Hyperlocomotion during Recovery from Isoflurane Anesthesia Is Associated with Increased Dopamine Turnover in the Nucleus Accumbens and Striatum in Mice

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Background: It was recently reported that isoflurane increases dopamine release in the striatum in rats both in vivo and in vitro, and that isoflurane inhibits uptake of dopamine in the rat brain synaptosomes. However, the functional role of these effects of isoflurane on dopamine neurons is uncertain. Dopaminergic mechanisms within the nucleus accumbens and striatum play an important role in the control of locomotor activity, and a change in dopamine turnover depends essentially on a change in impulse flow in the dopamine neurons. In this study, the effects of isoflurane on locomotor activity and on dopamine turnover were investigated in discrete brain regions in mice.

Methods: Mice were placed in individual airtight clear plastic chambers and spontaneously breathed isoflurane in 25% oxygen and 75% nitrogen (fresh gas flow, 4 L/min). Locomotor activity was measured with an Animex activity meter. Animals were decapitated after treatments with or without isoflurane, and the concentrations of monoamines and their metabolites in different brain areas were measured by high-performance liquid chromatography.

Results: During the 10 min after the cessation of the 20-min exposure to isoflurane, there was a significant increase in locomotor activity in animals breathing 1.5% isoflurane but not 0.7% isoflurane. This increase in locomotor activity produced by 1.5% isoflurane was abolished by a low dose of haloperidol (0.1 mg/kg), a dopamine receptor antagonist. Regional brain monoamine assays revealed that 1.5% isoflurane significantly increased the 3,4-dihydroxyphenylacetic acid/dopamine ratio (one indicator of transmitter turnover) in the nucleus accumbens and striatum, but a concentration of 0.7% did not. This significant increase in dopamine turnover in these regions continued during 20 min after the cessation of the administration of 1.5% isoflurane.

Conclusions: These results suggest that isoflurane-induced hyperlocomotion during emergence may be associated with increased dopamine turnover in the nucleus accumbens and striatum. (Key words: Anesthetics, volatile; isoflurane. Behavior: locomotor activity. Brain: frontal cortex; nucleus accumbens; striatum. Turnover, neurotransmitters: dopamine; noradrenaline; serotonin.)

GENERAL anesthetics have been shown to act on or moderate the endogenous neuroregulatory systems (e.g., ion channels, neurotransmitters and their receptors, and the intracellular second messenger systems) in the central nervous system.1 It was recently reported that isoflurane induces a significant, concentration-related increase in 3H-dopamine release from striatal synaptosomes of rats2; that isoflurane inhibits the specific synapticosomal uptake of 3H-dopamine in a concentration-dependent manner in the rat brain;3; that clinically relevant concentrations of isoflurane inhibit the high-affinity binding of 3H-2β-carboxmethoxy-3β-(4-fluorophenyl)tropane (H-CFT), a potent cocaine analog, in the rat brain synaptosomes3; and that in an in vivo microdialysis study, anesthetic concentrations of isoflurane increase the extracellular concentration of dopamine in the striatum of rats.4 However, the func-
tional role of these effects of isoflurane on dopamine neurons is uncertain.

Dopaminergic mechanisms within the nucleus accumbens and striatum play an important role in the control of locomotor activity, although the nucleus accumbens is primarily involved in the initiation and regulation of such activity. Stimulation of dopaminergic neurons in these areas induces an increase in locomotor activity, whereas decreased dopamine activity elicits a depression of spontaneous behavior.\(^6\)\(^7\) Although dopamine seems to be the primary neurotransmitter in inducing locomotor activity, the fine articulation and prolongation of locomotion undoubtedly involves transmitters such as norepinephrine. Furthermore, because other neurotransmitters such as 5-hydroxytryptamine (5-HT) interact directly with dopamine neurons in an inhibitory capacity, any of their pathways, if sufficiently stimulated or inhibited, could substantially alter locomotor activity in the absence of any direct experimental manipulation of dopamine neurons or receptors themselves.\(^7\)

The turnover of monoamines depends essentially on impulse flow in the neuron. An increase in impulse flow usually causes an increase in turnover, and a decrease in impulse flow causes a reduction in turnover. Turnover has been measured to gain some insight into the activity of various types of monoamine-containing neurons during different behavioral states.\(^8\)

In the present study, therefore, to elucidate the functional role of dopamine neurons in isoflurane-induced behavioral change, we examined the effects of isoflurane anesthesia on locomotor activity in mice. Furthermore, the content in dopamine, norepinephrine, and 5-HT of the frontal cortex, nucleus accumbens, and striatum and monoamine turnover in these brain areas were measured.

