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# Exposure to Halothane and Enflurane Affects Learning Function of Murine Progeny

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This study was conducted to assess the learning function of murine progeny born of mothers that had received either 1% or 2% halothane or 2% or 4% enflurane, on days 6 and 10 or days 14 and 17 of gestation. Their timed performance at the age of 6 to 7 weeks was compared in a maze with that of control mice of similar ages that had not been exposed to anesthetics prenatally. All mice exposed to halothane in utero performed poorly at first, especially the group with mothers exposed to 2% halothane on days 14 and 17 of pregnancy. By the 10th training period, the performance of all mice improved but remained significantly slower than control mice. The offsprings of mice exposed to enflurane also performed poorly on the first training period, but between the fifth and seventh training periods, made statistically significant progress. However, they too remained slower in maze performance than control mice. Although blood pressure and arterial blood gas studies were only performed on two pregnant mice, data obtained suggest that the anesthetics did not have sufficient effect on respiration to affect our results. Second generation offspring, born to dams exposed to 2% halothane in utero late in pregnancy and sired by normal unexposed males, were also consistently slower than control mice, indicating a possible genetic effect induced by the anesthetic.

**Key Words:** ANESTHETICS, Volatile: enflurane, halothane; ANESTHESIA: obstetric; BRAIN: function.

KELLOG et al (1) have shown that exposure to diazepam late in pregnancy affects the behavior of rat progeny. We conducted a preliminary study to assess whether halothane affected the behavior of young mice (pups) born of mothers exposed to 1% or 2% halothane, either on days 6 and 11 or on days 14 and 17 of gestation. At age 16 days, the offspring were isolated for 5 hours and then placed at one end of a 90-cm long linear passage way. They were timed to determine how long it took them to reach their mothers, which were placed behind a grid at the other end of the passage. Their performance was compared with that of control mice whose mothers were exposed to 100% oxygen for 30 minutes on days 6, 11, 14, and 17 of pregnancy. Pups born of mothers that had received 1% or 2% halothane on days 6 and 11 of gestation performed statistically similarly to control pups. Pups that had received 1% or 2% halothane later in pregnancy performed statistically slower than control pups (103 ± 19 seconds when 1% halothane was administered and 122 ± 20 seconds when 2% halothane was given, vs 16 ± 3 seconds for control pups, $p < 0.0005$). The present study was undertaken to ascertain whether the phenomenon persisted at a later age and whether similar findings could be obtained if 2% and 4% enflurane were substituted for 1% and 2% halothane.

## Methods

Fifty-four albino mice (Jackson Laboratories) aged between 6 and 7 weeks were subjects for the study. This age corresponds to adolescence in man as it is the time when mice become sexually mature. The mice were divided into nine equal groups (six mice each). After 24 hours of starvation they were tested in a maze formed of six compartments bisected by a central partition and with fixed and reversible exits (Figure). Three maze patterns could be set: (*a*) all exits

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on the right, (*b*) all exits on the left, and (*c*) alternating right and left exits. Mice in each group were tested three times a day, every 3 days for a total of 10 tests, and the time taken to cover all maze patterns was noted. Their mothers had been randomly chosen to receive 1% or 2% halothane or 2% or 4% enflurane, both in oxygen, at a rate of 5 L/min for 30 minutes, either on days 6 and 11 or on days 14 and 17 of gestation (equivalent to the first and early second trimester of pregnancy and late second and third trimester of pregnancy in humans). The ninth group (control) consisted of mice born of mothers that breathed 100% oxygen for 30 minutes on days 6, 11, 14, and 17 of gestation. Six additional pups born to two females exposed to 2% halothane on days 14 and 17 of gestation and sired by normal unexposed males were tested similarly at age 6 to 7 weeks. Mothers were not tested in the maze except the two dams of the second generation mice described above.

In all cases halothane or enflurane in oxygen and the oxygen were blown into a 13.5-L chamber containing the pregnant mice. The floor of the chamber was filled with 1.5 kg of barium hydroxide lime (USP) separated from a layer of straw by a perforated plate. Results were expressed as mean time to cover the maze by each group (±1 SEM) on days 1, 3, 5, 7, and 10 of training.

Arterial blood gas analysis was performed, using aortic blood, in two pregnant mice in one of which anesthesia was induced with 3% halothane, in the other 6% enflurane in oxygen. After the two mice were anesthetized in the 13.5-L chamber their heads were inserted through a diaphragm attached to an Ayre's T-piece receiving the gas mixture at a rate of 5 L/min. A three-way stopcock was inserted between the syringe used to obtain the blood sample and the 25-gauge butterfly needle inserted into the aorta. Before the sample was aspirated, anesthetic concentration was reduced to 2% halothane and 4% enflurane. The stopcock was switched to measure arterial blood pressure on a Statham transducer connected to a Gilson polygraph recorder.

