Ultrastructural effects of monocular deprivation in the neuropil of nucleus rotundus in the zebra finch: a quantitative electron microscopic study

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(Accepted 22 July 1986)

Key words: Synapse; Visual system; Bird; Monocular deprivation; Quantitative analysis

INTRODUCTION

The visual system of mammals has been widely used to study the influence of the environment on the normal course of neuronal development. It has been shown for several mammalian species that various forms of visual deprivation in early life can alter physiological, morphological and behavioral status of the animal as reviewed by Blakemore, Fregnac and Imbert, Movshon and Van Sluyters, and Sherman and Spear. At the ultrastructural level, for example, monocular deprivation affects neuronal circuitry in cats by changing the synaptic density. Disturbance of the establishing neuronal circuitry by depriving an animal of visual input also results in alterations of even more subtle synaptic features like the number of synaptic vesicles, the presynaptic terminal size of specific types of synapses, and number and size of synaptic grids.

In birds, the other homioothermic class of vertebrates, none of these ultrastructural features has been studied so far. Furthermore, very little is known about the effects of monocular deprivation in the visual system of birds. In birds, in contrast to mammals, retinal fibers from one eye entirely cross to the contralateral hemisphere. In one of the two visual systems, the so-called tectofugal pathway, information conveyed from one eye remains on the contralateral side. In the second system, the so-called thalamofugal pathway, however, recrossing fibers have been demonstrated. This provides binocular input for the visual wulst, the terminal of the thalamofugal pathway. Effects of monocular deprivation have been demonstrated by electrophysiological and histological methods.
physiological techniques in the visual wulst of the owl and by the autoradiographic [14C]2-deoxyglucose method in the pigeon. In the zebra finch effects of monocular deprivation on neuron size in the higher relay stations of the tectofugal pathway have been described. In the present study, we investigated the effect of monocular deprivation on ultrastructural parameters such as density of synapses, length of postsynaptic thickenings, and sizes of presynaptic terminals in the thalamic relay station of the tectofugal pathway. This pathway was chosen since, as a consequence of the lack of binocular input, no binocular competition should occur. As most of the deprivation studies in mammals were done on altricial animals like rats, cats, and rabbits, in which the visual system mainly develops postnatally, it seemed most promising to choose an altricial bird like the zebra finch.

**MATERIALS AND METHODS**

A total of 23 zebra finches (Taeniopygia guttata castanotis) of both sexes and different ages from the institute's stock were used for this study. Seven zebra finches were monocularly deprived shortly after hatching before natural eye opening. The animals were perfused at the age of 20 days (n = 4) or 100 days (n = 3). Sixteen zebra finches of different ages (1 day, n = 3; 5 day, n = 5; 10 day, n = 3; 20 day, n = 3, 100 day, n = 2) reared under normal conditions were used to compare the developmental stages of monocularly deprived birds with normal values.

**Deprivation technique**

In zebra finches the rim of the eyelids gradually develops during the first 10 days postnatally. This development can be prevented by applying a liquid adhesive plaster (Nobecutan) shortly after hatching on the closed eye in daily intervals. In addition the eyes were covered by black eyeliner to minimize incidence of light through the closed eyelids. In all experiments the left eye was covered.

**Tissue processing**

The animals were deeply anaesthetized and perfused via the left ventricle with 0.1 M sodium cacodylate buffer followed by a 3% formaldehyde–glutaraldehyde fixative. The brains were cut at 100 μm on an Oxford vibratome and nucleus rotundus was dissected with help of binocular optics from the 100-μm sections. The tissue pieces were postfixed in 1% Oso4-solution for 2 h at 4 °C, washed in buffer, dehydrated, and embedded in Epon 812. The ultrathin sections, cut with an Reichert ultramicrotome, were mounted on 300-mesh grids, double-stained with ethanolic uranyl acetate (2%), followed by concentrated lead citrate, and examined in a Hitachi H 500 electron microscope.

**Orientation in the electron microscope**

Semithin sections (1 μm) were cut from the Epon blocks and stained with Toluidine blue. The outline of one representative section from each block and the border of the nucleus rotundus were drawn using a drawing tube attached to a Zeiss light microscope. At the same magnification the bars of a 300-mesh grid were drawn onto a transparency. In order to locate a particular region of an ultrathin section in the electron microscope, the transparency was positioned on the outline drawing according to the image displayed in scan position, thus providing a coordinate system. For this procedure it is essential that the ultrathin sections are mounted exactly parallel to the grid bars.

