Acta Medica Okayama

Volume 48, *Issue* 3 1994 *Article* 2

JUNE 1994

Appearance of dark neurons following anodal polarization in the rat brain.

Nadira Islam* Akiyoshi Moriwaki†

Yukio Hattori‡ Yasuo Hori**

*Okayama University,

†Okayama University,

‡Okayama Univeristy,

**Okayama University,

Appearance of dark neurons following anodal polarization in the rat brain.*

Nadira Islam, Akiyoshi Moriwaki, Yukio Hattori, and Yasuo Hori

Abstract

An anodal direct current of 3.0 microA or 30.0 microA was unilaterally applied for 30 min or 3 h to the surface of the sensorimotor cortex of rats, and the effects of polarization on the morphology of brain cells were examined by light microscopy. After five repeated anodal polarization trials, dark neurons appeared mainly in the polarized neocortex regardless of the intensity and duration of the polarizing currents. Such dark neurons were scarce in the control animals or the animals receiving only one trial of polarization. The dark neurons were most abundant in the second to fourth layers of the ipsilateral superior-lateral convexity of the frontal cortex, but a few were present in the contralateral cortex. The dark neurons began to appear 24 h after the last polarization; thereafter almost all of these neurons gradually reverted to their normal morphological profiles through a transitory state within 1 month of the last trial of repeated polarization. No morphological changes were apparent in any of the brain structures other than the cerebral cortex. These findings indicate that repeated anodal polarization has reversible morphological effects on the cortical neurons, suggesting that the appearance of dark neurons after anodal polarization is an important index for evaluation of cortical plastic change induced by polarization.

KEYWORDS: anodal polarization, direct current, dark neuron, cerebral cortex, rat

---

*PMID: 7942070 [PubMed - indexed for MEDLINE]

ACTA MED OKAYAMA 1994; 48(3): 123-130

Appearance of Dark Neurons Following Anodal Polarization in the Rat Brain

NADIRA ISLAM*, AKIYOSHI MORIWAKI, YUKIO HATTORI AND YASUO HORI

Department of Physiology, Okayama University Medical School, Okayama 700, Japan

An anodal direct current of 3.0 μA or 30.0 μA was unilaterally applied for 30 min or 3 h to the surface of the sensorimotor cortex of rats, and the effects of polarization on the morphology of brain cells were examined by light microscopy. After five repeated anodal polarization trials, dark neurons appeared mainly in the polarized neocortex regardless of the intensity and duration of the polarizing currents. Such dark neurons were scarce in the control animals or the animals receiving only one trial of polarization. The dark neurons were most abundant in the second to fourth layers of the ipsilateral superior-lateral convexity of the frontal cortex, but a few were present in the contralateral cortex. The dark neurons began to appear 24 h after the last polarization; thereafter almost all of these neurons gradually reverted to their normal morphological profiles through a transitory state within 1 month of the last trial of repeated polarization. No morphological changes were apparent in any of the brain structures other than the cerebral cortex. These findings indicate that repeated anodal polarization has reversible morphological effects on the cortical neurons, suggesting that the appearance of dark neurons after anodal polarization is an important index for evaluation of cortical plastic change induced by polarization.

Key words: anodal polarization, direct current, dark neuron, cerebral cortex, rat

Anodal polarization, the passage of constant weak direct currents, of certain cortical areas such as the motor cortex is believed to influence the electrical activity of the cortex (1-4) and the peripheral motor activity (4-7). Earlier studies have shown that anodal polarization increases neuronal firing rate and the number and size of evoked potentials with persistent after effects lasting for hours (1-3, 8), and these effects progressively increase with repeated applications of the current (2). The characteristic changes in peripheral motor activity, such as contralateral forelimb flexions of rabbits induced by anodal polarization, have been reported to persist for several hours (4), or even as long as several weeks (5, 7, 9) with reproducible results. It has been suggested that these phenomena are due to a dominant focus induced at the polarized point (10). Moreover, repeated application of anodal direct current has been shown to decrease the threshold current for elicitation of cortical seizure activity (11). It has also been reported that glial and neurochemical changes, such as cyclic AMP generation, accumulation of reactive phosphate groups of RNA, and protein synthesis, occur in the polarized area (12-16). Therefore, anodal polarization is of considerable interest, as it produces a long-lasting change in neuronal activity by means of a small temporary alteration in the physical environment of neurons (1) and it has been thought to be useful for studying the mechanism of central plastic changes.

Granule cell discharges in the hippocampus evoked by sustained electrical stimulation have been suggested to cause neuronal death due to sustained excitability of the affected cells and excessive release of excitory neurotransmitters (17). Several pathological conditions, such as hypoxia, ischemia, epileptic seizures, severe hypoglycemia or deafferentation of vestibular nuclei, have also been reported to cause brain cell injury characterized by the appearance of dark neurons (17-24). Cellular homeostasis may be altered under these conditions, which can lead to either recoverable or nonrecoverable brain cell damage depending on the change in morphological profiles. Such homeostatic and morphological disturbance including the appearance of dark neurons may occur during cortical

* To whom correspondence should be addressed.

hyperactivity induced by anodal polarization; however, little is known on the occurrence, despite the extensive clinical (25, 26) and experimental uses of anodal polarization. Therefore, the present study was designed to assess the effects of cortical anodal polarization on the morphology of brain cells in rats, in which the recoverable appearance of dark neurons in the cerebral cortex was observed and characterized in relation to the plastic change of cortical function.

