Early postnatal development of functional ocular dominance columns in cat primary visual cortex

Stefan Rathjen[1,2] and Siegrid Löwel[1,CA]

[1]Leibniz-Institut für Neurobiologie, Brenneckestr. 6, D-39118 Magdeburg; [2]Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, D-60528 Frankfurt am Main, Germany

[CA]Corresponding Author

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During postnatal development of the visual cortex the thalamocortical afferents serving the two eyes segregate into alternating patches called ocular dominance (OD) columns. Interested in the dynamics of this segregation process we studied the appearance of functional OD columns in the primary visual cortex of normally raised and strabismic kittens aged 2–6 weeks using 2-deoxyglucose labelling in awake animals. In both experimental groups, OD columns covering the entire area 17 and spanning all cortical laminae are first visible at 3 weeks and appear already adult-like at 4 weeks, much earlier than thought on the basis of previous anatomical studies. We hypothesize that a small and anatomically undetectable imbalance between the afferents from the two eyes is amplified by intracortical interactions so that their activity patterns become different and may guide the segregation process of the afferents in cortical layer IV. *NeuroReport* 11:2363–2367 © 2000 Lippincott Williams & Wilkins.

Key words: Area 17; Cat visual cortex; Cortical development; 2-Deoxyglucose; Ocular dominance columns; Strabismus

INTRODUCTION

A prominent feature of the primary visual cortex is its segregation into different anatomical and functional modules. One aspect of this modular organization is represented by the segregation of left and right eye afferents into ocular dominance (OD) columns [1]. By labelling the thalamocortical afferents with transneuronal transport of [^{3}H]proline it was shown that the segregation process in cat primary visual cortex (area 17) takes place during early life between the third and sixth postnatal week [2]. In contrast, a recent optical imaging experiment in cat area 17 showed that a pattern of alternating eye preference reminiscent of ocular dominance columns is already visible at 2 weeks of age [3]. Since the optical images were restricted to 3×2 mm regions in the central visual field representation, we wondered whether functional ocular dominance segregation is detectable throughout area 17 and across all cortical laminae at these early ages. We therefore investigated the following three questions: 1. At what time do functional OD columns first occur in the entire area 17? 2. What is the time-course of their development? 3. Does strabismus change the kinetics of the segregation process? To this end, we visualized the complete pattern of functional ocular dominance columns in flat-mount sections of cat visual cortex by labelling these domains with 2-deoxyglucose after monocular stimulation in awake animals. Some of these results have been published in abstract form [4,5].

MATERIALS AND METHODS

In the present study, we used the 2-deoxyglucose (2-DG) technique [6] to examine the time-course of the development of functional OD patterns in the primary visual cortex (area 17). A total of 16 kittens was used from our institutes' colonies. In seven kittens, divergent strabismus was induced surgically at postnatal days 14 or 15. At an age of 2–6 weeks the kittens were subjected to a 2-DG experiment while being allowed to move freely around in the laboratory for effective monocular stimulation [7]. OD maps from five functionally adult animals (aged 9–13 weeks) from already published papers were also analyzed quantitatively [7,8].

Surgical procedure and visual stimulation: For the induction of strabismus, anaesthesia was induced with ketamine hydrochloride (20 mg/kg) mixed with xylazine hydrochloride (1.5 mg/kg), injected i.m. A divergent squint angle was induced by cutting the medial rectus muscle of the left eye. For the application of 2-DG a venous katheter was implanted under either halothane anaesthesia using an inhalation mask (2–4% halothane in a mixture of 70% nitrous oxide and 30% oxygen) or Saffan anaesthesia (15 mg/kg, i.m. injectable steroid, Pitman-Moore Ltd.). After full recovery from anaesthesia, one eye was occluded with a black contact lens and 2-deoxy-D-[U-^{14}C]glucose (Amersham, sp. act. 10.9–11.7 GBq/mmol) was injected i.v. at a dose of 3.7–4.2 MBq/kg. There were no differences in the quality of the 2-DG autoradiographs between the two anaesthesia protocols.

