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Adenosine A_1 and Benzodiazepine Receptors and Glucose Metabolism in the Visual Structures of Rats Monocularly Deprived by Enucleation or Eyelid Suture at a Sensitive Period

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Purpose: To determine the changes in the adenosine A_1 and benzodiazepine receptor density and in glucose metabolism in the visual centers of the rat brain following monocular enucleation or eyelid suture on postnatal day 10 (PN10).

Methods: Following monocular enucleation or eyelid suture on PN10 rats, the alterations in adenosine A_1 and benzodiazepine receptor density, and in glucose metabolism were evaluated in the superior colliculus (SC), the dorsal lateral geniculate body (DLG), and the visual cortex (VC) by ex vivo autoradiography with [^{11}C]MPDX, [^{11}C]flumazenil and [^{14}C]2-deoxyglucose, respectively.

Results: Enucleation reduced the [^{11}C]MPDX binding in the SC and DLG, and enhanced the [^{11}C]flumazenil binding in the SC. Eyelid suture reduced the [^{11}C]flumazenil binding in the VC at day 20. [^{14}C]2-deoxyglucose uptake was not decreased by enucleation in any region except in the SC and DLG at day 1, but was decreased by eyelid suture in the SC at days 20 and 55 and in the VC at day 55.

Conclusions: The decrease in the presynaptic adenosine A_1 receptors in the SC following enucleation is coupled with an upregulation of postsynaptic benzodiazepine receptors. These neural reactions are completely different from those following eyelid suture. The development of neural architecture for visual functions is not completed at PN10 in rats. **Jpn J Ophthalmol 2003;47:182–190** © 2003 Japanese Ophthalmological Society

Key Words: Enucleation, ex vivo autoradiography, eyelid suture, glucose metabolism, neuroreceptor.

Introduction

Rats and mice are widely used to investigate the effects of visual deprivation on the visual system, because 90–97% of the ganglion cell axons projecting from the retina to the superior colliculus (SC) and dorsal lateral geniculate body (DLG) decussate in the optic chiasma in adult rats and mice.[1,2] The effects of monocular deprivation by enucleation or eyelid suture are evaluated in the contralateral brain by conventional anatomical, immunohistochemical, and autoradiographic methods. It is known that the neural plasticity of the visual system is different be-

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tween the sensitive period of postnatal day 10 (PN10) to PN45 and in adult rats.

The effects of monocular deprivation on several neuroreceptors have been investigated in young animals at different postnatal ages and in adult rats by in vitro autoradiography of radioligand and receptor binding. After monocular enucleation in adult rats, the adenosine A_1 receptor density decreased significantly in the SC,[3–6] suggesting the presence of adenosine A_1 receptors on the axon terminals from the retina. The axonal degeneration also reduced the sero tonin$_1$ (5-HT$_1$) and quisqualate receptors in the SC significantly, while kainate and histamine H_3 receptors were significantly increased in the SC and in the DLG.[3,4,7] The 5-HT$_2$,[3] muscarinic,[3] N-methyl-D-aspartate,[4] and adenosine A_2[5,6] receptors were not altered.

In the developing stage of the visual system, on the other hand, visual form deprivation can give different results. Kiyosawa et al[8] found that the kainate binding sites were slightly but significantly decreased in the SC of rats unilaterally enucleated at PN10. A significant decrease in the β-adrenergic receptors in the SC contralateral to the enucleated eye at PN12 was not found in adults.[9] Recently, we studied the effects of monocular enucleation on the adenosine A_1 and benzodiazepine receptors and glucose metabolism in the visual centers by ex vivo autoradiography with a double tracing technique using the positron-emitting radionuclide ^{11}C and the conventional radionuclide ^{14}C in 6-week-old rats.[10] Monocular enucleation decreased the glucose metabolism significantly in the contralateral SC and visual cortex (VC) because of axonal degeneration. The density of presynaptic adenosine A_1 receptors was significantly decreased in the SC, and a transient upregulation of postsynaptic benzodiazepine receptor density occurred. Changes in these two neuroreceptors were not observed in the VC. A similar transient upregulation of benzodiazepine receptor density was also reported in the SC of adult mice after monocular enucleation,[11] but not in the SC of rats after monocular eyelid suture on PN10.[12] On the other hand, rats raised in complete darkness from birth until PN25 had significantly decreased binding levels of benzodiazepine receptors in the DLG and in the SC compared to normal rats.[13]

It is known that the benzodiazepine receptor density changes during postnatal development and that the changes are variable for the different visual structures.[13,14] Thomas and Westrum reported that the density of benzodiazepine receptor-binding sites in the piriform cortex was markedly increased following olfactory bulb removal at PN100, but not at PN0, suggesting plasticity of the benzodiazepine receptor-binding sites.[15] To examine the effect of time and maneuver, we have compared the changes in benzodiazepine receptor density in the SC following monocular enucleation to that following monocular eyelid suture in rats at PN10 by ex vivo autoradiography. The optic nerve from the retina to the SC degenerates in the former model, but remains intact in the latter model. We also examined the functional activity in the visual structures by the deoxyglucose technique following the two deprivation methods.