Materials and Methods

These studies were done according to protocols approved by the Committee on Animal Experimentation, Kagoshima University Dental School.

Animals

Adult male ddY mice (Kuroda Junkei Doushutsu, Kumanoto, Japan) weighing 31 - 42 g were used. Each animal was used only once, and we conducted only one trial per animal. The animals were housed with free access to food and water in an air-conditioned room maintained at 22 - 24°C and with a humidity level of 45 - 55% under a constant 12-h day/night cycle (lights on at 7:00 AM). All behavioral experiments were performed between 10:00 AM and 6:00 PM.

Anesthetic Apparatus

Mice were placed in airtight clear glass chambers that measured 30 cm wide × 30 cm deep × 17.5 cm high. Isoflurane (Abbott Laboratories, North Chicago, IL) in 25% oxygen and 75% nitrogen (fresh gas flow, 4 l/min) entered the chamber at one end and was vented at the other end. Isoflurane was delivered from a dedicated, calibrated vaporizer, and the anesthetic concentration in the chamber was continuously monitored. A gas sample was continuously drawn from the chamber, and the concentration of isoflurane was measured with a crystal sensor (ICOR Anesthetic Agent Monitor; ICOR AB Co., Bromma, Sweden). The anesthetized animals were kept warm with two overhead heat lamps.

In a preliminary study, anesthetic and subanesthetic concentrations of isoflurane were determined by an assessment for loss of righting reflex. After administration of isoflurane (0.5 - 1.5%), each animal was placed on its back at 5-min intervals for as long as 20 min. Failure to regain the upright posture within 60 s was taken as the criterion for indicating an anesthetic concentration. All five mice exhibited loss of the righting reflex at an isoflurane concentration of 1.5% within 5 min. In contrast, at 0.7%, none of the five animals showed any impairment of the righting reflex even after a 20-min exposure. Therefore, we used a concentration of 1.5% to indicate anesthesia and 0.7% for subanesthesia.

Locomotor Activity

Mice were placed in individual anesthetic chambers on top of an activity sensor (Varimex Activity Meter; Columbus Instruments, Columbus, OH). Locomotor activity was recorded every 5 min on electromechanical counters located a distance from the anesthetic chamber. Sensitivity was adjusted so that only locomotor activity was recorded. In the locomotor activity tests, three series of experiments were performed.

Experiment 1 (Alternate Isoflurane Concentrations)

Each mouse was placed in the chamber and allowed to become acclimated to the chamber for 30 min. A subanesthetic concentration of isoflurane (0.7%) was then administered for 20 min. Thereafter the concentration of isoflurane was increased to 1.5% for 10 min, after which the concentration was decreased to 0.7% for another 30 min. When the concentrations of isoflurane were changed at the vaporizer, the concentrations
in the chamber achieved the target concentrations within 2 to 4 min. Administration of isoflurane was stopped after the 60-min inhalation period. The animal then inhaled 25% oxygen–balance nitrogen without isoflurane for 20 min. Five animals were used in experiment 1.

**Experiment 2 (a Single Isoflurane Concentration)**

In experiment 2, we wanted to determine whether locomotor activity would change after isoflurane exposure was stopped. Mice were allowed to breathe either 0.7% or 1.5% isoflurane in 25% oxygen–balance nitrogen for 20 min. Thereafter isoflurane was stopped and the animals were allowed to breathe 25% oxygen–balance nitrogen for another 20 min. Control animals breathed 25% oxygen–balance nitrogen without isoflurane for 40 min. Five animals were used in each group.

**Experiment 3 (Haloperidol Pretreatment for Isoflurane-induced Hyperlocomotion)**

Experiment 3 examined the effect of the dopamine receptor antagonist haloperidol on 1.5% isoflurane-induced hyperactivity after cessation of exposure in mice. Haloperidol (Yoshitomi Pharmaceutical Co., Osaka, Japan) or saline was administered intraperitoneally 10 min before the mice inhaled 25% oxygen–balance nitrogen with or without 1.5% isoflurane for 20 min. The animals then breathed 25% oxygen–balance nitrogen for another 20 min. Five animals were used in each group. Locomotor activity was measured throughout each of the three experiments.

