-s ongoing activity in presence of etomidate (black) and without anesthesia (gray). Insets: cross-correlation index (binwidth 1 ms) between motor command and neuronal responses, normalized by number of intervals, i.e., contribution distribution. Contribution distribution on top is obtained in absence of etomidate and distribution below in presence of etomidate. D: summary of effects of etomidate (black bars) on spiking behavior of ELL neurons evoked by corollary discharge input, expressed as correlation coefficient (CC), contribution index (CI), and latency of peak in cross-correlation compared with the same measures in the absence of etomidate (light gray bars).
second spikelet had decreased. When the stimulus was turned off, the probability of firing an early spike was greatly increased compared with the prepairing period. The second spikelet was also evoked more frequently, although with a longer latency and more variable timing than during the pairing itself. Broad action potentials (gray dots) also appeared with a greater probability, and these changes persisted for 5 min.

Comparison of the intracellular responses shows an increase in the slope and amplitude of the EOCD EPSP after the pairing. A second MG cell is shown in Fig. 10B. This cell was inhibited by EOCD input and fired only a single broad spike in response to an artificial reafferent sensory stimulus. During pairing, the latency of this broad action potential increased, and after pairing, it can be seen that the two components of the EOCD-evoked IPSPs were enhanced.

Figure 10C shows associative plasticity in an LF efferent neuron that was mainly inhibited by EOCD input. During pairing, the sensory stimulus of the cell fired a burst of spikes whose latency gradually increased as pairing progressed. The latencies of the second and later spikes of the sensory response increased more than that of the first spike, and as pairing progressed, the later action potentials began to drop out. After pairing, it can be seen that the slope and amplitude of both components of the EOCD IPSP had increased.

These results show that the mechanism of associative plasticity is not abolished by anesthesia, although the increase in inter-EOD intervals that is induced under etomidate will necessarily affect the frequency of association.

DISCUSSION

Motor system

Etomidate did not alter either the timing of the EOD relative to the motor command or the form and amplitude of the EOD.
This means that the physical properties of the electric image projected on to the sensory surface are not changed, and this is important to future studies of natural electrosensory imaging under etomidate anesthesia. However, etomidate did have a major effect on the electric discharge rhythm, which was markedly slowed. The likely sites for this action are the medullary command generator, consisting of the relay and command nuclei, or the precommand nucleus (PCN) in the midbrain, which is the primary source of descending input to the command nucleus.

Under metomidate, a molecule similar to etomidate, the afterhyperpolarization of command neuron action potentials was markedly prolonged as the inter-EOD period lengthened (Clausse and Grant 1986). However, no recurrent synaptic inhibition in command neurons was found, and immunohistochemical studies targeting anti-GABA or anti-glutamic acid decarboxylase (GAD) activity in the command and relay nuclei have always given negative results. It remains to be studied whether the effect of etomidate is caused by potentiation of an as yet unidentified inhibition afferent to the command neurons or whether the effect is indirect, acting by reducing descending excitatory input.

Much of the afferent input to the command nucleus comes from the precommand nucleus whose neurons are densely covered by GAD-positive terminals (Grant et al. 1999) and that receive a powerful corollary discharge–driven feedback inhibition (von der Emde et al. 2000). In another mormyrid fish, Breionomyrus brachyistius, Carlson and Hopkins (2004) have shown that iontophoresis of GABA into the precommand nucleus lengthens the inter-EOD interval and injection of glutamate into the ventro-posterior nucleus of the torus semicircularis, which is afferent to both the PCN and the CN (Grant et al. 1999), produced similar results. It is therefore likely that etomidate slows the electromotor rhythm by potentiation of corollary discharge–driven GABAergic inhibition of the pre-command nucleus, with a consequent reduction of descending excitatory input to the medullary command nucleus. A direct action of etomidate on premotor circuits has also been described at several sites in the mammalian brain (Zeller et al. 2005; Zhang et al. 2001).

Sensory system

Primary afferent input was not changed by etomidate, suggesting that peripheral electroreceptor organs and sensory transduction were not affected. However, once within the electrosensory lobe, sensory integration can be subject to lateral inhibition and also depends strongly on motor context, i.e., on coincidence or not with corollary discharge feedback signals.