Materials and Methods

We purchased 8-Cyclopentyl-3,7-dihydro-1,3-dipropyl-1H-purine-2,6-dione (DPCPX) from Research Biochemicals (Natick, MA, USA). [^{14}C]2-Deoxyglucose (1.66–2.22 GBq/mmol) was purchased from Research Biochemicals International (Amersham International, Buckinghamshire, UK). [^{11}C]MPDX ([1-methyl-^{11}C]8-dicyclopropylmethyl-1-methyl-3-propylxanthine) (15–78 TBq/mmol)[16] and [^{11}C]flumazenil (18–130 TBq/mmol)[17] for mapping adenosine A_1 and benzodiazepine receptors, respectively, were prepared as previously described.[10]

Animal Preparation

Wistar rats were supplied from Tokyo Laboratory Animals Company (Tokyo), and housed in an air-conditioned, light-controlled environment (22°C, 12-hour light and 12-hour dark conditions) with free access to food and water. The study was conducted in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research, and approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology.

One group of rats (n = 58) was anesthetized with an intraperitoneal injection of pentobarbital and the right eye was enucleated at PN10.[10] The other group of rats (n = 67) underwent lid suture of the right eye also at PN10. At 1, 5, 20, and 55 days after the enucleation or eyelid suture, the animals were used for ex vivo autoradiography studies with [^{11}C]MPDX, [^{11}C]flumazenil and [^{14}C]2-deoxyglucose.

Ex Vivo Autoradiography

At 1 or 5 days after the surgery, one of the three tracers was injected into rats through a jugular vein under isoflurane anesthesia. The injected doses of [^{11}C]MPDX, [^{11}C]flumazenil, and [^{14}C]2-deoxyglucose were 1 GBq/kg, 0.5 GBq/kg and 3 MBq/kg, respectively. The animals were sacrificed 15 and 45

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minutes after the injection of the ^{11}C-labeled tracer and [^{14}C]2-deoxyglucose, respectively.

At 20 and 55 days after the surgery, the double tracer autoradiographic method was applied.[10] [^{14}C]2-Deoxyglucose was injected into rats through a tail vein, and 30 minutes later, [^{11}C]MPDX or [^{11}C]flumazenil was injected. The animals were then sacrificed 15 minutes later. Ex vivo autoradiography of the brain was carried out as described previously.[10] The brain was rapidly dissected, frozen, and cut coronally into 20-μm thick sections using a cryotome at −15°C (Bright Instrument, Huntingdon, UK). The brain sections were mounted on glass slides, dried on a hot plate at 60°C, and apposed to a storage phosphor screen (Phosphor Imager SI system; Molecular Dynamics, Sunnyvale, CA, USA). In single tracer autoradiography studies, the exposure times required to demonstrate the distribution of ^{11}C and ^{14}C were 2 and 72 hours. In the double tracer autoradiographic studies, the distribution of ^{11}C was demonstrated by a 1-hour exposure. Four hours later (ie, more than 10 times the half-life of ^{11}C), the brain sections were apposed again to another storage phosphor screen for 72 hours to demonstrate the distribution of ^{14}C. The brain sections were then stained with hematoxylin-eosin.

Data Analysis of Ex Vivo Autoradiography

Regions of interest (ROIs) were placed on the superficial layers of the SC, DLG, and the binocular and monocular areas of the visual cortex (bVC and mVC, respectively) based on histological images and the rat brain atlas.[18] The density of radioactivity in the ROIs was measured. For double tracer autoradiography, the density of ^{11}C in each ROI was corrected to that for ^{14}C [density of ^{11}C = (density after first exposure) − (density equivalent for 1-hour exposure of ^{14}C)].