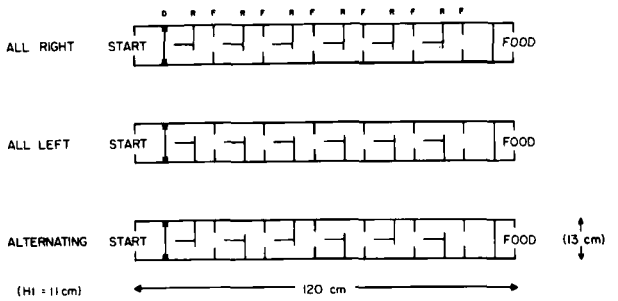


FIGURE. Maze used to assess learning function of mice. D, Donor admitting mice to maze; R, reversible door; F, fixed door; top, maze set so that all exits from six central compartments are to right; middle, maze set so that all exits are on left; bottom, maze set so that exits alternate from right to left.

Statistical significance was assessed by commonly used variance techniques (chi-square, *t*-test) at values of $p < 0.05$.

## Results

### First Generation Mice Born to Mothers Exposed to Halothane

Control mice made statistically significant progress in all maze settings by the third training period (Table 1), after which their performance varied little. Mice exposed in utero to 1% halothane on days 6 and 11 or days 14 and 17 of gestation made significant progress by the 10th training period. Mice exposed to 2% halothane in utero on days 6 and 11 of pregnancy failed to progress during the entire training period. The progeny of mice exposed to 2% halothane on days 14 and 17 of gestation made significant progress only by the seventh training period. This group alone was statistically slower than control mice on the first day of training, whereas all groups exposed to halothane in utero were significantly slower than control mice on all subsequent days.

### Mice Born to Mothers Exposed to Halothane in Utero (Second Generation)

The six pups born to the two females exposed to 2% halothane in utero on days 14 and 17 of gestation made significant progress in all maze settings only by the seventh training period (Table 2). They were significantly slower than control mice throughout training.

### Mice Born to Mothers Exposed to Enflurane

Mice born to mothers exposed to 2% enflurane on days 6 and 11 and on days 14 and 17 of gestation made significant progress in all maze settings by the 5th day of training (Table 3). The pups of mice exposed to 4% enflurane on days 14 and 17 of gestation made similar progress, but those exposed to it on days 6 and 11 of gestation only progressed by the seventh training period. During the first training day, the performances of the progeny of all mice exposed to enflurane were not statistically different from control mice in all maze settings. In all subsequent trainings, all groups were significantly slower than control mice.

**TABLE 1**

**First Generation Mice Born to Mothers Exposed to Halothane***

| Group | MS | Day of training | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 7 | 10 |
| Control | R | 96 ± 19‖ | 12 ± 1.6 | 10 ± 1.0 | 10 ± 2.0 | 11 ± 2 |
| | L | 63 ± 14‖ | 15 ± 3.0 | 17 ± 3.0 | 14 ± 2.0 | 13 ± 3 |
| | A | 63 ± 13‖ | 11 ± 2.0 | 11 ± 1.5 | 10 ± 1.5 | 13 ± 2 |
| Halothane 1% (days 6 and 11) | R | 68 ± 12 NS | 62 ± 12# | 64 ± 23‡ | 37 ± 5#‖ | 38 ± 8§ |
| | L | 75 ± 14 NS | 58 ± 14# | 72 ± 25† | 76 ± 25‖‖ | 37 ± 8¶ |
| | A | 72 ± 18 NS | 46 ± 5# | 72 ± 23§ | 55 ± 18‖‖ | 45 ± 11‖ |
| Halothane 2% (days 6 and 11) | R | 64 ± 8 NS | 40 ± 6# | 54 ± 3# | 48 ± 14§ | 40 ± 8¶ |
| | L | 67 ± 10 NS | 41 ± 5# | 44 ± 8¶ | 55 ± 5# | 66 ± 12# |
| | A | 91 ± 18 NS | 48 ± 9# | 49 ± 7# | 50 ± 5# | 55 ± 7# |
| Halothane 1% (days 14 and 17) | R | 66 ± 12 NS | 41 ± 7# | 52 ± 8# | 50 ± 14¶‖ | 26 ± 4¶ |
| | L | 60 ± 16 NS | 49 ± 7# | 50 ± 7# | 57 ± 12¶‖ | 36 ± 6¶ |
| | A | 84 ± 14 NS | 52 ± 9# | 49 ± 8# | 57 ± 11#‖ | 55 ± 7# |
| Halothane 2% (days 14 and 17) | R | 210 ± 22# | 164 ± 18#‖ | 166 ± 18#‖‖ | 81 ± 6# | 41 ± 8¶ |
| | L | 231 ± 25# | 190 ± 18#‖ | 160 ± 16#‖‖ | 83 ± 4# | 60 ± 12# |
| | A | 192 ± 17# | 197 ± 21#‖ | 175 ± 16#‖‖ | 76 ± 5# | 75 ± 7# |