Materials and Methods

Animals and surgery. Male Wistar rats weighing 180–230 g were used. They were allowed to free access to tap water and food pellets, and were maintained on a 12 h light/dark cycle at 20–24°C. Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital 35 mg/kg and placed in a stereotaxic frame. The scalp was incised along the midline and the subcutaneous tissue was removed to expose the cranium. Two silver electrodes (1 mm in diameter) were bilaterally implanted into the cranial bone so as to set the tips on the dura mater over the sensorimotor cortex at symmetrical points 1.5 mm rostral and 3.5 mm lateral to the bregma. A stainless steel electrode was implanted in the midline of the nasal bone. These electrodes were secured in place with dental resin.

Anodal polarization. After a postoperative recovery period of 7 days or more, rats were divided into the experimental and control groups. For anodal polarization, the experimental rats were transferred from their home cages to large cages with no food or water and left for at least 1 h for acclimatization. The polarization was performed without anesthesia or restraint. A 3.0 μA or 30.0 μA current from a dry battery was applied continuously for 30 min or 3 h to the left sensorimotor cortex, using the cortical and nasal electrodes as the anode and cathode, respectively. Two polarization paradigms were developed in the present study. In one paradigm, the 30-min or 3-h polarization was applied only once (single polarization), and in the other it was repeated five times at an interval of 24 h (repeated polarization). The control rats were implanted with electrodes but received no current (sham-operated).

Sampling for morphology. Rats were perfused at 1, 6, 12, 24, 72 h, 1, 2 weeks and 1 month after the single or the last trial of repeated polarization, as follows: Under anesthesia with sodium pentobarbital 50 mg/kg, i.p., a tracheostomy was performed and the animal was supplied with a gas mixture of CO_2 in oxygen through a glass cannula. A quick thoracotomy was performed and 0.5 ml heparin (1000 IU/ml) was injected into the left ventricle. A narrow cannula with short tip was inserted into the root of the ascending aorta through the left ventricle and secured to the position with a ligature. The right atrium was incised and the descending aorta was clamped. After a quick rinse with physiological saline, 400 ml of fixative containing 10 % formaldehyde solution in 0.1 M phosphate buffer (pH 7.4) was allowed to perfuse the cephalic circulation using a gravity-fed system. The first half of the fixative was allowed to perfuse the brain in 6–8 min for rapid fixation and then a drip clamp was adjusted to achieve a total perfusion time of 20–25 min. After the brain was left *in situ* for 2–4 h to stabilize, it was carefully collected and post-fixed overnight in the same fixative. The fixed brains were processed for the frozen microtome sectioning or paraffin embedding. Frozen microtome sections (10 μm) were stained with 1 % cresyl violet stain, and paraffin sections (5 μm) with 1 % cresyl violet or hematoxylin-eosin stain.

Morphological analysis. In subserial coronal sections, all the brain structures including the cerebral cortex were examined for morphological changes. The cerebral cortex was quantified in 7 different levels (Fig. 1) using a light microscope at a magnification of × 175. In each level, an area of 300 × 300 μm was counted by means of an ocular grid, which was moved from end-to-

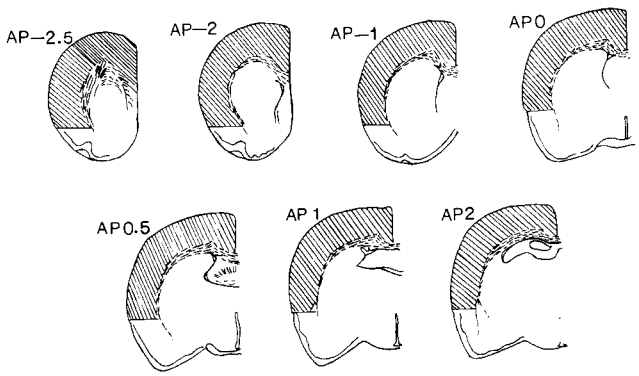

Fig. 1 Schematic drawing of sections at various levels through the rat brain demonstrating the standardized levels chosen from the subserial sections for neuronal quantification. Neurons in the hatched areas on both hemispheres were counted and summed. The AP numbers indicate the distance from frontal zero plane (34).

end and/or side-to-side inside the cerebral cortex; from the cingulate cortex above the corpus callosum over the lateral hemispheric surface to the area corresponding to the rhinal sulcus. Edge error was eliminated by counting only the neurons which touched two of the four grid sides. Neurons observed in all fields were classified into three different types according to their profiles: normal neurons had the typical owl's eye nuclei with clear nucleoplasm and a prominent nucleolus surrounded by a thin rim of cytoplasm; dark neurons had condensed, dark, angular or irregular shaped nuclei and cytoplasm with dark, tortuous processes; and transitory neurons had almost normal shapes and sizes, but as a whole were darker than normal appearing neurons. The neurons were counted in seven different levels separately, summed and then taken as a single data unit for each rat. The average density of total, dark and transitory neurons in a unit area (300×300 μm), and the percentage of dark and transitory neurons out of the total number of neurons were calculated. Both hemispheres were treated in the same way. Statistical analysis was carried out by analysis of variance, followed by Student's t-test.