**Data collection**

Electron micrographs at 7000 x were made systematically throughout the center of nucleus rotundus. For ultrastructural stereological analysis of subcellular components, prints are usually prepared at final magnifications of 15,000–30,000 x. The final magnification of our prints was 24,000 x. Photographs were taken exclusively from the neuropil; somata, capillaries, and myelinated axons were ignored. Fig. 2 shows a typical micrograph of the neuropil of nucleus rotundus. A synapse was identified by its presynaptic terminal with accumulation of synaptic vesicles near the presynaptic membrane, its postsynaptic structure which mostly exhibits a characteristic postsynaptic density (PSD), and a clearly visible synaptic cleft separating the pre- and postsynaptic element. The vesicles associated with synapses were spheroidal or pleomorphic. Synapses with flattened vesicles were rarely observed. No attempt was made to classify synapses according to their vesicle type. All synapses identified according to the criteria described above were marked on the micrographs.
used for final measurements. Synapses were only counted if the presynaptic terminal and the paramembranous specialization were completely within the micrograph plane. In 20-day-old zebra finches, monocularly deprived at hatching, a total of 1009 synapses on an area of 5313 μm² were counted and measured on a graphics tablet (Digikon) connected to a microcomputer (Digital equipment, PDP 11). In adult birds 730 synapses on an area of 4000 μm² came into quantitative evaluation. The length of PSDs were also determined with the graphics tablet. All micrographs were coded and the code was not broken before the end of all measurements.

Statistics

The distribution of the sizes of presynaptic terminals, i.e., area of axon endings in cross section, and the lengths of postsynaptic thickenings (PSDs) were plotted in a histogram. The profile-size frequency histogram of synapses in the deprived nucleus rotundus of adult zebra finches, monocularly deprived at hatching, is illustrated in Fig. 1 as a typical example for the size distribution of synapses (see also Nixdorf and Bischof35).

Fig. 1 clearly shows that the data are not distributed normally. This is also proved by the χ² test at the 0.1% level41. Therefore, we used a non-parametric test procedure, the Mann–Whitney U-test, for quantitative analysis. This test procedure is sensitive to differences of the median values, less sensitive to different skewness, and not affected by variation of the variance39.

Synapse number, size of presynaptic terminals, and length of PSDs were evaluated from the micrographs. The data of each brain were tested for a right/left-asymmetry (i.e., deprived vs non-deprived nucleus rotundus). Then the data of all brains were pooled according to age (20 and 100 days) and hemisphere (deprived and non-deprived) and were tested again. Monocularly deprived birds were also compared to normally reared zebra finches. The development of the above mentioned parameters in normally reared birds has been published in detail35. Therefore, not only differences between the deprived and non-deprived rotundus have been quantified, but also the developmental stages in experimentally treated birds compared to those in normal development.

RESULTS

Density of synapses

Synaptic density, as used here, represents the actual number of synapses observed in a given area of tissue. Mean and median of the distribution of number of synapses do not differ within each experimental group. Therefore, a Gaussian distribution of the number of synapses can be expected in the investigated area. On the deprived side of 20-day-old birds, the mean synapse number was determined to be 190 synapses per unit area (1000 μm²); for the non-deprived side synapse density amounted to 189 synapses per unit area. In the neuropil of nucleus rotundus in 20-day-old monocularly deprived zebra finches synaptic density in both deprived and non-deprived nucleus is 35% higher (P < 0.001) than in zebra finches reared without monocular deprivation (Fig. 3a).

