Background: Anesthesia and sleep share physiologic and behavioral similarities. The anesthetic requirement of the recently identified Drosophila mutant minisleeper and other Drosophila mutants was investigated.

Methods: Sleep and wakefulness were determined by measuring activity of individual wild-type and mutant flies. Based on the response of the flies at different concentrations of the volatile anesthetics isoflurane and sevoflurane, concentration-response curves were generated and EC_{50} values were calculated.

Results: The average amount of daily sleep in wild-type Drosophila (n = 64) was 965 ± 15 min, and 1,022 ± 29 in Na{bar}\textsuperscript{+}\textsuperscript{1} (P > 0.05; n = 32) (mean ± SEM, all P compared to wild-type and other shaker alleles). \textit{Shmns} flies slept 584 ± 13 min (n = 64, P < 0.01). \textit{Sh120} flies 412 ± 22 min (n = 32, P < 0.01), and \textit{Sh290} flies 782 ± 25 min (n = 32, P < 0.01). The EC_{50} values for isoflurane were 0.706 (95% CI 0.649 to 0.764, n = 661) and for sevoflurane 1.298 (1.180 to 1.416, n = 522) in wild-type Drosophila; 1.599 (1.527 to 1.671, n = 508) and 2.329 (2.177 to 2.482, n = 282) in \textit{Sh120}; 1.306 (1.212 to 1.400, n = 393) and 2.013 (1.868 to 2.158, n = 550) in \textit{Shmns}, 0.957 (0.860 to 1.054, n = 297) and 1.619 (1.508 to 1.731, n = 386) in \textit{Sh290}, and 0.6154 (0.581 to 0.649, n = 360; P < 0.05) and 0.9339 (0.823 to 1.041, n = 274) in Na{bar}\textsuperscript{+}\textsuperscript{1}, respectively (all P < 0.01).

Conclusions: A single-gene mutation in Drosophila that causes an extreme reduction in daily sleep is responsible for a significant increase in the requirement of volatile anesthetics. This suggests that a single mutation affects both sleep behavior and anesthesia and sedation.

Materials and Methods

Animals

\textit{Drosophila melanogaster} were bred in the laboratory at 21°C, 68% humidity, on yeast, corn syrup, and agar food. For determination of sleep and wakefulness, male and female fruit flies were used in equal numbers. To exclude age-associated effects, only young flies (≤ 2 weeks) were tested for all experiments. \textit{Drosophila} stocks used were \textit{Shmns}, \textit{Sh162}, \textit{Sh120}, Na{bar}\textsuperscript{+}\textsuperscript{1}, and wild-type Canton-S. To remove modifiers, stocks were consequently outcrossed for at least five rounds to Canton-S background as described before. Canton-S is not known to be resistant to volatile anesthetics. \textit{Shmns}.
$Sb^{102}$, and $Sb^{120}$) are different mutant alleles of the $Shaker$ locus, encoding the alpha subunit of a tetrameric voltage-dependent potassium channel. The $Na$ gene encodes a sodium leak channel, which exerts opposite effects on excitability to the $Shaker$ gene; e.g., $Na[har38]$ is known to be hypersensitive to volatile anesthetics.

**Determination of Locomotor Activity**

Sleep and wakefulness were determined from individual fruit flies placed in a *Drosophila* activity monitoring system (Trikinetics, Waltham, MA) at constant environmental conditions. Activity measurement was recorded for consecutive 1-min periods for 1 week after 1 day of adaptation, and analyzed with custom-designed software developed in our laboratory. As described before, sleep was defined as any period of uninterrupted behavioral immobility (0 counts per minute) lasting > 5 min. The total duration of sleep episodes was then calculated exactly to the minute.

**Measurement of Anesthetic Sensitivity**

Anesthetic sensitivity was tested in a custom-made *Drosophila* anesthesia chamber ($V = 200$ ml) connected to isoflurane or sevoflurane vaporizers, respectively, with a constant flow of 1.6 l/min. For each experiment, at least 10 young (≤ 2 weeks) wild-type or mutant strain fruit flies were placed inside the chamber and exposed to distinct anesthetic concentrations. After a 10-min exposure the chamber was rotated and shaken for 2 s under the control of a motor, which caused the flies to fall from their current position to the bottom of the chamber. With this accepted method of sleep deprivation, we were able to distinguish between sleep and anesthesia. The numbers of mobile and immobile flies were counted by a blinded observer, but a convulsion was not considered movement. The results were recorded for subsequent statistical analysis. All experiments were carried out at constant environmental temperature of 21°C, and concentrations of the volatile anesthetics were continuously monitored at the chamber outflow with a Datex-Ohmeda Capnomac Ultima (GE Healthcare, Chalfont St. Giles, England).

