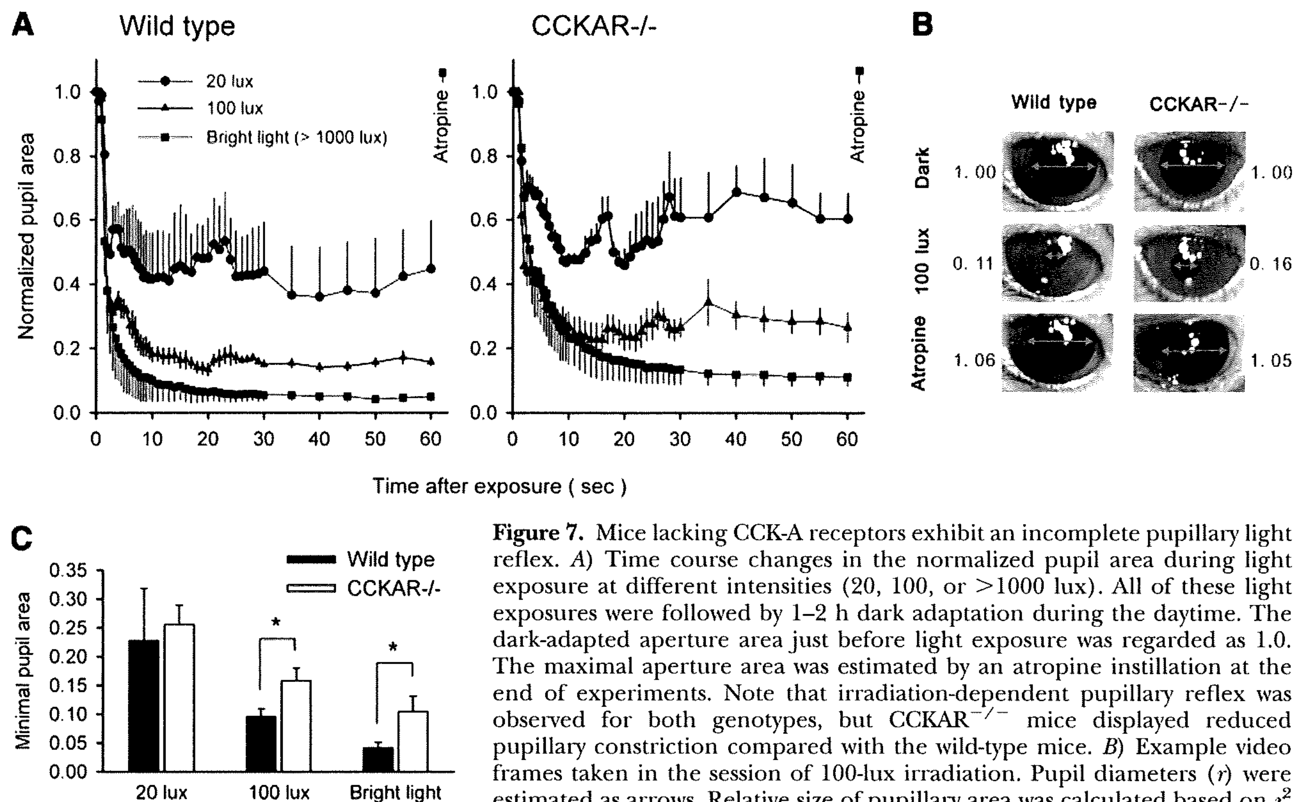

Figure 7. Mice lacking CCK-A receptors exhibit an incomplete pupillary light reflex. *A*) Time course changes in the normalized pupil area during light exposure at different intensities (20, 100, or >1000 lux). All of these light exposures were followed by 1–2 h dark adaptation during the daytime. The dark-adapted aperture area just before light exposure was regarded as 1.0. The maximal aperture area was estimated by an atropine instillation at the end of experiments. Note that irradiation-dependent pupillary reflex was observed for both genotypes, but CCKAR$^{-/-}$ mice displayed reduced pupillary constriction compared with the wild-type mice. *B*) Example video frames taken in the session of 100-lux irradiation. Pupil diameters (r) were estimated as arrows. Relative size of pupillary area was calculated based on r^2 of dark-adapted pupil image. *C*) The minimal pupil area during 1 min of light exposure was significantly larger in CCKAR$^{-/-}$ mice than in the wild-type mice when they were exposed at 100 lux or >1000 lux. n = 4–5 in each group. $^*P < 0.05$ by Student's t test.

cells. Inhibition of GABAergic amacrine cells would then disinhibit the bipolar ganglion cell synapse. Retinal ganglion cells, which project to the SCN, could then be activated by ON bipolar cell activity (**Fig. 8**). It is also possible that the excitation of glycinergic amacrine cells by activation of CCK-A receptors may inhibit OFF bipolar terminals, which may also allow activation of retinal ganglion cells. It has been suggested, however, that intrinsically photosensitive retinal ganglion cells, which are the predominant type (~75%) of SCN-projecting retinal ganglion cells (43) only receive ON bipolar terminals and amacrine cell terminals (8).

Rhythmic *mPer1* expression has been reported to occur in the majority of GABAergic amacrine cells but not glycinergic amacrine cells (44). Also, the AII amacrine cells exhibit circadian rhythms in parvalbumin expression in constant darkness (45). Therefore, a subpopulation of amacrine cells may contain an intrinsic molecular clock mechanism. It has not been shown previously, however, that amacrine cell-mediated pathways have a critical role in circadian photoentrainment. The present results showed that CCKAR$^{-/-}$ mice exhibited significantly reduced bright light-induced behavioral phase shifts; thus, we propose that the CCK-A receptor-mediated amacrine cell pathway has an important role in circadian photoentrainment *via* cone photoreceptor pathways, although we cannot exclude the possible involvement of rods in this signaling pathway only with the results of behavioral phase shifts. In specific photoreceptor-deficient mutants, such as midwavelength coneless mice (46), exposure to full-spectrum light or saturating bright light (>100 lux) masks their deficiency in circadian photoentrainment. In the present study, however, CCKAR$^{-/-}$ mice exhibited a significantly reduced saturating bright light response, suggesting that multiple cone-mediated pathways may be processed *via* amacrine cells that express CCK-A receptors. This hypothesis is consistent with the dense lateral distribution of CCK-A receptors within the inner nuclear layer that we observed in the present study using X-gal staining.

CCK-A receptors may control outputs and inputs of the circadian clock

The present data show that CCK-A receptors are involved in the photic input to the SCN for photoentrainment. This finding raises the possibility that CCK receptors in related structures may have additional functions in other aspects of circadian control of behaviors. For example, the CCK peptide is known as an important regulator for feeding behaviors, and CCK-A receptors may participate in control of satiety both *via* central and gastrointestinal systems (47). As described above, efferent fibers from the SCN contain CCK peptides (16), and they may terminate on satiety-controlling hypothalamic nuclei such as the paraventricular nucleus and dorsal medial hypothalamus that express CCK-A receptors (47), observable also in our results (Fig. 1). This suggestion raises the possibility that CCK outputs from the SCN contribute to the circadian rhythms in feeding behaviors. If so, the CCK-A receptor-mediated pathway could not be the sole output from the SCN because CCKAR$^{-/-}$ mice maintain normal nocturnal feeding rhythms (48). Consistent with this hypothesis,

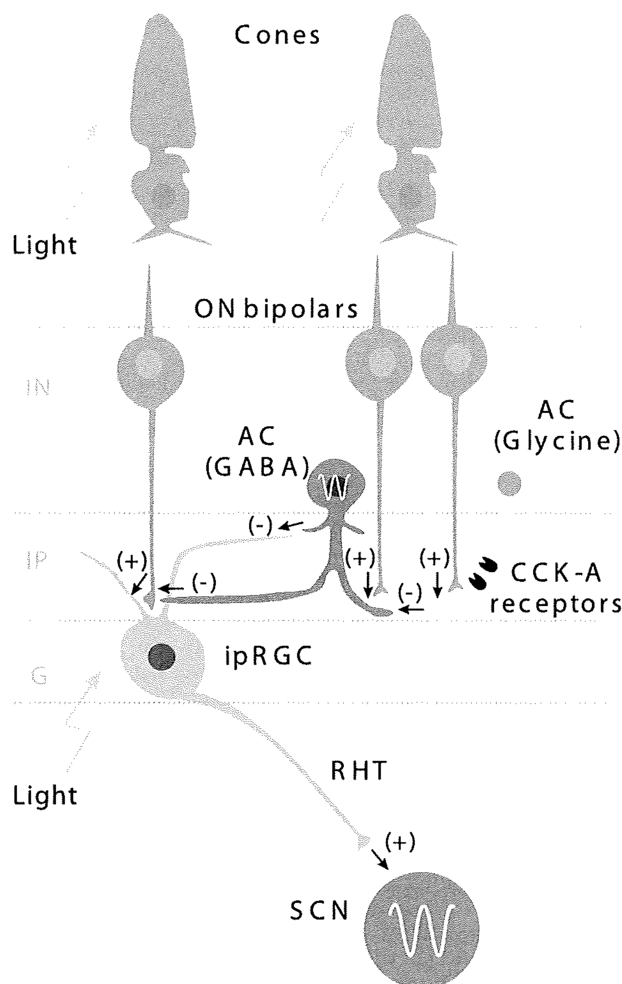

Figure 8. Diagram of the possible retinal circuitry underlying the role of amacrine cells and CCK-A receptors in photoentrainment of the circadian clock. Activation of CCK-A receptors excites glycinergic amacrine cells [AC (Glycine)]. These cells may then inhibit activation of GABAergic amacrine cells [AC (GABA)], which would disinhibit ON bipolar-ganglion cell synapses, allowing activation of intrinsically photosensitive retinal ganglion cells (ipRGC) and then SCN neurons. We propose that glycinergic amacrine cells modulate cone-mediated pathways because CCKAR$^{-/-}$ mice exhibited significantly reduced phase shifts in response to bright light pulses and functional CCK-A receptors were located primarily on glycinergic amacrine cells. Sine waves indicate cell types in which intrinsic intracellular clock gene oscillations have been described (GABAergic amacrine cells and SCN neurons). IN, inner nuclear layer; IP, inner plexiform layer; G, ganglion cell layer; RHT, retinohypothalamic tract. (+) denotes excitatory synaptic transmission, and (−) denotes inhibitory synaptic transmission in the presence of light.

our locomotor activity recordings also showed strong circadian rhythms in CCKAR$^{-/-}$ mice.

