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AROUSAL ENHANCES [¹⁴C]2-DEOXYGLUCOSE UPTAKE IN FOUR FOREBRAIN AREAS OF THE ZEBRA FINCH

HANS-JOACHIM BISCHOF and KATHRIN HERRMANN

Lehrstuhl Verhaltensphysiologie, Fakultät Biologie, Universität Bielefeld, Bielefeld (F.R.G.)

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The activity pattern of the forebrain of male zebra finches was investigated by the [¹⁴C]2-deoxyglucose (2-DG) method in 4 different behavioral situations. (1) Sitting alone in the cage (control); (2) courtship by experienced birds; (3) first courtship of inexperienced birds (100 days of age), and (4) chasing the birds around the cage. The primary sensory areas (ectostriatum, field L) were active above background in each experiment. Vocal-motor control areas were at background activity (RA, HVc, MAN), or below background (area X), all unaffected by the type of experiment. In contrast, 4 different areas were active in Expts. 3 and 4, but not in 1 and 2: (a) part of the neostriatum intermedium; (b) part of the lateral neostriatum, both with adjacent parts of the hyperstriatum ventrale, (c) hyperstriatum accessorium and hyperstriatum dorsale, (d) a portion of the caudal neo/archistriatum. It is concluded that the enhanced activity of these areas is not due to distinct external stimulation or enhanced motor activity, but correlates with high arousal levels of the animals.

INTRODUCTION

In a recent paper Maier and Scheich¹⁶ demonstrated that acoustic imprinting leads to differential [¹⁴C]2-deoxyglucose (2-DG) uptake in the chick forebrain. Three rostral areas showed an increased 2-DG level in imprinted chicks compared to naive animals. These 3 areas were the so-called visual wulst, an area of the medial neostriatum/hyperstriatum ventrale, and another area of the lateral neostriatum/hyperstriatum ventrale. The 2-DG experiments were carried out after the imprinting procedure, and Maier and Scheich concluded that these areas need not necessarily be involved in the storage process, but in the processing of important acoustic stimuli. These stimuli gain their behavioral importance in the imprinting process, and, as a consequence,

lead to enhanced attention and arousal of the animal.

Courtship behavior in zebra finches is stimulated by the sight of a female³. The features of this stimulus are learnt in early development (sexual imprinting)¹¹. Thus, a certain stimulus gains its behavioral importance by the imprinting process, like in the acoustic imprinting paradigm provided by Maier and Scheich. If similar physiological mechanisms and anatomical locations are involved in both cases, one should find comparable activity patterns in the male zebra finch brain in situations with a female as a courtship-eliciting stimulus. We tested this hypothesis with the 2-DG method according to Sokoloff et al.²².

Behavioral experiments (Immelmann, unpublished results) indicate that zebra finches can alter their previously acquired preference for a

Correspondence: H.-J. Bischof, Lehrstuhl Verhaltensphysiologie, Fakultät Biologie, Universität Bielefeld, Postfach 8640, 4800 Bielefeld 1, F.R.G.

sexual partner during the first courtship sequence of its life, but not in later courtship events. Therefore, imprinting-like learning might be involved in the first courtship in contrast to subsequent courtship events. We wanted to know whether this difference is reflected by a different activity pattern of the forebrain. Therefore, we tested inexperienced birds too.

Müller and Scheich¹⁷ provided evidence that in chicks the activity of the same areas as those described by Maier and Scheich are enhanced if the birds are separated from their siblings. They conclude that stress may be the factor which corresponds to the enhanced glucose uptake in the 3 brain areas. We wanted to test whether very unspecific arousal has the same effect on the brain activity pattern in the zebra finches.

This was tested in a third series of experiments by chasing the birds around the cage.

MATERIALS AND METHODS

Twelve adult male zebra finches were used for this study. Four of these birds were isolated from their parents at 35 days of age and housed in separate cages. These birds could not see other males or females up to the time of the experiment and thus did no courting. The other birds were kept either in large aviaries together with other males and females or were housed together with a female for breeding.

In most cases the tests were carried out in cages identical to the cages housing the birds. The room was artificially lighted at normal levels, and no precautions were taken to eliminate background noise. At the beginning of the experiment the birds were given an injection in the pectoral muscle with 12 μ Ci 2-DG in 0.1 ml physiological saline, and returned to the experimental cage. During the experimental session, the behavior of the bird was monitored from a distance of about 3 m for 55 min.

