neuroReport

NeuroReport 2, 711 – 714 (1991)

Rearing conditions affect neuron morphology in a telencephalic area of the zebra finch

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THE morphology of ANC (Archi-neostriatum caudale) neurons in zebra finches is affected by arousal and rearing conditions. Branching index and spine density of ANC neurons are decreased in isolated birds and enhanced in cage reared animals, compared to aviary reared animals. Chasing the birds around the cage, or seven days of social contact with a female, raises these indices in birds isolated until adulthood relative to those of the aviary reared animals. We conclude that branching index and spine density of ANC neurons are determined during development by the amount of social contact, arousal, and activation of ANC. The changes observed after short term treatments in adult bird may depend on the same factors.

Key words: Birds, Development, Neuronal plasticity, Forebrain, Associative areas, Spine density, Arousal, Golgi method

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Introduction

It is widely accepted that influences from the environment affect the shaping of the central nervous system during development of an animal. For example, increasing or decreasing the complexity of the visual environment during development leads to changes of morphological features of the whole cortex and single cortical neurons in rats.[1,2] Recent studies in birds have shown that changes like those described above are not only obtained in sensory areas, but also in regions of the forebrain which are likely to have more associative functions. Wallhäusser and Scheich,[3] for example, demonstrated that the spine density of neurons in the medial neostriatum/hyperstriatum is reduced in three-day-old chicks which are exposed for a short time to a mother hen (filial imprinting). Experiments with ^{14}C-2-deoxyglucose (2-DG) demonstrated that this area, like the two other forebrain regions, is activated in arousing situations like the above described exposure to the mother hen,[3,4] or if the chick is separated from its siblings.[5]

Such 'arousal' areas have also been demonstrated by ^{14}C-2-Deoxyglucose (2-DG) experiments in the zebra finch. Besides the three areas which have been found in the chick, a fourth area in the caudal archi-neostriatum (ANC) of the zebra finch is also activated in arousing situations, for example by chasing the birds around the cage for one hour. Activation of the four areas can be also demonstrated if a young zebra finch male, which has been isolated from day 40 (after being independent from the parents) to day 100 (when the bird is adult), is exposed to a female for one hour after being injected with 2-DG.[6,7]

This is a remarkable finding because we found in behavioural experiments that this exposure to a female is important for stabilization of the preference for females of the parent species, which the young males develop during the first 40 days of life.[8] We argue in this paper that a high arousal level, which has been shown in the 2-DG experiments, is important for the stabilization to occur.

In this study we were interested to see whether the morphology of neurons of the ANC is altered by short exposure to a female after some time in isolation. We also wanted to know whether rearing conditions affected ANC neuron morphology. If these experiments showed an effect, this would be the first demonstration of plastic changes in an adult bird. Moreover, it would be a first hint that alteration of the neuron morphology is in some way linked to neuronal activity.

Materials and Methods

A total of 17 male zebra finches (*Taeniopygia guttata*) were used for this study. The birds were reared under five different conditions: (1) 'Isolates': reared singly in a cage from day 40 (the day the fledglings are independent from their parents) to day 107 without visual and social contact ($n = 4$); (2) 'Cage': reared in a cage (80 × 10 × 30 cm) together with three other males and one female ($n = 3$); (3) 'Aviary': reared in an aviary (2 × 2 × 2.70 m) together with a flock of about 15 to 20 conspecific males and females ($n = 4$); (4) 'Unspecific arousal': reared like group 1, except that the birds were chased around the cage between day 100 and 107 for one hour a day ($n = 3$) and (5) '1 W ♀': reared like group 1, except that the birds were given contact with a female between day 100 and day 107 ($n = 3$).

At day 108 the birds were sacrificed with an overdose of Nembutal; 100 μm transverse sections were cut after processing the brains according to a modification of the Bubenaite Golgi method which

has been described elsewhere.[9] At least 5 fully impregnated ANC type I neurons (see results) from each bird were analyzed from drawings, made with the help of a drawing tube attached to a Zeiss microscope, at a magnification of ×1125 under oil. In addition to ANC, the same number of neurons from the acoustic field L of the forebrain were examined as a control.

