induced to walk actively along a circular path of three full turns of 360° each before collecting food items, but fail to do so after active rotations of five full turns (n = 4). 1b) The hamsters compensate passive rotations incurred during the collection of food on a centrally located platform up to one or two full turns, but they are no longer able to do so when the rotations reach three turns (n = 3). Thus, both active and passive rotations are compensated up to different limits, the range of possible compensations being larger for active displacements, which involve not only vestibular, but all categories of idiothetic information. 2) Another series of experiments involved misleading the animals as to the time and place of departure. This was achieved by transferring them from the nest exit to the food source in a narrow transportation tube. Under these conditions, 10 subjects invariably returned to the arena's periphery by inverting the direction in which they had left the transportation tube at the food source by 180°, most probably because they made a confusion between the tube and the nest exit (a narrow channel in natural conditions).

These complementary findings confirm that under IR light the homing behaviour of our subjects is based exclusively on path integration. They also illustrate the cumulative effect of errors on the integration of path-dependent information as long there is no possibility of applying appropriate corrections provided by stable cues from the environment. Thus, the dusk and night active golden hamster may well navigate over limited distances by path integration, but uses peripheral visual information as soon as it becomes available.

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Intraocular 6-hydroxydopamine prevents the persistent estrus induced by continuous light

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Summary. Following the intraocular injection of 6-hydroxydopamine, which can destroy the retinal dopaminergic neurons, female rats showed a normal estrous cycle in LD 12:12 but not a persistent estrus in continuous light.
Key words. 6-Hydroxydopamine; intraocular administration; continuous light; persistent estrus; female rat.

In adult mammals, the retina is the only route for reception of light information[1,2]. Dopamine (DA) is the principal catecholamine in a specific subpopulation of amacrine cells localized within the innermost part of the inner nuclear layer[3]. Since there is no dopamine-containing axon in the optic nerves[4], all dopaminergic neurons are intraretinal. The DA-accumulating amacrine cells synapse with other amacrine cells which connect the bipolar and ganglion cells[5,6]; some light information may be mediated by DA acting as a retinal neurotransmitter[7].

In continuous light (LL), many circadian rhythms in nocturnal mammals initially show a free-running rhythm followed by a loss of apparent rhythmicity, a state known as 'periodicity fade-out'[8,9]. Since the rat's estrous cycle is based on the circadian rhythm[10], exposure to LL leads first to a free-running rhythm and then a fading out of the estrous cycle[9,11]. The latter condition is eventually characterized by persistent estrus.

Intraocularly administered 6-hydroxydopamine (6-OHDA) can destroy the retinal dopaminergic neurons; this can be confirmed by ultrastructural[12,13] and histofluorescence[13] methods. In this study, we used 6-OHDA to investigate whether the retinal dopaminergic neurons are involved in the induction of persistent estrus in LL.

Material and methods. All animals used were female Wistar strain rats provided with food and water ad libitum. 6-Hydroxydopamine (6-OHDA, Sigma) was dissolved in 0.9% saline containing 1 mg/ml of ascorbic acid. 6-OHDA was injected into the bilatral vitreous bodies under ether anesthesia with a Hamilton microsyringe (50 µg/10 µl). Some animals received 10 µl of saline containing 1 mg/ml of ascorbic acid under the same conditions. Vaginal smears were taken at random times (09.00–17.00 h) to avoid providing a nonphotic 24 h time signal. Persistent estrus was defined as the condition where vaginal cornification was found on more than 7 consecutive days.