In adult zebra finches (100 days) hypertrophy of synapse number in the deprived rotundus remains, whereas the non-deprived side returns to normal values (Fig. 3b). All 3 animals with monocular deprivation exhibited the same trend of having more synapses per unit area on the deprived side than on the non-deprived. The differences between the two sides were 17%, 23%, and 67%, respectively. The mean number of synapses per unit area amounted to 208 in the deprived rotundus, and 156 in the non-deprived
Fig. 2. Electron micrograph representing typical neuropil of nucleus rotundus of the zebra finch brain. This photograph was taken from the deprived nucleus of the 20-day-old bird, monocularly deprived at hatching. There are no obvious qualitative differences compared to the non-deprived side, nor to normally developed material. Some axon endings (At) making synaptic contacts with the dendrite, are marked. The length of the PSD is indicated by two arrow-heads. Note the regular appearance of the spheroidal vesicle type in nearly all terminals. At, axon terminal; den, dendrite; mit, mitochondria; mvb, multivesicular body; PSD, postsynaptic density; sER, smooth endoplasmic reticulum; sv, synaptic vesicles.
rotundus. In zebra finches reared under normal conditions synaptic density was 147 synapses per unit area. Therefore, synaptic density on the deprived side of the experimental animals is 41% higher than in normally reared birds ($P < 0.001$). No significant difference in synaptic density was detected between the non-deprived rotundus and the rotundus of zebra finches reared under normal conditions.

**Presynaptic terminal size**

In 20-day-old zebra finches the presynaptic terminals of the deprived nucleus rotundus were smaller by 8% than those of the non-deprived side (0.370 $\mu m^2$ vs. 0.401 $\mu m^2$, Figs. 4a, 5). This difference proved to be significant ($P < 0.02$), although one of the 4 birds did not show this result. In contrast, the 13% difference in the median value of presynaptic terminal size between non-deprived and normal rotundus proved to be not significant ($P > 0.3$, Fig. 4a). The median value of presynaptic terminals in zebra finches without monocular deprivation measures up to 0.461 $\mu m^2$. Thus, a difference of 20% was observed compared to the deprived rotundus ($P < 0.02$).

During normal development presynaptic terminals grew until the 20th day posthatching, when they reach adult values$^{35}$. Since the presynaptic terminals in the deprived rotundus did not reach the normal value, the median was tested against younger stages to determine at which stage growth of presynaptic terminals of the deprived nucleus comes to a stop. The median value of the presynaptic area of the deprived nucleus (0.370 $\mu m^2$) proved to be significantly larger compared to 1-day- (47%, $P < 0.0001$) and 5-day- (34%, $P < 0.0001$) -old zebra finches. No differences was detected in comparison to normally reared 10-day-old zebra finches (1%, $P > 0.3$), indicating that the development of the size of presynaptic terminals stops at about 10 days posthatching in the deprived rotundus. This suppression of development lasts until adulthood.

In 100-day-old zebra finches reared with monocular deprivation from hatching the median value of the presynaptic terminal size in the deprived nucleus amounted to 0.357 $\mu m^2$, thus being 18% smaller compared to the non-deprived side (Fig. 4b, 5). This difference proved to be highly significant ($P < 0.001$), and all 3 birds showed the same trend of smaller presynaptic terminals on the deprived side than on the non-deprived (i.e. 7%, 17% and 30%, respectively).
Fig. 5. Profile-size frequency histogram of presynaptic terminals in the neuropil of nucleus rotundus in juvenile (20-days, top) and adult (100-days, bottom) zebra finches monocularly deprived at hatching. Presynaptic terminals on the deprived side (lined area) are significantly smaller in juvenile (8%, top) and adult (18%, bottom) zebra finches than those on the non-deprived side (white area).

The median values of presynaptic terminal size of the deprived rotundus were distributed within a very small range (0.353, 0.358, 0.360 \( \mu \text{m}^2 \)). The presynaptic terminal size in non-deprived areas, on the other hand, spread more widely (0.389, 0.430, 0.505 \( \mu \text{m}^2 \)) with the median value of 0.436 \( \mu \text{m}^2 \). No difference was detected to the value measured in normally reared birds (P > 0.4). This median of presynaptic terminals amounts to 0.436 \( \mu \text{m}^2 \).

Length of PSD
In 20-day-old zebra finches no significant difference in the length of PSD was observed between the two sides of monocularly deprived animals (P > 0.5) or compared to the median value of 20-day-old normally reared birds (deprived vs normal, P > 0.3; non-deprived vs normal, P > 0.4).