The following parameters were calculated: the radius of the dendritic field, the branching index, and the spine density. The radius of the dendritic field was defined as the distance between the center of the soma and the tip of the longest dendrite. The branching index was calculated by dividing the number of free dendritic endings by the number of primary dendrites (the dendrites emerging from the soma). The spine density was expressed as the mean number of spines per 10 μm of dendritic length. For the measurements we selected only those of the most peripheral (terminal) sections of the dendritic tree, which were parallel to the surface. Terminal sections were chosen because previous studies show that these are most strongly affected by early experience.[3,9,10] These terminal sections were marked during drawing, the length measured with help of a graphics tablet, and the number of spines counted. The spines were counted without regard to their shape, and no correction was made for hidden spines.[11]

Because the number of neurons analyzed per bird varied, we first compared the values obtained from different birds. As no significant difference between birds could be found, the data were pooled. Differences between groups were then tested by Mann–Whitney U-tests.

Results

Four types of neurons can be identified within the ANC of male zebra finches. The classification of neurons depends on shape, size of the soma, thickness of the dendrites and spine density (Fig. 1). Only type I neurons will be described here, because only this neuron type was evaluated quantitatively.

The somata of type I neurons (Fig. 1, I) are 5–10 μm in diameter. Most of the 4–5 primary dendrites are orientated radially, arborize 3-4 times, and show a relatively low spine density. The dendrite diameter is about 1.2–1.4 μm at the primary sections, the radius of the dendritic field is about 100 μm. Most of the spines have a thin spine stem and a spine head. An axon with fine collaterals emerges from the soma. The same type of neuron can be identified within the acoustic field L.

The measurements of the dendritic field radius of type I neurons are depicted in Fig. 2a. No significant differences ($p \geq 0.4$) were obtained for the different rearing conditions either within ANC or field L, respectively. In each group (within ANC and field L) the radius of the dendritic field is between 90 and 100 μm.

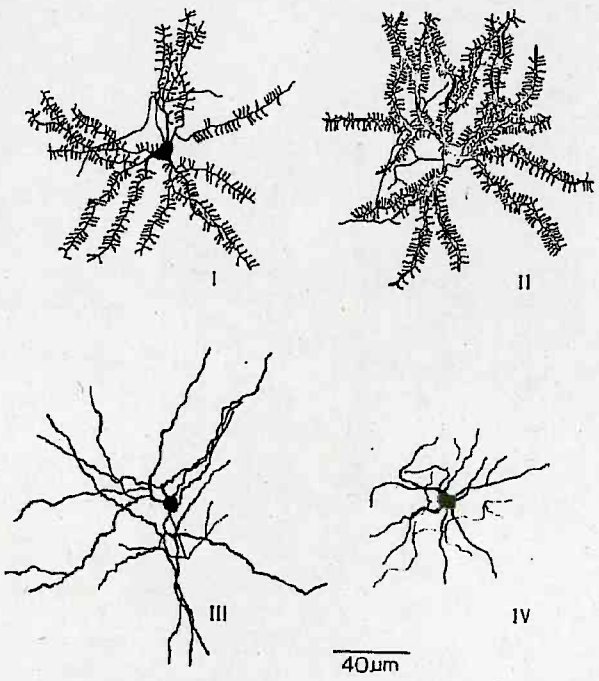

FIG. 1. The four types of neurons identified within ANC by Golgi techniques. Further explanation see text.

The means of the branching index for the different rearing conditions are shown in Fig. 2b. Again, no significant effects could be obtained in all experimental groups for field L neurons. The branching index of type I neurons ranges from 3 to 3.5 terminals per primary dendrite. Within ANC, neurons of isolated birds have significantly ($p \leq 0.0004$) fewer terminals per primary dendrite (2.01 ± 0.13) compared to cage or aviary reared birds (3.66 ± 0.10 and 3.61 ± 0.15, respectively). ANC neurons of birds which were chased around the cage for some time (after a period of isolation until day 100) or exposed to a female for 7 days, also have a lower branching index compared to cage or aviary reared birds (2.40 ± 0.14 '1 W ♀' and 2.18 ± 0.28 'Chasing'). The values obtained in these two groups are not significantly different from the isolated birds ($p \geq 0.5$).