Histological procedures: After 45 min, the animals were given a lethal i.v. dose of Nembutal. The occipital poles and the lateral geniculate nuclei (LGN) of the brains were removed and the visual cortices flat-mounted prior to freezing the tissue on dry ice [9]. Subsequently, 25 μm serial cryostat sections (at −17°C) were cut. Blocks containing the visual cortex were cut parallel to the cortical surface; those containing the LGN were cut in the frontal plane. The sections were mounted on glass slides, immediately dried on a hot plate and exposed to X-ray film (Agfa Structurix) for 3–4 weeks.

Image processing and quantitative analyses: Magnified photographs (×6) were taken from representative 2-DG autoradiographs of the visual cortical hemispheres and then scanned at 300 d.p.i. resolution using a flatbed scanner (Umax Astra 610S) connected to a Macintosh G3 Power PC. Using NIH Image the following two parameters were calculated: First, density profile plots were generated using a straight line selection (length: 200 pixels, corresponding to about 8.5 mm) along the anterior/posterior axis of area 17 (see Fig. 2a). Second, quantitative analyses of the grey value distributions were calculated within a rectangular selection (200 × 100 pixels; corresponding to about 36 mm^2; see Fig. 2b). The contrast of the 2-DG patterns was calculated using the standard deviations of the grey value distributions: high contrast pictures contain a wide range of grey values and have large s.d. whereas low contrast images are more restricted in their range of grey values and have smaller s.d. The values for the investigated hemispheres were averaged and displayed for the different ages (see Fig. 2c,d). We checked that variation in exposure times of the photographs only slightly affected the distribution of grey values: the within-animals variance was much smaller than the variance between the different animals of the same age.

RESULTS

In area 17 of 2 week-old normally raised kittens, we observed homogeneous 2-DG labelling (Fig. 1a,e). Only 1 week later, at 3 weeks of age, monocular stimulation induced a clearly discernible 2-DG pattern that extended through all cortical layers (data not shown) and covered the entire area 17 (Fig. 1b). Pattern contrast was however lower than in older animals (compare with Fig. 1c,d). An adult-like pattern of functional OD columns was already observed in 4-week old animals (Fig. 1c) and thus at an age at which the segregation of the thalamocortical afferents is still in progress [2]. The following weeks of postnatal development (weeks 5–6) did not show further changes (Fig. 1d). The labelling patterns on the autoradiographs were comparable to those of previously investigated older animals [7].

The strabismic kittens showed a similar time-course in functional OD column development. One week after squint induction, at the age of 3 weeks, OD columns were already visible (Fig. 1f) and, as in normally raised kittens, the columnar pattern extended through all cortical layers and covered the entire area 17. Again, the contrast of active and inactive territories was lower than in older animals (compare with Fig. 1g,h). At the age of 4 weeks (Fig. 1g), the 2-DG pattern was no longer discernible from that of 1- or 2-week older animals (Fig. 1h) or even adult cats [7].

In the LGN, the monocularly induced 2-DG patterns were quite similar in all experimental animals (data not shown). Irrespective of rearing conditions, 2-DG uptake in the LGN ipsilateral to the open eye was highest in layers A1 and C1. In the LGN contralateral to the open eye, 2-DG uptake was highest in layers A and C thus confirming effective monocular stimulation of the animals [7]. To substantiate our qualitative descriptions of OD column development we analyzed the 2-DG autoradiographs quantitatively. Optical density measurements (profile plots, see Materials and Methods) along vectors across area 17 showed (i) that discernible 2-DG domains first appear at 3 weeks of age and (ii) that the contrast of the 2-DG autoradiographs increased between the second and fourth postnatal week (Fig. 2a). The increased contrast is further illustrated by a broadening of the range of grey values contained in the autoradiographs (Fig. 2b) and thus in an increased s.d. of the grey value distributions during the first few postnatal weeks. Mean s.d. (i.e. the contrast of the autoradiographs, see Materials and Methods) increased between the second and fourth postnatal week and then remained more or less constant for both normal and strabismic animals (Fig. 2c). Pooling the data of these animals for a statistical analysis revealed a significant increase in contrast between the second and fourth postnatal week (Spearman rank correlation coefficient $r_s = 0.906$, $p < 0.001$) but no significant difference in contrast between the fourth postnatal week and adulthood ($r_s = 0.273$, $p > 0.1$). In addition, averaging the values of both experimental groups but analyzing hemispheres ipsilateral and contralateral to the open eye separately revealed a similar increase in contrast between the second and fourth postnatal week (Fig. 2d). Furthermore there was no difference in the contrast of the 2-DG autoradiographs for the ipsilateral and contralateral hemispheres from 2 weeks of age to adulthood.

