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Cochlear ablation in *deafness* mutant mice: 2-deoxyglucose analysis suggests no spontaneous activity of cochlear origin

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Deafness mutant mice show no stimulus-related cochlear potentials as well as abnormal electrically-evoked responses recorded from the inferior colliculus. Abnormal spontaneous activity in the auditory periphery could result in abnormal development and/or maintenance of the central auditory pathways. We therefore assessed spontaneous activity of cochlear origin in the central nuclei of the mutants by ablating one cochlea and subsequently using the 2-deoxyglucose (2DG) technique to study metabolic activity. Any asymmetries in labeling in a given nucleus should be due to spontaneous activity in the cochlear nerve on the unoperated side. In control animals (+/dn mice undergoing unilateral cochlea ablation), statistically significant decreased 2DG labeling was observed in the ipsilateral PVCN and AVCN, and contralateral MNTB and IC; all receive primary excitatory input from the ablated ear. No significant differences in labeling between right and left sides were observed in any of the nuclei studied in the mutant animals. These findings suggest that there is no spontaneous activity of cochlear origin in these mutants, even though many cochlear nerve fibers and spiral ganglion cells survive.

Deafferentation; Genetic deafness; Central auditory system

Introduction

Altered auditory input during critical periods of development can result in profound anatomical and physiological abnormalities in the central auditory nuclei (e.g. Trune, 1982a,b; Born and Rubel, 1985; Sanes and Constantine-Paton, 1985). An understanding of how these abnormalities arise may be relevant in determining strategies for treating newborn and very young children with hearing impairment. Stimulus-related neural activity may influence normal sensory system development, but spontaneous activity may also be important. Spontaneous activity has been shown to influence the development of neural connections in the central visual system (Casagrande and Condo, 1988; Stryker and Harris, 1986; Dubin et al., 1986) and to influence the metabolism of central auditory nuclei in chickens and gerbils (Tucci et al., 1987; Born and Rubel, 1988; Robb et al., 1988; Pasic and Rubel, 1989). The aim of the study reported here is to assess the extent of spontaneous activity of cochlear origin in the central auditory pathways in the *deafness* mutant mouse.

Hereditary deafness is a major cause of profound hearing impairment in children, accounting for approximately 40–50% of cases (e.g. Fraser, 1976). The *deafness* mutant mouse has been the subject of a number of studies as a potential model for some forms of autosomal recessive deafness in humans. This mutant shows the neuroepithelial type of cochlear abnormality, with a primary organ of Corti defect (Steel and Bock, 1983). The hair cells show retarded development and extensive degeneration from about two weeks of age onwards (Bock and Steel, 1983; Pujol et al.,

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1983). Practically no recognizable hair cells remain by 6 months of age (Steel, unpublished observations). Nerve endings below inner hair cells are swollen and largely devoid of cytoplasmic contents in the newborn mutants and are degenerating by seven days of age. Outer hair cell synapses remain immature and degenerate early, so that very few are even recognizable by twenty days after birth (Pujol et al., 1983; Bock and Steel, 1983). Spiral ganglion cells are ultrastructurally abnormal at birth and progressively degenerate; half of them remain at 6 months, and only 21% are left at 16 months of age (Pujol et al., 1983; Steel and Bock, 1984; Webster, 1985). No cochlear microphonics or compound action potential responses can be recorded from the round window, even in young mutant mice before extensive hair cell degeneration has occurred (Steel and Bock, 1980; Bock and Steel, 1983).

The complete lack of stimulus-related cochlear nerve activity during the development and maturation of the central auditory pathways in *deafness* mutants might be expected to result in anomalies in central nuclei. Such anomalies have been found: electrical stimulation of the cochlear nerve produces *larger* responses in the contralateral inferior colliculus in the *deafness* mutant than in control mice (Steel and Bock, 1984). Single units in the contralateral inferior colliculus in the mutants show an abnormally larger mean number of spikes in response to electrical stimulation, and there is a greater preponderance of multiple-spike units compared with single-spike response units in the mutants than in the controls (Horner and Bock, 1984). No spontaneous activity was recorded in either mutants or controls in the single unit study, possibly because the cochlea had been destroyed so that the cochlear nerve could be stimulated directly. Destruction of the cochlea has been shown previously to eliminate cochlear nerve spontaneous activity (Born and Rubel, 1984; Koerber et al., 1966).