During normal development the median length of PSDs decreases by about 14\% between hatching and adulthood (P < 0.005). The following median lengths of PSDs were observed in the different age groups in normally reared zebra finches: 1 day, 0.361 ± 0.017 \( \mu \text{m} \); 5 days, 0.335 ± 0.009 \( \mu \text{m} \); 10 days, 0.333 ± 0.011 \( \mu \text{m} \); 20 days, 0.315 ± 0.011 \( \mu \text{m} \); 100 days, 0.309 ± 0.005 \( \mu \text{m} \). Some of these values differ slightly, but not significantly from those published in a previous paper\(^3\), since additional data were used for the present study. The median lengths of PSDs during normal development were tested against the adult value at the age of 100 days (1 day vs 100 days: P < 0.005; 5 vs 100 days: P < 0.005; 10 vs 100 days: P < 0.03; 20 vs 100 days: P > 0.3). Hence, the main reduction in length of PSDs takes place during the first 20 days of life (13\%).

In 20-day-old monocularly deprived zebra finches the lengths of PSDs are 0.336 ± 0.006 \( \mu \text{m} \) (median ± S.E.M.) in the deprived nucleus and 0.330 ± 0.006 \( \mu \text{m} \) in the non-deprived nucleus (P > 0.5). Hence, the reduction of the length of PSDs is only 7\% and 9\%, respectively, compared to 13\% in normally reared birds. However, in monocularly deprived animals there is a further significant reduction between the age of 20 and 100 days (11\% in deprived nucleus, P < 0.04; 10\% in non-deprived nucleus, P > 0.04). The final length of PSDs in monocularly deprived adult animals is 0.298 ± 0.007 \( \mu \text{m} \) in the deprived and 0.298 ± 0.008 \( \mu \text{m} \) in the non-deprived nucleus (Fig. 6).

Similar to presynaptic terminal sizes, individual variance is small in deprived areas, with all values of PSDs within a range of 30 nm. The values for PSDs of normally reared zebra finches are also found close together, ranging from 0.300 \( \mu \text{m} \) to 0.332 \( \mu \text{m} \) with a median value of 0.309 \( \mu \text{m} \). Like in juvenile birds (20 days) no significant difference in the length of PSDs was observed between experimentally and normally reared zebra finches at the age of 100 days (P > 0.5).

Percentage of presynaptic terminals per unit area
During normal development presynaptic terminals
Fig. 6. Histogram showing the effects of monocular deprivation on the length of the PSD between 20 and 100 days of age in deprived, non-deprived, and normal nucleus rotundus. A significant reduction (P < 0.04) in the length of PSDs can be observed in experimentally treated birds (deprived, non-deprived), but not in normally reared zebra finches.

grow in size and synapses increase in number up to 20 days of age\textsuperscript{35}. Presynaptic terminal size and synaptic density constitute the 'synaptic space' that is available for a system. The percentage of occupation of neuropil by synaptic space per unit area increases during development under normal conditions. The calculations for the different age groups amount to a 9-fold increase of synaptic space during development. In 20-day-old monocularly deprived zebra finches the available synaptic space of the neuropil of the deprived nucleus rotundus amounts to 8.7\%, of the non-deprived area to 9.2\%. Twenty-day-old zebra finches with monocular deprivation have thus almost the same amount of synaptic space at their disposal as normally reared birds have at the age of 100 days (9.1\%)\textsuperscript{35}. In adult zebra finches no difference in the amount of synaptic space in the non-deprived rotundus (9.1\%) can be detected compared to normally reared birds. In the deprived nucleus 9.8\% of the neuropil were covered by the synaptic space, in the non-deprived side 9.1\%, and in normal material also 9.1\%. The deprived rotundus exhibits thus 0.7\% more synaptic space than in normally reared birds.

The observation that presynaptic terminals are small in deprived areas and, on the other hand, synaptic density is high, may indicate a certain correlation of these two parameters. It may be that, in adult birds, a smaller number of synapses can be compensated by a larger size of the single synapse and vice versa, so that the overall coverage of presynaptic terminal space is kept constant. A linear regression analysis of the median values of each age group of experimentally treated tissue confirms this assumption. The correlation factor was calculated to be $r = 0.965$.

DISCUSSION

The results of the present study demonstrate that visual input influences the development of synapses in the neuropil of nucleus rotundus in the zebra finch. Our observations do not differentiate between specific types of synapses. For that reason it is possible, that only some synapses are affected, while others stay unchanged. Therefore, this report only concerns general effects in ultrastructural changes of synaptic features such as synaptic density, presynaptic terminal size, and length of PSD in monocularly deprived birds. At the age of 20 days these parameters are mature in normally reared zebra finches\textsuperscript{35}, but in monocularly deprived birds this development is disturbed on both sides of the brain. In contrast, only the deprived nucleus rotundus remains affected by monocular deprivation in the adult zebra finch, whereas the non-deprived nucleus in adult experimentally treated birds shows normal values for synaptic density, presynaptic terminal size, and length of postsynaptic density.