**Assay Procedure**

In a monoamine assay, two series of experiments were done. In experiment A (0.7% or 1.5% isoflurane administration, alone or in combination), mice were assigned to one of the following four groups. (1) Control animals (n = 6) spontaneously breathed 25% oxygen–balance nitrogen without isoflurane for 20 min. (2) Mice (n = 6) breathed 0.7% isoflurane for 20 min. (3) Mice (n = 6) breathed 1.5% isoflurane for 20 min. (4) Animals (n = 6) breathed 1.5% isoflurane for 20 min. At 10 min, animals (n = 6) breathed 1.5% isoflurane for 20 min, and then 25% oxygen–balance nitrogen without isoflurane for 10 min. At 20 min, animals (n = 6) breathed 1.5% isoflurane for 20 min, and then 25% oxygen–balance nitrogen without isoflurane for 20 min. Electrical stimulation of the nigrostriatal or mesolimbic dopamine pathway increases dopamine turnover in the striatum and olfactory tubercles, and this increase in turnover occurs within 5 min after treatment and reaches a maximum at about 20 min. Therefore, a 20-min exposure to isoflurane would seem to be enough to observe the change in turnover.

The mice were decapitated immediately after the treatments. The brain was quickly removed and dissected to an ice-cooled glass plate into the frontal cortex, nucleus accumbens (including olfactory tubercle), and striatum according to the method of Heffner et al., which was slightly modified. Briefly, these regions were dissected with a razor blade from the coronal brain slice that included the most nucleus accumbens. The brain parts were weighed, frozen on dry ice, and stored at −40°C until the assay. Monoamines and their metabolites in the discrete brain regions were measured by high-performance liquid chromatography with electrochemical detection, as follows. Tissue samples were homogenized in 0.1 M perchloric acid containing 5 mM ethylene diaminetetraacetic acid and 25 pg/μl 3,4-dihydroxybenzylamine using an ultrasonic cell disruptor (40% pulsed power for 30 s, model 185, Branson, Danbury, CT), and centrifuged at 28,000g for 20 min at 4°C (KR-20000T, Kubota Seisakusho, Tokyo, Japan). The supernatant was filtered through a 0.45-μm membrane filter (LC3A, Gelman Sciences, Tokyo, Japan), and a 20-μl aliquot of the filtered solution was injected into the high-performance liquid chromatography apparatus, which consisted of a delivery pump (Waters 510, Waters Associates, Milford, MA), a reverse-phase column (250 mm length × 4.6 mm internal diameter; Eicompak MA-ODS, Eicom Co., Kyoto, Japan), an electrochemical detector (LC-4B, Bioanalytical Systems, Tokyo, Japan) set at a potential of + 0.8 V versus an Ag/AgCl reference electrode, and a computing integrator-printer (Waters 740, Waters Associates). The analytical column temperature was controlled at 40°C. The mobile phase consisted of 12% (v/v) methanol containing 0.1 M sodium acetate, 0.1 M citric acid, 0.23 mM sodium octyl sulfate, and 1.6 mM ethylene diaminetetraacetic acid adjusted to a pH of 3.90, and was pumped through the column at a rate of 1 ml/min.

**Statistical Analysis**

The data were analyzed by one-way analysis of variance with the least significant difference test or using
a paired t test. The results were considered significant when $P < 0.05$.

**Results**

**Time-course Effects of Isoflurane on Locomotor Activity (Experiment 1)**

Administration of isoflurane did not produce any change in locomotor activity. Activity tended to increase after cessation of exposure, but no significant difference was found ($0.05 < P < 0.10$ compared with locomotor activity counts during the last 10 min of the acclimatization period, by paired t test; fig. 1).

**Locomotor Activity after Cessation of Isoflurane Administration (Experiment 2)**

During the first 10 min after cessation of the 20-min exposure to 1.5% isoflurane, there was a significant increase in locomotor activity ($F[2, 12] = 17.084, P < 0.01$), but not after 0.7% isoflurane. At the second 10 min period, however, the activity returned to the control level ($F[2, 12] = 1.736, P > 0.05$; fig. 2).

**Effect of Haloperidol on Isoflurane-induced Hyperlocomotion after Cessation of Administration (Experiment 3)**

As shown in figure 3, 1.5% isoflurane significantly increased locomotor activity during the first 10 min after cessation of exposure. This hyperactivity produced by 1.5% isoflurane was significantly suppressed by a low dose of haloperidol (0.1 mg/kg) ($F[3, 16] = 20.927, P < 0.01$). This dose of haloperidol did not, on its own, interfere with the animals’ spontaneous locomotor activity.