Four different groups were compared.

(1) *Controls*. Males sat quietly in the cage throughout the experiment ($n = 3$).

(2) *Experienced males*. A female was introduced into the test cage of a male with courtship experience. The female elicited courtship behavior

in the male ($n = 3$).

(3) *Inexperienced males*. Same experiment as group 2 with inexperienced birds ($n = 4$).

(4) *Unspecific arousal*. The males were chased around the cage for 55 min by the experimenter ($n = 2$).

After the experiment the birds were decapitated and the brains were quickly removed from the skull and immediately frozen onto a cryostat head at -80°C and cut into 30- μm transverse sections at -17°C the next day. The mounted sections were quickly dried on a hot plate of $+60^{\circ}\text{C}$ and exposed to Kodak X-ray film at 4°C for 4 weeks. The film sheets were then processed by standard development methods. Sections were stained with thionin for identification of the labelled areas. Quantitative measurements of labeling densities were obtained by a computer-aided densitometric system. Access to this system was kindly provided by Prof. W. Singer, MPI für Hirnforschung, Frankfurt. At least 8 measurements were made in each area mentioned in the study, in either hemisphere of each bird.

Relative densities were calculated in order to standardize the measurements, which varied according to factors such as the amount of glucose injected, the time taken for removing the brain, or the length of exposure to the photographic paper. These were based on a scale reaching from the area with the lowest density in all experiments (area X, 0), and one with a high, consistent density throughout all experiments (MLD, 100). Areas with higher densities than MLD, consequently, could reach values above 100 (see Fig. 2). Differences were tested by Student's two-tailed *t*-test.

RESULTS

1. Controls

The pattern of 2-DG labelling in the control birds was similar to that previously described by Braun et al.^{4,5} (Fig. 1/1A–F). Therefore, only the main features will be mentioned here. Very strong labelling was obtained in the various nuclei of the tectofugal visual system and the auditory system. In contrast to the uniformly labelled nucleus rotundus and the ectostriatum of the tectofugal