There are several reasons for investigating whether *deafness* mutant mice exhibit spontaneous activity in their auditory system. First, information about spontaneous activity in the cochlear nerve of *deafness* mutants would be helpful in interpreting physiological and anatomical anomalies seen in the central auditory pathways. Second, as spontaneous activity may be a correlate of some types of tinnitus, an enhanced level of such activity in the auditory brainstem of *deafness* mice might indicate that these mutants could be useful for studying tinnitus. Finally, it would also be interesting to know whether a cochlear nerve fiber can exhibit spontaneous activity in the absence of any apparently normal synapses with hair cells.

We used the 2-deoxyglucose (2DG) technique (Sokoloff et al., 1977) to assess spontaneous activity indirectly. The left cochlea and spiral ganglion were destroyed in order to eliminate any spontaneous activity unilaterally. Following 2DG exposure in a quiet environment, we examined 2-deoxyglucose labeling patterns in central auditory nuclei. In normal rodents, this procedure results in marked asymmetry in brainstem and midbrain auditory nuclei labeling (Woolf et al., 1983; Sasaki et al., 1980). Any such asymmetries in the mutant mice should be due to spontaneous activity in the cochlear nerve on the unoperated side.

Materials and Methods

Five mutant (dn/dn) and five littermate control (+/dn) mice between 6 and 7 months of age were the subjects of these experiments. Mice were obtained from a breeding colony maintained at the MRC Institute of Hearing Research (Nottingham, U.K.). The mice were not inbred but were maintained on a heterogeneous genetic background within a closed colony. The use of littermate +/dn mice as controls should minimize the risk of any systematic genetic difference between mutants and controls other than at the dn locus. Animals were raised with ad libitum access to food and water in a normal acoustic environment.

Animals in both groups were anesthetized with Nembutal intraperitoneally (0.1 mg/g body weight), supplemented with local topical application of Lidocaine. An incision was made behind the pinna, the bulla was opened, and the left cochlea was destroyed surgically, taking care to leave the stapedial artery intact. The incision was closed with cyanoacrylate glue, and the mice were allowed to recover.

Twenty-four h after cochlea ablation, each mouse was given a tail vein injection of 5 μCi of

^{14}C-2DG (2-deoxy-D-glucose, New England Nuclear, specific activity 45–55 mCi/mmol, suspended in 0.1 ml saline) and placed alone in a sound-attenuated chamber with forced air flow. Sound levels in the chamber, measured in 50 Hz frequency bands with a calibrated microphone and a B and K spectrum analyzer, were found to be 40 ± 5 dB SPL from 212 Hz to 20 kHz. One control (+/dn) mouse with unilateral cochlea ablation was exposed to 85 dB SPL white noise in a large IAC double-walled sound-attenuated booth.

After 45 min in either chamber, mice were deeply anesthetized with Nembutal and briefly perfused with 10% formalin buffered to pH 7.4 with 0.1 M phosphate. Brains were then removed from the heads, blocked and frozen in heptane cooled with liquid nitrogen. An average of 12 min elapsed between the beginning of the perfusion and the time at which the brain was frozen. Brains were stored at $-60°$ C until sectioning.

Brains were sectioned on a cryostat at $-15°$ C. A one-in-three series of 16 μm coronal sections through the brainstem auditory nuclei was thaw-mounted onto chrom-alum subbed coverslips heated to 55° C. Coverslips were mounted onto cardboard, placed inside X-ray cassettes, and exposed either to SB5 or XTL-2 film (Kodak). After exposures ranging from 5 to 15 days, films were developed with GBX developer and fixed with Rapid Fixer (Kodak). After acceptable X-ray films had been obtained, the sections were stained with thionin, dehydrated, and mounted onto slides with DPX (Gallard Schlessinger).

