Anesthesiology 2008; 109:790–8

Sevoflurane Anesthesia Alters Exploratory and Anxiety-like Behavior in Mice Lacking the β_2 Nicotinic Acetylcholine Receptor Subunit

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Background: **Preexisting cognitive impairment and advanced age are factors that increase the risk of developing postoperative cognitive dysfunction. Because anesthetic agents interfere with cholinergic transmission and as impairment of cholinergic function is associated with cognitive decline, the authors studied how the volatile anesthetic sevoflurane affects exploratory and anxiety-like behavior in young and aged animals with a genetically modified cholinergic system.**

Methods: **Young and aged wild-type and mutant mice lacking the β_2 subunit of the nicotinic cholinergic receptor (β_2KO) were anesthetized for 2 h with 2.6% sevoflurane in oxygen and compared with nonanesthetized controls. Locomotor activity and organization of movement in the open field model were assessed before and 24 h after anesthesia. Locomotor activity and anxiety-like behavior in the elevated plus maze were assessed 24 h after anesthesia. High- and low-affinity nicotinic receptor and cholinergic uptake site densities were evaluated in the hippocampus, amygdala, and forebrain regions using receptor autoradiography.**

Results: **Sevoflurane anesthesia significantly reduced locomotor activity, altered temporospatial organization of trajectories, and increased anxiety-like behavior in young β_2KO mice, whereas no such changes were observed in young wild-type mice. Aged wild-type and β_2KO mice displayed reactions that were similar, but not identical, to the reactions of young mice to sevoflurane anesthesia. However, behavioral changes were not associated with differences in nicotinic receptor or cholinergic uptake site densities.**

Conclusion: **In conclusion, sevoflurane anesthesia altered exploratory and anxiety-like behavior in mice lacking the β_2 nicotinic acetylcholine receptor subunit.**

CHOLINERGIC neurotransmission is involved in the orchestration of cognitive functions in the central nervous system,[1,2] and alteration of cholinergic functions has been suggested to play an important role in cognitive deterioration in aging and neurodegenerative disorders such as Alzheimer disease.[3,4] Moreover, pharmacologic modulation of nicotinic cholinergic functions in various psychiatric disorders can modify cognitive function and reduce symptoms.[5–7]

Regarding postoperative cognitive dysfunction, several risk factors, such as advanced age[8] and preoperative cognitive impairment,[9] in combination with general or regional anesthesia have been associated with an increased incidence.[9,10] Although impaired cholinergic neurotransmission has been proposed to play a key role in postoperative cognitive dysfunction,[11] the underlying mechanisms remain obscure.[12]

Volatile anesthetics such as sevoflurane have a high affinity to nicotinic acetylcholine receptors (nAChRs) and may thus interfere with cholinergic nicotinic neurotransmission.[13–17] Furthermore, recent studies link exposure to volatile anesthetics to enhanced aggregation and toxicity of the Alzheimer disease–associated amyloid β protein.[18,19]

Mice lacking the β_2 subunit of the nAChR (β_2KO) have a preexisting cholinergic dysfunction and display a distinct behavioral phenotype.[20–24] Consequently, β_2KO mice have been proposed to serve as a suitable animal model for the study of cognitive deficits, particularly those influenced by nicotinic cholinergic transmission.[22,25,26]

The aim of this investigation was to study sevoflurane-induced alterations of exploratory and anxiety-like behavior in young and aged mice with preexisting nicotinic cholinergic dysfunction.

Materials and Methods

Animals

After approval by the Karolinska Institutet Ethical Committee on Animal Research (Stockholm, Sweden; Dnr N154/05), male wild-type C57BL/6JICO (WT) mice and male mutant knockout SOPF HO ACNB2 $\beta_2^{-/-}$ (β_2KO) mice were obtained from Charles Rivers Laboratories France (L'Arbresle Cedex, France). β_2KO mice were backcrossed onto a C57BL/6 background for at least 19 generations. Young animals arrived to the animal facility at 10 weeks of age and were housed individually in cages 2 weeks before the start of the experiment. Aged mice (15–18 months) were group housed in cages in the animal facility from the age of 10 weeks until the time of the experiment. Animals were housed under

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Received from the Department of Physiology and Pharmacology, Section for Anesthesiology and Intensive Care Medicine, Karolinska Institutet, Stockholm, Sweden, and the Karolinska University Hospital, Stockholm, Sweden. Submitted for publication March 25, 2008. Accepted for publication July 9, 2008. Supported by grants from the Swedish Research Council (K2008-53X-13405-09-3), Vetenskapsrådet, Stockholm, Sweden; Institut Pasteur, Paris, France; Karolinska Institutet, Stockholm, Sweden; Karolinska University Hospital, Stockholm, Sweden; Stockholm County Council, Stockholm, Sweden; Swedish Society for Anesthesia and Intensive Care Medicine, Stockholm, Sweden; and the Alliance Française (Svensk-Franska Stiftelsen), Stockholm, Sweden.

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Table 1. Animal Group Characteristics

	Young				Old			
	WT		β_2KO		WT		β_2KO	
	Control	Anesthesia	Control	Anesthesia	Control	Anesthesia	Control	Anesthesia
Number	19	11	19	9	10	10	11	11
Age, mo	3–4	3–4	3–4	3–4	15–18	15–18	15–18	15–18
Weight, g	26 ± 1.2	25 ± 1.2	29 ± 2.9*	28 ± 1.6*	33 ± 0.6*	32 ± 3.3*	29 ± 0.9*†	29 ± 1.1*†
Sevo, %	NA	2.6 ± 0.15	NA	2.6 ± 0.12	NA	2.6 ± 0.12	NA	2.6 ± 0.18
RR, breaths/min	NA	109 ± 18	NA	112 ± 21	NA	97 ± 29	NA	101 ± 23
Temperature, C°	NA	36.9 ± 0.3	NA	36.8 ± 0.4	NA	37.0 ± 0.7	NA	37.1 ± 0.3
RORR, s	NA	78 ± 78	NA	178 ± 130*	NA	138 ± 87	NA	199 ± 110*

Values are presented as mean ± SD.