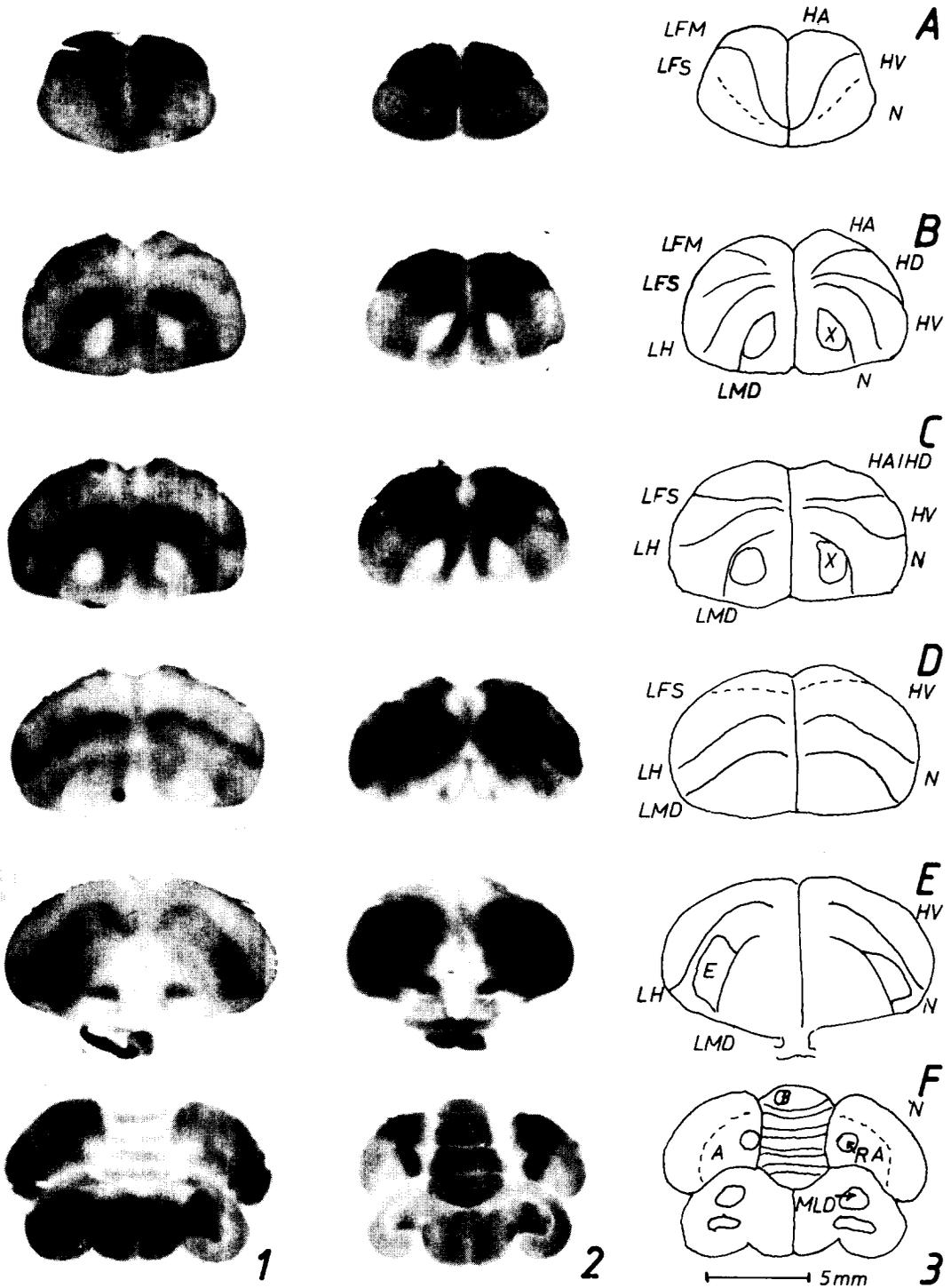


Fig. 1. Transverse sections of representative brains for experimental groups 1 (controls, 1) and 3 (courtship, 2). Groups 2 and 4 are not presented, as they are identical to 1 and 3, respectively. A–F: sections in order from rostral to caudal. Third row: idealized outlines of the sections drawn from thionin-stained material.

system (Fig. 1/1E), the tectum opticum was labelled only in one layer (II b, according to ref. 5). No enhanced 2-DG uptake was detected in the thalamofugal visual pathway. The thalamic nuclei as well as the visual wulst, the telencephalic station of this pathway, showed densities at background level.

In contrast to the auditory system, the song control nuclei of the male zebra finches revealed labels at or below background. Activity in area X was remarkably low (Fig. 1/1B,C), whereas the other nuclei (MAN, RA, and HVc) were at or slightly below background levels.

Besides these structures, which are known to belong to one or the other sensory or motor system, a series of other telencephalic structures of almost unknown function were labelled above background in the control birds. A common feature of these areas was that they were found in the vicinity of the various laminae of the forebrain, and in most cases included the area of the lamina.

At the rostral pole of the telencephalon, enhanced activity was found in the lamina frontalis superior (LFS), which separates HA from HV (Fig. 1/1A). More caudally, a strong patch dorsal to area X was detected which has been described as a part of the tractus fronto-medialis (TFM) by Braun et al.^{4,5} (Fig. 1/1B). However, on more caudal sections (Fig. 1/1C) this patch is divided into two portions along the two laminae LH and LMD, leaving the TFM and the adjacent parts of the neostriatum intermedium free of higher labels. Therefore, it is more likely that the pattern observed in Fig. 1/1B is a composition of the stronger labels of the two laminae, which extends into the neostriatum, but, in the control birds, leaves the TFM free. More caudally (Fig. 1/1D), the label along the lamina hyperstriatica extends to the lateral ends of the brain. However, this stronger labelling is not uniform, but appears to be patchy in the control birds.

2. Courtship of experienced birds

Compared to the control group, no obvious differences in the 2-DG pattern were found in the experienced animals exposed to a female during the experiment. In some areas of the forebrain,

which are described in detail in the next paragraph, as well as in the sensory areas (exemplified by field L in Fig. 2), glucose consumption seemed to be slightly enhanced. This effect, however, was never significant (Fig. 2).

The mean of occurrences of courtship song uttered throughout the experiment was 5.33 ± 3.01 bouts/experiment. Copulation occurred very rarely (mean = 0.5 ± 0.5 /experiment).