We have previously observed that light pulse-induced phase shifts and *c-fos* expression in the SCN were reduced in obese mutant OLETF rats (26, 27), which lack multiple genes, including genes encoding CCK-A receptors (28, 29). The present study further demonstrated that CCKAR$^{-/-}$ mice exhibited impaired circadian photoentrainability similar to that observed in OLETF rats. The CCKAR$^{-/-}$ mice are not obese, presumably due to the basal energy balance of mice, although food intake activity is up-regulated in mutant mice (30, 48, 49). Therefore, a lack of CCK-A receptors, but not an obese phenotype, underlies impaired circadian photoentrainability. The present study demonstrated that in CCKAR$^{-/-}$ mice a key cause of impaired photoentrainability is a deficiency in CCK-A receptor expression in amacrine cells.

As in cerebral CCK-receptive neurons, amacrine cells may receive CCK peptides from neighboring retinal neurons, because CCK peptides have been found in retina (18–22), and blood-retinal barriers may prevent peptide transport from the gastrointestinal system. It has recently been suggested, however, that there may be direct regulation of satiety control centers by peripheral nutritional signals, such as CCK, ghrelin, and leptin, *via* "leaky" portions of the blood-brain barrier and circumventricular organs (47, 50). This suggestion raises the possibility that peripheral CCK harmonistically activates cerebral and retinal CCK-A receptors, adding an interesting aspect to possible circadian clock mechanisms, especially at a systems level and in relation to metabolic control.

In conclusion, our data suggest a novel function for retinal CCK in nonimage-forming visual functions, including circadian photoentrainment and pupillary light reflex. CCK-A receptors on glycinergic amacrine cells may have a key role in the process of photoentrainment, probably modulating retinal ganglion cell activation of the SCN. FJ

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THE OLFACTORY CONDITIONING IN THE EARLY POSTNATAL PERIOD STIMULATED NEURAL STEM/PROGENITOR CELLS IN THE SUBVENTRICULAR ZONE AND INCREASED NEUROGENESIS IN THE OLFACTORY BULB OF RATS

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Abstract—The olfactory memory acquired during the early postnatal period is known to be maintained for a long period, however, its neural mechanism remains to be clarified. In the present study, we examined the effect of olfactory conditioning during the early postnatal period on neurogenesis in the olfactory bulb of rats. Using the bromodeoxyuridine–pulse chase method, we found that the olfactory conditioning, which was a paired presentation of citral odor (conditioned stimulus) and foot shock (unconditioned stimulus) in rat pups on postnatal day 11, stimulated the proliferation of neural stem/progenitor cells in the anterior subventricular zone (aSVZ), but not in the olfactory bulb, at 24 h after the conditioning. However, the number of newborn cells in the olfactory bulb was increased at 2 weeks, but not 8 weeks, after such conditioning. Neither the exposure of a citral odor alone nor foot shock alone affected the proliferation of neural stem/progenitor cells in the aSVZ at 24 h after and the number of newborn cells in the olfactory bulb at 2 weeks after. The majority of newborn cells in the olfactory bulb of either the conditioned rats or the unconditioned rats expressed the neural marker NeuN, thus indicating that the olfactory conditioning stimulated neurogenesis in the olfactory bulb. These results suggest that olfactory conditioning during the early postnatal period temporally stimulates neurogenesis in the olfactory bulb of rats. **© 2008 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: neural stem cells, proliferation, SVZ, bromodeoxyuridine, olfactory memory.

The olfactory function is well known to play an important role in the survival of newborn animals as well as in humans. The long-term olfactory memory acquired during the early postnatal period is involved in these olfactory functions, and therefore they are able to learn their mother's odor and successfully approach her nipple without any visual information (Teicher and Blass, 1976). In general, the paired presentation of odor and somatosensory stimulation is known to be crucial in establishing olfactory learning. In rat pups, the pairing of odor and foot shock is able to establish olfactory learning (Okutani et al., 1999; Sullivan et al., 2000). Okutani et al. (1999) have reported that rat pups that had been exposed to citral odor associated with shock treatment on postnatal day (PD) 11, showed an aversive response to that odor. At present, the mechanism underlying the olfactory learning in early postnatal rats is considered to involve the modulation and plasticity of the synapse in the olfactory bulb (Wilson and Sullivan, 1994; Sullivan and Wilson, 2003), which involves either GABA (Okutani et al., 1999, 2003), noradrenaline (Sullivan et al., 1989, 1992; Yuan et al., 2003) or serotonin (Yuan et al., 2003).

On the other hand, recent studies have revealed that the neural stem/progenitor cells, which possess the ability of proliferation and differentiation into neurons and glial cells (Ono et al., 2001), are located not only in the embryonic brain but also in the postnatal brain, including the anterior subventricular zone (aSVZ), subgranular zone of the hippocampal dentate gyrus (Gage, 2002) and the olfactory bulb (Fukushima et al., 2002; Gritti et al., 2002). The neural stem/progenitor cells in the aSVZ have been proven to migrate via the rostral migratory stream (RMS) and finally differentiate into interneurons, such as granule cells and periglomerular cells in the olfactory bulb, and it takes approximately 2 weeks for neural stem/progenitor cells to migrate and differentiate in the olfactory bulb (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Kato et al., 2001; Coskun and Luskin, 2002). Furthermore, the proliferative and differentiative activities of the neural stem/progenitor cells in the aSVZ dynamically change under various physiological conditions such as pregnancy (Shingo et al., 2003) and enriched odor exposure (Rochefort et al., 2002).

Interestingly, a number of studies have reported hippocampus-dependent learning to enhance adult neurogenesis in the hippocampal dentate gyrus and the newborn neurons integrated in the hippocampal network exhibit synaptic plasticity and are also involved in memory formation (Gould et al., 1999a,b; Shors et al., 2001; Shors, 2004; Pham et al., 2005). These reports suggest that the certain types of learning and memory might be formed by the replacement of newborn neurons derived from the

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Abbreviations: aSVZ, anterior subventricular zone; BrdU, bromodeoxyuridine; CS, conditioned stimulus; PB, phosphate buffer; PBS, phosphate-buffered saline; PBSGT, phosphate-buffered saline containing 1% normal goat serum and 0.3% Triton X-100; PD, postnatal day; RMS, rostral migratory stream; US, unconditioned stimulus.

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neural stem/progenitor cells in the postnatal brain. From these lines of evidence, it is possible that early olfactory learning involves newborn neurons in the olfactory bulb which originates from neural stem/progenitor cells, which develop in the aSVZ and thereafter migrate via the RMS to the olfactory bulb. To explore this possibility, we therefore investigated whether the olfactory conditioning during the early postnatal period affects the neurogenesis in the olfactory bulb of the rats, using the bromodeoxyuridine (BrdU) –pulse chase method.

EXPERIMENTAL PROCEDURES

Animals

Male and female pups of Long-Evans rats (SLC, Shizuoka, Japan) were used. Dams were housed in polypropylene cages (41×25×19 cm) with wood shavings, and were kept in an environment with controlled temperature (23 °C) and light (12-h light/dark). Food and water were available *ad libitum*. The litters were culled to 11 on PD 1 (PD 0 is defined as day of birth). All procedures were conducted in accordance with the guidelines of the Institution for Animal Care and the Use Committee of the Nagasaki University. All experiments conformed to international guidelines on the ethical use of animals. All efforts were made to minimize the number of animals used and their suffering.

Olfactory conditioning and sampling schedule

Olfactory conditioning was performed on PD11. During a 30 min training session, the conditioned subjects received continuous exposure of citral odor [conditioned stimulus (CS)] with concurrent electrical foot shock [unconditioned stimulus (US)] (CS/US group). For odor exposure, absorbent cotton (2×2 cm) with 1 μl of citral (Wako, Osaka, Japan) was attached to the ceiling of the training chamber. The foot shock consisted of 15 presentations of a 5-s electrical shock (0.5 mA) which were given at 2 min intervals. For controls, unconditioned subjects received only citral odor (CS/− group). Additional unconditioned subjects that were naïve subjects (−/− group) and shock-only subjects (−/US group), naïve subjects received neither citral odor nor foot-shock and shock-only subjects received only foot shock. The pups were trained in translucent Plexiglas training chamber with a stainless steel grid floor. Immediately after the training, pups were intraperitoneally injected with BrdU (100 mg/kg). Twenty-four hours after the olfactory conditioning, half the pups were deeply anesthetized with diethyl ether and then were perfused intracardially with 25 ml of chilled saline followed by 25 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB) and thereafter their brains were quickly removed. The remaining pups were returned to their dams and they were maintained for 2 weeks. Two weeks after such olfactory conditioning, the pups were anesthetized and perfused as described above and their brains were quickly removed. To investigate the survival of newly generated cells during the olfactory conditioning, a group of pups was allowed to survive 8 weeks after the odor conditioning. They were anesthetized with diethyl ether and then were perfused intracardially with 100 ml of chilled saline followed by 100 ml of 4% paraformaldehyde in 0.1 M PB and thereafter their brains were quickly removed.