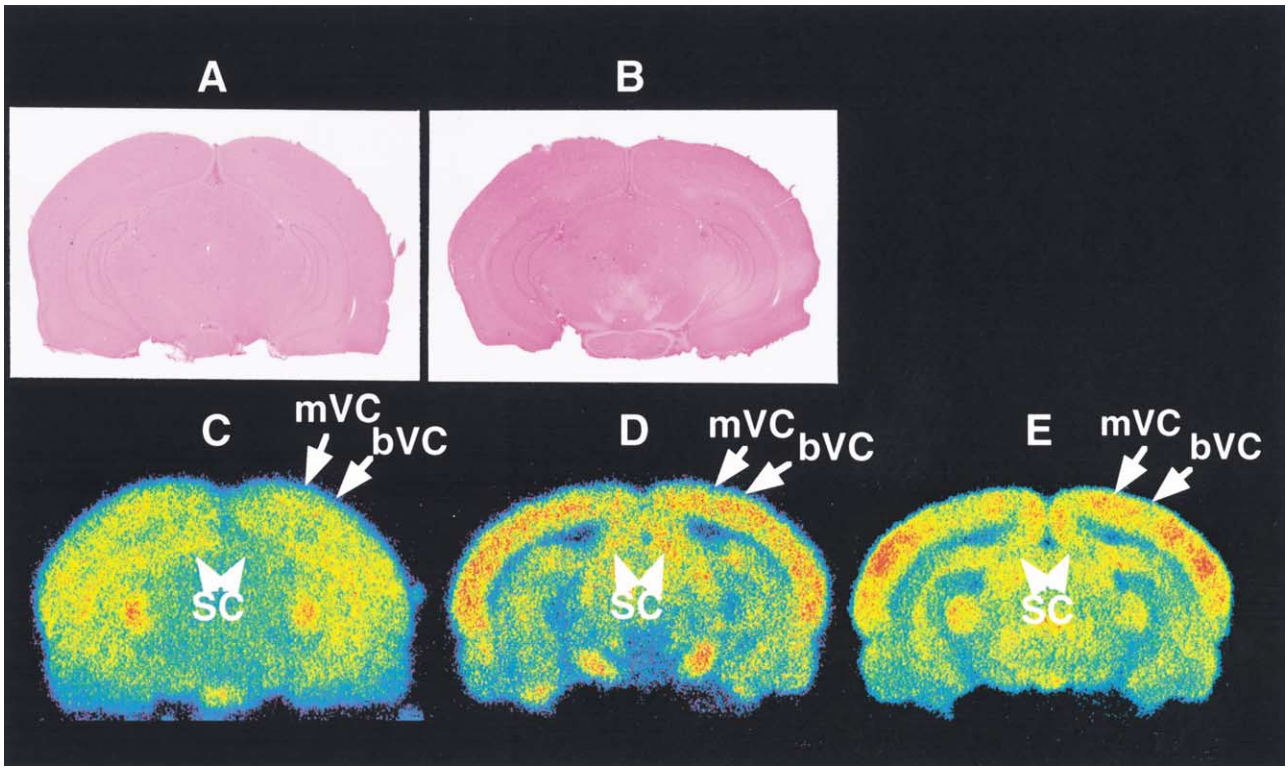

Figure 1. Autoradiograms and histological sections of the rat brain 20 days after enucleation of the right eye. bVC: binocular area of visual cortex, mVC: monocular area of visual cortex, SC: superior colliculus. (**A**) and (**B**) Representative brain sections stained with hematoxylin-eosin. (**C**) Autoradiogram of [^{11}C]MPDX binding corresponding to the brain section A. The specific binding was 27% less in the left SC than in the right SC. This indicates the loss of presynaptic adenosine A_1 receptors. (**D**) and (**E**) Autoradiograms of [^{11}C]flumazenil binding and [^{14}C]2-deoxyglucose uptake, respectively, corresponding to the brain section B. No alteration in the [^{14}C]2-deoxyglucose uptake was found in any visual structures, whereas the specific binding of [^{11}C]flumazenil was 10% higher in the left SC than in the right SC. This indicates an upregulation of postsynaptic benzodiazepine receptors.

The ratio of the [^{14}C]2-deoxyglucose uptake in the contralateral ROI to the ipsilateral ROI was calculated. The receptor specific binding of each of the ^{11}C-labeled ligands was determined as described below, and the receptor-ligand specific binding ratio of the contralateral ROI to the ipsilateral ROI was also calculated.

In our previous study, the percentage receptor-specific uptake of each ligand was calculated from the total uptake in the control and that in the blocked-uptake group using 8-week-old male rats (n = 4). The percentage of the receptor-specific uptake of [^{11}C]flumazenil 15 minutes after the injection was estimated to be 91%, 88% and 92% of the total uptake in the SC, DLG and VC, respectively, from the total uptake in the control and that in the blocked-uptake group.[10] The specific binding of [^{11}C]MPDX to adenosine A_1 receptors was re-evaluated as the difference between the uptake in control rats and that in the rats pretreated with the selective adenosine A_1 receptor ligand DPCPX (2 μmol/kg) 15 minutes before the tracer injection as previously described.[10] The estimated percentages of the specific uptake of [^{11}C] MPDX were 61%, 68%, and 64% of the total uptake in the SC and DLG and VC, respectively. In the individually treated rats, the nonspecific uptake was calculated from the total uptake on the ipsilateral side of the brain. The receptor-specific binding of radioligands in each of the brain regions was determined as the difference between the nonspecific uptake and the total uptake on the contralateral side.