Results

Behavioral and morphological characteristics. During and after anodal polarization, rats were quiet without showing any abnormal behavior at any intensity or duration of the polarizing currents tested.

The perfused brains were firm and the sections were uniformly white, free of erythrocytes; there was no hydropic cell change or perivascular vacuolation at any time interval studied.

In non-polarized sham-operated rats ($n = 8$), no neuronal change was observed. After single polarization ($n = 96$, 3 in each polarization condition), the sections were generally considered normal, and no abnormal neurons were seen in either case under any polarization conditions or at any time interval studied. However, in both groups, all animals showed one or two occasional scattered dark neurons in the entire field of examination. The morphological change noted in the polarized brain was the dramatic appearance of dark neurons in the cerebral cortex when polarization was repeated five times ($n = 168$, 5 in each polarization condition except 3.0 μA for 3 h, where $n = 6$). No obvious morphological changes outside the cerebral cortex were seen in any control or polarized brains, such as hippocampus, thalamus, pyriform cortex or amygdala.

Dark neurons in the cerebral cortex after repeated polarization. Thirty-two of the 42 rats (76 %) killed by perfusion-fixation 24 h to 72 h after the last trial of repeated polarization exhibited structural alterations (Fig. 2). The dark neurons, which were observed both in paraffin and frozen sections, showed marked shrinkage with a triangular or irregular shape, and condensation of both their nucleus and cytoplasm with

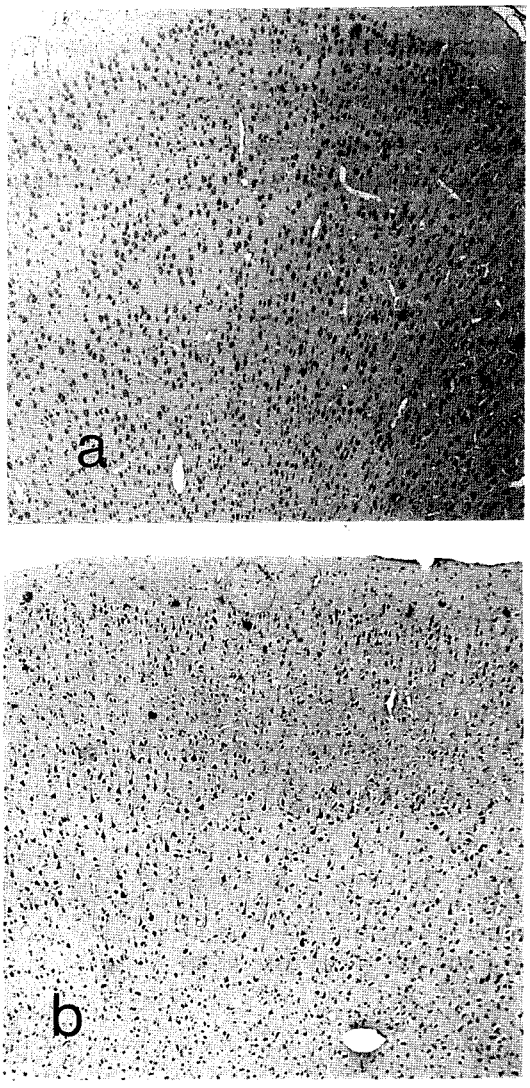

Fig. 2 Non-polarized and polarized neocortex. **a**: Non-polarized control showing normal profiles of neurons; **b**: Dark neurons appearing 24 h after the last trial of repeated polarization with 30.0 μA for 3 h. Almost all the neurons in layers 2 to 6 are rendered dark and punctate in this field. Hematoxylin-eosin stain, × 125.