**Effects of Isoflurane on Monoamine Turnover (Experiment A)**

The concentrations of monoamines and their metabolites were examined in discrete brain regions after the four different treatments (see Materials and Methods). In the frontal cortex, none of the isoflurane treatments altered the 3,4-dihydroxyphenylacetic acid (DOPAC):dopamine ratio, which is one indicator of transmitter turnover ($F[3, 20] = 1.224, P > 0.05$; fig. 4A). In contrast, in the nucleus accumbens, the 1.5% concentration of isoflurane significantly increased dopamine turnover by 27% of the control group value ($F[3, 20] = 11.871, P < 0.01$), and the 1.5% + 0.7% isoflurane tended to increase the turnover by 12%. However, 0.7% alone did not have a significant effect. Furthermore, the 0.7% isoflurane pretreated with the 1.5% concentration significantly increased the dopamine turnover to 118% of the value of

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Figure 5 shows the effects of isoflurane on the 5-hydroxyindoleacetic acid:5-HT ratio in the discrete brain regions of the mice used in this experiment. There was no significant difference after any of the isoflurane

A. Frontal cortex

B. Nucleus accumbens

C. Striatum

the group that was only treated with a concentration of 0.7% isoflurane (fig. 4B). In the striatum, 1.5% isoflurane also produced a significant increase in the DOPAC:dopamine ratio by 38% (F(3, 20) = 12.520, \( P < 0.01 \); fig. 4C). The homovanillic acid:dopamine ratio showed a similar tendency (data not shown). The levels of dopamine did not change after any of the treatments in the nucleus accumbens (F(3, 20) = 0.688, \( P > 0.05 \)) or in the striatum (F(3, 20) = 1.257, \( P > 0.05 \)), but they did change in the frontal cortex (F(3, 20) = 6.77, \( P < 0.01 \); table 1). Therefore the changes in the levels of DOPAC primarily reflect those in the DOPAC:dopamine ratios in the nucleus accumbens and striatum. The concentrations of dopamine, DOPAC, and homovanillic acid after each treatment in the discrete brain regions are shown in table 1.

The concentrations of norepinephrine in the frontal cortex and striatum significantly increased in the 1.5% + 0.7% isoflurane-treated group (F(3, 20) = 4.696, \( P < 0.05 \) and F[3, 20] = 4.308, \( P < 0.05 \), respectively; table 1).

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Table 1. Effects of Isoflurane on Concentrations of DA, NE, and 5-HT, and Their Metabolites in Discrete Mouse Brain Regions