* Values are means ± SEM. Abbreviations used are: MS, maze setting; R, right; L, left; A, alternate. N = 6 × 3 = 18 in each instance. Progress made by mice exposed to halothane in utero (all maze settings). Paired data: two vertical lines, $p < 0.025$; three vertical lines, $p < 0.0025$ (vs day 1). Comparison between the performance of all groups exposed to halothane and that of controls. Unpaired data: NS, not significant; † $p < 0.025$; ‡ $p < 0.0125$; § $p < 0.01$; ‖ $p < 0.005$; ¶ $p < 0.0025$; # $p < 0.0005$.

**TABLE 2**

**Second Generation Mice Born to Mothers Exposed to Halothane in Utero***

| Group | MS | Day of training | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 7 | 10 |
| Control | R | 96 ± 19‖ | 12 ± 1.6 | 10 ± 1.0 | 10 ± 2.0 | 11 ± 2 |
| | L | 63 ± 14‖ | 15 ± 3.0 | 17 ± 3.0 | 14 ± 2.0 | 13 ± 3 |
| | A | 63 ± 13‖ | 11 ± 2.0 | 11 ± 1.5 | 10 ± 1.5 | 13 ± 2 |
| Halothane 2% (days 14 and 17, 2nd generation) | R | 167 ± 17‖ | 46 ± 6# | 31 ± 3.8#‖ | 15 ± 1.5** | 20 ± 2.6** |
| | L | 119 ± 15‖ | 49 ± 8# | 33 ± 3.2#‖ | 25 ± 2.3# | 28 ± 2.5# |
| | A | 84 ± 12** | 50 ± 7# | 32 ± 3.2‖ | 22 ± 6.0# | 24 ± 2.0# |

* Values are means ± SEM. Abbreviations used are: MS, maze setting; R, right; L, left; A, alternate. N = 6 × 3 = 18 in each instance. Progress made by mice born to mothers exposed to 2% halothane in utero on days 14 and 17 of gestation (all maze settings). Paired data: two vertical lines, $p < 0.025$ (vs day 1). Comparison between the performance of these mice to that of controls. † Unpaired data; ** $p < 0.05$; ‖ $p < 0.005$; # $p < 0.0005$.

### Blood Pressure and Blood Gases

The aortic blood pressure of the pregnant mouse exposed to 2% halothane was 100/80 torr. In this animal, aortic arterial pH was 7.31, $Pa_{CO_2}$ was 51 torr, and $Pa_{O_2}$ was 237 torr. The blood pressure of the pregnant mouse exposed to 4% enflurane was 105/80 torr. Its pH was 7.25, with a $Pa_{CO_2}$ of 55 torr and a $Pa_{O_2}$ of 236 torr.

## Discussion

On day 1 of training, the performance of most pups born to mothers that had received halothane in pregnancy was not significantly different from that of control mice. An exception to the rule was noted in pups exposed to 2% halothane in utero on days 14 and 17 of pregnancy; they performed significantly slower than control mice. By the third training day, all mice with mothers exposed to halothane during pregnancy performed significantly slower than control mice. This was due to a marked improvement in the performance of control mice (Table 1). The difference persisted during the whole training period. The progeny of mice exposed to enflurane during pregnancy performed equally poorly and their performance was significantly slower than that of control mice by day 3 of training (Table 3).

Aortocaval compression could not have influenced our results because: (*a*) the murine uterus, unlike the human, is bicornuate. As a rule only one horn becomes a long coiled structure in which the fetuses are lined up like peas in a pod (each with its own placenta and umbilical cord); (*b*) the aorta and inferior vena