3. First courtship of inexperienced birds

In this experiment, the glucose consumption of 4 different forebrain areas was drastically enhanced (Fig. 2):

(a) Hyperstriatum accessorium and hyperstriatum dorsale (Fig. 1/2A, Fig. 2/HA).

(b) An area above area X, which belongs to the neostriatum and includes the TFM (Fig. 1/2B,C, Fig. 2/Nint.). In this area the two separate labels along the laminae observed in the control birds merge into one patch of high density. Within this

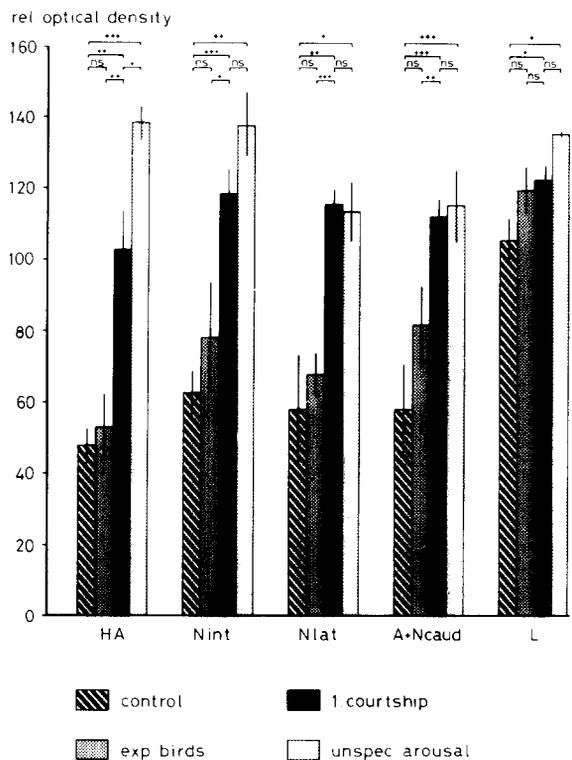


Fig. 2. Relative optical densities of the 4 forebrain areas affected by the experimental variations, and of one primary sensory forebrain area (field L). The 4 different shadings are related to the 4 experiments. T-tests: * $P \leq 0.01$; ** $P \leq 0.005$; *** $P \leq 0.001$.

area, the song control nucleus MAN sometimes appeared as a light spot, indicating that this structure was much less active than surrounding ones. In more caudal areas, the light patches along the LH disappeared and the 2-DG label extended more ventrally into the neostriatum (Fig. 1/2D). This area extended slightly into the adjacent parts of the hyperstriatum ventrale.

(c) A portion of the neostriatum lateral to the ectostriatum. This label appeared to be nearly as strong as the ectostriatum label. Therefore, the boundary between the ectostriatum and the surrounding neostriatum, which is relatively sharp in the controls, was blurred (Fig. 1/2E, Fig. 2/N lat.). Again, this area extended into the adjacent hyperstriatum ventrale.

(d) A wedge-shaped area of the caudal telencephalon, which extends from the dorsal to the ventral aspect of the brain, including archistriatal as well as neostriatal portions. RA is situated at the medial border of this area. Like MAN, RA activity is not enhanced. Therefore, it also appears as a light spot in the dark surroundings (Figs. 1/2F and 2/A + Ncaud.).

As shown in Fig. 2, the enhancement of density in these 4 areas is significant at the $P \leq 0.01$ or the $P \leq 0.001$ level. In contrast to experienced birds, the enhancement of activity of the sensory field L in inexperienced birds is slightly higher ($P > 0.05$). Courtship song and copulations were enhanced compared to experiment two (courtship song: mean = 24.71 ± 14.97 ; copulations: mean = 1.43 ± 0.65).

4. Unspecific arousal

In this experiment, the pattern of enhanced uptake of 2-DG was very similar to the previous experiment. Again, the same 4 forebrain areas were activated. There were certain differences in the amount of enhancement. These differences, however, were not significant, with the exception of HA, where the activity was slightly higher than in the previous group ($P > 0.05$).