Immunohistochemistry

The brains were post-fixed in 4% paraformaldehyde in 0.1 M PB overnight at 4 °C, followed by immersion in 20% sucrose in 0.1 M PB for 48 h. The brain sections that were processed for immunohistochemistry were sampled at four distinct antero-posterior levels. The frozen coronal sections with a thickness of 30 μm were made with a cryostat (Leica, Nussloch, Germany) at the olfactory bulb, aSVZ, dentate gyrus and basolateral amygdala levels. The sections were incubated in 2× SSC/formamide at 65 °C for 2 h, and then were treated with 1 N HCl at 37 °C for 20 min, followed by neutralization with 0.15 M sodium borate (pH 8.5) at room temperature for 10 min. After three washes with phosphate-buffered saline (PBS), the sections were incubated with rat anti-BrdU antibody (1:100; Oxford Biotechnology, Oxford, UK) diluted with phosphate-buffered saline containing 1% normal goat serum and 0.3% Triton X-100 (PBSGT) at 4 °C overnight, followed by AlexaFluor568-conjugated goat anti-rat IgG (1:200; Molecular Probes, Eugene, OR, USA) and 0.1 μg/ml of Hoechst33258 for a nuclear counterstaining, diluted with PBSGT at room temperature for 2 h. For the double-labeling of the olfactory bulb, the sections were processed for BrdU-immunostaining and then were incubated in primary antibodies at the following dilutions: mouse anti-NeuN antibody 1:500 (Chemicon, Temecula, CA, USA), mouse anti-GFAP antibody 1:150 (Sigma-Aldrich, St. Louis, MO, USA). The sections were incubated with AlexaFluor488-conjugated goat anti-mouse IgG (1:200; Molecular Probes), and 0.1 μg/ml of Hoechst33258 for nuclear counterstaining. After washing with PBS, the sections were mounted, dried and coverslipped with Gel/MountTM, aqueous mounting gel (Biomeda Corporation, Foster City, CA, USA).

Quantification of the number of BrdU-positive cells and the proportion of BrdU-positive cells that co-express neural marker or astroglial marker

Three to five sections in each anatomical region were analyzed. The images were photographed by a digital fluorescent microscope camera (DP70, Olympus, Tokyo, Japan) equipped with a fluorescent microscope (ECLIPSE E600, Nikon, Tokyo, Japan). The number of BrdU-positive cells in the subgranular zone of the dentate gyrus and basolateral amygdala was counted by an observer without any knowledge of the groups. In the olfactory bulb, the number of BrdU-positive cells in the granule cell layer, internal plexiform layer, and mitral cell layer was counted as described above. In the case of aSVZ 24 h after olfactory conditioning, the number of BrdU-positive cells was counted using the Scion image software (Scion Corporation, Frederick, MD, USA) as previously reported (Aida et al., 2002). Double-labeling experiments were analyzed by confocal scanning microscopy (LSM510, Carl Zeiss, Jena, Germany).

Statistical analysis

The data in the present study were statistically analyzed by one-way ANOVA followed by either Fisher's protected LSD post hoc test or unpaired Student's *t*-test.

RESULTS

The effect of olfactory conditioning on the proliferation of the neural stem/progenitor cells

To determine whether the olfactory conditioning affects the proliferation of the neural stem/progenitor cells in the olfactory bulb, we quantified the number of BrdU-positive cells in the olfactory bulb of the conditioned (CS/US group) and unconditioned (CS/− groups) pups 24 h after the olfactory conditioning followed by BrdU injection. Fig. 1A shows the representative immunofluorescence images of BrdU-positive cells in the olfactory bulb of CS/− and CS/US pups. There was no statistical difference in the number of BrdU-positive cells in the olfactory bulb between CS/US and CS/− groups (Fig. 1B). We examined whether olfactory conditioning affects the proliferation of the neural stem/progenitor cells in the aSVZ. We counted the number

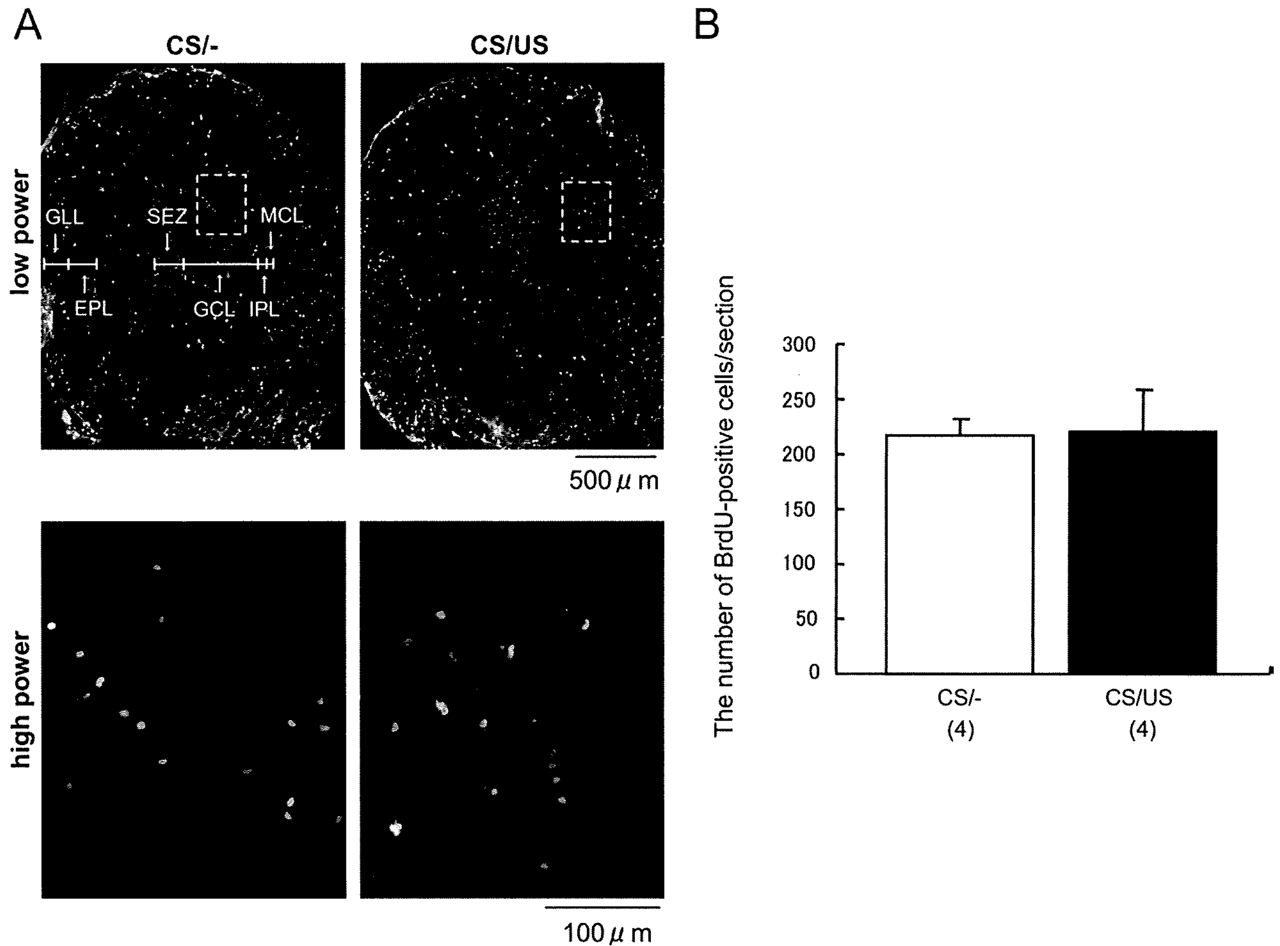

Fig. 1. Olfactory conditioning fails to affect the proliferation of the neural stem/progenitor cells in the olfactory bulb. (A) The representative immunofluorescence images of BrdU-positive cells in the olfactory bulb of CS/− and CS/US pups 24 h after the conditioning. The upper images represent entire olfactory bulb and lower images are the enlargement of the granule cell layer enclosed by dashed rectangle in upper images. SEZ, subependymal zone; GCL, granule cell layer; IPL, interplexi layer; MCL, mitral cell layer; EPL, external plexi layer; GLL, glomerular layer. (B) The number of BrdU-positive cells in the olfactory bulb. The number in parentheses indicates the number of pups.

of BrdU-positive cells in the aSVZ of the conditioned (CS/US group) and unconditioned (CS/−, −/− and −/US groups) pups at 24 h after the olfactory conditioning followed by BrdU injection. Fig. 2A shows the representative immunofluorescence images of BrdU-positive cells in the aSVZ of CS/− and CS/US pups. The number of BrdU-positive cells within the aSVZ in the CS/US group was significantly more abundant in comparison to those in the CS/− control groups (Fig. 2B). In the other groups, no statistical difference was observed in the number of BrdU-positive cells in the aSVZ between the −/− group [758.6±66.50 (n=4)] and −/US group [816.4±97.31 (n=3)], or between the −/− group [758.6±66.50 (n=4)] and CS/− group [744.7±76.59 (n=7)]. In the subgranular zone of the hippocampal dentate gyrus, there was no statistical difference in the number of BrdU-positive cells between the CS/− group [68.3±3.16 (n=4)] and CS/US group [72.2±2.45 (n=4)]. Since basolateral amygdala is reported to contain BrdU-positive cells in adult rodents (Wennstrom et al., 2004), we examined the effects of olfactory conditioning on the number of BrdU-positive cells in the basolateral amygdala. However, no significant difference was found in the number of BrdU-positive cells in the basolateral amygdala between the CS/− group [19.4±0.65 (n=4)] and the CS/US group [18.3±1.45 (n=4)].