Density of synapses

Comparing the influence of monocular deprivation on synaptic density our results are in good agreement with studies on rabbits\textsuperscript{47}. In both species the number of synapses per unit area increases as a consequence of monocular deprivation on the deprived side. In cats, the opposite effect appears, since synaptic density decreases on the deprived side (ref. 43, but see also refs. 52, 53). Vrensen and DeGroot\textsuperscript{47} demonstrated that the increase in synaptic density in the rabbit is restricted to the monocular segment of the retina. However, the explanation that this difference may be due to the degree of binocularity in the different species, which is high in cats and very low in zebra finches and rabbits, is ruled out by the fact that in rats, also a species with a small binocular field, synaptic density decreases on the deprived side\textsuperscript{58} as in cats and does not increase as in zebra finches and rabbits. It seems likely that binocular competition mech-
anisms similar to those obtained in cats do not play a role in the zebra finch tectofugal pathway. However, some unknown hemispheric interaction influences the effects of monocular deprivation in this species, as will be discussed later.

**Size of presynaptic terminals**

The presynaptic terminal size in monocularly deprived zebra finches remains small in the deprived rotundus. The non-deprived side develops normally in this respect. Presynaptic terminals of the deprived side in 20-day-old birds are significantly smaller than those of the non-deprived side or those of normal animals (Fig. 4). They are the same size as those of 10-day-old normal zebra finches. This retarded development persists until adulthood. Similar effects can be detected in other species\(^1,13,33,43,45,52,53\). In dark-reared rats, for example, presynaptic terminals remain small (4%) in the superficial layers of the visual cortex\(^13\). In monocularly deprived cats the size of presynaptic terminals is even more reduced, being 25% smaller compared to non-deprived terminals\(^43\).

As mentioned above, there exists a correlation between size and number of synapses such that small terminals are correlated with a higher synaptic density. A similar relationship between size and number of neurons has been reported by Cragg\(^15\) in the visual cortex of binocularly deprived cats. He found that neuronal somata are 8% smaller while neuronal density is 28% higher. A reverse relationship between presynaptic terminal size and synaptic density has been observed in the visual cortex and lateral geniculate nucleus (LGN) of dark-reared rats\(^13,14\). In the LGN of rats synaptic density decreases by 34% while synaptic terminals become up to 15% larger. A similar relation between size and number has also been described by Fifkova\(^18\) for monocularly deprived rats. Cragg\(^14\) postulated a regulating mechanism between size and number of synapses, when he suggested that the size of terminals might be a function of the number of terminals. Our results seem to support this suggestion.

**Length of PSD**

It is generally accepted that the postsynaptic density plays a crucial role in structural plasticity (for review see Siekevitz\(^22\)). In zebra finch brains monoculcular deprivation slows down the normal development of the length of PSDs in the neuropil of the deprived and non-deprived nucleus rotundus. The main reduction of length of PSDs does not take place during the first 20 days of life like in normally reared zebra finches; there is a further substantial reduction (> 10%) between the ages of 20 and 100 days in monocularly deprived birds (Fig. 6).

Length of contact zones have been measured in monocularly deprived monkeys\(^36\), dark-reared and monocularly deprived rabbits\(^46-48\), dark-reared rats\(^14\), monocularly deprived cats\(^45\), and in dark-reared chickens\(^9\). In none of these studies, except those of Bradley et al.\(^9\), changes in the length of PSDs after visual deprivation are reported. In dark-reared chickens the length of PSDs in the left hemisphere was shorter than on the right side. This asymmetry, however, disappears with further training. In visually trained rabbits, no change in length of PSDs was observed either\(^49\), although the thickness of PSDs increased. Rearing animals in enriched or impoverished environments or changing the animal's internal environment has substantial effects on the postsynaptic density in various species\(^5,17,21,31\). Visual deprivation, on the other hand, does not seem to affect the length of PSDs in adult animals. This seems to be in good agreement with our observations in adult zebra finches, where no effects of visual deprivation on the length of PSDs in the adult brain were detected. Our results, however, also demonstrate that monocular deprivation does indeed affect the PSDs during development in zebra finches. This effect, on the other hand, can only be detected in a study of the time course of deprivation. There are no data available to evaluate if this time-dependent deprivation influence is a common feature in other species, too.