<table>
<thead>
<tr>
<th>Region and Treatment</th>
<th>DA (ng/g tissue)</th>
<th>DOPAC (ng/g tissue)</th>
<th>HVA (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex Control</td>
<td>41 ± 2</td>
<td>47 ± 3</td>
<td>113 ± 5</td>
</tr>
<tr>
<td>0.7%</td>
<td>43 ± 2</td>
<td>53 ± 5</td>
<td>155 ± 5†</td>
</tr>
<tr>
<td>1.5%</td>
<td>50 ± 3†</td>
<td>60 ± 7</td>
<td>153 ± 7†</td>
</tr>
<tr>
<td>1.5% + 0.7%</td>
<td>54 ± 2†</td>
<td>76 ± 4†</td>
<td>194 ± 10†</td>
</tr>
<tr>
<td>Nucleus accumbens Control</td>
<td>6,188 ± 360</td>
<td>1,290 ± 77</td>
<td>835 ± 41</td>
</tr>
<tr>
<td>0.7%</td>
<td>6,341 ± 480</td>
<td>1,438 ± 186</td>
<td>1,062 ± 123</td>
</tr>
<tr>
<td>1.5%</td>
<td>6,674 ± 62</td>
<td>1,765 ± 76</td>
<td>1,198 ± 45†</td>
</tr>
<tr>
<td>1.5% + 0.7%</td>
<td>6,501 ± 267</td>
<td>1,521 ± 90</td>
<td>1,207 ± 83†</td>
</tr>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9,465 ± 467</td>
<td>1,751 ± 71</td>
<td>1,502 ± 64</td>
</tr>
<tr>
<td>0.7%</td>
<td>8,497 ± 256</td>
<td>1,525 ± 93</td>
<td>1,700 ± 83</td>
</tr>
<tr>
<td>1.5%</td>
<td>9,049 ± 348</td>
<td>2,319 ± 57†</td>
<td>2,056 ± 75†</td>
</tr>
<tr>
<td>1.5% + 0.7%</td>
<td>9,305 ± 410</td>
<td>1,824 ± 92</td>
<td>2,018 ± 99†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NE (ng/g tissue)</th>
<th>5-HT (ng/g tissue)</th>
<th>5-HIAA (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>329 ± 11</td>
<td>620 ± 26</td>
</tr>
<tr>
<td>0.7%</td>
<td>306 ± 8</td>
<td>584 ± 30</td>
</tr>
<tr>
<td>1.5%</td>
<td>333 ± 22</td>
<td>626 ± 35</td>
</tr>
<tr>
<td>1.5% + 0.7%</td>
<td>385 ± 17†</td>
<td>702 ± 31</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>554 ± 34</td>
<td>1,175 ± 57</td>
</tr>
<tr>
<td>0.7%</td>
<td>502 ± 55</td>
<td>1,219 ± 145</td>
</tr>
<tr>
<td>1.5%</td>
<td>538 ± 34</td>
<td>1,257 ± 21</td>
</tr>
<tr>
<td>1.5% + 0.7%</td>
<td>525 ± 40</td>
<td>1,158 ± 64</td>
</tr>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>163 ± 10</td>
<td>627 ± 23</td>
</tr>
<tr>
<td>0.7%</td>
<td>145 ± 10</td>
<td>571 ± 35</td>
</tr>
<tr>
<td>1.5%</td>
<td>169 ± 13</td>
<td>636 ± 21</td>
</tr>
<tr>
<td>1.5% + 0.7%</td>
<td>194 ± 4†</td>
<td>654 ± 24</td>
</tr>
</tbody>
</table>

Mice were decapitated after the four different treatments (see the legend in fig. 4). Values are mean ± SEM of six animals.

*p < 0.05, †p < 0.01 versus control group, one-way ANOVA with the least-significant-difference test.

treatments in the frontal cortex (F[3, 20] = 1.201; P > 0.05; fig. 5A). In contrast, all of the treatments with isoflurane significantly increased 5-HT turnover with similar potency by 12–16% of the control value in the nucleus accumbens (F[3, 20] = 3.260, P < 0.05; fig. 5B) and by 18–23% in the striatum (F[3, 20] = 7.677, P < 0.01; fig. 5C). The levels of 5-HT did not change after any of the treatments in any brain region. The concentrations of 5-HT and 5-hydroxyindoleacetic acid after each treatment in the different brain areas are also shown in table 1.

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Time-course Effects of Isoflurane on Monoamine Turnover (Experiment B)

Figure 6 shows the time-course effects of 1.5% isoflurane after the cessation of the administration on DOPAC/dopamine ratio in the different brain areas. At 10 min after the 20-min exposure to 1.5% isoflurane

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**Fig. 5. Effects of isoflurane on the 5-hydroxyindoleacetic acid:5-HT ratio in the frontal cortex (A), nucleus accumbens (B), and striatum (C) of mice. Animals were decapitated after the four different treatments (see the legend in fig. 4). Each bar represents the mean ± SEM of six animals. *P < 0.05, **P < 0.01 compared with the control group, NS (= no significant difference) between the two groups linked with a solid line, by one-way analysis of variance with the least-significant-difference test.**
was stopped, when isoflurane-induced locomotor activity during emergence was approximately at a peak, there was a significant increase in the DOPAC: dopamine ratio in the frontal cortex $(F[3, 20] = 3.450, P < 0.05; \text{fig. } 6A)$, the nucleus accumbens $(F[3, 20] = 7.049, P < 0.01; \text{fig. } 6B)$, or the striatum $(F[3, 20] = 11.850, P < 0.01; \text{fig. } 6C)$. This significant increase in dopamine turnover in these regions continued even at 20 min after the isoflurane exposure was terminated (fig. 6).

The concentrations of norepinephrine did not change in any brain region at any time after the 1.5% isoflurane administration was stopped (table 2).

As shown in figure 7A, the 5-hydroxyindoleacetic acid-5-HT ratio in the frontal cortex did not change during emergence from 1.5% isoflurane $(F[3, 20] = 0.769, P > 0.05)$. In contrast, the increased 5-HT turn-
over induced by 1.5% isoflurane continued during 20 min after the discontinuation of isoflurane in the nucleus accumbens (F[3, 20] = 3.816, P < 0.05; fig. 7B) and the striatum (F[3, 20] = 4.566, P < 0.05; fig. 7C). The contents of dopamine, 5-HT, and their metabolites after each treatment in the discrete regions are shown in table 2.