The effect of olfactory conditioning on the differentiation of neural stem/progenitor cells

We examined whether the olfactory conditioning has any effect on the differentiation of the neural stem/progenitor cells. Therefore, we quantified the number of BrdU-positive cells in the olfactory bulb of conditioned (CS/US group) and unconditioned (CS/−, −/− and −/US groups) pups at 2 weeks after the olfactory conditioning followed BrdU injection, since it takes approximately 2 weeks for neural stem/progenitor cells to migrate and differentiate in the olfactory bulb (Lois and Alvarez-Buylla, 1994). Fig. 3A shows the representative immunofluorescence images of BrdU-positive cells in the olfactory bulb in CS/− and CS/US pups 2 weeks after the conditioning and BrdU injection. The number of BrdU-positive cells in the olfactory

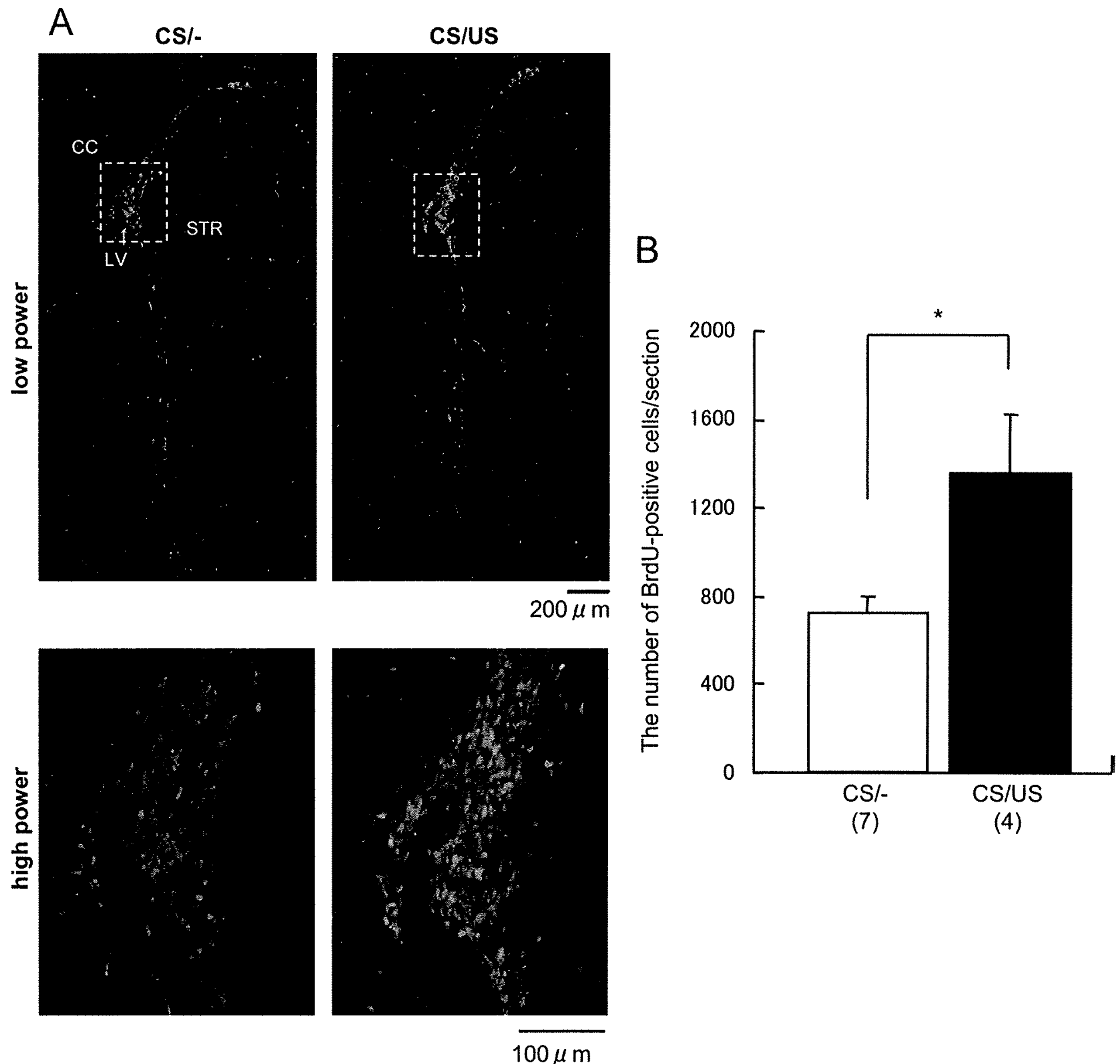

Fig. 2. Olfactory conditioning stimulates the proliferation of the neural stem/progenitor cells in the aSVZ. (A) The representative immunofluorescence images of BrdU-positive cells in the aSVZ of CS/− and CS/US pups 24 h after conditioning. The position of sections that used for cell counting is between Bregma levels +0.3 and +1.2 mm. The upper images represent the entire aSVZ and the lower images show the higher magnification of enclosed area by a dashed rectangle in the upper images. CC, corpus callosum; LV, lateral ventricle; STR, striatum. (B) The number of BrdU-positive cells in the aSVZ. The number in parentheses indicates the number of pups. The asterisk indicates a significant difference ($P<0.01$, one-way ANOVA followed by Fisher's PLSD test).

bulb of CS/US group [578.7±39.62 ($n=14$)] was significantly more abundant in comparison to those in CS/− control groups [477.8±26.12 ($n=18$)] (Fig. 3B). In the other groups, no statistical difference was observed in the number of BrdU-positive cells in the olfactory bulb between the −/− group [485.5±34.54 ($n=9$)] and the −/US group [515.4±8.63 ($n=3$)], or between the −/− group [485.5±34.54 ($n=9$)] and the CS/− group [477.8±26.12 ($n=18$)]. The number of BrdU-positive cells in the olfactory bulb of CS/US group [578.7±39.62 ($n=14$)] tended to increase in comparison to those in −/− control group [485.5±34.54 ($n=9$)] ($P=0.078$), though this difference did not reach a significant level.

We also counted the number of BrdU-positive cells in the aSVZ of conditioned (CS/US group) and unconditioned (CS/− groups) pups 2 weeks after the olfactory conditioning and BrdU injection. There was no statistical difference in the number of BrdU-positive cells between the CS/− group [30.64±6.27 ($n=4$)] and the CS/US group [38.19±8.03 ($n=4$)].

We next examined the effect of olfactory conditioning on the fate of the neural stem/progenitor cells. Therefore, we quantified the proportion of BrdU-positive cells that co-ex-

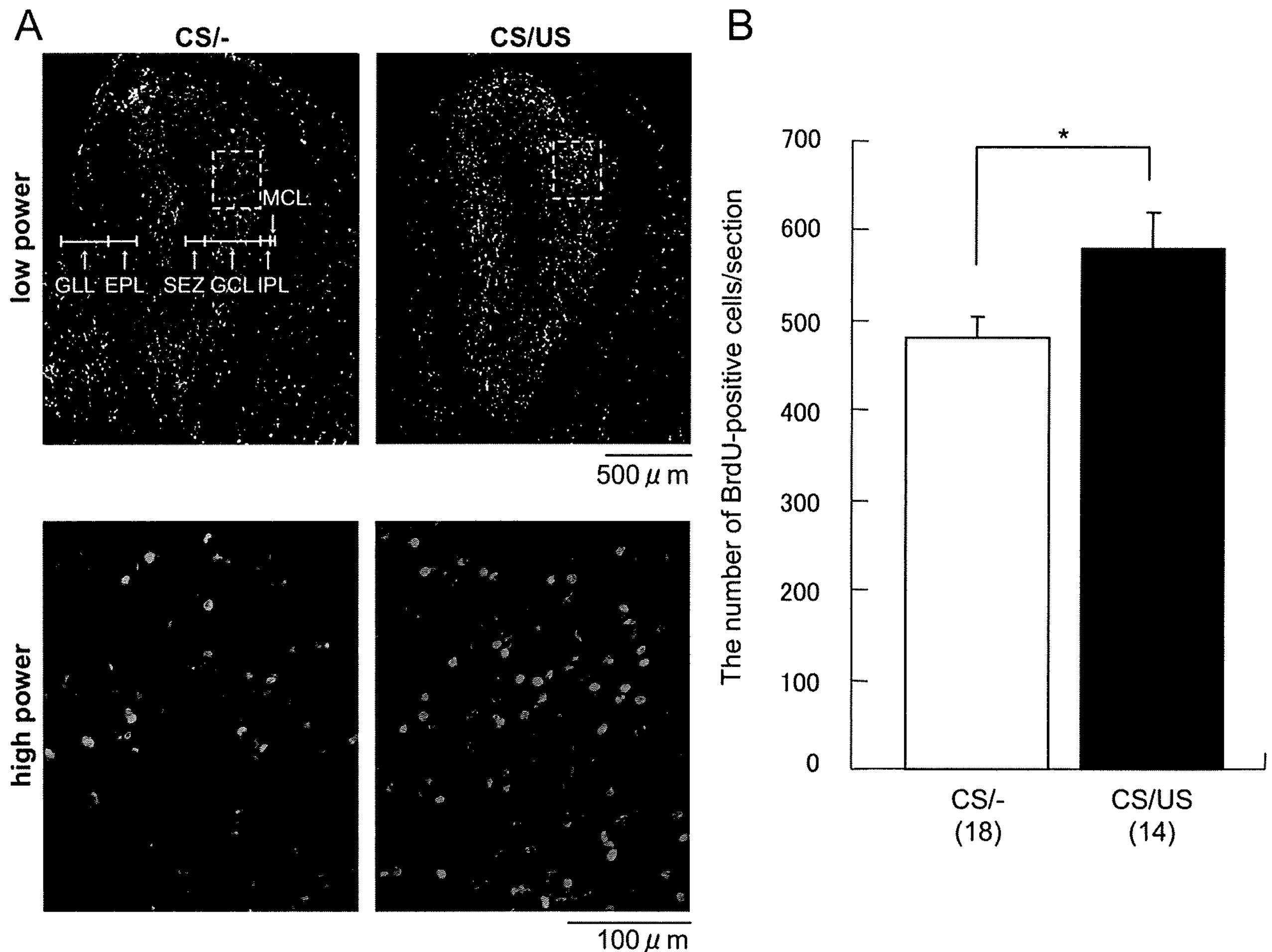

Fig. 3. Olfactory conditioning increases the production of newborn cells in the olfactory bulb. (A) Representative immunofluorescence images of BrdU-positive cells in the olfactory bulb of CS/− and CS/US pups 2 weeks after conditioning. The upper images represent the entire olfactory bulb while the lower images show the enlargement of the granule cell layer enclosed by a dashed rectangle in the upper images. SEZ, subependymal zone; GCL, granule cell layer; IPL, interplexi layer; MCL, mitral cell layer; EPL, external plexi layer; GLL, glomerular layer. (B) The number of BrdU-positive cells in the olfactory bulb. The number in parentheses indicates the number of pups. An asterisk indicates a significant difference ($P<0.05$, one-way ANOVA followed by Fisher's PLSD test).