Results

Representative autoradiograms of ^{11}C-labeled ligands and [^{14}C]2-deoxyglucose uptake, and histological images of the brain of rats 20 days after monocular enucleation are shown in Figure 1. The uptake of [^{11}C]MPDX was lower in the contralateral SC than in the ipsilateral SC at 20 days (Figure 1C),

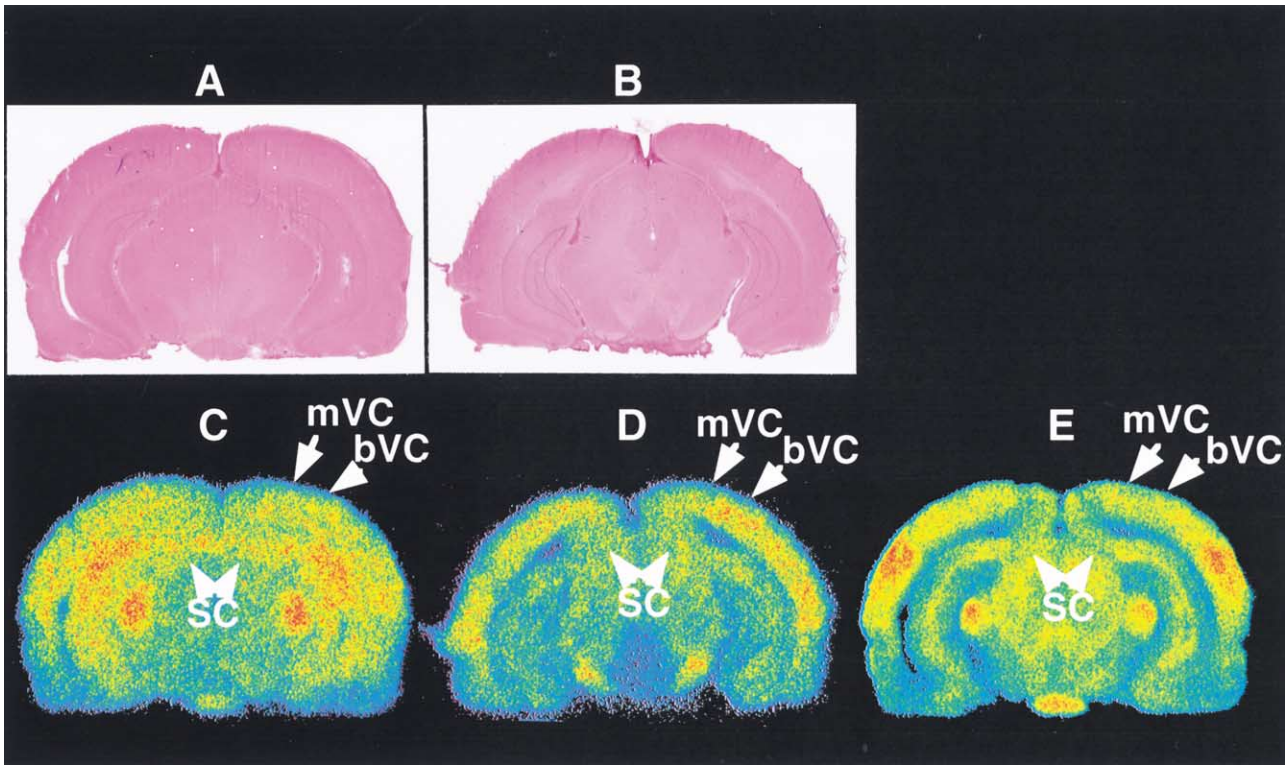

Figure 2. Autoradiograms and histological sections of the rat brain 55 days after right eyelid suture. bVC: binocular area of visual cortex, mVC: monocular area of visual cortex, SC: superior colliculus. (**A**) and (**B**) Representative brain sections stained with hematoxylin-eosin. (**C**) and (**E**) Autoradiograms of [^{11}C]MPDX binding and [^{14}C]2-deoxyglucose uptake, respectively, corresponding to the brain section A. (**D**) Autoradiogram of [^{11}C]flumazenil binding corresponding to brain section B. No asymmetrical alterations can be seen for the two ^{11}C-labeled tracers in any visual structures; 5% reduction in the uptake of [^{14}C]2-deoxyglucose was found in the left VC and left SC compared with the corresponding regions in the right brain hemisphere.

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whereas the uptake of [^{11}C]flumazenil was higher in the contralateral SC than in the ipsilateral SC (Figure 1D). Such a differential uptake of [^{14}C]2-deoxyglucose was not found in the contralateral and ipsilateral SCs 20 days after the enucleation (Figure 1E). No significant difference in the results obtained by the three tracers was found between the contralateral and ipsilateral sides in any other visual structures.