**Discussion**

The present study showed that 1.5% isoflurane significantly increased locomotor activity during the first 10 min after the cessation of the administration, whereas 0.7% isoflurane did not (fig. 2). However, isoflurane did not induce any change in activity during the exposure period (fig. 1). The hyperlocomotion produced by 1.5% isoflurane was abolished by a low dose of haloperidol, a dopamine receptor antagonist (fig. 3). Regional brain monoamine assays revealed that 1.5% isoflurane significantly increased the DOPAC:dopamine ratio (one indicator of transmitter turnover) in the nucleus accumbens and striatum, but a concentration of 0.7% did not (fig. 4). This increase in dopamine turnover in these regions induced by 1.5% isoflurane continued during 20 min after cessation of exposure (fig. 6). These results suggest that isoflurane-induced hyperactivity during emergence may be associated with an increase in dopamine turnover in the nucleus accumbens and striatum.

It has been reported that anesthetic concentrations of isoflurane induce an increase in extracellular striatal dopamine in rats in an in vivo microdialysis study.\(^5\) We showed in this study that an anesthetic concentration of isoflurane significantly increased dopamine turnover in the nucleus accumbens and striatum. This increase may be attributed to an increase in impulse flow in dopamine neurons. Therefore our studies of dopamine turnover seem to support the findings of the microdialysis study, because an increase in impulse flow produces an increase in transmitter release from the nerve terminals. However, this study has not verified any functional role of the dopamine neurons stimulated by isoflurane.

Isoflurane has been shown to inhibit uptake of \(^3\)H-dopamine into rat brain synaptosomes and to replace \(^3\)H-CFT, a cocaine analog, at the dopamine transporter in the rat brain.\(^7\) This effect of isoflurane on dopamine reuptake, therefore, may increase extracellular dopamine concentration demonstrated by microdialysis.\(^5\) Uptake inhibitors of dopamine elevate synaptic concentrations of endogenous dopamine by inhibiting the reuptake of released dopamine into presynaptic terminals. Consequently, dopamine uptake blockers may reduce dopamine turnover in vivo because of the inhibition of activity of dopamine neurons resulting from activation of transynaptic feedback pathways. This has been supported by the electrophysiologic finding that dopamine uptake blockers inhibit the firing of the dopaminergic cells in the substantia nigra and ventral tegmental area.\(^11,12\) Neurochemically, however, the
potent and selective dopamine uptake inhibitors GBR 12909 (which most specifically binds to the dopamine uptake site and does not stimulate the release of dopamine), amfetamine acid and mazindol do not change dopamine turnover in the rat brain. In contrast, it has been reported that amfetamine acid and the other dopamine uptake blockers, nomifensine and methylphenidate, increase dopamine metabolism in rats. The reason for the discrepancy among these findings is unknown. Despite such conflicting findings, however, it has consistently been demonstrated that coadministration of a certain dopamine uptake inhibitor, either GBR 12909, amfetamine acid, or mazindol, and a dopamine receptor antagonist induces synergistically an increase in dopamine turnover. This fact suggests that an impulse-induced facilitation of dopamine release is responsible for this synergistic effect because the blocking of postsynaptic dopamine receptors by a dopamine receptor antagonist interferes with neuronal feedback loops, thus increasing impulse flow and turnover. However, it is unlikely that the isoflurane-induced increase in dopamine turnover is mediated by its dopamine receptor antagonistic action, because functionally isoflurane produced an increase in both locomotor activity and dopamine turnover during emergence, but this hyperactivity was blocked by a dopamine receptor antagonist (figs. 2, 3, and 6). Therefore these findings suggest that the ability of isoflurane to both inhibit dopamine uptake and increase impulse flow in dopamine neurons might synergistically increase dopamine turnover.