Our quantitative analyses of the grey value distributions thus confirmed the developmental steps as inferred from the qualitative description of the 2-DG autoradiographs.

DISCUSSION

When kittens are born, the thalamocortical afferents serving the two eyes overlap in layer IV, the input layer of primary visual cortex. Anatomical investigation of these projections using transneuronally transported [^{3}H]proline showed that segregation is a postnatal process occurring between the third and sixth week of life [2]. Our findings now demonstrate that functional OD columns that extend through all cortical layers and cover the entire area 17 appear already at 3 weeks of age and can look adult-like only one week later, at an age at which the anatomical segregation of the thalamocortical afferents is still in progress. Interestingly, a detailed analysis of the development of individual thalamocortical axon arbors indicated that the most striking morphological changes, i.e. an increase in arbor density and the formation of distinct patches of terminals occur at the same postnatal period, namely between postnatal days 23 and 30/31 [10]. Our observation of an early postnatal development of OD

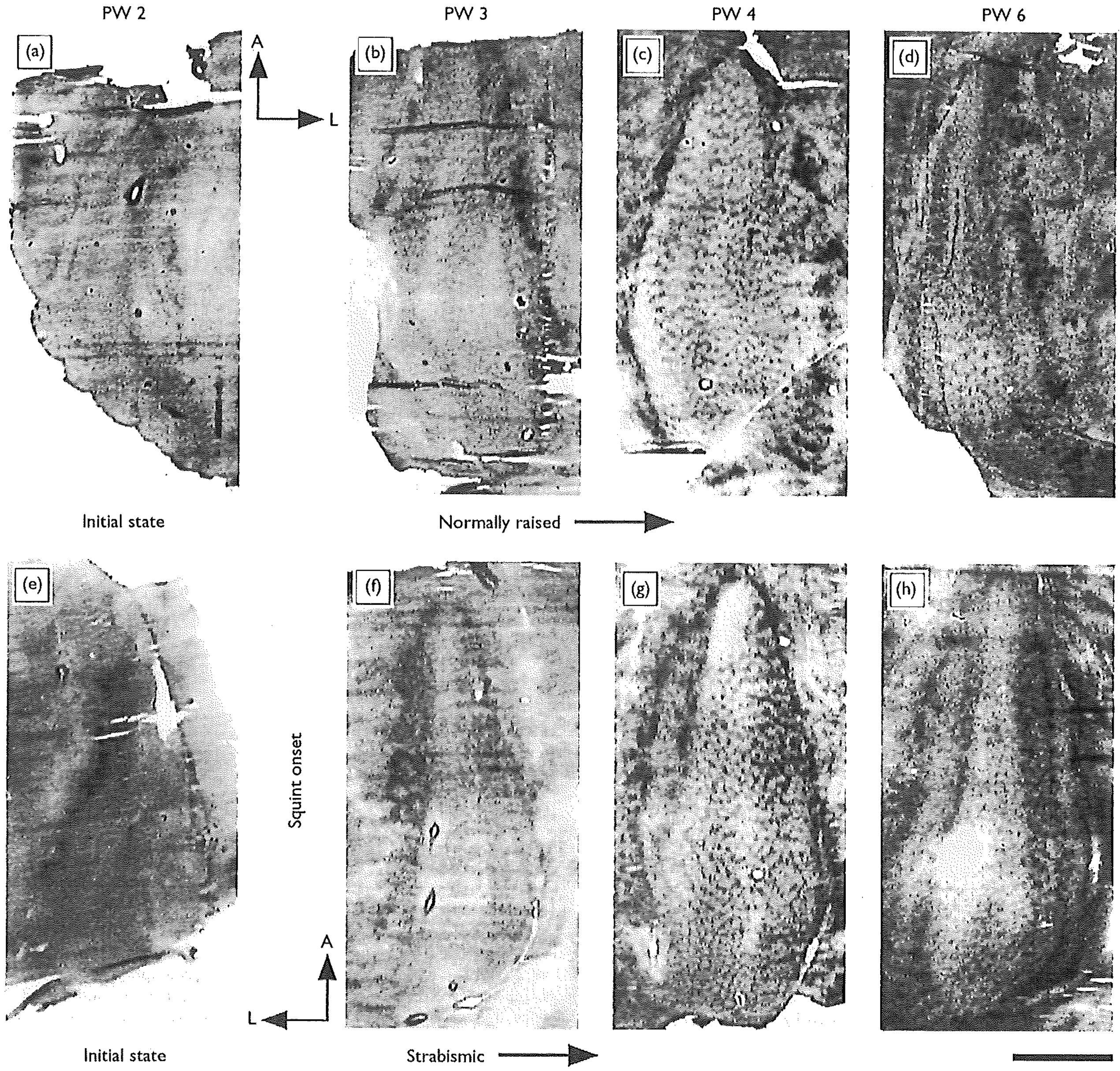

Fig. 1. Development of OD columns in the primary visual cortex of normally raised (**b–d**) and strabismic kittens (**f–h**) from the same initial state at 2 weeks (**a,e**). OD columns were visualized by 2-DG labelling in awake animals. 2-DG autoradiographs of supragranular flat-mount sections of unfolded right (upper row) and left (lower row) hemispheres. All animals had been stimulated through the right eye. (a,e) 2-DG labelling in area 17 of a 2-week-old kitten. The 2-DG distribution is rather homogeneous. (b) 3-week-old