After eyelid suture, the two ^{11}C-labeled tracers showed symmetrical images without any detectable changes, while the uptake of [^{14}C]2-deoxyglucose was decreased in the SC (5% reduction) and VC (5% reduction) at day 55 (Figure 2). The time courses of the left-to-right ratios of the receptor-specific binding of [^{11}C]MPDX and [^{11}C]flumazenil and of [^{14}C]2-deoxyglucose uptake in the visual centers of monocularly enucleated rats are shown in Figure 3. The left-to-right ratio of the receptor-specific binding of [^{11}C]MPDX decreased in the SC and DLG immediately after the monocular enucleation, and was 0.75 ($P < .01$) and 0.93 ($P < .05$) after day 20 in the SC and DLG, respectively (Figure 3A). The ratios for [^{11}C]flumazenil binding, on the other hand, increased immediately after the surgery and remained at a higher level; a 10% increase ($P < .01$) at day 20 and an 8% increase ($P < .01$) at day 55 (Figure 3B). The ratios for the two radioligands did not change in any other visual centers investigated. The ratios for [^{14}C]2-deoxyglucose uptake decreased in the SC and DLG only at day 1 after the enucleation (Figure 3C).

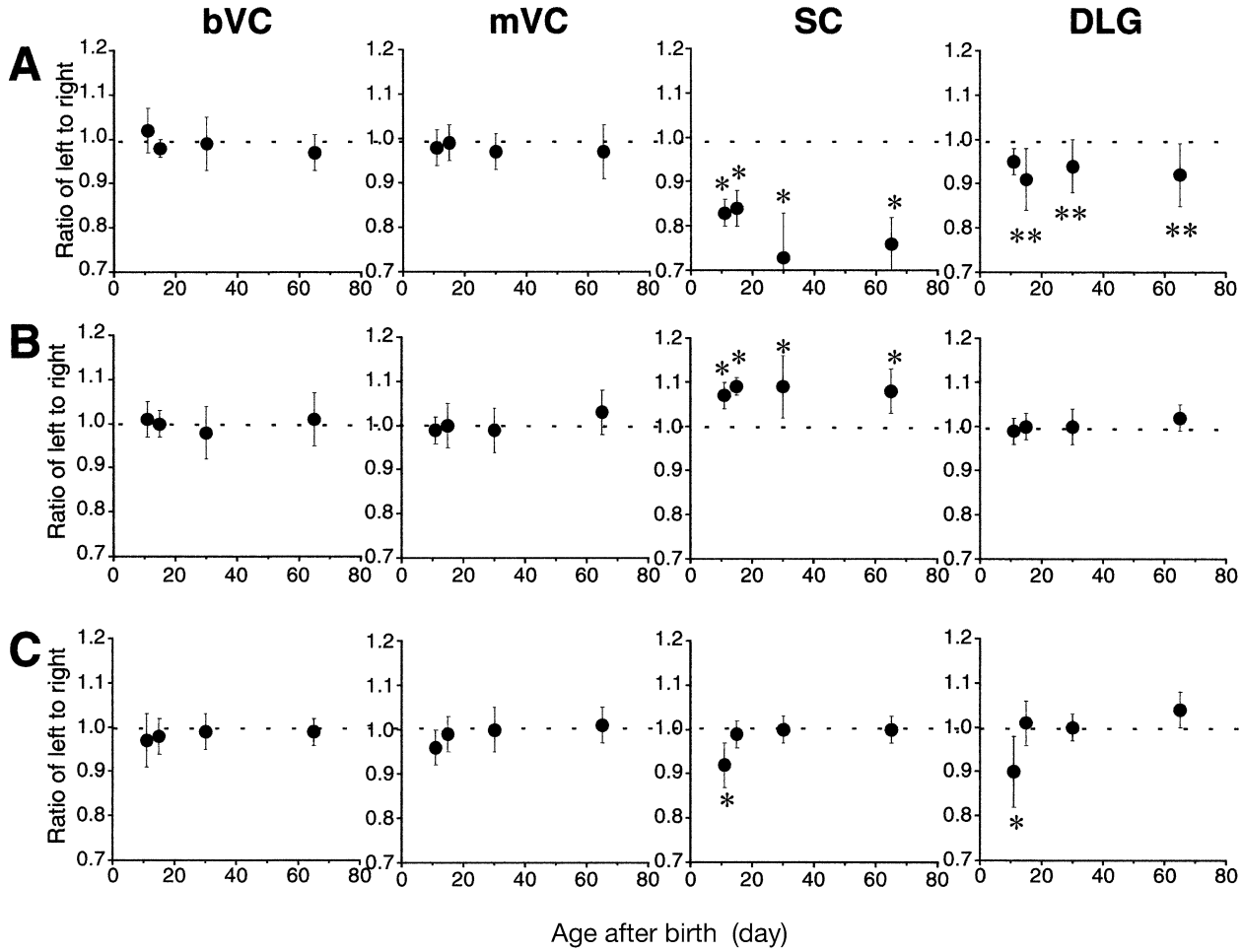

Figure 3. Time course of the left-to-right ratios of the receptor-specific binding of [^{11}C]MPDX (**A**) and [^{11}C]flumazenil (**B**) and of the uptake of [^{14}C]2-deoxyglucose (**C**) in the visual structures of the rats after the monocular enucleation. bVC: binocular area of visual cortex, mVC: monocular area of visual cortex, SC: superior colliculus, DLG: dorsal lateral geniculate nucleus. Enucleation was performed on postnatal day 10. *$P < .01$, **$P < .05$ (Student t-test).