As shown in figure 4, the administration of 0.7% isoflurane did not alter the DOPAC:dopamine ratio in any brain region. However, it is of interest that the 0.7% isoflurane pretreated with the anesthetic concentration (1.5%) significantly increased the DOPAC:dopamine ratio in the nucleus accumbens compared with the 0.7% concentration used alone (fig. 4B). This finding suggests that the effects of 0.7% isoflurane on dopamine turnover during recovery may differ from those during induction. In contrast, pharmacokinetic data have shown that the major factors affecting the rate of elimination of inhalational anesthetics are the same as those crucial in the uptake and distribution phases, and thus recovery from the anesthetics is essentially a reverse of induction. The reason for the discrepancy between the pharmacokinetic data and our finding on dopamine turnover is uncertain. However, we considered the possible reason for the dissociation: the activation of dopamine neurons caused by an anesthetic concentration of isoflurane may return to steady state more slowly than with the elimination of the anesthetic.

Behaviorally, locomotor activity significantly increased during the first 10 min after exposure to 1.5% isoflurane, but soon returned to the control level at the second 10-min period (fig. 2). On the other hand, biochemically, the increase in dopamine turnover occurred even at 20 min after the cessation of the 1.5% isoflurane exposure (fig. 6). It has been reported that electrical stimulation of dopaminergic axons results in two biochemical effects in dopaminergic projection fields: (1) enhanced dopamine release and metabolism, reflected by increased DOPAC levels, and (2) an increased rate of tyrosine hydroxylation, reflected by enhanced dihydroxyphenylalanine (DOPA) accumulation after administration of a decarboxylase inhibitor. The enzyme isolated from rat striatum after electrical stimulation exhibits a kinetic activation characterized by increased affinity for the pterin cofactor and decreased affinity for the end-product inhibitor, dopamine. These allosteric changes are observed even 15 min after the electrical stimulation was stopped. Further, it has been shown that low doses of dopamine receptor blockers increase dopamine release and metabolism, but the same doses do not induce catalepsy. Catalepsy is defined as an immobile behavioral state in which an animal keeps a constant, bizarre posture, and it is believed to be mediated by postsynaptic dopamine receptor antagonism. High doses increase dopamine metabolism and catalepsy. The increased dopamine metabolism, however, continues longer than the catalepsy. These findings suggest that once dopaminergic neurons are activated, it may take the increased dopamine turnover longer to return to a steady state compared with the behavioral changes.

Locomotor activity is an extremely complex entity, being the result of the mutual interaction of several neurotransmitter systems. However, dopamine appears to be first among these, inasmuch as in the absence of central dopamine receptor stimulation, locomotor activity will not and cannot occur to any significant extent. This appears also to be the case in disease states, specifically akinesia in Parkinson's disease. The same cannot be said for the receptors of any other neurotransmitter system, because locomotor activity will occur in the presence of dopamine receptor stimulation no matter how markedly the receptors of other central neurotransmitters, including norepinephrine, are inhibited. The fine articulation and prolongation of locomotor activity, however, undoubtedly involves transmitters such as norepinephrine. It has been reported that norepi-
neprine affects dopamine via a pathway between the locus ceruleus and the nigrostriatal system that enhances impulse flow between the substantia nigra and the striatum. Furthermore, an agent that promotes the release of norepinephrine enhances dopamine synthesis and use in the striatum and the rest of the forebrain. Conversely, drugs that antagonize norepinephrine function inhibit the synthesis and use of dopamine in these structures. Thus a reduction of the norepinephrine influence may lead to reduced dopamine impulse flow. We showed in this study that norepinephrine in the frontal cortex and striatum significantly increased in the mice treated with 1.5% + 0.7% isoflurane (table 1), suggesting a decrease in norepinephrine turnover in these regions. Therefore, this decrease in norepinephrine turnover might suppress the 1.5% isoflurane-induced hyperactivity during emergence. It is uncertain whether such a state can occur, however, because norepinephrine was unchanged during emergence from 1.5% isoflurane (table 2). In addition, because the major metabolites of norepinephrine, trimethoxy-4-hydroxyphenylglycol and normetanephrine could not be detected, and thus the norepinephrine turnover was not determined, it is also unknown whether a norepinephrine turnover change actually occurs.