**TABLE 3**
**Mice Born to Mothers Exposed to Enflurane***

| Group | MS | Day of training | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 7 | 10 |
| Control | R | 96 ± 19‖ | 12 ± 1.6 | 10 ± 1.0 | 10 ± 2.0 | 11 ± 2 |
| | L | 63 ± 14‖ | 15 ± 3.0 | 17 ± 3.0 | 14 ± 2.0 | 13 ± 3 |
| | A | 63 ± 13‖ | 11 ± 2.0 | 11 ± 1.5 | 10 ± 1.5 | 13 ± 2 |
| Enflurane 2% (days 6 and 11) | R | 145 ± 23 NS | 36 ± 4.7#‖‖ | 18 ± 1.5# | 19 ± 2.4¶ | 21 ± 2.5¶ |
| | L | 236 ± 22# | 39 ± 6.3¶‖‖ | 29 ± 3.5§ | 29 ± 5.0‖ | 30 ± 4.6¶ |
| | A | 116 ± 26** | 34 ± 7.3¶‖‖ | 22 ± 3.0¶ | 24 ± 3.5¶ | 24 ± 2.6¶ |
| Enflurane 4% (days 6 and 11) | R | 196 ± 23¶ | 61 ± 13# | 65 ± 14.0#‖‖ | 25 ± 3.2# | 22 ± 3.5‖ |
| | L | 252 ± 18# | 64 ± 15¶ | 38 ± 4.5#‖‖ | 37 ± 6.5¶ | 43 ± 4.0# |
| | A | 101 ± 19 NS | 74 ± 14# | 33 ± 3.5#‖‖ | 32 ± 5.0# | 28 ± 3.2¶ |
| Enflurane 2% (days 14 and 17) | R | 143 ± 27 NS | 29 ± 3.5#‖‖ | 25 ± 3.4# | 26 ± 1.7# | 17 ± 2.0† |
| | L | 259 ± 17# | 52 ± 7.6#‖‖ | 51 ± 7.0# | 21 ± 2.6† | 22 ± 2.8** |
| | A | 155 ± 24‖ | 39 ± 4.8#‖‖ | 35 ± 3.8# | 27 ± 3.4# | 22 ± 3.0§ |
| Enflurane 4% (days 14 and 17) | R | 146 ± 24 NS | 47 ± 8#‖ | 27 ± 3.9# | 43 ± 5.4¶ | 29 ± 3.8# |
| | L | 212 ± 23# | 73 ± 13#‖ | 66 ± 9.0# | 49 ± 7.6# | 36 ± 6.8§ |
| | A | 93 ± 21 NS | 45 ± 6#‖ | 44 ± 5.9# | 55 ± 9.0# | 44 ± 6.2# |

* Values are means ± SEM. Abbreviations used are: MS, maze setting; R, right; L, left; A, alternate. N = 6 × 3 = 18 in each instance. Progress made by mice exposed to enflurane in utero (all maze settings). Paired data: two vertical lines, $p < 0.025$; three vertical lines, $p < 0.0025$; four vertical lines, $p < 0.0005$ (vs day 1). Comparison between the performance of all groups exposed to enflurane and that of control mice. Unpaired data: NS, not significant; ** $p < 0.05$; † $p < 0.025$; § $p < 0.01$; ‖ $p < 0.005$; ¶ $p < 0.0025$; # $p < 0.0005$.

cava are, unlike the situation in humans, contained in a peritoneal sheath which moves freely away from the vertebral column; and (*c*) mice exposed to halothane lay prone or on either side, whereas mice exposed to 2% enflurane walked incessantly in an inebriated fashion and those exposed to 4% enflurane slept but frequently assumed the upright position.

The possibility of hypotensive or hypoxic episodes as causative factors appears unlikely because of the high arterial pressures (normally 85 to 107 torr systolic) (2) and high oxygen tensions noted in the two mice studied under 2% halothane and 4% enflurane. The respiratory acidosis found in those mice was probably due to pressure of abdominal viscera against the diaphragm during spontaneous respiration under anesthesia, when ventilation is entirely diaphragmatic. The relatively low $Pa_{O_2}$ with an $F_{IO_2}$ close to 1 is probably similarly explained.

Noted during the study, but not included in the protocol, was the slow physical development of the progeny of mice exposed to both anesthetics. They also opened their eyes and responded to a monotone sound 3 days later than control mice, and were weaned 4 days later than control mice. In addition, their growth was retarded and their weight through adult life remained 35% less than that of control mice. Finally, the learning retardation noted in the second generation born to mice exposed to halothane in utero, suggests that the anesthetic agent may have caused a genetic aberration.

Our findings may be due to "the high metabolic stability of constituents of myelin sheaths. $^{14}$C-labeled cholesterol is known to persist in the white matter of the rabbit for a year when administered in the newborn period. It is probable that there is a similar stability for other substances of the brain, such as DNA. Thus, exposure to agents exerting an untoward effect on the formation of myelin (such as halothane or enflurane) could have long lasting sequelae" (3).

The maze test used was based on deprivation. Starved mice had to learn a preset maze pattern. Altering the maze evaluated adaptability to change. These tests are based on spatial discernibility rather than on more advanced cognitive processes. The mouse is not a primate. It is, therefore, presumptuous to apply findings described in this study to the behavior of higher mammals, especially man. It would not be ethical to use information offered in this paper to explain learning deficiencies found in the off-spring of pregnant or parturient women exposed to halothane or enflurane, although the present data do indicate the need for further studies in this field.

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