After eyelid suture, no significant alterations were found in the left-to-right ratios of the [^{11}C]MPDX binding in any brain region at any period (Figure 4A), and the ratios for the [^{11}C]flumazenil binding decreased slightly but significantly at day 20 (0.95, $P < .05$) in the mVC. The uptake ratios of [^{14}C]2-deoxyglucose were 0.96 ($P < .05$) and 0.95 ($P < .01$) in the SC at day 20 and 55, respectively, and it was 0.95 in the mVC at day 55 ($P < .01$).

Discussion

We have compared the effects of monocular enucleation to those of eyelid suture on the adenosine A_1 and benzodiazepine receptor density and glucose metabolism in different visual centers. These two methods of visual deprivation induced very different changes for the two receptors and for glucose metabolism. Monocular enucleation at PN10 induced asymmetrical changes of the three markers in the contralateral visual structures, as was previously shown in 6-week-old rats.[10] However, the effects were much weaker in the 10-day-old rats. Unilateral enucleation reduced the specific binding of [^{11}C]MPDX to adenosine A_1 receptors in the contralateral SC to approximately 50% of that in the ipsilateral SC in the 6-week-old rats.[10,16] However, the reduction was approximately 30% in the 10-day-old rats. Because enucleation resulted in the loss of the retinal projection terminals in the contralateral SC, the difference observed in the two groups of rats suggests two possibilities; first, in 6-week-old rats, 50% of the adenosine A_1 receptors in the SC are present on the presynaptic retinal projection terminals, and the remaining are present on other neu-