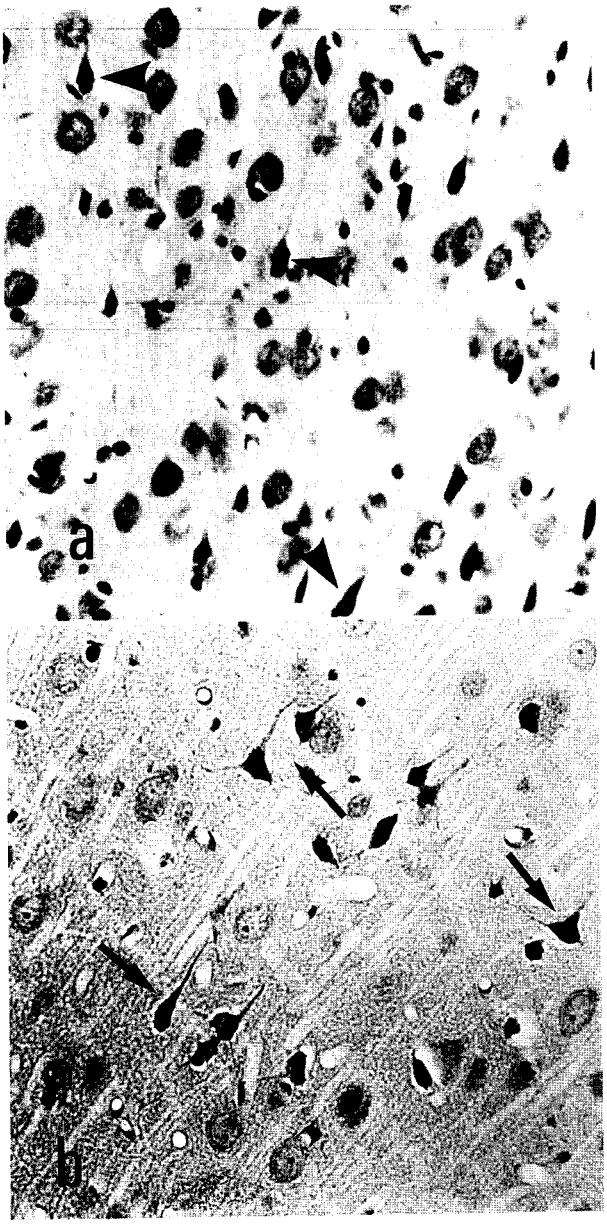

Fig. 3 Dark neurons in layers 2-3 of neocortex appearing 24h after the last trial of repeated polarization with 30.0μA for 3h. **a**: Arrowheads indicate condensed, angular neurons with perineuronal vacuolations; **b**: Arrows indicate darker nuclei as compared with cytoplasm. Hematoxylin-eosin stain, × 500.

perineuronal vacuolations. The processes of these condensed neurons were visible as dark, tortuous bands traversing the neuropil (Fig. 3a). Both the nucleus and cytoplasm were stained quite dark compared with those of normal neurons in the same field. In the dark neurons, the nuclei were darker than the cytoplasm whereas the surrounding normal neurons had the typical owl's eye nuclei with clear nucleoplasm and a prominent nucleolus (Fig. 3b). The most vulnerable neurons, found in layers 2-4, were affected in every cases regardless of the intensity and duration of polarizing currents. In layers 5-6, affected neurons were observed at a survival time of 24h after polarization with 30.0μA for 3h (Fig. 2b). The dark neurons were most abundant over the superior-lateral convexity of the cortex, but did not extend up to the pyriform cortex or entorhinal cortex on the ventral cortical surface. There were many dark neurons in the frontal cortex, relatively few in the temporo-parietal cortex, and

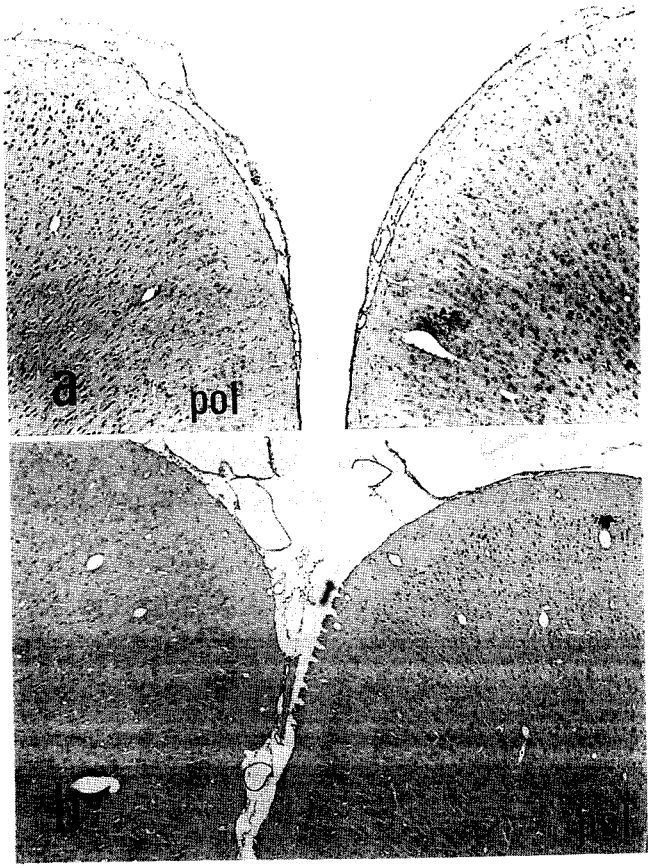

Fig. 4 Lateral differences in the number of dark neurons appearing 24h after the last trial of repeated polarization showing more dark neurons on the polarized side as compared with the contralateral hemisphere. **a**: polarization with 3.0μA for 3h; **b**: polarization with 30.0μA for 3h. pol: polarized hemisphere. Hematoxylin-eosin stain, × 125.

they were very scarce in the occipital cortex. Although the dark neurons appeared in both hemispheres, they were more abundant ipsilateral to the polarization under all polarization conditions tested (Fig. 4). Qualitatively, 3.0 μA and 30.0 μA caused a similar neuronal change, but a non-significant quantitative difference was observed in the number of the affected neurons ($P > 0.05$).