kitten. A pattern of OD-columns is already visible (small dark-grey patches on a light grey background). Note that the three dark horizontal lines are cutting artefacts. (c) 4-week-old kitten. The OD pattern has a higher contrast than in the 3-week-old animal in (c) and appears already adult-like. (d) 6-week-old kitten. Active and inactive territories are as sharply delineated as in 4-week-old animals. (f–h) The time-course of the OD development in strabismic kittens is similar to that of normally raised animals (b–d). (f) 3-week-old strabismic kitten. OD columns are discernible. (g) 4-week-old strabismic kitten. The OD-pattern appears adult-like (enhanced contrast compared with the pattern in f). (h) 6-week-old strabismic kitten. There are no further changes. Note that due to an artefact, 2-DG labelling is missing in the grey area. A, anterior; L, lateral; PW, postnatal week. Bar = 1 cm.

columns extends results from recent optical imaging experiments [3], in which functional maps obtained at 2 weeks of age showed a pattern of alternating eye preference in kitten area 17 reminiscent of OD columns. Taken together, these data indicate that the development of functional OD columns or upper layer cortical organization in general [3] (see also [11,12]) precedes the anatomical segregation and elaboration of the thalamocortical afferents in cortical layer IV. We suggest that a small and anatomically undetectable imbalance between the afferents from the two eyes is amplified by intracortical interactions so that their activity patterns become different and may in turn guide (rather than follow) the segregation process. This observation is compatible with the hypothesis that segregation is driven by activity-dependent self-organizing processes.