For all animals, glucose metabolism in various auditory nuclei was estimated by measuring the optical density (OD) in X-ray films (Sharp et al., 1983). Standard topographic locations within the dorsal, anteroventral and posteroventral cochlear nuclei (DCN, AVCN, PVCN), the lateral and medial superior olivary nuclei (LSO,MSO), the medial nucleus of the trapezoid body (MNTB) and the central nucleus of the inferior colliculus (IC) were chosen for measurement. For each brain, Nissl-stained sections were used to choose the corresponding X-ray films for measurement. Measurements for right and left auditory nuclei were always made from the same section. OD measurements were made using a Leitz Ergolux variable aperture microdensitometer. Aperture size was held constant for each nucleus and one measurement was taken for each nucleus. In each section from which auditory nuclei measurements were made, the OD of the nearby brainstem trigeminal nucleus also was measured on each side of the brain. A 'corrected' OD for each auditory structure measurement was calculated by dividing the OD of the auditory nucleus by the OD of the trigeminal nucleus on the same side of the brain. For each animal the ratio of the corrected OD for a given auditory structure on the right side of the brain to that on the left side was calculated. These ratios of ODs were averaged and examined for control and mutant animals. For statistical analysis, corrected optical densities and not right to left OD ratios were compared. First, a two-tailed paired *t*-test was used to compare the mean left and mean right corrected OD measurements in both mutants and controls, for each auditory nucleus measured. In addition, the mutant and control groups also were compared directly with each other; a two-tailed group *t*-test was performed to compare for each nucleus the difference in OD between left and right sides for mutants and controls.

Results

We examined specific nuclei in the auditory pathway for 2DG labeling patterns. For each nucleus, we looked for differences in labeling intensity between the two sides of the brain, indicating a difference in spontaneous activity. Fig. 1 shows stained sections and corresponding autoradiograms from control mice exposed in quiet (left two columns). In control mice, differences in labeling between the right and left sides of the brain can be seen in PVCN (Fig. 1e), AVCN (Fig. 1h), MNTB (Fig. 1h) and IC (Fig. 1k). In each case, labeling is reduced on the side of the brain receiving a major projection from the ablated cochlea.

The right column in Fig. 1 shows autoradiograms from the same auditory nuclei in *deafness* mice. No differences in labeling on the two sides of the brain were observed in the mutants (Figs. 1 c,f,i,l).

The results of densitometric analysis of autoradiograms from control and *deafness* animals