Experiment 1. 30 rats, 3–4 months of age, were kept for 20 days in LD 12:12 (lights on at 06.00 h) and then exposed to LL (an average of 400 lux on the floor of their cages) for 20 days. The injection of 6-OHDA (n = 10) and saline (n = 8), respectively, were performed at the beginning of LL (09.00 h). Following the injection, vaginal smears were taken for 20 consecutive LL days. Intact rats (n = 12) were used as controls. For judging the photoreceptive ability of rats treated with 6-OHDA, the period of the free-running rhythm in LL (400 lux) was analyzed[8]. Five rats from each injected and control group

were used. The number of total wheel rotations were integrated and printed every 30 min (Integrating indicator, Tosoku Co., Tokyo). Since the activity rhythm disappears as time of exposure to LL increases[14], the period of the free-running rhythm was estimated by visual inspection of the activity record of each animal for first 10 consecutive days. If an activity rhythm disppeared within 10 days, this activity record was omitted. A period of the activity rhythm for the last 4 consecutive LD 12:12 days, one estrous cycle, was used as a base line value. The smear records of these rats were added to those of treated animals.

Experiment 2. 14 rats, 3–4 months of age, were kept in LD 12:12 as before. The injection of 6-OHDA (n = 7) was performed at beginning of LL (09.00 h). Following the injection, vaginal smears were taken for 30 consecutive LL days and then for 20 consecutive LD 12:12 days. Intact animals (n = 7) were used as controls.

Experiment 3. 10 rats, 3–4 months of age, were kept in LD 12:12 (06.00 h on). The injections of 6-OHDA was performed at 09.00 h. Following the injection, vaginal smears were taken for 20 consecutive LD 12:12 days.

Experiment 4. 15 rats, 2 months of age, were exposed to LL for 60 days. The injection of 6-OHDA (n = 8) and saline (n = 7), respectively, was performed at 09.00 h. Following the injection, vaginal smears were taken for 20 consecutive LL days. Rats treated with saline were used as controls (n = 7).

Statistical analysis. A period of estrous state (day) was analyzed by the χ^2-test; estrous state was summarized as 1 day, 2, 3, 4, 5, 6, and 7 or more. The period of the free-running rhythm of wheel running was analyzed by the two-tailed Mann-Whitney U test.

Results. Vaginal estrus was evident every 4th day for rats in a LD 12:12 photoperiod. The 4-day estrous cycle was not affected by the 6-OHDA injection in the LD 12:12 photoperiod (experiment 3).

A persistent estrous state was found in intact animals (experiment 1, 67%, 8/12; experiment 2, 100%, 7/7) and in saline-treated animals (experiment 2, 25%, 2/8) but not in 6-OHDA-treated animals (experiment 1, 0%, 0/10; experiment 2, 0%, 0/7) (fig. 1 and 2). The persistent estrus induced by LL was not reversed in LL by the 6-OHDA injection (experiment 4). After changing from LL to LD 12:12, the 4-day estrous cycle soon reappeared in 6-OHDA animals as in intact animals (fig. 2).

When the period of the estrous state was compared, there was a significant difference between intact and 6-OHDA animals (experiment 1, $\chi^2 = 22.70$, $p < 0.01$; experiment 2, $\chi^2 = 17.43$, $p < 0.01$) and between saline and 6-OHDA animals (experiment 1, $\chi^2 = 22.87$, $p < 0.01$), but not any difference between intact and saline animals (experiment 1, $\chi^2 = 5.57$, $p > 0.50$). After changing from LL to LD 12:12, no difference between intact and 6-OHDA animals was found (experiment 2, $\chi^2 = 0.74$, $p > 0.50$). There was no difference between intact and 6-OHDA animals in LL (experiment 4, $\chi^2 = 6.31$, $0.50 > P > 0.30$).