pressed the neuronal marker NeuN or the astroglial marker GFAP in the granule cell layer of the olfactory bulb in the conditioned (CS/US group) and unconditioned (CS/− group) pups 2 weeks after the olfactory conditioning and BrdU injection. Fig. 4A and 4B exhibit the representative confocal images double-labeled with BrdU-positive cells and NeuN or GFAP in the olfactory bulb. The majority of the BrdU-positive cells co-expressed NeuN, while the BrdU-positive cells with GFAP expression were only sparsely observed. Fig. 4C shows the proportion of the cells double-labeled with either NeuN or GFAP in the olfactory bulb. No difference was observed in the proportion of the BrdU-positive cells co-expressing either NeuN or GFAP between the conditioned (CS/US) group and the unconditioned (CS/−) group.

To investigate the survival of cells newly generated during the olfactory conditioning, we quantified the number of BrdU-positive cells and the proportion of BrdU-positive cells that co-expressed the neuronal marker NeuN or the astroglial marker GFAP in the granule cell layer of the olfactory bulb in the conditioned (CS/US group) and unconditioned (CS/− group) pups 8 weeks after the olfactory conditioning and BrdU injection. Even 8 weeks after the olfactory conditioning followed by BrdU injection, newly generated cells which incorporated BrdU during the olfactory conditioning was observed in the olfactory bulb. There was, however, no statistical difference in the number of BrdU-positive cells between the CS/− [215.4±15.56 (n=4)] and the CS/US group [203.4±6.89 (n=4)]. In regard to the proportion of the BrdU-positive cells co-expressing NeuN, no difference was observed between the CS/− [83.1±1.1% (n=4)] and the CS/US group [84.6±1.2% (n=4)]. Likewise, we could not detect any difference in the proportion of the BrdU-positive cells co-expressing GFAP between the CS/− [14.3±0.6% (n=4)] and the CS/US groups [15.9±1.0% (n=4)].

DISCUSSION

The present study was designed to investigate whether the olfactory conditioning during the early postnatal period

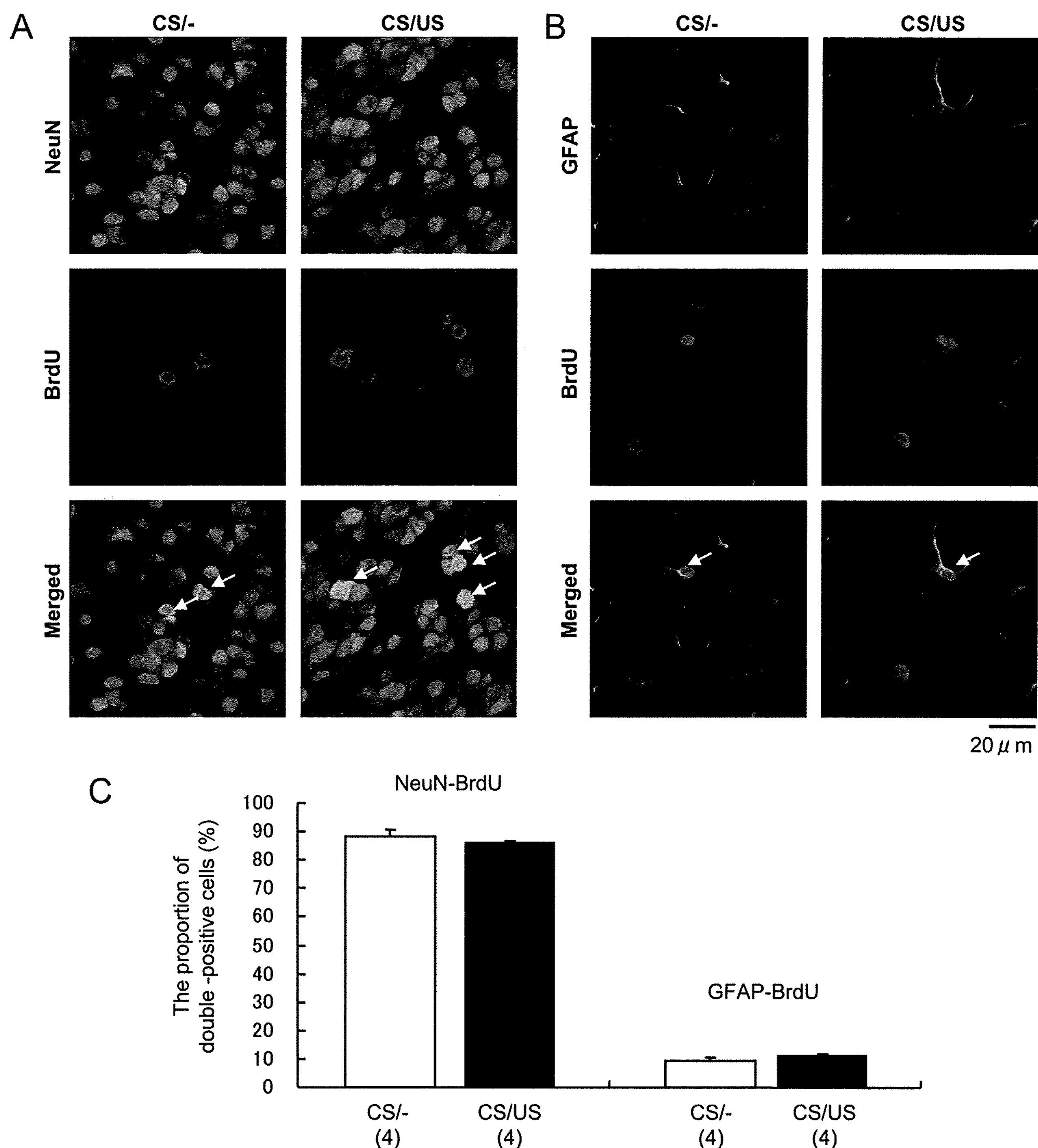

Fig. 4. Most of the newborn cells express the neural marker NeuN. (A) Representative confocal images of the double labeling of BrdU-positive cells with the neural marker NeuN in the olfactory bulb of CS/− and CS/US pups at 2 weeks after conditioning. The arrows in the merged image indicate double-positive cells. (B) The representative confocal images of double labeling of BrdU-positive cells with the astroglial marker GFAP in the olfactory bulb of CS/− and CS/US pups 2 weeks after the conditioning. The arrows in the merged image indicate double-positive cells. (C) The proportion of BrdU–NeuN and BrdU–GFAP double-positive cells in the granule cell layer of the olfactory bulb. The number in parentheses indicates the number of pups.

affects the neurogenesis in the olfactory bulb of rat pups. Using the BrdU–pulse chase method, we found early olfactory conditioning in rats to stimulate the proliferation of the neural stem/progenitor cells in the aSVZ followed by an increase in the number of newborn neurons in the olfactory bulb 2 weeks, but not 8 weeks, later. These results suggest that olfactory conditioning activated the neural stem/progenitor cells in the aSVZ and that the neural stem/progen-

itor cells might migrate rostrally thereby inducing an increase in neurogenesis in the olfactory bulb. To our knowledge, this is the first report to show that olfactory conditioning during the early postnatal period temporally stimulates neurogenesis in the olfactory bulb of rats.

It is well known that the olfactory bulb plays a critical role in odor learning (Wilson and Sullivan, 1994; Sullivan and Wilson, 2003). Since a number of studies have revealed neurogenesis to occur in the postnatal olfactory bulb (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Coskun and Luskin, 2002), it is therefore possible that neurogenesis could be involved in the neural plasticity in the olfactory bulb. To explore this possibility, we examined whether the olfactory conditioning in early postnatal rats stimulates the neurogenesis of the neural stem/progenitor cells in the olfactory bulb. As a result, early olfactory conditioning was proven to increase neurogenesis in the olfactory bulb temporally.

We determined where the newborn neurons in the olfactory bulb came from. Since recent studies have revealed that neural stem/progenitor cells exist in the olfactory bulb (Fukushima et al., 2002; Gritti et al., 2002), we examined whether olfactory conditioning in early postnatal rats stimulates the proliferation of neural stem/progenitor cells in the olfactory bulb 24 h after such olfactory conditioning. However, such olfactory conditioning failed to affect the proliferation of neural stem/progenitor cells in the olfactory bulb. Next, we investigated the effect of olfactory conditioning on the proliferation of neural stem/progenitor cells in the aSVZ, in which neural stem/progenitor cells are known to migrate along the RMS and differentiate into interneurons in the olfactory bulb (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Kato et al., 2001). As a result, the neural stem/progenitor cells were observed to remarkably proliferate in the aSVZ 24 h after olfactory conditioning, thus suggesting that neurogenesis in the olfactory bulb originated from the neural stem/progenitor cells in the aSVZ.