For sensory responses to stimuli falling outside the corollary discharge window, CSD analysis generally showed no changes in the first stage of central integration of electrosensory input in the granular cell layer. At this level, primary afferents contact granule cells with electrotonic synapses, and stimulus intensity is coded by both spike latency and the number of spikes in the afferent volley (Bell 1989, 1990). It might be expected that etomidate would potentiate lateral inhibition among granule cell populations, generated through connections with GABAergic large myelinated interneurons (Han et al. 2000a; Meek et al. 2001) and could thus alter the balance of the center-surround structure of receptive fields. However, although potentiated lateral inhibition arriving one (chemical) synaptic delay later might curtail the integration of the later spikes of the primary afferent volley, it probably would not alter the coding of local stimulus intensity represented by the timing of the first spike of the primary afferent sensory response (Gomez et al. 2004).

A more complex situation is present in the case of reafferent sensory input that coincides with the corollary discharge gating window. Preliminary records (Engelmann and Bacelo, unpublished results) indicate that GABAergic large myelinated in-
interneurons (LMIs), present in the granular and intermediate cell layers, fire a single spike at the time of the electric organ discharge. The axon and dendrites of LMI cells synapse with granule cell bodies over a wide area (Han et al. 2000a; Meek et al. 2001), and it is therefore possible that this sets up a basal level of GABAergic inhibition over the granule cell population, coincident with the arrival of reaффerent electroreceptive input. Excitatory sensory responses are superimposed on this. In this case, by potentiating LMI inhibition of granule cells, etomidate could alter the threshold for granule cell integration of sensory input. More experiments and modeling studies will be necessary to understand the balance of excitation and inhibition in the granule cell layer and the full complexity of inhibitory gain control of sensory integration at this level.

**Corollary discharge input**

Field potentials and CSD analysis suggested that there was little change to the early components of the EOCD input to ELL under etomidate. We therefore conclude that the principal central inputs to the ELL, coming from the preeminential and juxtalobar nuclei and the caudal lobe parallel fiber pathway, are largely unaffected. This is of major importance regarding the use of etomidate anesthesia for studies of sensory processing in the ELL, where integration of sensory input depends on the precise timing and complex temporal structure of the EOCD and primary aффerent signals.

The visible effects of etomidate appeared in the later components of the corollary discharge responses, for example, affecting the n3 and p3 complex of the EOCD field potentials that were significantly prolonged by etomidate. This prolongation of field potentials coincided closely with the timing of the late IPSP seen in intracellular recordings from ganglionic layer neurons. These components of the field potentials most likely represent intrinsic processing of the EOCD input by cells within ELL, rather than late-arriving EOCD input (Mohr et al. 2003b).

Under etomidate, the importance of inhibitory connections within ELL became very obvious in almost all neurons. Synaptically mediated IPSPs increased in amplitude and duration, and in some cases, synaptic inhibition became apparent where none was visible before. An increase in synaptic inhibition under etomidate has now been described for specific cell types in a number of preparations, including hippocampus, cortex, and cerebellum in culture (Jurd et al. 2003; Wafford et al. 2004; Yang and Uchida 1996), targeting postsynaptic phasic GABA\(_A\) receptors. An increase in the probability of receptor channel opening and prolonging opening time is probably mediated at B2 and B3 subtype receptor sites (Angel and Arnott 1999; Proctor et al. 1986; Yang and Uchida 1996; Zhang et al. 2002). Intracellular records from these experiments also showed that, under etomidate, almost all cells in ELL became hyperpolarized, intrinsic spiking activity was reduced, and input resistance decreased. These effects are consistent with an enhancement of tonic inhibitory conductance at extrasynaptic GABA\(_A\) receptors (Bieda and MacIver 2004; Farrant and Nusser 2005), possibly because of an increase in GABA overflow from the very large number of inhibitory synapses present in the molecular and cell layers of ELL. Tonic inhibitory conductances mediated by the \(\alpha5\) GABA\(_A\) receptor subunit in hippocampal pyramidal neurons are highly sensitive to etomidate, and their enhancement has been associated with the amnesic effect of this drug (Caraiscos et al. 2004). However, in different cell types, tonic conductances can also be carried by other GABA\(_A\) receptor subunits, for instance by the d receptor subtypes in cerebellar granule cells (Farrant and Nusser 2005; Mody and Pearce 2004). It is not yet known which receptor subtypes are present in the different types of neurons in ELL but because most neurons are affected in this way by etomidate, it is likely that tonic GABA\(_A\) conductances are widely expressed.