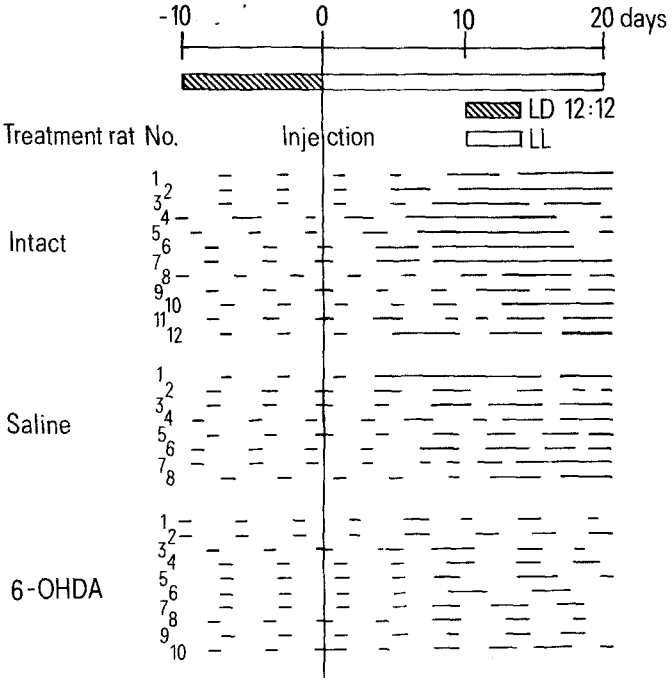

Figure 1. Smear records before and after the bilateral intraocular injection of saline and 6-hydroxydompamine (6-OHDA), respectively. The time of injection is indicated as injection in figure. Vaginal estrous day is indicated as solid bar. Persistent estrus was found in intact animals (67%, 8/12), in saline-treated (25%, 2/8) but not in 6-OHDA-treated (0%, 0/10) for 20 consecutive LL days (an average of 400 lux).

A period of wheel running in the LD 12:12 photoperiod (baseline value) was 23.7 ± 0.2 h in intact animals, 24.1 ± 0.4 h in saline, and 24.5 ± 0.2 h in 6-OHDA (mean ± SE). The period of a free-running rhythm of wheel running in LL was 24.8 ± 0.1 h in intact animals, 24.9 ± 0.2 h in saline, and 25.5 ± 0.1 h in 6-OHDA. There was no difference among the three groups (intact group versus saline, $U = 8$, $p > 0.05$; intact versus 6-OHDA, $U = 21$, $p > 0.05$; saline versus 6-OHDA, $U = 15$, $p > 0.05$).

Discussion. The period of the free-running rhythm in 6-OHDA animals was not different from that of both intact and saline animals (experiment 1), indicating that rats treated with 6-OHDA had a normal photoreceptive ability.

Persistent estrus was induced by LL in a large proportion of both intact and saline animals (fig. 1 and 2). 6-OHDA prevented the induction of persistent estrus by LL (fig. 1 and 2). However, the persistent estrus which had already been induced by LL could not be reversed by the injection of 6-OHDA (experiment 4). After changing from LL to LD 12:12, vaginal

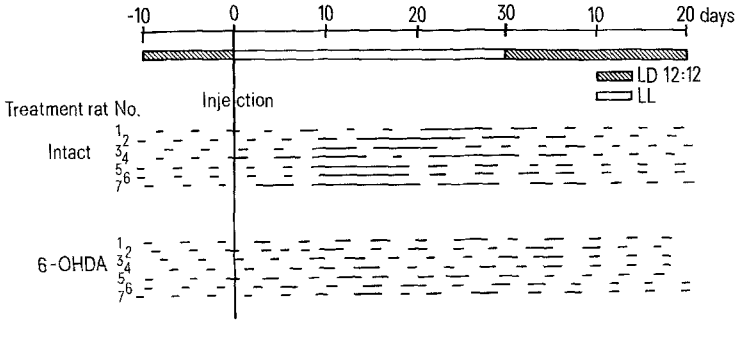

Figure 2. Smear records before and after the bilateral intraocular injection of 6-hydroxydopamine (6-OHDA). The time of injection is indicated as injection in figure. Vaginal estrous day is indicated as solid bar. Persistent estrus was found in intact animals (100%, 7/7) but not in 6-OHDA (0%, 0/7) for 30 consecutive LL days (an average of 400 lux). After changing from LL to LD 12:12, the 4-day estrous cycle soon reappeared in 6-OHDA animals as in intact animals.

estrus reappeared every 4th day in 6-OHDA animals as in intact animals (fig. 2). The 4-day estrous cycle continued in LD 12:12 after the 6-OHDA injection (experiment 3). Following destruction of the retinal dopaminergic neurons, persistent estrus could not be induced by LL.