It is possible that the CS (citral odor) alone or the US (foot shock) alone influenced the proliferation of these neural stem/progenitor cells. However, this possibility is unlikely because we found neither the CS alone nor the US alone to affect the proliferation of neural stem/progenitor cells in the aSVZ.

It has been reported that a considerable number of newborn neural stem/progenitor cells die during migration from SVZ to olfactory bulb (Brunjes and Armstrong, 1996) and a small portion of newborn cells in the SVZ could reach cerebral cortex (Gould et al., 2001; Gould and Gross, 2002). Kato et al. (2001) indicated that two-thirds of newly generated neurons in the granule cell layer of the olfactory bulb were lost during the short survival time (6 weeks). In our present study, it should be noted that the difference in the number of BrdU-positive cells in the aSVZ 24 h after the olfactory conditioning between the CS/− group and CS/US group was more apparatus than that in the olfactory bulb 2 weeks after the olfactory conditioning. Furthermore, we demonstrated that a part of newly generated neurons in the olfactory bulb survived 8 weeks after the olfactory conditioning, but a number of BrdU-positive cells were less than a half of those 2 weeks after the olfactory conditioning and there was no significant difference in the number of BrdU-positive cells between the CS/− group and the CS/US group. Based on both the findings of previous reports and our present results, a part of newly generated cells in the aSVZ during the olfactory conditioning might arrive and survive for a long time in the olfactory bulb, leading the decrease in the difference in the number of BrdU-positive cells 2 and 8 weeks after the conditioning.

Hippocampus-dependent learning, such as water maze learning, trace eyeblink conditioning and contextual fear-conditioning, modulates the neurogenesis in the dentate gyrus, but not in the aSVZ (Gould et al., 1999a; Pham et al., 2005). Shors et al. (2001) demonstrated the neurogenesis in the adult dentate gyrus to be causally involved in the formation of trace eyeblink conditioning using a reagent to diminish the number of adult-generated cells. On the other hand, olfactory conditioning was found to affect the proliferation of neural stem/progenitor cells in the aSVZ, but not in the dentate gyrus in the present study. Thus, the neural stem/progenitor cells in the aSVZ and the dentate gyrus are considered to be independently regulated according to individual learning tasks.

In the present study, we showed early olfactory conditioning to be associated with increases in the proliferation of the neural stem/progenitor cells within the aSVZ followed by increases in neurogenesis in the olfactory bulb at 2 weeks after olfactory conditioning, but its precise mechanism remains unknown. In rats that undergo an olfactory bulbectomy, the neural stem/progenitor cells in the aSVZ continued to proliferate and migrate rostrally (Kirschenbaum et al., 1999). In other studies, olfactory deprivation by naris closure did not affect the proliferation or migration of the majority of neural progenitor cells in the SVZ and RMS (Frazier-Cierpial and Brunjes, 1989; Corotto et al., 1994). Our present study demonstrated that the exposure of the citral odor (CS) alone failed to affect the proliferation of neural progenitor cells in the aSVZ. Based on both the findings of previous reports and our present results, the neural activation in the olfactory bulb by odor stimulation is thus suggested to not be sufficient for the increased neurogenesis caused by olfactory conditioning and olfactory conditioning is suggested to necessarily appear to be associated with odor. Since a number of factors such as growth factors and neuropeptides have been shown to regulate the proliferation of neural stem/progenitor cells in the aSVZ (Gritti et al., 1999; Wagner et al., 1999; Shingo et al., 2003), these factors might associate CS with US in order to regulate the proliferation of neural stem/progenitor cells.

It is known that the neural stem/progenitor cells in the aSVZ migrate long distances and differentiate into interneurons, namely granule cells and periglomerular cells in the olfactory bulb (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Zigova et al., 1996). However, it remains to be clarified exactly how newborn neurons are involved in the neural networks of the olfactory bulb. The

granule cells form GABAergic inhibitory synapse to the mitral cells which are the main output neurons from the olfactory bulb. Mutant mice lacking neural cell adhesion molecule have been reported to be deficient in the migration of neural stem/progenitor cells, thus resulting in an impairment of odor discrimination (Gheusi et al., 2000). Similarly, Rochefort et al. (2002) reported an enriched odor environment to increase the number of the newborn granule cells in the olfactory bulb, thereby improving olfactory memory in adult mice. In the present study, we demonstrated the number of newborn neurons in the olfactory bulb to increase by the olfactory conditioning at 2 weeks after, but not 8 weeks after the olfactory conditioning. Further experiments will be required to clarify the role of the temporal stimulation of the neurogenesis by the olfactory conditioning in the brain functions such as olfactory learning and memory.

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■ 特集 低出生体重児の外科 Update

壊死性腸炎の最近の管理

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はじめに

近年，消化管穿孔における壊死性腸炎（necrotizing enterocolitis：NEC）と限局性腸管穿孔（localized intestinal perforation：LIP）は臨床像，組織像の違いから異なる病態として認識されるようになっている。日本小児外科学会が行った2003年の新生児外科全国集計では，NECの登録症例数は1998年の51例から43例と若干減少しているものの，2,500g以下の低出生体重児におけるNECの死亡率は50%であり，それ以外の消化管穿孔の死亡率を上回り，もっとも生命予後不良な疾患となっている[1)]。

本項では，当院の治療方針を提示しつつ，最近の文献に基づいてNECの病因・予防，内科的治療，外科的治療の適応と方法などについて述べる。

I．病　因

NECは，新生児，ことに早産児の未熟な腸管における血流障害と病原性細菌の異常増殖による感染・壊死が主な病態と考えられる[2)]。本症の発症には腸管細菌の増殖が必須であるが，経腸栄養開始の遅れや抗生剤投与がビフィズス菌を主とする正常細菌叢の定着を妨げ，環境からの特定の病原菌のみが腸管内で異常増殖することが引き金となると推測される。腸管機能の未熟性としては，粘液産生・修復能・抗酸化作用・腸管血流の調節能の欠乏，腸管局所の感染防御機構の不全・腸管蠕動異常などがあげられる。NEC発症時には炎症性サイトカインの上昇が報告されているが[3)]，未熟な腸管に病原性細菌による感染や周産期の循環障害による軽微な粘膜障害が刺激となり，局所への好中球集積・炎症性変化，種々のサイトカインの放出がひき起こされる。その結果ノルエピネフリンの急激な上昇，腸間膜動脈収縮による腸管血流低下・虚血・再還流が起こり，組織障害・壊死性変化にいたるものと考えられている[4,5)]。

II．予防（図1）

1．循環管理

NECの発症は腸管の虚血も一因であるため，全身の循環管理は急性期〜慢性期を通して重要と考えられる。とくに未熟児動脈管開存症は本症の危険因子である。当院では出生早期の予防的インダシン投与は行っていないが，心臓超音波検査により動脈管開存症症候化の兆しがみえたところでインダシン投与を開始している。インダシン投与は0.1 mg/kg/doseを8〜24時間ごと3回までを原則とし，インダシン不応性と考えられた場合は，可及的速やかに結紮術を行っている。肺血流増加型先天性心疾患もまた本症発症のhigh risk群であり，状態に応じて鎮静・低酸素療法による体血流維持を図っている。

2．長期絶食の回避と正常細菌叢の確立

未熟な腸管機能の成熟のためには，出生後可及的速やかに母乳栄養を開始することが推奨されている。母乳に含まれる分泌型IgA，ラクトフェリン，リゾチーム，オリゴ糖などの感染防御物質，およびEGF (epidermal growth factor)，TGF-α (transforming growth factor-α)，IGF-1 (insulin-like growth factor-1)，HGF (hepatocyte growth factor)，FGF (fibroblast growth factor) などの細胞増殖因子が未熟な消化管細胞の増殖・成熟を促進する[6)]。またNECの発症にplate-

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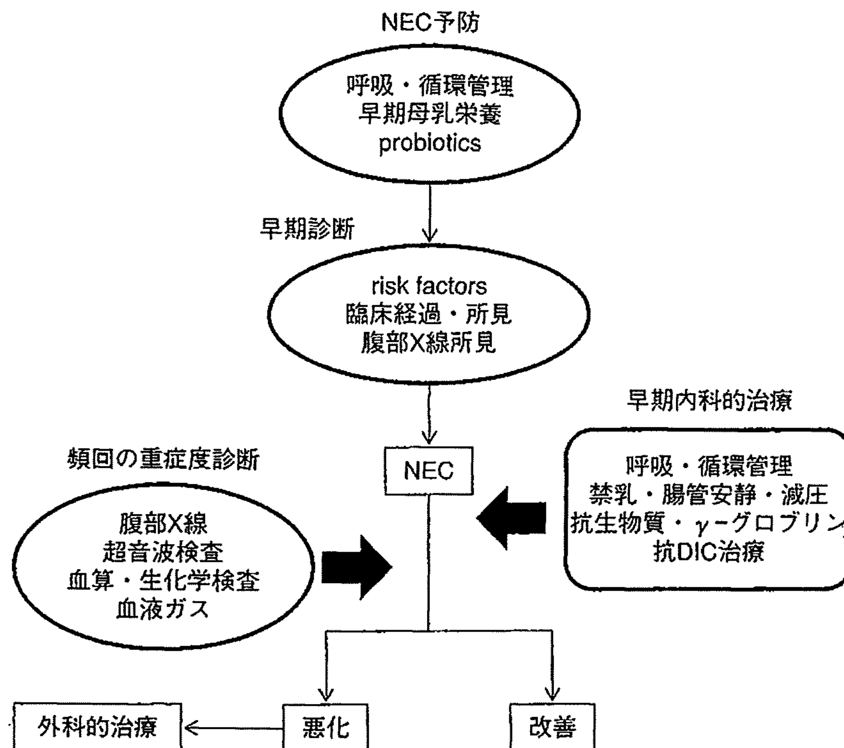