DISCUSSION

Three of the four forebrain areas which we found to be activated in our experiments, have

previously been described to be active in a rather different context. Maier and Scheich¹⁶ demonstrated that the areas described above, with the exception of the caudal neo/archistriatal region, revealed higher 2-DG uptake in young guinea fowl chicks exposed to an acoustic stimulus to which they had been imprinted earlier. As stated above, Maier and Scheich suggest that the areas with enhanced uptake may be involved in the processing of auditory stimuli once they have become meaningful by experience. We cannot tell from our experiments, what sort of sensory cues are involved in the enhancement of 2-DG uptake in the areas we found. However, our results clearly demonstrate that the enhanced activity of the 4 forebrain areas cannot be solely due to the presence of certain external stimuli. It appears in two situations. In the semi-natural situation, where the male is exposed to a female, and also in the situation where the bird was frightened by the experimenter. Moreover, the label is different in Expts. 2 (experienced birds) and 3 (unexperienced birds), where the external stimulation was the same.

Another explanation for the enhanced activity of the 4 areas may be a correlation to higher motor activity. This is also unlikely, as motor activity was almost the same in Expts. 2 and 3, and much higher in Expt. 4. In Expt. 4 the birds were forced to move around the cage for the entire 55 min; in the two other experiments the birds were never active more than about 20% of the time. In contrast, the high 2-DG uptake was observed in Expts. 3 and 4, but not in Expt. 2.

The third, and from our point of view most plausible explanation for the occurrence of high activities in the 4 areas is a correlation with high arousal levels of the animals. This idea was also raised by Müller and Scheich¹⁷. In Expt. 4, the reason for enhanced arousal is obvious. In Expt. 3, the birds courted females for the first time in their lives, which might be more arousing than courtship is for an experienced bird. The higher rates of courtship song (5 times as many) and copulation attempts (3 times as many) in this group may be an indicator for this higher level of arousal.

As not much information is available on the

function of the 4 areas, estimation of the role of arousal for neuronal processing can only be speculative. Three of the four areas have been described as receiving sensory input^{6,7,10,12,18,19} (also Engelage and Bischof, in preparation). No information is available for the medial part of the neostriatum (Nint). Likewise, for two of the three areas (HA/HD and A + N caud), additional input from the reticular formation has been demonstrated^{1,2} (also Herrmann et al., in preparation).

The effect of arousal on the processing of sensory input has been investigated several times, for example in area 17 of the cat by comparing neuronal responses to visual stimulation in awake and sleeping cats¹⁵, or by application of norepinephrine¹³, and by stimulation of the reticular formation^{20,21}. In all experiments, arousal enhances the vigor of responses to visual stimulation and the signal/noise ratio of the neurons. Gonzalez-Lima and Scheich^{8,9} demonstrated that combined reticular and auditory stimulation leads to higher 2-DG uptake in parts of the inferior colliculus and auditory cortex of the rat, which were also labelled by pure acoustic stimuli, but to a smaller degree.

One can speculate that the high activity measured in the 4 forebrain areas in this study may also be due to enhanced processing of sensory stimulation, which concentrates the bird's attention on a behaviourally important stimulus.

This should be important for reactions to frightening stimuli (Expt. 4) as well as for courtship (Expts. 2 and 3). The fact that we did not obtain significant enhancements in the group of experienced birds may simply be due to the shorter isolation times of the birds of this group, which leads to less frequent courtship behavior (Böhner, personal communication). This is presently under investigation in our lab.

In contrast to the above-mentioned studies with chicks^{14,16,17}, our results cannot directly decide whether imprinting-like learning occurs in the first courtship experiment. However, as a high arousal level, as is indicated in Expt. 2 by the high activity of the 4 areas, is probably an important prerequisite for learning^{8,9,16,17,20,21}, one could

speculate that at least one of the preconditions for learning is fulfilled during the first courtship of the birds. Further experiments are necessary to decide this question.

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ABBREVIATIONS

02-DG	[¹⁴ C]2-deoxyglucose
A + N caud	portion of the caudal archi/neostriatum
HA	hyperstriatum accessorium
HD	hyperstriatum dorsale
HV	hyperstriatum ventrale
HVc	nucleus hyperstriatum ventrale pars caudale
LFS	lamina frontalis superior
LH	lamina hyperstriatica
LMD	lamina medullaris dorsalis
MAN	nucleus magnocellularis neostriatalis anterioris
MLD	nucleus mesencephalicus lateralis dorsalis
Nint	neostriatum intermedium
Nlat	neostriatum laterale
RA	nucleus robustus archistriatalis
TFM	tractus frontomedialis

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