DA turnover in the retina is mediated through a stimulation of photoreceptors by light[15,16] and undergoes a circadian rhythm[17]. The role of the retinal DA system might be to mediate continuous light information which induces the persistent estrus. Since 6-OHDA animals showed a normal estrous cycle in LD 12:12, the retinal DA system might not transmit periodic light information to which the reproductive system entrains.

In summary, our results suggest that the retinal dopaminergic neurons are involved in the induction of the persistent estrus in LL.

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Genetic load and effective size of natural populations of *Drosophila melanogaster* in Korea

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Summary. The effect of 750 second chromosomes of *Drosophila melanogaster* on viability was studied. 19.3% of them proved lethal or semilethal (= drastics) in homozygous condition. Compared to data obtained in previous years at the same sampling site, a significant frequency decrease of drastics during the past decade could be observed. The dynamic processes taking place in the Korean wild populations of *D. melanogaster* are discussed.

Key words. Drosophila melanogaster; second chromosome; drastics; genetic load; population size.

Information concerning the amount of genetic load hidden in natural populations of *Drosophila* is available for a few species; *D. pseudoobscura*[2], *D. subobscura*[3,4], *D. willistoni*[5], and *D. melanogaster*. Natural populations of *D. melanogaster* from various localities in the USA[6,7], Europe[8], Japan[9] and Korea[10-12] hav been studied in more detail.

There are two main hypotheses regarding the maintenance of genetic load in natural populations of *Drosophila*; 1) The balance theory: the genetic load is assumed to be maintained primarily by balancing selection. 2) The classical theory: the existence of the load is primarily due to mutation pressure. Although it is not easy to discriminate between these alternative hypotheses, a number of population parameters can be used for the estimation of the proportions of the balanced to the mutational components of the load[13,14].

Genetic load and viability variation in the population of *D. melanogaster* in Anyang, Korea, was already measured in 1969[10] and 1978[11]. The purpose of the present paper is to provide further information about the mechanisms maintaining the genetic burden in natural populations.

Materials and methods. Samples of wild male and female flies of *Drosophila melanogaster* were taken from the same trapping site as in 1976 and 1981[11,12]. Wild females were transferred individually to separate vials to establish isofemale lines. Wild males or one male of each isofemale line were crossed to *Cy/Pm* females from a strain whose genetic background (X- and III-chromosomes) had been previously substituted by chromosomes from the wild Anyang population. The usual crossing procedure[15] results in a F_3 generation composed of heterozygous *Cy*/+ and homozygous +/+ genotypes (+0 = identical wild second chromosomes). Cy (Curly) and Pm (Plum) are dominant markers of second chromosomes traditionally used in this kind of experiment. They prevent crossing over effectively, and are lethal when homozygous.

In the test for homozygous viability, the expected F_3 offspring should consist of $\frac{2}{3}$ *Cy*/+ individuals and $\frac{1}{3}$ +/+ flies. Hence, on the basis of the relative frequencies of +/+ flies in the test generation, the wild second chromosomes can be classified into the following five groups; complete lethals (less than 1% +/+ genotypes), semilethals (< 16.6%), subvitals (< 26.7%), quasinormals (< 39.9%), and supervitals (more than 39.9%)[12].

To estimate the viability of random heterozygotes the crossing procedure was performed in the same way as for homozygous viabilities, but in the last test crosses the *Cy*/+ flies were taken from two different lines (e.g. $Cy/+^n \times Cy/+^{n+1}$). Thus, the normal individuals (+/+) in the F_3 test generation represented random heterozygotes for wild second chromosomes. The percentage of these normal genotypes was then employed as a measure of heterozygous viabilities as was done for the homozygotes.

For the estimation of effective population size, allelism tests were performed between the various different complete lethal chromosomes kept in balance over *Cy*. Those lethals of allelism crosses which yielded no normal individuals were considered to be allelic.

Results. The homozygous viabilities for the sample of 750 second chromosomes of *D. melanogaster* from Anyang 1982 are presented in table 1 and compared to those of previous investi-