図 1　NEC に対する予防・診断・内科的治療

let-activating factor (PAF) が重要な役割を果たしていると考えられており，母乳中の PAF-acetylhydrase が本症発症を抑制する可能性が動物実験で示唆されている[4]。

ビフィズス菌をはじめとする probiotics 投与により正常細菌叢の早期確立[7]と，本症発生率の低下が報告されているが[8]，投与する probiotics の種類・量・期間についてはまだ一定の見解はない。当院では donor's milk は取り入れていないため，まずは生後 24 時間以内にビフィズス菌製剤投与を開始し，own mother's milk が得られしだい母乳投与を開始している。消化管感染症あるいは敗血症などに対して長期抗生剤投与を必要とした場合は，ミコナゾール併用を行う。ミコナゾールの投与経路は経腸栄養継続中は原則経腸的に，禁乳中は経静脈的に行っている。

Ⅲ. 内科的治療 (図 1)

胃内残乳の増加 (ことに胆汁様吸引)，腹満，腹部色調変化，血便などが認められた場合 (Bell stage Ⅰ) には，NEC を疑い腹部 X 線写真，便培養，血液培養をふくめ各種細菌検査を行う。NEC が疑われた場合は禁乳とし，胃管を開放し，腸管の安静・減圧，抗生剤投与を行う。児の保菌状況に応じて抗生剤を選択するが，菌が同定されるまでは広域性スペクトラムのカルバペネム系抗生剤を使用することが多い。γ-グロブリン低値の場合はγ-グロブリン補充も行う。NEC は数時間の経過で重症化するため，疑った段階で積極的な介入を行うことが大切で，多少の over treatment はやむをえないと考えている。

全身状態の悪化を伴うため，呼吸・循環管理は不可欠である。NEC 発症はほとんどが超低出生体重児であり，通常慢性肺疾患に対しては，軽度の低酸素・高二酸化炭素は容認する呼吸管理を行っているが，本症の管理中は酸素化・換気とも正常に保たれるよう呼吸器設定は高めとする。循環不全に対しては volume 負荷，カテコールアミン投与などによる循環補助を行う。貧血・血小板減少・AT Ⅲ低下を伴うことが多いため，状態に応じて積極的に補充療法・抗 DIC 治療などを行う。

腹部所見の注意深い経過観察と頻回の X 線写真・腹部超音波検査を行い，外科的介入の時期を逸しないようにする。消化管穿孔を起こしていて

もX線写真でfree airが確認できないこともあり，内科的治療で腹部所見が改善しない場合や，超音波検査で腹水の増加傾向や腹水所見の変化を認めた場合は，いたずらに内科的管理に固執するべきではないと考えている。

IV. 外科的治療

1. 手術適応

NECに対する外科的治療の絶対的適応としては消化管穿孔所見が認められる場合であるが，内科的治療が奏功せず腸管壁が全層性壊死（未穿孔状態）となった段階で介入することができれば理想的であると考えられる。重症度については，1978年のBellら[9]の報告したstage分類が現在でも使用されているが，従来NECとされていた病態のなかにはLIPやその他の腸炎も含まれており，NECの治療方針に重要なのはBell stage IIからstage IIIへと進行する症例をいかに予見するかであると考えられる。要するに，Bell stage IIの段階で外科的介入を決定するために有用な画像・検査所見を同定できれば，さらに本症の予後を改善させることが可能と考えられる。

外科的介入のタイミングを図る指標としては，腹部所見のほかに重症度を反映する血液検査所見（CRP，血小板数，血液ガス所見）の推移が重要である。画像診断の指標としては門脈内ガス像（portal venous air：PVA）は以前から外科的介入の指標となるか否かについて議論がなされており，PVAが指標となりうるとの報告も散見される。Sharmaら[10]はPVAを伴うNEC患児64例のうち非手術例33例では30例が生存していることからPVA単独では手術の適応とはならないと報告している。しかし，彼らの報告ではPVAを伴う症例では，PVAを伴わない症例に比して静脈栄養期間が有意に長く，腸管狭窄の発生も有意に高いとの結果が得られており，PVAが腸管の壊死程度を反映する指標である可能性を裏付けている[10]。

また，近年では超音波検査機器の性能が向上するにつれて，低出生体重児でも腹部超音波所見が良好な画像としてとらえることが可能となっている。Silvaら[11]はfree gas, focal fluid collections, 腸管血流の途絶などが予後と関連すると報告している。当院においても，経時的なfluid collectionの評価，Doppler USによる腸管血流の評価を積極的に行う方針としており，今後NECの外科的介入を決めるうえでのさらに重要性を増す検査になると考えている。

2. 外科的治療方針

NECの外科的治療方針については図2のごとくであるが，広範囲壊死が認められない限りsecond-look operationの適応はないことを考えると，まず腹腔ドレナージとするのか，開腹壊死腸管切除とするのか，さらに開腹壊死腸管切除を選択した場合にストーマ造設とするのか，一期的に腸管吻合を行うのかという点が論点になる。

まず，腹腔ドレナージと開腹術のどちらを選択するかであるが，最近のまとまった報告をみても有意な結論は得られていない。Blakelyら[12,13]は，156例（1,000 g以下のNEC患児96例，LIP患児60例）を疾患と治療に基づいて検討し，NEC 96例の腹腔ドレナージ後と開腹治療後の死亡率はそれぞれ63%と50%と報告している。しかし，両群のNECのみに関する治療群別の症例比較がなされておらず有意な結果を示すものではない。また，Mossら[14]はNEC患児117例（34週未満，1,500 g未満）に対して腹腔ドレナージと開腹壊死腸管切除・ストーマ造設のrandomized trialを行い，いずれの方法で介入しても生命予後に有意な影響を与えないと報告している。

開腹術を選択した場合の，ストーマ造設と一期的吻合については，諸家からの報告があるが生存率が良好である報告が多い[15]。しかし，一期的吻合を行う条件については，術前の臨床経過と腸管切除断端の状態が良好であることなどが述べられているが明確ではない。近年では，1,000 g未満の症例に対する一期的吻合も報告されている[16]。

3. 当院におけるNECの外科的治療（表2）

当院は2003年11月に開院した新しい施設であるが，2009年7月までの約5年間の間に，低出生体重児の消化管穿孔性腹膜炎は9例であり，そのうちNECは5例であった（表1）。5例のうち当院での出生例が4例，他院からの搬送例が1例であった。Bell stage IIすなわち壁内ガス像，

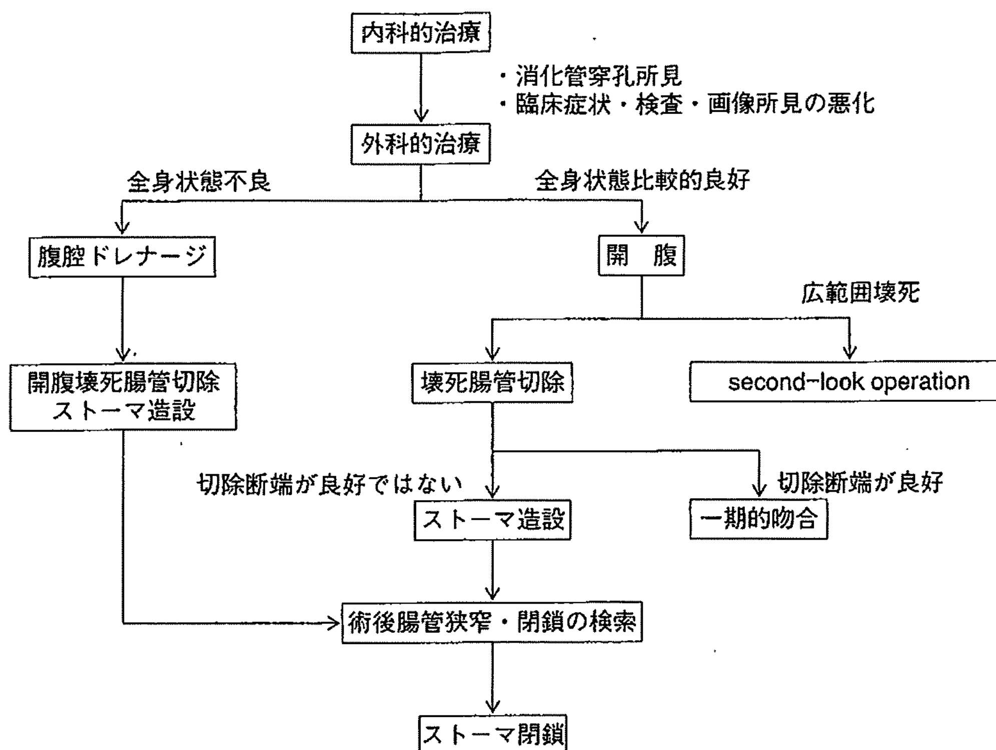

図 2　NEC に対する外科的治療

PVA が確認された症例は，残念ながら内科的治療で改善せず外科的治療となっている。

われわれの基本的な方針としては，呼吸・循環の安定性，検査所見の推移を新生児科医とディスカッションしたうえで，全身状態が不良であれば腹腔ドレナージを優先させ，比較的良好であれば開腹壊死腸管切除・ストーマ造設術を行うこととしている。腹腔ドレナージを優先させた場合には，全身状態の改善傾向を確認して速やかに壊死腸管切除・ストーマ造設術を行う。ストーマは主に回腸を用いて造設し，二連銃の場合と口側は単孔式で肛門側断端は術後の造影・洗浄・整腸剤注入に用いるために腸瘻とする場合がある。一期的吻合については術後結腸狭窄の可能性を危惧して行っていない。実際，われわれの症例では，回腸ストーマ造設を行った生存 4 例のうち壊死・穿孔部以