**Hemispheric interaction**

The effects of monocular deprivation in adult birds might lead to the conclusion that only neurons of the deprived side are affected. However, examinations of birds at a younger age, e.g. 20 days, show that the nuclei of the non-deprived hemisphere are also affected. In juvenile birds, deprivation effects on both sides of the brain seem to be a general phenomenon detected at the light-microscopical and ultrastructural level\(^6\). Soma size measurements in both the thalamic relay station (nucleus rotundus) and the telen-
cephalon (ectostriatum) indicate hypertrophy of soma size after short term deprivation on both sides of the brain.$^{22,23}$ Furthermore, at the light microscopic level, we observed that the volume of the ectostriatum remains small on both sides of the brain in monocularly deprived young zebra finches (Nixdorf and Bischof, in preparation). At the ultrastructural level, synaptic density per unit area increases in the nucleus rotundus on both sides, and the length of the postsynaptic thickening shows a bilaterally retarded development. Similar effects on synaptic structure have been observed in the ectostriatum (Nixdorf and Bischof, in preparation), where, due to monocular deprivation, both sides of the brain are affected in juvenile birds. These observations indicate that there is some sort of interaction between the two sides of the visual system. This interaction can be demonstrated directly by electrophysiological recordings (Engelage and Bischof, in preparation).

Three possibilities of such interaction affecting synaptic development in monocularly deprived zebra finches can be discussed. First, in young birds transient ipsilateral projections can be demonstrated, which disappear later in life, but remain if the contralateral eye is removed.$^{28}$ We have not tested yet whether monocular deprivation affects possible transient ipsilateral projections. Second, two systems of recrossing fibers have been described for the visual system: a tectotectal and a tectocontralateral rotundal projection. These two projections have been stated to be very small.$^4$ However, autoradiographic studies in our research group demonstrate a considerable projection from the rectum opticum to the contralateral nucleus rotundus (Niemann and Bischof, in preparation). Therefore, one cannot exclude a substantial effect on the development of the contralateral nuclei. Third, the tectofugal system is influenced at different levels by the thalamofugal system, which is binocularly driven.$^{2,29}$ Again, it is possible that these projections contribute to the effects we see in the monocularly deprived zebra finches.

Our results do not allow to differentiate between these 3 possibilities. However, they demonstrate that there must be such an interaction and may stimulate further research in that direction. Moreover, they show that the effects of monocular deprivation are not uniform at different ages of the animal. Hence, the mechanism underlying the effects of monocular deprivation seems to be even more complicated than was thought before.

Further research will show whether the observed effects of monocular deprivation are restricted to certain types of synapses, e.g. inhibitory synapses or synapses belonging to a certain input to nucleus rotundus. Moreover, it will have to be examined whether the deprivation effects can be found in higher as well as in lower stations of the tectofugal pathway. Finally, it would be interesting to know whether other synaptic features such as presynaptic dense projections or presynaptic grids are affected by monocular deprivation, too. Experiments to investigate these problems are in progress in our laboratory.

ACKNOWLEDGEMENTS

We like to thank H. Stein for writing the computer programs and Prof. Dr. Willimczik for providing the computer equipment. We are grateful to Prof. Dr. Jockusch for the use of the Oxford vibratome. We also like to thank Prof. Dr. Cruse for his helpful discussion on the statistics part. Mr. R. Church’s improvement of the English is gratefully acknowledged. This work was supported by grants from the ‘Minister für Wissenschaft und Forschung des Landes Nordrhein-Westfalen’ and the Deutsche Forschungsgemeinschaft (Bi 245/2.3) to H.-J.B. and by a fellowship of the Studienstiftung des deutschen Volkes to B.N.

REFERENCES


44 Turlejksi, K. and Kossut, M., Decrease in the number of synapses formed by subcortical inputs to the striate cortex of binocularly deprived cats, Brain Research, 331 (1985) 115–125.  
45 Valverde, F., Rate and extent of recovery from dark rearing in the visual cortex of the mouse, Brain Research, 33 (1971) 1–11.  