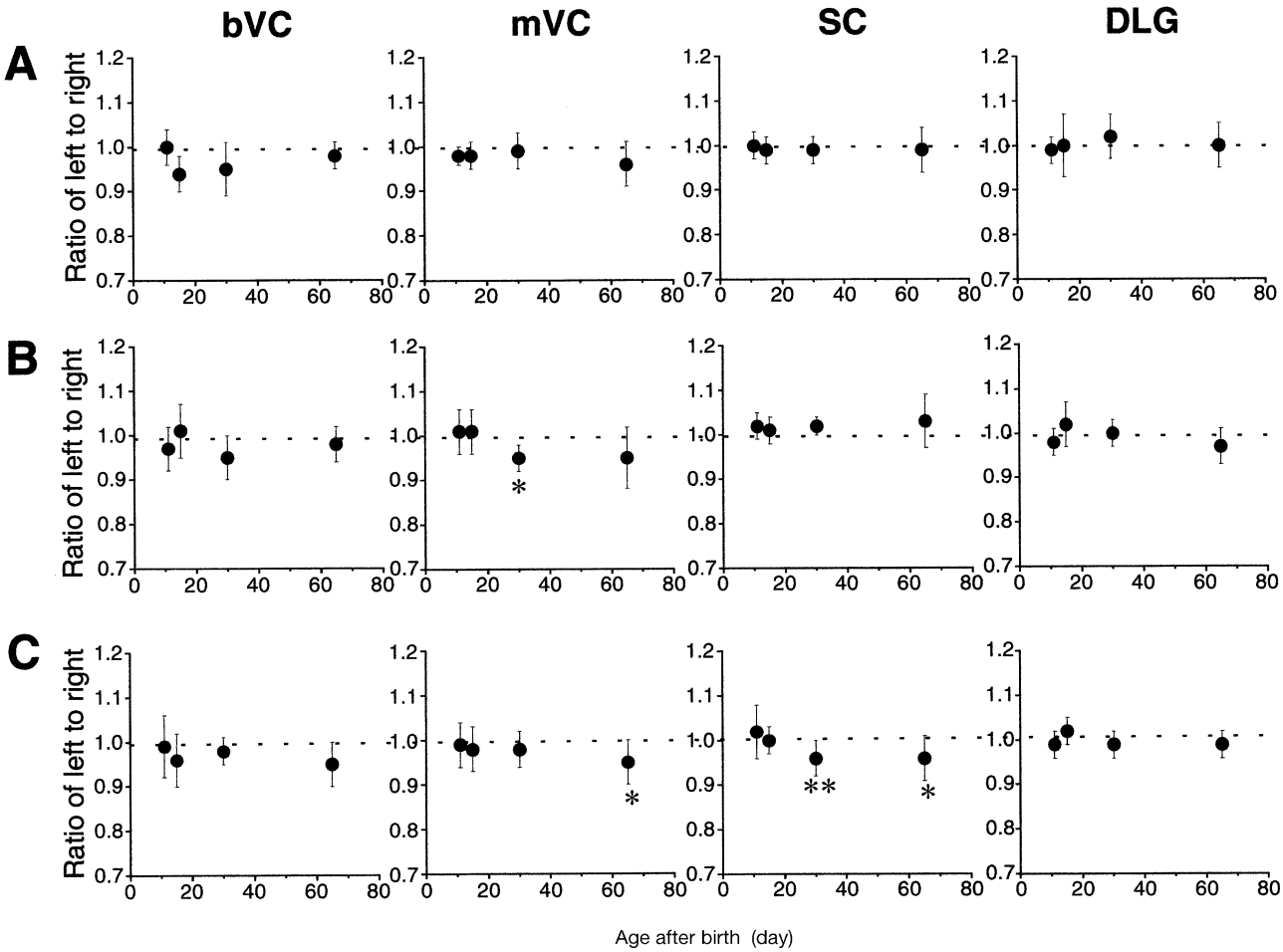

Figure 4. Time course of the left-to-right ratios of the receptor-specific binding of [^{11}C]MPDX (**A**) and [^{11}C]flumazenil (**B**) and of the uptake of [^{14}C]2-deoxyglucose (**C**) in the visual structures of the rats after eyelid suture. Suture was performed on postnatal day 10. *$P < .01$, **$P < .05$ (Student *t*-test). bVC: binocular area of visual cortex, mVC: monocular area of visual cortex, SC: superior colliculus, DLG: dorsal lateral geniculate nucleus.

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rons. In the 10-day-old rats, on the other hand, the corresponding ratio is 30%. The second possibility is that the percentage of ganglion cell axons decussating in the optic chiasma is lower in the 10-day-old rats than in 6-week-old rats, and the percentage of decussating axons after the enucleation was lower in 10-day-old rats than in adult rats. Lund et al[19] have reported that the uncrossed optic nerve terminals increased only when enucleation was performed within the first 2 weeks after birth. Yagi et al[20] also demonstrated an expansion of uncrossed visual fibers from the retina to ipsilateral visual structures and normal uncrossed visual pathways by vertical-horizontal discrimination studies.

After unilateral enucleation, an increase in the specific binding of [^{11}C]flumazenil to benzodiazepine receptors in the contralateral SC was observed in 10-day-old rats just as previously found in 6-week-old rats.[10] The increase in the ratio of the benzodiazepine receptor density in the contralateral SC to that in the ipsilateral SC was lower in the 10-day-old rats than in the 6-week-old rats. However, the upregulation continued during the entire period investigated, while a transient upregulation was found at 2–3 weeks after the enucleation in the 6-week-old rats.

Interesting findings were made in the functional activity of the visual structures by the deoxyglucose technique. The [^{14}C]2-deoxyglucose uptake was reduced in the contralateral SC and DLG at day 1 after monocular enucleation, but thereafter the level recovered to the normal level. No reduction was found in the mVC and bVC at any time investigated after the enucleation in young rats. These results are markedly different from those in 6-week-old rats where the [^{14}C]2-deoxyglucose uptake was reduced in the contralateral VC and SC to the level of approximately 50% of the ipsilateral regions after the enucleation and was followed by a gradual recovery. The destruction of the anterior visual pathway caused a depression of glucose metabolism in the visual centers of the brain.[3,10] These findings may be partially explained by the incomplete crossover of ganglion cell axons projecting to the SC and DLG in immature rats as described above. Another possibility is that the visual deprivation by enucleation in rats at PN10, when eyes are not yet open, interferes with the development of visual function. Fagiolini et al found that all visual cortical functions were immature in the young rats at PN17 to PN19, and visual acuity was half of the adult value.[21] Consequently, the contralateral visual structures may have unknown functions that are comparable to the visual functions in the ipsilateral visual structures as judged by a deoxyglucose technique. Veraart et al reported that the glucose metabolism in the visual cortex is higher in the early onset blind humans than in controls despite immature visual functions, suggesting that some extraretinal activation of the visual cortex could originate from other pathways or else the density of supranumerary synapses remained high during development.[22,23]