表 1　当院における NEC 経験例

	在胎週数	出生体重	性別	発症日齢	壊死・穿孔部位	risk factors
症例 1	24w4d	712	F	16	全結腸壊死 S 状結腸穿孔	PPHN*
症例 2	25w2d	746	F	35	S 状結腸穿孔	PDA 手術（日齢 34）
症例 3	25w0d	710	M	43	回腸末端部壊死	PDA（インダシン投与；日齢 1） ESBL*大腸菌感染 総動脈幹症
症例 4	29w0d	1,194	M	22	回腸末端部壊死 虫垂壊死	
症例 5	32w5d	1,512	F	16	遠位側回腸 ～上行結腸壊死	

PPHN：persistant pulmonary hypertension of newborn
ESBL：extended-spectrum β-lactamase

表 2 手術術式，術後結腸狭窄，予後

		手術時日齢	術式	部位	術後狭窄・閉鎖	予後
症例 1	OP1	16	穿孔部閉鎖，回腸ストーマ造設	全結腸壊死 S 状結腸穿孔		死亡
症例 2	OP1	37	腹腔ドレナージ	S 状結腸穿孔		
	OP2	39	穿孔部閉鎖，回腸ストーマ造設			
	OP3	90	回腸ストーマ閉鎖，回盲部形成 結腸ストーマ造設		回盲部閉鎖 S 状結腸の瘢痕化閉鎖	
	OP4	153	結腸ストーマ閉鎖			生存
症例 3	OP1	50	壊死腸管切除， 回腸ストーマ造設，虫垂瘻	回腸末端部壊死		
	OP2	94	回腸ストーマ閉鎖 結腸狭窄部切除・吻合		下行結腸狭窄（図 3）	生存
症例 4	OP1	24	腹腔ドレナージ	回腸末端部壊死 虫垂壊死		
	OP2	28	壊死腸管切除， 回腸ストーマ造設，チューブ回腸瘻			生存*
症例 5	OP1	17	腹腔ドレナージ，腸瘻造設	遠位側回腸上行結腸壊死		
	OP2	104	壊死腸管切除，回腸ストーマ造設			
	OP3	179	回腸ストーマ閉鎖，下行結腸ストーマ造設		S 状結腸の瘢痕化閉鎖（図 4）	生存*

*ストーマ閉鎖予定

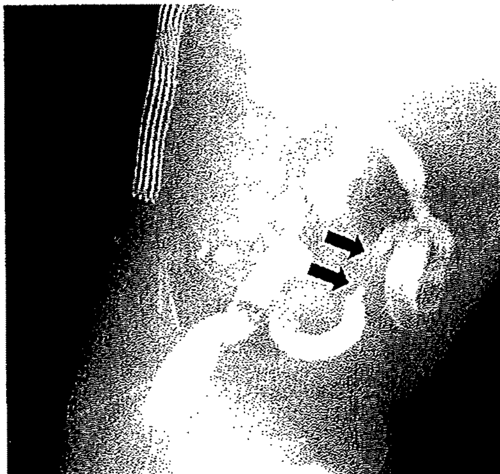
図 3 症例 3，下行結腸限局性狭窄

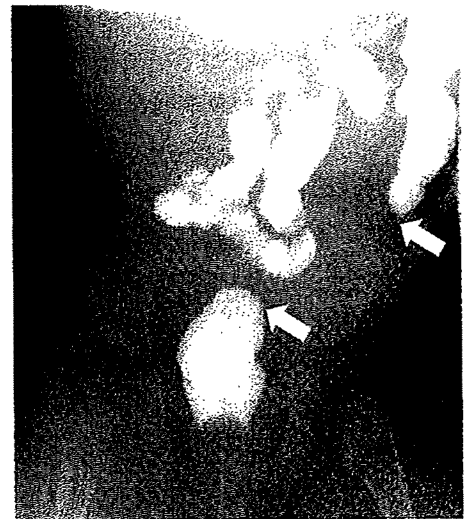
図 4 症例 5，下行結腸から直腸上部に及ぶ広範囲の閉鎖

外の結腸狭窄・閉鎖を 3 例に認めており（表 2），回腸ストーマの造設は有用であったと考えている。ストーマ閉鎖前には，注腸造影を行い結腸狭窄・閉鎖の検索を行う。結腸狭窄・閉鎖に対しては，範囲が限局しているもの（図 3）であれば切除・端々吻合，範囲の広いもの（図 4）であれば口側結腸でストーマの再造設を行っている。現在のところ，5 例中，4 例が生存しており，2 例（症例 4，5）はストーマ閉鎖の予定である。

V. 術後管理

1. 全身管理

術後急性期は術前と同様，呼吸・循環管理を行う。循環不全を伴うため状態に応じて volume 負荷，カテコールアミン投与による循環補助を，また貧血，血小板減少，AT Ⅲ低下を伴うことが多いため，補充療法は積極的に行い，必要に応じて抗 DIC 治療を行う。炎症反応の陰性化まで十分な抗生剤治療を行う。抗真菌剤（ミコナゾール）の予防投与を，禁乳中は経静脈的に，経腸栄養再開後は経腸的に併用する。

2. 栄養管理

急性期の呼吸・循環が安定し，術後腸管蠕動が改善したら速やかに経腸栄養を開始する。母乳栄養で開始し，胃内残乳量，ストーマからの腸液排泄量を観察しながら経腸栄養を増量していくが，本症の好発部位が回腸末端であり，ストーマは回腸に造設されることが多いことから，経腸栄養のみでは体重増加が困難な場合が多い。穿孔例のほとんどは超低出生体重児であり，投与可能な水分量も限られるため，経験的に経腸栄養と経静脈栄養が半々程度となることが多い。経腸栄養は原則として母乳で開始しているが，ストーマが空腸に造設された場合は消化・吸収は難しく，消化態経腸栄養剤（50%エレンタール P）との併用を選択することもある。経静脈栄養は，アミノ酸製剤は手術翌日から，脂肪製剤は感染が鎮静化に向かい血小板が回復するころを目安に開始している。経腸栄養併用のため，胆汁鬱滞性肝障害が重篤となることは少ないが，経静脈栄養>経腸栄養となる場合は，cyclic PN を行っている。腸管機能温存のため，炎症反応が鎮静化後はストーマ肛門側から母乳・整腸剤などの投与と，肛門よりグリセリン浣腸を行っている。しかし，ストーマより肛門側腸管は虚血性変化による狭窄・閉塞を起こしていることもあるため注意を要する。

おわりに

低出生体重児の壊死性腸炎の管理について当院の症例を提示しつつ，文献を交えて概説した。低出生体重児における壊死性腸炎の予後は依然厳しい状況にあるが，さらなる予防方法の研究，外科的介入の最適なタイミングの検討がなされることにより，予後の向上が期待できるものと考えている。

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Medical and Surgical Management for Necrotizing Enterocolitis in Low-birth-weight Infants

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Key words : Low-birth-weight infants, Necrotizing enterocolitis, Medical treatment, Surgical intervention, Surgical treatment.
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The mortality rate of necrotizing enterocolitis (NEC) is very high in low-birth-weight infants. In the management of this life-threatening disease, prevention, early diagnosis, and early medical treatment are the most important strategies. Intestinal perforation is accepted as the absolute indication of surgical intervention. However, the relative indications and initial optimal surgical procedure—peritoneal drainage or laparotomy—have been controversial. We will describe our strategies of prevention and medical and surgical management for NEC and discuss issues of prevention, medical treatment, indications for surgical intervention, and optimal surgical treatment.

*　　　*　　　*

特集 脳・神経系の画像診断

Ⅲ．疾患各論

脳室周囲白質軟化症

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要旨

周産期医療の発展に伴い，極低出生体重児の生存率は改善しているが，その神経学的合併症は減少していない．周産期におけるおもな中枢神経系合併症は出血性病変から白質病変へと変化しており，focal PVLとdiffuse WMIに二分される．focal PVLは予定日前後ないし18カ月頃の頭部MRIでおおむね診断可能であり，後に脳性麻痺をきたす．一方，diffuse WMIには確実な画像診断法がないのが現状である．

はじめに

周産期医療の発展により，出生体重1,500 g未満の極低出生体重児の生存率は著しく改善した．その一方で，生存児の神経学的後遺症は減少しておらず，10％以上が脳性麻痺，学習障害，知的障害などを呈す．かつては，脳室内出血ならびに出血後水頭症が早産低出生体重児における重要な神経合併症であったが，現在では低酸素性虚血性損傷に基づく白質損傷（white matter injury, 以下WMIと略す）が主要な病型である．WMIは巣状病変からびまん性病変（以下，diffuse WMIと略す）まで広範なスペクトラムをもち，脳室周囲白質軟化症（periventricular leukomalacia, 以下PVLと略す）はその一病型である．一般的にPVLは深部白質病変をさし，先に述べた局所的なWMIないしVolpeの定義するfocal PVLに相当するため，以下，単語をfocal PVLで統一する．

病理学的特徴と神経学的後障害

元来，focal PVLは剖検脳の神経病理学的所見に基づいて診断されてきた．1962年BankerとLarroccheは，剖検脳において脳室周囲白質に肉眼的に“white spots”として認められる軟化巣を“periventricular leukomalacia”と名づけた[1]．軟化巣の組織反応を経時的に追うと，まず凝固壊死や軸索の腫大が数時間みられ，次にミクログリアの活性化がおこる．その後，反応性アストロサイトが数日～7日前後に出現し，新生血管が1～2週間で増生する．壊死巣の中心は融解し，空洞形成が10日～2週間以降に認められる．好発部位は側脳室前角周囲，半卵円中心，三角部周囲の深部白質である．乳幼児期には脳性麻痺がほぼ必発し，focal PVLでは皮質脊髄路の内側を走る下肢への神経伝達路が障害されるため

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