**Table 1. Isoflurane EC50 of Short-sleeping $Na[bar^{38}]$ and Wild-type *Drosophila* Calculated from Dose-response Curves**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isoflurane EC50 Value (95% CI)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (n = 661)</td>
<td>0.706 (0.649 to 0.764)</td>
<td>&lt; 0.0001 as compared with wild-type</td>
</tr>
<tr>
<td>$Sh^{102}$ (n = 297)</td>
<td>0.957 (0.860 to 1.054)</td>
<td>&lt; 0.0001 as compared with $Sh^{102}$</td>
</tr>
<tr>
<td>$Sh^{mns}$ (n = 393)</td>
<td>1.306 (1.212 to 1.400)</td>
<td>&lt; 0.0001 as compared with wild-type</td>
</tr>
<tr>
<td>$Sh^{102}$ (n = 308)</td>
<td>1.599 (1.527 to 1.671)</td>
<td>&lt; 0.0001 as compared with wild-type</td>
</tr>
<tr>
<td>$Na[har^{38}]$ (n = 360)</td>
<td>0.6154 (0.581 to 0.649)</td>
<td>&lt; 0.0001 as compared with wild-type</td>
</tr>
</tbody>
</table>

* Isoflurane EC50 values are presented as means with 95% CI, and were calculated from dose-response curves generated in multiple experiments at different concentrations.

**Statistical Analysis**

A Student $t$ test was used to assess statistically significant differences for periods of sleep and wakefulness between *Drosophila* strains. Based on the response of the flies at different concentrations of isoflurane and sevoflurane, concentration-response curves were generated according to the method of Waud for quantal biological responses. The half-maximum effective concentration (EC$_{50}$) values and 95% CIs were calculated and compared for statistically significant differences using GraphPad Prism version 4.03 for Windows (GraphPad Software, La Jolla, CA).

**Results**

For determination of locomotor activity, male and female *Drosophila* were used in equal numbers. As described before, the duration of sleep and wakefulness was different in wild-type *Drosophila* and $Shaker$ mutants. The average amount of daily sleep in wild-type *Drosophila* (n = 64) was 965 ± 15 min (mean ± SEM), as compared with 584 ± 13 min for $Sh^{mns}$ flies (n = 64, $P < 0.01$), and as compared with wild-type $Sb^{120}$ and $Sb^{102}$; 412 ± 22 min for $Sb^{102}$ flies (n = 32, all $P < 0.01$) and 782 ± 25 min for $Sb^{120}$ (n = 32, all $P < 0.01$). Thus, the short-sleeping phenotype was most pronounced in $Sb^{102}$, moderately less expressed in $Sh^{mns}$ and weakest in $Sb^{120}$. $Na[bar^{38}]$ showed a sleeping phenotype comparable to wild-type (1,022 ± 29 min, n = 32, $P > 0.05$).

Response of different *Drosophila* strains to the volatile anesthetics isoflurane and sevoflurane measured at various concentrations ranging from 0.13 to 5% for isoflurane and from 0.21 to 4% sevoflurane, respectively, yielded specific concentration-response curves. The EC$_{50}$ values for both volatile anesthetics, isoflurane and sevoflurane, were significantly increased statistically in fruit flies expressing the short-sleeping phenotype, and decreased in $Na[bar^{38}]$, as compared to wild-type *Drosophila*. Moreover, EC$_{50}$ values for isoflurane and sevoflurane were associated with the severity of the short-sleeping phenotype. The differences in the anes-
Table 2. Sevoflurane \( EC_{50} \) of Short-sleeping Na\[har38\] and Wild-type Drosophila Calculated from Dose-response Curves

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sevoflurane ( EC_{50} ) (95% CI)</th>
<th>( P ) Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (n = 522)</td>
<td>1.298 (1.180 to 1.416)</td>
<td>&lt; 0.0005 as compared with wild-type</td>
</tr>
<tr>
<td>( Sh^{120} ) (n = 386)</td>
<td>1.619 (1.508 to 1.731)</td>
<td>&lt; 0.0005 as compared with ( Sh^{120} )</td>
</tr>
<tr>
<td>( Sh^{mns} ) (n = 550)</td>
<td>2.013 (1.868 to 2.158)</td>
<td>&lt; 0.0001 as compared with wild-type</td>
</tr>
<tr>
<td>( Sh^{102} ) (n = 282)</td>
<td>2.329 (2.177 to 2.482)</td>
<td>&lt; 0.0001 as compared with ( Sh^{102} )</td>
</tr>
<tr>
<td>Na[har38] (n = 274)</td>
<td>0.934 (0.823 to 1.041)</td>
<td>&lt; 0.0001 as compared with wild-type</td>
</tr>
</tbody>
</table>

Sevoflurane \( EC_{50} \) values are presented as means with 95% CI, and were calculated from dose-response curves generated in multiple experiments at different concentrations.