Not only 1.5% isoflurane, but also 0.7% alone and 0.7% pretreated with 1.5% isoflurane produced a significant increase in the 5-hydroxyindoleacetic acid:5-HT ratio with similar potency in the nucleus accumbens and striatum (fig. 3). This suggests that the activation of serotonergic neurons due to isoflurane occurs even at a subanesthetic concentration, and that serotonergic neurons, rather than dopaminergic neurons, may be susceptible to isoflurane. It has been shown that the precursors of 5-HT, L-tryptophan and 5-hydroxytryptophan, inhibit spontaneous locomotor activity. L-Tryptophan also has been found to inhibit hyperlocomotion induced by amphetamine, an indirect dopamine receptor agonist. Furthermore, inhibition of 5-HT synthesis potentiates spontaneous locomotor activity and hyperactivity induced by L-DOPA, a precursor of dopamine, or by amphetamine, a dopamine receptor agonist. Lesions of the midbrain raphe (which contains many serotonergic neurons) significantly enhance spontaneous locomotor activity. Depletion of central 5-HT is also found to potentiate amphetamine-induced hyperlocomotion. Central serotonergic mechanisms, therefore, appear to play an inhibitory role in controlling locomotor activity. These findings and our data on serotonin turnover suggest that the activated serotonergic neurons interact with dopamine neurons to attenuate 1.5% isoflurane-induced hyperlocomotion during emergence, perhaps via stimulation of dopaminergic neurons.

In rodent brains DOPAC is the major metabolite, and the short-term accumulation of DOPAC may provide an accurate reflection of activity in dopaminergic neurons. Drugs that increase impulse flow also increase DOPAC and dopamine turnover. Drugs that block or decrease impulse flow reduce DOPAC and dopamine turnover. Thus there appears to be an excellent correlation between changes in impulse flow in dopaminergic neurons and changes in the levels of DOPAC and in dopamine turnover. As might be predicted, however, drugs that act in the central nervous system can have various effects, which can alter the turnover without necessarily altering impulse flow in the dopaminergic system being studied. Therefore, the most direct way to determine if a drug alters impulse flow in the dopaminergic system is to measure the activity of that system while the animal is under the influence of the drug.

Intracerebral drug injections and lesion studies have revealed that both mesolimbic and nigrostriatal dopamine pathways and their terminal regions, particularly the nucleus accumbens and the striatum, mediate locomotor activity, although the nucleus accumbens is a more active site than the striatum to induce locomotor activity. Stimulation of dopaminergic neurons in these areas induces an increase in locomotor activity in rodents. Isoflurane at a concentration of 1.5% produced anesthesia in mice, and thus locomotor activity was inhibited during the administration. When exposure was discontinued, however, the locomotion significantly increased at least for the 10-min period that followed. Neurochemically, the 20-min exposure to 1.5% isoflurane significantly increased dopamine turnover in the nucleus accumbens and striatum. In contrast, during the isoflurane anesthesia, dopamine turnover in the frontal cortex was unchanged. The reason for the regional differences in dopamine turnover is uncertain, but we suggest several possible explanations. First, interactions between dopamine neurons and other neurons in the mesocortical dopamine pathway may be different than those in the mesolimbic and nigrostriatal dopamine pathways. Second, autoreceptors may be absent on the mesocortical dopamine neurons but present on the mesolimbic and nigrostriatal dopamine neurons. Furthermore, both dopamine and 5-HT turnover were unchanged in the frontal cortex, suggesting that all neurons in the cortex might be depressed during isoflurane anesthesia. This speculation is supported by the fact that isoflurane depresses neuronal excitability by hyper-
polarizing the cell membrane of all nocortical neurons tested in humans. Based on this speculation, glutamatergic neurons, which originate from the cortex and terminate in the basal ganglia, might also be depressed by isoflurane. This glutamatergic system has been shown to activate dopaminergic neurons in the nucleus accumbens and striatum in a behavioral study. Furthermore, in an in vitro study, anesthetic concentrations of isoflurane suppress the [3H]-dopamine release evoked by glutamate from rat striatal synaptosomes. These findings suggest that during anesthesia brought about by isoflurane, the depression of the upper brain region (possibly the neocortex) masks the activation of dopaminergic neurons in the nucleus accumbens and striatum, and thus during the recovery phase locomotor activity may be increased as a result of liberation from the depression.

Drugs such as amphetamine, cocaine, and L-DOPA, which produce hyperactivity in rodents, induce agitation, hallucinations, and psychosis in humans. The symptoms induced by these drugs are believed to be mediated by the stimulation of dopamine neurons. A clinical study has revealed a greater incidence of agitation and delirium with emergence from isoflurane than from desflurane in humans. Our behavioral and biochemical studies in mice showed that isoflurane-induced hyperlocomotion during emergence may be associated with an increase in dopamine turnover in the nucleus accumbens and striatum. Therefore, our data in this study seem to support, at least in part, the involvement of dopamine neurons in agitation or delirium during emergence from isoflurane anesthesia in humans.

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