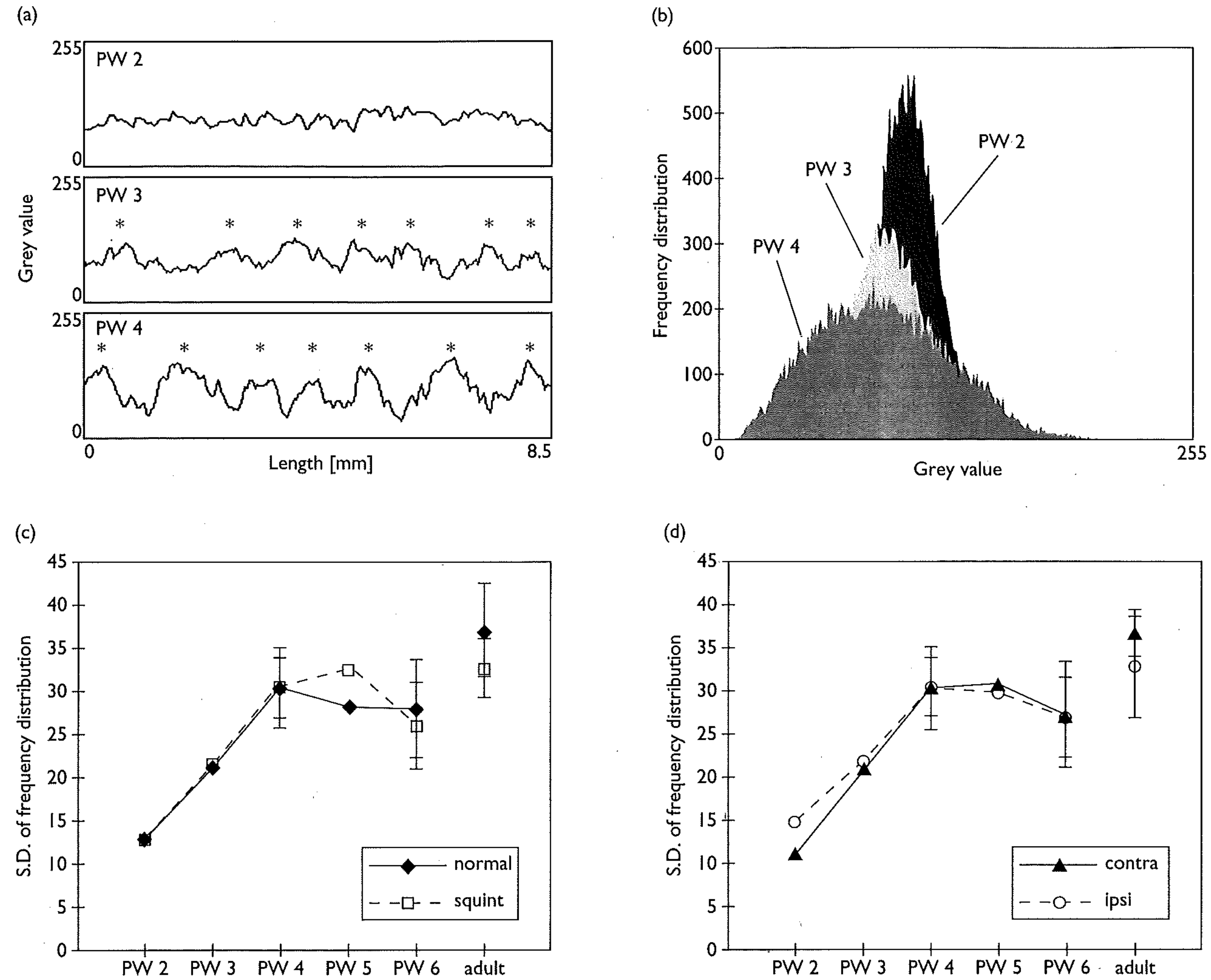

Fig. 2. Quantitative analyses of the 2-DG labelled ocular dominance patterns. Grey value 0 corresponds to white, grey value 255 to black. **(a)** Density profile plots along the anterior/posterior axis of area 17 in 2- (PW 2), 3- (PW 3) and 4-week-old (PW 4) animals (autoradiographs shown in Fig. 1a-c). The grey value differences (contrast) between more and less labelled regions (upward/downward deflexions in the graphs) increase until PW 4. Note that discernible OD domains, represented by larger upward deflexions (labelled by asterisks) appear first at PW 3. **(b)** Histograms of the grey value distribution within the same autoradiographs. The increased contrast of the 2-DG autoradiographs between PW 2 and PW 4 is reflected in a broadening of the frequency distribution of grey values: at PW 2, only a limited set of medium grey values is present on the autoradiographs (small black histogram), whereas at PW 4, darker and lighter grey values are added (broadening of the histogram). **(c)** Averaged s.d. of the grey value distributions from PW 2 to adulthood, taken as a measure of image contrast. Contrast increases between PW 2 and PW 4 and remains more or less constant thereafter (no significant differences between PW4 and adulthood). There are no differences between normally raised and strabismic animals. **(d)** Same data as in (c) but now plotted for hemispheres contra- or ipsilateral to the open (stimulated) eye. As in (c), contrast increases between PW 2 and PW 4. There is no difference between image contrast in ipsi- and contralateral hemispheres and thus no detectable contralateral bias. Error bars represent s.d. (for $n \geq 4$). Contra, contralateral; ipsi, ipsilateral; PW, postnatal week.

In strabismic animals, the correlation of activity between the eyes is severely reduced because the images on the two retinae cannot be brought into register. As a consequence the segregation of thalamocortical afferents into alternating ocular dominance columns is enhanced in area 17 of strabismic compared to normally raised cats [13,14] and neurons become responsive almost exclusively to stimulation of either the left or the right eye [15]. On the basis of theoretical models about the development of ocular dominance columns the elimination of correlated activity suggested an enhanced velocity of ocular dominance segregation in strabismic animals [16–19]. However, our present results do not provide evidence for an experience-dependent change in the dynamics of this process by showing that in both normally raised and strabismic cats functional OD patterns are first detectable at the same postnatal age, namely at 3 weeks. Thus the time-course of OD column development was similar in the two experimental groups. The fact that the period during which a functional pattern is formed seems to be in the range of days or even hours and thus much faster than previously supposed (e.g. [2]) could, however, obscure possible differences between the two experimental groups, since temporal differences less than 1 week were not analysed in the present experiments.