Time-dependent changes in number of dark neurons. There was almost no dark neuron in the rats 1, 6 and 12 h after the last trial of polarization. The dark neurons began to appear in the neocortex 24 h after the last trial, and the number remained almost constant until 72 h, irrespective of the intensity and duration of polarizing currents. Then the number of dark neurons decreased gradually until there were almost no dark neurons remaining 1 month after polarization (Table 1, Fig. 5). At 1 week after the last trial, the profiles of some neurons appeared normal in their shape and size, but the cytoplasm, nuclei and the processes in some cases were darker than their adjacent normal counterparts, representing a transitory form with volumetric restoration (Fig. 5c). At 2 weeks of recovery, there were more transitory type neurons than dark neurons (Fig. 5d). The density of the total neuronal population was compared

Table 1 Time course of the average number of total, dark and transitory neurons within an area of 300 × 300 μm of the cerebral cortex

Polarization condition and type of neuron	Cortical side	Time after last polarization trial				
		24 h	72 h	1 week	2 weeks	1 month
3.0 μA for 30 min						
Total	Left	397 ± 14.1	376 ± 16.9	421 ± 12.9	377 ± 15.1	390 ± 16.1
	Right	401 ± 13.9	380 ± 20.0	407 ± 11.7	384 ± 14.3	410 ± 15.9
DN	Left	91 ± 6.5 (22.8%)*	85 ± 5.9 (22.7%)*	60 ± 5.7 (14.4%)*	8 ± 0.70 (2.2%)*	0
	Right	16 ± 2.1 (4.0%)	11 ± 1.3 (3.0%)	10 ± 2.1 (2.5%)	2 ± 0.01 (0.4%)	0
TN	Left	0	0	23 ± 1.4 (5.4%)*	39 ± 1.7 (10.4%)*	0
	Right	0	0	6 ± 0.5 (1.4%)	17 ± 1.9 (4.5%)	0
3.0 μA for 3 h						
Total	Left	401 ± 17.5	438 ± 10.1	393 ± 17.1	423 ± 22.0	426 ± 17.1
	Right	408 ± 15.4	419 ± 11.2	406 ± 16.4	419 ± 19.7	429 ± 16.8
DN	Left	86 ± 7.4 (21.4%)*	92 ± 6.4 (21.2%)*	50 ± 3.1 (12.8%)*	7 ± 0.80 (1.6%)*	0
	Right	18 ± 1.3 (4.3%)	16 ± 1.4 (3.8%)	9 ± 1.0 (2.2%)	2 ± 0.02 (0.3%)	0
TN	Left	0	0	22 ± 1.7 (5.7%)*	39 ± 1.9 (9.2%)*	0
	Right	0	0	6 ± 0.9 (1.4%)	16 ± 1.7 (3.9%)	0
30.0 μA for 30 min						
Total	Left	397 ± 18.1	403 ± 12.8	402 ± 14.3	389 ± 17.5	399 ± 15.1
	Right	404 ± 17.6	397 ± 11.9	393 ± 18.9	394 ± 16.1	404 ± 16.0
DN	Left	96 ± 8.1 (24.1%)*	104 ± 9.2 (26.0%)*	61 ± 4.8 (15.1%)*	11 ± 0.90 (2.9%)*	0
	Right	17 ± 3.1 (4.3%)	11 ± 1.1 (2.9%)	10 ± 0.9 (2.6%)	2 ± 0.01 (0.3%)	0
TN	Left	0	0	25 ± 1.8 (6.3%)*	45 ± 3.6 (11.5%)*	0
	Right	0	0	6 ± 0.7 (1.6%)	19 ± 1.1 (4.8%)	0
30.0 μA for 3 h						
Total	Left	410 ± 17.7	407 ± 19.1	397 ± 12.9	401 ± 19.1	398 ± 11.9
	Right	405 ± 16.7	404 ± 20.2	403 ± 11.3	399 ± 18.7	397 ± 18.9
DN	Left	113 ± 7.9 (27.5%)*	111 ± 6.9 (27.3%)*	60 ± 5.4 (15.1%)*	8 ± 0.70 (2.1%)*	0
	Right	19 ± 1.4 (4.8%)	12 ± 1.3 (3.1%)	11 ± 1.1 (2.7%)	2 ± 0.01 (0.4%)	0
TN	Left	0	0	27 ± 2.4 (6.9%)*	45 ± 4.0 (11.3%)*	0
	Right	0	0	4 ± 0.3 (1.0%)	20 ± 1.8 (5.0%)	0

Total, DN and TN represent total, dark and transitory neurons, respectively.
Values are the number of neurons, expressed as the mean ± SEM of 5 or 6 animals.
Values in parentheses indicate the percent of the number of dark or transitory neurons to that of total neuron.
Asterisks (*) indicate statistically significant differences from the contralateral cortical side at $P < 0.001$.