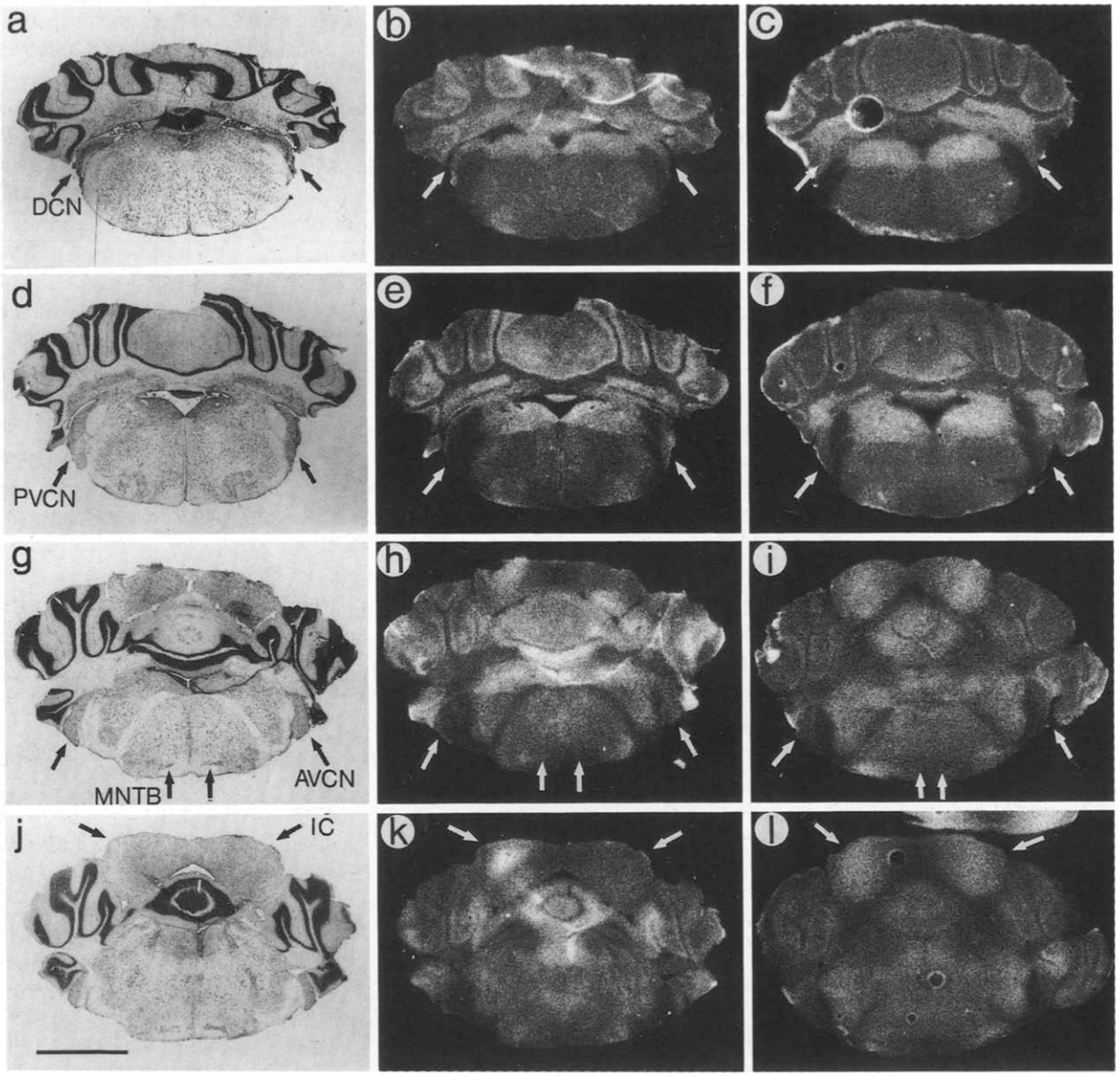

Fig. 1. Photomicrographs of Nissl-stained sections through the brainstem auditory nuclei and their corresponding autoradiograms from a control animal (left two columns) and autoradiograms only from the same nuclei from a *deafness* animal (right column). All autoradiograms have been photographically reversed so that white areas indicate regions of increased labeling. In each panel, arrows show the location of the auditory nuclei identified in Nissl-stained sections. At each level, nuclei examined were: (a, b, and c) DCN; (d, e, f) PVCN; (g, h, i) AVCN, LSO, MSO, MNTB; and (j, k, l) IC. In both animals, the left cochlea was ablated. Note reduced 2DG labeling in the left PVCN and AVCN and right MNTB and IC of the control animal. No labeling asymmetry is apparent in the *deafness* animal.

exposed in quiet are shown in Fig. 2. For each auditory nucleus, the average right to left corrected OD ratios are shown for control and *deafness* animals. The quantitative results confirm our qualitative impressions. In *deafness* animals, average ratios for all nuclei were close to unity, indicating no change in labeling following cochlea removal. In control animals, ratios for PVCN,

TABLE I

RESULTS OF TWO TYPES OF STATISTICAL ANALYSES DONE ON THE CORRECTED OD VALUES FOR EACH NUCLEUS

In the first analysis, average values for corrected OD on the left side of the brain and the right side of the brain were compared in each nucleus for both mutant and control animals. A 2-tailed paired *t*-test was used for the left vs. right comparisons. For dn/dn animals (first line), no left vs. right comparisons were significantly different. In the second analysis (bottom line), for each nucleus the differences between left and right corrected OD were obtained for each animal, averaged within groups and the averages for mutants vs. control animals were compared using a 2-tailed group *t*-test. Reliable differences were observed in PVCN, MNTB and IC.