Interesting in the context of the present results is a recent anatomical study showing that in binocularly enucleated ferrets thalamocortical afferents form patches in area 17 [20]. The authors concluded from these data that activity is less important for OD column segregation than previously thought. However, removal of the eyes does not necessarily eliminate neural activity in thalamocortical and cortical circuits [21,22]. Binocular enucleation of ferrets at postnatal day 21 only modestly affected the development of clustered horizontal connections in the cortex whereas silencing the cortex abolished clusters [23]. Thus, even in the absence of retinal drive, activity in thalamocortical circuits may contain information that can drive the initial patterning of cortical circuits. In addition, given the wealth of studies demonstrating activity-dependent cortical development [24] it is at present a highly debated issue how important molecular cues are for OD column development or more generally for the development of cortical maps [25]. Further experiments are required to determine whether neural activity is instructive, permissive or unnecessary [24] for the establishment of correct neural connections in various species.

CONCLUSION

We have shown that, in cat primary visual cortex, functional ocular dominance columns first appear at 3 weeks of age and can be adult-like at 4 weeks. The development of functional OD columns thus precedes the anatomical segregation of the thalamocortical afferents in cortical layer IV. These data indicate that intracortical activity patterns may guide rather than follow the segregation process of the thalamocortical afferents. Thus the present observations support the hypothesis that segregation is driven by activity-dependent self-organizing processes. Strabismus induced at 2 weeks of age does not seem to influence the kinetics of the segregation process. Since the period during which functional OD maps develop seems to be in the range of days or even hours, the precise onset of altered visual experience may be crucial in determining whether activity-dependent effects are observed.

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Erratum

S. Löwel, a co-author of 'Early postnatal development of functional ocular dominance columns in cat primary visual cortex', *NeuroReport* Volume 11 Number 11, pages 2363–2367, wishes it to be known that Figure 2(c) appears in a corrupted state, as the symbol for squinting animals should appear for the first time at PW3, not PW2.