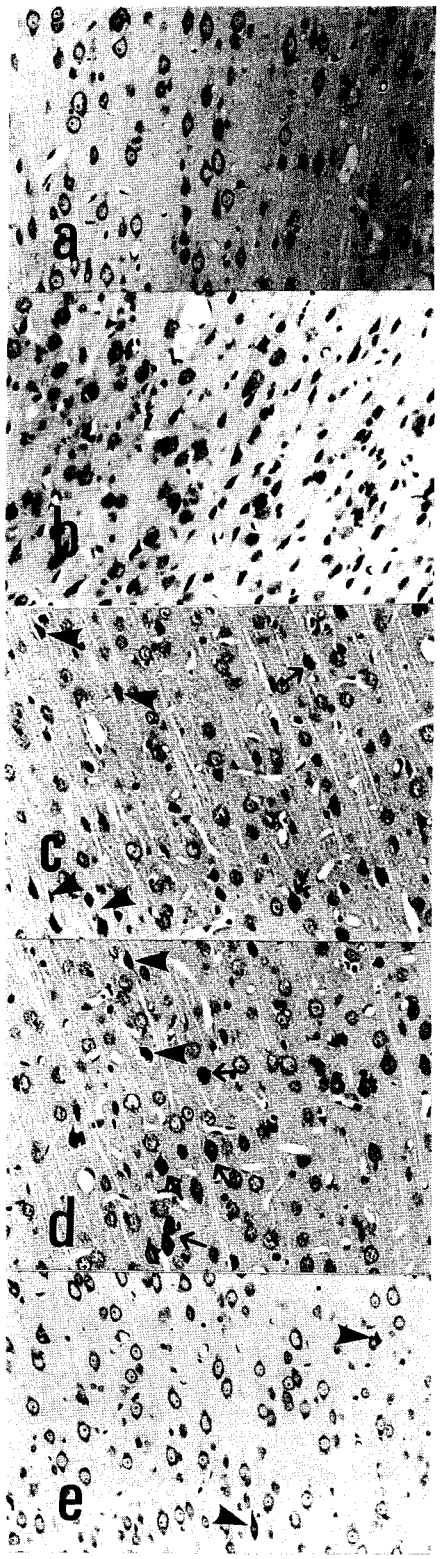

Fig. 5

with the control count (mean ± SEM = 399 ± 17.0/ 300 × 300 μm), but no apparent depopulation of cortical neurons was observed at any time interval studied ($P >$ 0.05, Table 1).

Discussion

The results of the present study reveal that dark neurons appeared 24 h after repeated anodal polarization of the unilateral sensorimotor cortex predominately in the polarized area of the neocortex, and then almost all of these neurons gradually reverted to their normal morphological profiles through a transitory state within 1 month. Interpretation of the appearance of dark neurons is probably one of the most controversial subjects in neurohistological experiments. Dark neurons have been attributed to a wide variety of causes (17–24), including physical trauma and poor fixation (27, 28). In the present study, the experimental procedures did not injure the brain directly. A careful perfusion technique was used and the brain was left undisturbed for 2–4 h following perfusion to avoid artifacts (27, 28). Our fixation as well as staining procedure is believed to be satisfactory based on following evidences: (a) no dark neurons were observed in our sham-operated control animals which were fixed and processed identically to the experimental animals; (b) the number of dark neurons gradually decreased over time after the last trial of repeated polarization; (c) although dark neurons were found in both hemispheres, obvious lateral differences in the number of dark neurons were present; and (d) the distribution of dark neurons in both frozen and paraffin-embedded sections was similar regardless of the staining techniques. These findings suggest that the dark neurons observed in the present experiments were not artifacts.

The mechanism of neuronal morphological alterations by polarization is not clear. Although no previous study describing neuronal morphology after anodal polarization is available for comparison with the present results,

Fig. 5 Histological changes in neurons in the ipsilateral neocortex after the last trial of repeated polarization with 30.0 μA for 3 h. **a**: non-polarized control; **b**: 72 h; **c**: 1 week; **d**: 2 weeks; **e**: 1 month. Arrowheads and arrows indicate the dark and transitory neurons, respectively. The number of dark neurons is gradually decreased with the appearance of transitory types. Hematoxylin-eosin stain, × 250.

several other histopathological studies (17-21, 23, 24) have shown that various kinds of stimuli cause the appearance of dark cells in different structures of the brain, which is thought to reflect some cellular functional changes, resulting in cellular damage or death. Regardless of the various stimuli, affected cells with common morphological alterations suggest that the mechanisms responsible for the appearance of dark cells ultimately share several features in common. According to the hypothesis of Siesjö (29), excessive influx of calcium ions into the cells following either ischemia, hypoglycemia or epileptic seizures induces a series of chemical reactions which alter the cell function; and which in turn produces the histologically characterized dark cells. Pathological changes in postsynaptic neurons have been suggested to be caused by excessive presynaptic release of excitatory neurotransmitter by electrical stimulation (17). Anodal polarization has been reported to influence migration of divalent cations and membrane resistance, as well as neuronal firing and neurotransmitter release (30). In our study, the most vulnerable neurons were in the middle cortical layers, which are credited with high calcium ion conductance in their apical dendrites (29). Therefore, repeated polarization may affect the intracellular calcium concentration and neurotransmitter ralease which results in neuronal hyperactivity leading to the appearance of dark neurons. This possibility seems to be evidenced by the fact that in the direct current electric field excessive influx of extracellular calcium followed by contraction of the cytoskeleton modifies the behavior of cultured amphibian neuronal crest cells (31).