The most prominent change observed in MG cells and efferent neurons was the amplification of the late IPSP evoked by EOCD input. In cells of this type where no IPSP was initially visible, membrane potential showed reduced variability during this period and thus when an IPSP appeared under etomidate it is likely that this was indeed an amplification and not an IPSP generated “de novo”. The most likely source of this IPSP is from MG cells which represent 70–80% of the cell population in the ganglionic and molecular layers of ELL and are driven by EOCD input. MG-cells respond with bursts of small action potentials to the EOCD and these bursts begin 5 to 10 ms before the onset of the late IPSP seen in projection neurons and MG cells themselves. Their axons have a GABAergic terminal arborization densely covering the soma of neighboring output neurons (LG and LF neurons) within a span of about 300 microns either side of their cell body and probably also reciprocally contacting neighboring MG cells (Meek et al. 1996). Mohr et al. (2003b) also noted that the only cells in ELL that did not show an IPSP in response to nucleus preeminentialis stimulation were the TSD and medium fusiform cells. In our records, these were also the only cell types that never showed a late, long-lasting IPSP under etomidate. However these neurons were often hyperpolarized under etomidate, suggesting the presence of tonic GABA\(_A\) receptors.

Despite increased inhibition under etomidate, the major components of the subthreshold EOCD synaptic responses were still present in the anesthetized fish. The principal effect of increased inhibition was to reduce the time window in which ELL neurons were able to fire an action potential in response to excitatory EOCD and reaффerent sensory input. This gave the impression of reduced jitter and increased precision in spike timing. Together with the overall reduction in intrinsic activity, the result was an apparent increase in the contrast of excitatory responses to EOCD or reaффerent sensory input, which stood out against the following increased inhibition and tonically reduced background activity.

Similar results have been reported for a variety of anesthetic and in different preparations (Edeline 1999; Kisley and Gerstein 1999; Massaux et al. 2004). In many studies, a reduction in cortical activity has been described at anesthetic doses lower than those affecting sensory-evoked responses in thalamus or other low-level sensory nuclei (Antkowiak and Heck 1997; Antkowiak and Helfrich-Forster 1998; Antkowiak...

**Sensory receptive fields**

The spatial structure of sensory receptive fields within the ELL is probably largely a property of the organization of primary afferent projections and intrinsic connections within ELL and is not primarily dependent on descending input to the ELL. The phenomenon of lateral inhibition in the mormyrid ELL under conditions of natural stimulation has not yet been studied in sufficient detail, making it difficult to give a functional interpretation of the possible effects of potentiation by etomidate. However, the importance of lateral inhibition in defining receptive fields, in controlling information transfer, and in enabling stimulus discrimination has been shown in the rather similarly organized ELL of the wave-emitting gymnotid electric fish *Apteronotus* (Bastian et al. 2002; Chacron et al. 2003). The reported actions of anesthetics on the size of receptive fields vary depending on the animal species, the chemical and pharmacological nature of the anesthetics, the brain area, and the sensory system studied, and remains a topic of much discussion that has been well reviewed by Dougherty et al. (1997).

In this study of ELL, neither receptive field width nor the center-surround organization (if present) was altered under a moderate depth of anesthesia with etomidate. However, because intrinsic spontaneous firing was reduced, etomidate frequently increased the signal-to-noise ratio measured in the center of the receptive field. At rather higher concentrations of anesthetic, the receptive field width increased, together with a further increase in the signal-to-noise ratio. This dose-dependency is in accordance with results obtained under etomidate anesthesia in the rat (Angel and Arnott 1999).

**Synaptic plasticity**

Our data show that, under etomidate anesthesia, plasticity was still present, and this plasticity led to changes in synaptic efficacy in all MG cells tested, as well as in LF cells. Thus, although in an in vitro study (E. van den Burg, J. Engelmann, J. Bacelo, and K. Grant, unpublished data) showed that the probability of backpropagation of dendritic action potentials that are necessary for the generation of parallel fiber synaptic plasticity in MG cells (Han et al. 2000b) can be greatly reduced by etomidate, such plasticity can be induced in vivo. Because etomidate slows the EOD cycle, the repetition rate of EOCD sensory associations is markedly reduced, and it is likely that this change in frequency compared with the unanesthetized animal, and the consequent increased interval between successive associations, is responsible for the differences in plasticity observed under anesthesia. However, the basic mechanism of associative plasticity clearly remains intact.

In conclusion, it seems that while etomidate anesthesia markedly reduces motor activity, it has only a relatively small effect on the processing of reafferent sensory input in the early central stages. This anesthetic does not alter the basic structure of sensory responses or of corollary discharge–driven active filtering of sensory integration that provides the mechanism for distinguishing reafferent and efferent sensory input in the mormyromast region of ELL. However, sensory input occurring at times other than reafferent input generated by the fish’s own electric discharge will arrive during the potentiated inhibition that follows the initial facilitation set up by the corollary discharge–mediated active filter and therefore response threshold is likely to increase. The mechanisms for synaptic plasticity implicated in the formation and update of central sensory expectation remain intact.

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