Spine density measurements are shown in Fig. 2c. No significant effect ($p \geq 0.5$) could be obtained for spine density in field L neurons. Within ANC, neurons of isolated birds have significantly ($p \leq 0.005$) fewer spines per 10 μm than neurons of birds of the other groups. ANC neurons of birds which were chased around the cage after a period of isolation show an increase of spine density compared to isolated birds. An increase of spine density can also be seen in birds with 7 days contact with a female (after a period of isolation until day 100). The spine density of these birds is only slightly lower (n.s., $p \geq 0.5$) than the spine density of birds which were

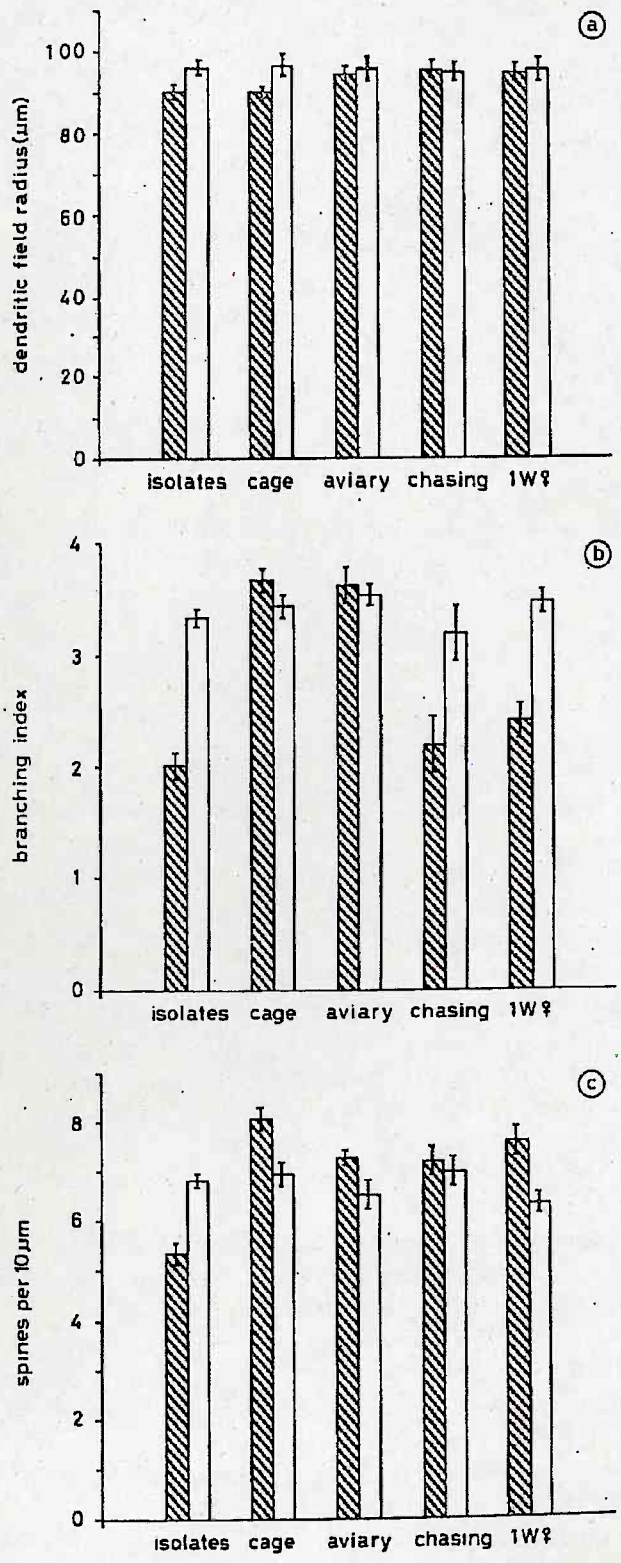

FIG. 2 Measurements of the dendritic field radius (a), branching index (b) and spine density (c) within ANC (hatched bars) and field L (open bars). Means and standard deviations. Abbreviations see methods section.

socially reared in cages. Aviary reared birds are significantly ($p \leq 0.0082$) lower in spine density than cage reared animals.

Discussion

Our experiments clearly demonstrate that ANC type I neuron morphology is affected by different rearing conditions, and that under certain circumstances the morphology of these neurons can be altered even in adult birds.