In this study, a few dark neurons appeared in the neocortex contralateral to the polarization. Transsynaptic modulation has been reported to be involved in the increased cortical excitability by anodal polarization (32). Similar effects of anodal polarization have also been provided by neurochemical studies, in which cyclic AMP generation and protein synthesis are altered in the polarized cortex (13, 14, 16). These findings suggest that the effects of anodal polarization are not restricted to the polarized area, and the effects spread transsynaptically to the neighbouring neurons, even to distant neurons. The appearance of dark cells in deafferentated vestibular nuclei also represents a transneuronal phenomenon (24). Therefore, contralateral evolution of dark neurons may be due to transsynaptic activation through the callosal projections from the hyperactive ipsilateral cortex. In this context, it is not clear why no neuronal changes were observed in any other brain structures outside the cerebral cortex in the present study. There is good evidence for the contralateral spread of cortical activity through the callosal projection, such as in cortical epileptic activity (33), but little is known about its subcortical spread. The appearance of dark neurons in the restricted cortical areas, but not in the subcortical structures, following anodal polarization, therefore, may be related to unknown factors.

Earlier studies have shown that cytoplasmic shrinkage and hyperchromatic karyoplasm are reversible pathological changes in neuron of the central nervous system (19, 23). Hyperchromatic dark neurons with conspicuous mitochondrial swellings may also be compatible with the survival of the cells (19). As for neuronal survival, the present findings reveal that the number of dark neurons which appeared after repeated anodal polarization decreased gradually over time without marked total depopulation. These findings indicate that anodal polarization exerts recoverable, nonlethal effects on the cortical neurons. The increase in behavioral motor activity induced by anodal polarization has been shown to last for several weeks, in which case repeated polarization is necessary to establish stable motor activity (6, 9). Taken together, these findings suggest that the morphological and behavioral changes may occur concurrently during the repeated polarization trials, although there is a slight difference between their retention time. Therefore, the cumulative effects of cortical polarization may promote a neurochemical process which results in homeostatic alteration, the appearance of dark neurons, and increased behavioral activity. In this context, the changeover mechanism seems to play a role in the fundamental events for the increased behavioral activity, by which the dark neurons enter the recovery process. Thus, the appearance of dark neurons is probably an important index for evaluation of cortical plastic change induced by anodal polarization.

In conclusion, repeated polarization of the rat cerebral cortex was found to induce recoverable dark neurons, which seemed to reflect the increased cortical excitability. Thus, it is likely that repeated anodal polarization exerts recoverable effects on the morphology of cortical neurons, which is an important index for evaluation of central plastic changes induced by polarization.

Acknowledgments. We would like to thank Prof. A. Tokunaga for kind permission to use his laboratory facilities, Dr. K. Ono for advice regarding the histological methods, and Dr. K. Taguchi for helpful criticism.