TYPE	DCN	PVCN	AVCN	MNTB	MSO	LSO	IC
dn/dn L vs. R	NS	NS	NS	NS	NS	NS	NS
+/dn L vs. R	NS	**	*	*	NS	NS	**
dn/dn L-R vs. +/dn L-R	NS	***	NS	*	NS	NS	***

NS = Not significant.
* = Significant at $P < 0.05$; ** = Significant at $P < 0.01$; *** = Significant at $P < 0.001$.

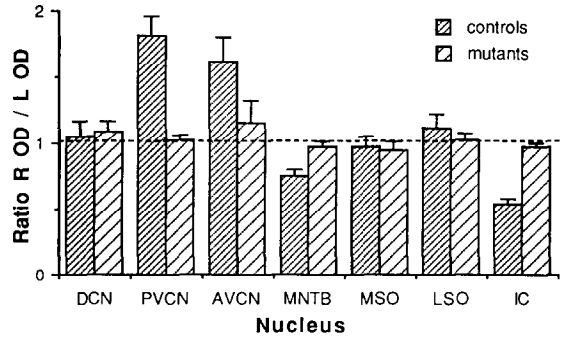

Fig. 2. Graph showing ratios of right corrected OD divided by left corrected OD for auditory nuclei in *deafness* and control animals exposed in quiet. Average ratios ± S.E.M. are plotted; dotted line indicates a ratio of 1. Note that ratios for mutants are near unity.

AVCN, MNTB, and IC indicated a decrease in glucose incorporation for ipsilateral PVCN and AVCN and contralateral MNTB and IC.

Statistical analyses of corrected OD data are shown in Table I. In *deafness* animals, differences between corrected OD in the right and left nuclei were not significant. For control animals, the right side OD was significantly higher in PVCN and AVCN and lower for MNTB and IC. When average right-left differences for *deafness* animals are compared to those for control animals, the two groups of mice differ significantly for measurements from PVCN, MNTB, and IC. Small sample size and higher variability of differences in AVCN probably account for the lack of significance when average left to right differences are compared.

The OD ratios for one control (+/dn) animal exposed to white noise during 2DG administration are DCN — 1.63, PVCN — 2.71, AVCN — 1.73, MNTB — 0.56, MSO — 0.69, LSO — 1.01, and IC — 0.47. The results are qualitatively similar to those seen for animals exposed in quiet. However, in the control animal the ratios appear more divergent from unity, and two additional nuclei appear to show changes: DCN shows a decrease ipsilateral to ablation and MSO a decrease contralateral to ablation.

Discussion

Controls

Previous studies in mammals (Sasaki et al., 1980; Woolf et al., 1983) and birds (Lippe et al., 1980; Heil and Scheich, 1986) have examined the effects of cochlea removal on 2DG labeling in the auditory system. Our results in control animals are in general agreement with these studies. Decreases in 2DG labeling are observed in those portions of the auditory pathway known to receive input from the ablated cochlea (see Webster and Aitkin, 1975

for a review). The only exception to this pattern was DCN. In animals exposed in a quiet environment, no differences were seen between left and right sides, despite a direct projection from the ipsilateral cochlea. DCN does receive inhibitory descending input (e.g. Palmer, 1987) which would presumably be unaffected by ipsilateral cochlea removal. This inhibitory activity would contribute to 2DG labeling in DCN (Nudo and Masterton, 1986) and may mask any decreases in eighth nerve input. However, Woolf and colleagues (1983) did observe a change in DCN 2DG uptake in the gerbil after cochlea removal for exposure in both quiet and noise. Minor differences in the organization of the DCN between mice and other species (Martin, 1981; Webster and Trune, 1982) may explain this observation.

The single control animal stimulated with 85 dB noise demonstrated labeling patterns similar to those seen following exposure to a 40 dB environment, but differences between the two sides of the brain appeared more pronounced. These results are consistent with experiments showing increased 2DG uptake in auditory neurons with increasing stimulus intensity (Sharp et al., 1981; Canlon and Schacht, 1983; Ryan et al., 1985). At this higher intensity of stimulation, DCN does show increased labeling contralateral to the ablated cochlea.