Concerning the direction of the changes, it is conceivable to regard the 'aviary' condition as the most natural one. The results of this rearing condition can therefore serve as a baseline for the estimation of the data. The number of branchings as well as the number of dendritic spines per 10 µm was lower in isolated animals than in aviary reared birds. Cage reared birds, in contrast, have ANC neurons with a higher density of spines than aviary reared animals. Social contact during development may therefore be necessary to reach a 'normal' spine density. This idea is supported for the following reasons. Firstly, the 'isolate' group in our experiments was isolated in single cages which allowed them to see the bird room environment, but no conspecifics. Acoustic contact between the birds of the colony was not prevented. Thus, the isolation was mainly from seeing social partners. Secondly, the only difference between the 'cage' group and the 'isolate' group was the number of birds per cage; both groups were housed in cages of the same type and dimension, and in both groups, birds in other cages could be heard, but not be seen. Thirdly, cage reared animals have most probably more social contacts than aviary reared birds, because they have less opportunity, for example, to avoid aggressive encounters, and the number of spines is also larger in the cage reared animals.

Our results further show that the effect of isolation is not necessarily permanent. If the birds were exposed as adults to a female or were chased around the cage an hour daily for one week, the spine density increased to values of aviary reared birds. Phenomena like this are quite common during development.[9] Retardation of development as a consequence of some sort of deprivation, (e.g. undernutrition), is quickly compensated if the deprived factor is provided. However, this is normally limited to periods of development of the animal. Our study shows that, at least partly, restoration of normal values is even possible in adult birds. The fact that this is only true for spine density, not for the branching index, shows that spine density is most easily affected by environmental changes. Whether the branching index will also rise, if the arousing conditions last longer than seven days, is at present under investigation.

Our idea that social contact is important for the development of normal spine density has to be revised slightly if one considers the fact that chasing the birds around the cage for seven days, one hour daily, has the same effect on spine density as exposing them to a female for a week. This treatment is

obviously no normal social contact, although it may belong to social interactions in the wider sense. We interpret this result as showing that it may be one endogenous component of social contact, namely the enhanced arousal level of the animals, which mediates the effect on ANC neuron morphology. As already mentioned in the introduction, Bischof and Herrmann[6,7] demonstrated with 2-DG experiments that the ANC, under exactly the same conditions which we used in these experiments ('chasing' and '1 week female'), showed very high activation. Taken together with the results of the present paper, these data suggest that the increase in spine density may be a direct effect of higher activation of this area.

The idea, that activation of a given area is a precondition for changes to occur, is supported by another result. In contrast to experiments on rats and other mammals[13] showing effects of differential rearing in sensory areas of the cortex, we could not demonstrate alterations within field L, the telencephalic end station of the acoustic pathway in birds. Preliminary results of ours show that a visual sensory area, the ectostriatum, is also not affected by rearing conditions. This indicates that sensory isolation *per se* cannot be the reason for the observed alterations. One therefore has to consider that ANC (and probably other non sensory telencephalic areas) react differently from areas which receive direct sensory input. Probably, this differential effect may be due to the necessity of additional activation from other areas for the changes to occur: ANC receives input from the reticular formation and other nuclei of the brainstem, which are thought to be involved in the control of the internal arousal level of the animal,[14] while ectostriatum and field L do not receive such input, and did not show different levels of activation in the 'isolate' or the 'exposure to a female' condition in the 2-DG-experiments.[6,7]

Renner and Rosenzweig[15] prefer to explain the differential effects of enriched and impoverished environments on cortical neurons by different opportunities to learn about the environment. We, as stated in the introduction, have found that during the seven days exposure to a female in the '1 W ♀' group, it is important to stabilize a previously labile preference for females of the parent species.[8] Such relationships between alterations of spine density and imprinting-like learning, have been reported several times.[3] However, we do not at present see how to prove whether this is a real correlation or just a coincidence.

Conclusion

We conclude from our experiments that branching index and spine density of ANC type I neurons are determined during development by the amount of social contact a bird receives. Social contact, in turn, affects the overall arousal level of the bird, and this might be the physiological mediator of the anatomical effects of the different rearing conditions. In addition, we conclude that alterations of spine densities in adult birds are also correlated with high arousal levels, and with alterations of the overall activity of ANC neurons: it may be tempting to relate arousal, neuronal activity, and spine density changes, which all occur at the same time in a distinct area of the forebrain of the zebra finch, to imprinting-like learning.

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ACKNOWLEDGEMENTS: Our thanks are due to Mrs. E. Geissler for preparing the figures. Supported by the Deutsche Forschungsgemeinschaft (Bi 245/3)

Received 19 July 1991;
resubmitted 29 August 1991;
accepted 31 August 1991