Discussion

The main findings of our study are that mutations that cause a short-sleeping phenotype in Drosophila are also responsible for a significant difference in anesthetic requirement, and that the quantity of volatile anesthetic required to anesthetize Drosophila is associated with the severity of the short-sleeping phenotype.

Although sleep and general anesthesia are not identical, there has been increasing consensus that both states are neurophysiologically related. It has been shown that anesthetics may act partly by duplicating activities of brain regions important in initiating or maintaining sleep, and the effects on regional neuronal activity suggest activation of endogenous sleep-promoting pathways.\(^{12-14}\) Sleep deprivation potentiates anesthetic-induced loss of the righting reflex, and anesthetic agents increase sleep when administered into brain regions known to regulate sleep.\(^{15,16}\) In addition, the neurotransmitter adenosine increases the sleep requirement, enhances anesthetic potency, and delays recovery from halothane anesthesia.\(^{17-19}\) Animal experiments suggest that anesthetic agents induce loss of consciousness, at least in part, via activation of endogenous, nonrapid eye movement, sleep-promoting hypothalamic pathways.\(^{20,21}\)

Differences in the anesthetic sensitivity to volatile anesthetics have been reported for several mutations in genes affecting ion channels, and neurotransmitters and their receptors in Caenorhabditis elegans and Drosophila.\(^{22-25}\) However, until now there have been no reports of common mechanisms of naturally occurring sleep and anesthesia on a molecular level.

Drosophila is an ideal model for investigating mechanisms involved in anesthesia in humans, as these flies have a complex nervous system and possess many of the same ion channels, neurotransmitters, and neurotransmitter receptors as vertebrates. Recently, some of us identified \( Sh^{mns} \), a Drosophila strain exhibiting an extreme reduction in sleep requirement, as compared with wild-type flies. We also found that other severe loss-of-function mutations of \( Shaker \), including \( Sh^{102} \), were short sleepers, while weak hypomorph alleles such as \( Sh^{120} \) show only little variance.\(^{4}\) Previous electrophysiological and molecular studies found that the \( Shaker \) current and a normal-sized protein product were completely absent in short-sleeping mutants such as \( Sh^{102} \), whereas in \( Sh^{120} \) mutants the \( Shaker \) current is present, although reduced.\(^{26,27}\) With the present study, we demonstrate that a single-gene mutation affecting sleep regulation in Drosophila is also associated with an increased anesthetic requirement in these fruit flies. Moreover, the severity of the short-sleeping phenotype among different alleles was consistent, with an increased anesthetic requirement in Drosophila. The fact that the hypersensitive strain Na\[har38\] does not show a significant long-sleeping phenotype underscores the relation-
ship between the Shaker gene, sleep, and anesthetic requirement. Moreover, it should be mentioned that other authors have identified Na\textsuperscript{11001}/H\textsubscript{11001} as a long-sleeper.\textsuperscript{28} This might be as a result of differences in the presence of genetic modifiers.

In contrast to intravenous anesthetics and opioids that have been shown to exert their anesthetic and analgesic properties mainly because of specific receptor-ligand interactions, the mechanism of action of volatile anesthetics remains largely elusive. In this study we showed that a mutation in a voltage-gated potassium channel powerfully affects the anesthetic requirement of Drosophila. Shaker controls membrane repolarization after action potentials and presynaptic transmitter release.\textsuperscript{6}

Neurotransmitters and their receptors have been well conserved during evolution, and homologous ion channels in vertebrates have similar properties.\textsuperscript{29} Also, our study and previous quantitative comparisons of the EC\textsubscript{50} values of volatile anesthetics reveal an impressive correlation between Drosophila and humans,\textsuperscript{5} although the EC\textsubscript{50} calculated after a 10-min exposure may reflect a complex mixture of pharmacokinetic and pharmacodynamic effects of the mutation. Furthermore, it is important to know that looking at different anesthetic endpoints in Drosophila may lead to completely different results.\textsuperscript{2,30}

Our findings may have implications for at least two reasons: They demonstrate a link between sleep and anesthesia on a molecular level, and they show that a single-gene mutation can have a drastic effect on the susceptibility to volatile anesthetics.

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References