Mutants

The main aim of the study reported here was to assess the extent of spontaneous activity of cochlear origin in the *deafness* mutant mouse. There were no significant differences in OD values between left and right sides in any of the auditory nuclei studied in *deafness* animals. It is reasonable to interpret this observation as evidence that there is no spontaneous activity of cochlear origin measurable with 2DG in the auditory system of these mutant mice. This interpretation is based on several assumptions: (1) that cochlea ablation eliminates spontaneous activity in the cochlear nerve (Koerber et al., 1966; Born and Rubel, 1984); (2) that the elimination of spontaneous activity persists for the survival time of 24 h used in these experiments; and (3) that there is no stimulus-related cochlear nerve activity in *deafness* mutants (Steel and Bock, 1980) such that any asymmetry in activity would reflect spontaneous activity associated with the intact cochlea. Experimental evidence indicates that these assumptions are warranted.

An additional assumption we made concerns the use of 2DG uptake as an indirect measure of electrical activity (Sokoloff, 1977; Serviere and Webster, 1981; Schoppmann and Stryker, 1981; Serviere et al., 1984). The relationship between 2DG labeling and electrical activity is complex (e.g. Nudo and Masterton, 1986). For example, it has been a common observation in 2DG studies that absolute 2DG labeling is high in auditory regions such as inferior colliculus (e.g. Sokoloff, et al., 1977; Ryan et al., 1985). However, spontaneous activity levels are generally believed to decrease as one moves from caudal to rostral levels in the central auditory pathway (Ryan et al., 1984). Therefore, absolute levels of 2DG uptake probably reflect general metabolic activity as well as electrical activity. In our experiments, 2DG labeling greater than other brainstem areas was observed in IC, even in mutant mice. One interpretation of this observation would ascribe spontaneous electrical activity in IC to sites other than the cochlea. Direct correlation between electrophysiological measurements of spontaneous activity and 2DG labeling in the central auditory nuclei of *deafness* mutants would be needed to clarify this issue.

Two other reasons for our interest in spontaneous activity in *deafness* mutant mice were the possible use of these mutants as a tinnitus model and to resolve whether eighth nerve spontaneous activity is possible in the absence of normal hair cell synapses. The apparent absence of spontaneous activity in the *deafness* mutants suggests that they probably do not show that type of tinnitus which has been suggested to be associated with increased spontaneous firing rates. Our observations also suggest that there is no spontaneous activity in the absence of hair cells and their synapses, even though many cochlear nerve fibers and spiral ganglion cells survive.

There may be differences in the connectivity of neurons between mutants and controls as a result of the sensory deprivation during development, which might explain in part the finding of a larger gross evoked response in IC in the mutants. However, individual inferior colliculus units have also

been found to have a larger mean spike rate in response to electrical stimuli than controls (Horner and Bock, 1984). It is not clear why neurons with no apparent spontaneous activity should show a greater driven activity in these mutants; in normal animals, cochlear nerve or cochlear nucleus neurons generally show larger driven firing rates if they have a higher spontaneous rate, at least at moderate sound intensities (Liberman, 1978; Palmer, 1987). It may be that those neurons which would normally have a high firing rate are the only ones to survive in the mutants, which would change the population of inferior colliculus neurons available for sampling. This explanation is one of many possibilities for greater than normal evoked activity. Other possible explanations include increased stores of neurotransmitter or some change in inhibitory inputs to these neurons.

The *deafness* mutant has been used to study the effects of total auditory deprivation throughout development on both the anatomy (Webster, 1985) and physiology (Steel and Bock, 1984) of the central auditory system. We chose to examine the mutants at 6 months of age because at this age the central auditory neurons seem to be particularly responsive to electrical stimulation (Steel and Bock, 1984). Our results show that at this age, there is neither spontaneous activity nor stimulus-related activity in central auditory nuclei. It would also be useful to know whether there is spontaneous activity in younger *deafness* mutants. Absence of any electrical activity during development might be expected to have more serious consequences than simply absence of stimulus-related activity (Tucci et al., 1987; Casagrande and Condo, 1988; Stryker and Harris, 1986; Born and Rubel, 1988; Robb et al., 1988; Pasic and Rubel, 1989).

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