References

1. Bindman LJ, Lippold OCJ and Redfearn JWT: Long-lasting changes in the level of the electrical activity of the cerebral cortex produced by polarizing currents. Nature (1962) **196**, 584-585.
2. Bindman LJ, Lippold OCJ and Redfearn JWT: The prolonged after-action of polarizing currents on the sensory cerebral cortex. Nature (1962) **162**, 45P-46P.
3. Morrell F: Effect of anodal polarization on the firing pattern of single cortical cells. Ann NY Acad Sci (1961) **92**, 860-876.
4. Novikova LA, Rusinov VS and Semiokhina AF: Electrophysiological analysis of closing function in the cerebral cortex of rabbit in the presence of a dominant focus. Zh Vyssh Nervn Deyat (1952) **2**, 844-861.
5. Hori Y and Yamaguchi K: Prolonged formation of a cortical dominant focus by anodal polarization. Med J Osaka Univ (1975) **26**, 27-38.
6. Lu Y-F, Hattori Y, Hayashi Y, Moriwaki A and Hori Y: Further characterization of cortical polarization-induced motor behavior in rabbits. Physiol Behav (1992) **52**, 1197-1200.
7. Sokolova AA and Bu KS: Electrophysiological study of the dominant area in the cerebral cortex of a rabbit produced by the action of a continuous current. Zh Vyssh Nervn Deyat (1957) **7**, 135-145.
8. Bindman LJ, Lippold OCJ and Redfearn JWT: The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. J Physiol (1964) **172**, 369-382.
9. Yamaguchi K and Hori Y: Long lasting retention of cortical dominant focus in rabbit. Med J Osaka Univ (1975) **26**, 39-50.
10. Rusinov VS: The Dominant Focus: Electrophysiological Investigations, Consultants Bureau, New York (1973).
11. Hayashi Y, Hattori Y, Moriwaki A, Asaki H and Hori Y: Effects of prolonged weak anodal direct current on electrocorticogram in awake rabbit. Acta Med Okayama (1988) **42**, 293-296.
12. Brazovskaya FA, Malikova AK and Pavlygina RA: After-effects of anodal polarization in the cat cerebral cortex. Neurofiziologia (1972) **4**, 194-199.
13. Gartside IB: Mechanisms of sustained increase of firing rate of neurons in the rat cerebral cortex after polarization: Role of protein systhesis. Nature (1968) **220**, 383-384.
14. Hattori Y, Moriwaki A, Pavlygina RA and Hori Y: Regional difference in the histamine-elicited accumulation of cyclic AMP in rabbit cerebral cortex with a cortical dominant focus. Brain Res (1983) **279**, 308-310.
15. Hattori Y, Moriwaki A and Hori Y: Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic-AMP in rat cerebral cortex. Neurosci Lett (1990) **116**, 320-324.
16. Moriwaki A: Polarizing currents increase noradrenaline-elicited accumulation of cyclic AMP in rat cerebral cortex. Brain Res (1991) **544**, 248-252.
17. Sloviter RS: Epileptic brain damage in rats induced by sustained electrical stimulation of the perforant path: I. Acute electrophysiological and light microscopic studies. Brain Res Bull (1983) **10**, 675-697.
18. Agardh C-D, Kalimo H, Olsson Y and Siesjö BK: Hypoglycemic brain injury: I. Metabolic and light microscopic findings in rat cerebral cortex during profound insulin-induced hypoglycemia and in the recovery period following glucose administration. Acta Neuropathol (Berl) (1980) **50**, 31-41.
19. Auer RN, Kalimo H, Olsson Y and Siesjö BK: The temporal evolution of hypoglycemic brain damage: I. Light and electron microscopic findings in the rat cerebral cortex. Acta Neuropathol (Berl) (1985) **67**, 13-24.
20. Brierley JB, Meldrum BS and Brown AW: The threshold and neuropathology of cerebral "Anoxic-Ischemic" cell change. Arch Neurol (1973) **29**, 367-374.
21. Brown AW, Brierley JB: The nature, distribution and earliest stages of anoxic-ischemic nerve cell damage in the rat brain as defined by the optical microscope. Br J Exp Pathol (1968) **49**, 87-105.
22. Dam AM, Hertz M and Bolwing TG: The number of hippocampal neurons in rats after electrically induced generalized seizures. Brain Res (1980) **193**, 268-267.
23. Ito U, Spatz M, Walker JT Jr and Klatzo I: Experimental cerebral ischemia in Mongolian gerbils. Acta Neuropathol (1975) **32**, 209-223.
24. Johnson JE Jr: The occurrence of dark neurons in the normal and deafferentated lateral vestibular nucleus in the rat: Observations by light and electron microscopy. Acta Neuropathol (Berl) (1975) **31**, 117-127.
25. Baker AB: Brain stem polarization in the treatment of depression. S Afr Med J (1970) **44**, 473-476.
26. Redfearn JWT, Lippold OCJ and Costain R: A preliminary account of the clinical effects of polarizing the brain in certain psychiatric disorders. Br J Psychiatry (1964) **110**, 773-775.
27. Cammermeyer J: The importance of avoiding dark neurons in experimental neuropathology. Acta Neuropathol (1961) **1**, 245-270.
28. Stensaas SS, Edwards CQ and Stensaas LJ: An experimental study of hyperchromic nerve cells in the cerebral cortex. Exp Neurol (1972) **36**, 472-487.
29. Siesjö BK: Cell damage in the brain: A speculative synthesis. J Cereb Blood Flow Metab (1981) **1**, 155-185.
30. Morrell F: Electrophysiological effects of direct currents. Neurosci Res Prog Bull (1974) **12**, 132-138.
31. Cooper MS and Keller RE: Perpendicular orientation and directional migration of amphibian neural crest cells in dc electrical fields. Proc Natl Acad Sci USA (1984) **81**, 160-164.
32. Gartside IB: Mechanisms of sustained increase of firing rate of neurons in the rat cerebral cortex after polarization: Reverberating circuits of modification of synaptic conductance. Nature (1968) **220**, 382-383.
33. Moriwaki A, Hattori Y, Hayashi Y, Islam N and Hori Y: Development of primary and secondary epileptic foci following intracortical injection of ferrous chloride solution in the rat. Neurosciences (1992) **18**, 151-155.
34. Fifkova E and Marsala J: Stereotaxic atlases for the cat. rabbit and rat; in Electrophysiological Methods in Biological Research, Bures, Petran and Zachar eds, Academia, Prague (1967) pp653-731.

Received December 17, 1993; accepted February 22, 1994.