Monocular deprivation by unilateral eyelid suture did not affect the adenosine A_1 receptors in any visual structures investigated up to 55 days after the surgery. In contrast to monocular enucleation, eyelid suture did not produce an upregulation of the density of benzodiazepine receptors in the SC. Rothe et al also reported no alteration of the density of the benzodiazepine receptors in the SC, DLG, and VC 15 days after eyelid suture at PN10 by in vitro binding assay using a membrane preparation.[12,24] In a preliminary study on 6-week-old rats, we also found that monocular eyelid suture did not alter the adenosine A_1 receptors or the benzodiazepine receptors in any visual centers (not published). Together, these results suggest that interference with visual experience by eyelid suture does not change either the presynaptic adenosine A_1 receptors or the postsynaptic benzodiazepine receptors in the SC in both 10- and 6-week-old rats. Thus, degeneration of the retinal ganglion cell axons following enucleation produces a decrease in adenosine A_1 receptors and a consequent upregulation of the benzodiazepine receptors in the SC. In contrast, Schliebs et al reported that rearing in complete darkness from birth until PN25 induced a significant decrease in the binding levels of benzodiazepine receptors in the DLG and in the SC by 29% and 17%, respectively.[13] Presumably, rats still receive a higher level of stimulation by eyelid suture than by complete darkness, and visual functions may develop to a certain degree.

In the mVC, on the other hand, a slight decrease in the density of benzodiazepine receptors was found at later times after the eyelid suture; 5% reduction at day 20 ($P < .05$) and at day 55 (not significant). It is known that the benzodiazepine receptor density changes during postnatal development in the visual centers.[13,14] Eyelid suture may partially interfere with the normal development of visual function, which may account for the slight decrease (5%) in the density of benzodiazepine receptors. Werner et al found that monocular deprivation by eyelid suture from PN12 to PN80 reduced the number of apical dendrites on the pyramidal cells in layer V of the contralateral mVC.[25]

Eyelid suture at PN10 did not affect the glucose metabolism in any contralateral visual structures in-

vestigated at day 1 and day 5, but resulted in a slight decrease later: a 5% reduction in the SC at day 20 and day 55, and a 5% reduction in the mVC at day 55. The reduction in the bVC was not significant. In contrast with the 10-day-old rats, monocular eyelid suture in 6-week-old rats decreased glucose metabolism much more in the contralateral visual structures than in the ipsilateral ones: a 4% and 12% decrease in the SC and mVC, respectively, at day 1 after surgery, and 3% and 14% in the SC and mVC, at day 7 (unpublished data). The differences observed in two groups of rats suggest the following possibilities. Usually rat eyes open between PN14 and PN16, when the visual functions are not completely developed.[21] Consequently, there was no effect on the glucose metabolism in the visual system at day 1 and day 5 after eyelid suture at PN10. During the development of the visual functions after the eye opening, light stimuli were weaker in the lid-sutured eye than in the untreated eye, which might have resulted in the reduction of glucose metabolism in the contralateral SC and mVC at day 20 and day 55 after the eyelid suture. However, it is also possible that the incomplete development of visual functions caused a smaller reduction in the metabolic activity in the rats that were eyelid-sutured at PN10 than in those rats treated at 6 weeks of age.

We have evaluated the effects of visual deprivation on the neuroreceptors and glucose metabolism as the difference between the changes in the contralateral and ipsilateral visual structures. It should be noted that the effects of the deprivation on both sides were not evaluated. Schliebs et al[26] had reported that the binding of [^{3}H]quinuclidinyl benzylate to muscarinic cholinergic receptors was significantly increased on both sides of the SC but reduced in both the VC of rats after unilateral eyelid suture at PN10. Aurich et al[27] also reported that [^{3}H]rauwolscine binding to alpha 2-adrenergic receptor sites was decreased in both the ipsilateral and contralateral DLG of 3-month-old rats by unilateral eyelid suture at PN11.

In conclusion, axon degeneration in rats monocularly enucleated at PN10 resulted in a decrease in the density of the adenosine A_1 receptor in the SC and a subsequent upregulation of postsynaptic benzodiazepine receptor density. These results suggest that both adenosine A_1 and benzodiazepine receptors are vulnerable to deafferentiation. Compared with the responses in the 6-week-old rats, the decrease in the adenosine A_1 receptors was less significant in the 10-day-old rats, and the extent of the upregulation of benzodiazepine receptors was also less, but prolonged. Enucleation at PN10 did not decrease glucose metabolism in any visual structures at any time up to day 55, except in the SC and DLG at 1 day after the treatment. These findings are very different from the significant reduction in the 6-week-old rats reported previously. However, eyelid suture slightly decreased the glucose metabolism in the contralateral VC and SC on day 20 and later after the treatment, probably because of the development of the visual functions. These results suggest that neural reactions following enucleation are completely different from those following eyelid suture, and that the visual functions are not completely developed in rats at PN10.

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