* $P < 0.05$ compared with control group of young wild-type (WT) mice. † $P < 0.05$ compared with control group of aged WT mice.

β_2KO = mice lacking the β_2 subunit of the nicotinic acetylcholine receptor; NA = not applicable (no measurement was performed); RORR = time to return of the righting reflex from discontinuation of sevoflurane administration; RR = respiratory rate; Sevo = mean sevoflurane concentration of the inspired gas mixture during the 2-h duration of anesthesia.

standard conditions with food and water *ad libitum* and with a circadian light cycle of 12 h light–12 h dark in the housing room. All experiments were performed during the light cycle; between 10:00 AM and 16:00 PM. Animal group characteristics are presented in table 1. At the end of the experiment, animals were killed with carbon dioxide. Brains were dissected and immediately frozen on dry ice. This study, including care of the animals involved, was conducted according to the official edict by the French Ministry of Agriculture (Paris, France) and the recommendations of the Declaration of Helsinki. Experiments were performed according to the European Community Council guidelines[27] and in conformity to the guidelines in the *Guide for the Care and Use of Laboratory Animals*.[28] Therefore, the experiments were conducted in an authorized laboratory and under supervision of authorized researchers (A.W. and S.G.).

Anesthesia

Young mice were randomly assigned to control, sham, and anesthesia groups. On the day of anesthesia, control group mice remained in their cages. Mice in the sham groups were introduced into a clear plastic cylinder (Plexiglas®; Evonik Röhm GmbH, Darmstadt, Germany), 25 cm long and 7 cm in ID, in which they remained for 2 min, and were subsequently returned to their home cages. Mice in the anesthesia groups were introduced into the same plastic cylinder and remained there for 2 min, before administration of sevoflurane commenced. Mice were subsequently anesthetized for 2 h before being returned to their home cages. No significant behavioral differences were observed between the control and sham groups among young mice, which thus were merged into one single control group for each genotype. Hence, the old mice were randomly assigned to either control or anesthesia groups. Anesthesia was provided by administration of the volatile anesthetic sevoflurane (Sevorane®; Abbott France, Saint Remy sur Avre, France) *via* a calibrated vaporizer (Penlon Sigma Elite; Penlon Ltd., Abingdon, United Kingdom). Oxygen (100%) was fed through the vaporizer at a constant flow rate of 2 l/min, and the gas mixture was humidified before flushed into the plastic chamber. Sevoflurane concentration in the chamber was continuously measured (AION; Artema Medical AB, Stockholm, Sweden). During induction, the vaporizer was set to 8% for 30 s, and then to 5% for 3 min, and vaporizer settings were thereafter adjusted to maintain 2.6% sevoflurane in the inspired gas mixture during 2 h. The body temperature of anesthetized mice was monitored by a rectal probe (RET-3; Physitemp Instruments Inc., Clifton, NJ) connected to a thermometer (Kimo TK-2; Kimo, Montpon, France) and was maintained between 36° and 38° by the use of a heating lamp. The rectal probe was inserted after induction, as soon as the mice had lost the righting reflex. Respiratory rate was counted by observing chest movements every 15 min. After 2 h, sevoflurane administration was discontinued and mice were allowed to recover, breathing oxygen in the anesthesia chamber for 10 min. When moving spontaneously in the chamber, the mice were returned to their home cage for further recovery. Oxygen saturation was measured (Nonin 8500AV; Nonin Medical Inc., Plymouth, MN) in the hind limbs of two WT and two β_2KO mice. Oxygen saturation remained above 95% throughout the anesthetic procedure.

Behavioral Assessments

Spontaneous novelty exploration was assessed in a circular open field on two occasions, the first 24 h before anesthesia and the second 24 h after anesthesia. The open field (Manutan SA, Gonesse, France) was made of white opaque plastic material and measured 100 cm in diameter with 40-cm-high borders. Illumination was set to 110 lux in the center of the open field. Visual cues were placed on the walls of the room. Animals were

originally placed in the center of the arena, and their trajectories were recorded during 30 min using an automated video tracking system (Videotrack; View-Point, Lyon, France). The speed at which the animal moved was used to break down the trajectory into navigation (speed > 11.8 cm/s), fast exploration (*i.e.*, small movements with speed ranging from 6.8 to 11.8 cm/s) and slow exploration (*i.e.*, very small movements at a speed below 6.8 cm/s), as previously described.[23] An index of slow *versus* fast movements, denominated as the exploration index, was obtained by dividing time in slow exploration with time in navigation. Refined temporospatial analysis of the trajectories was performed using digitized video recordings taken at 25 frames/s. Trajectories within the open field were deconstructed into active (A) or inactive (I) periods, and peripheral (P) or central (C) positions. The center (C) was defined as a virtual area of 50 cm diameter, in the middle of the open field, and periphery (P) was the area outside of the center to the borders of the open field.

Active and inactive periods were defined by the instantaneous velocity of the mouse within the open field, averaged over time windows of 0.2 s. Each time frame was thus associated with two symbols, which when combined formed a four-symbol code (PI, CI, PA, and CA) describing the state of the mouse at each time point of the experiment. Each individual experiment was then transformed into a sequence of 45,000 state symbols (30 min at 25 frames/s), which was subsequently reduced to a matrix of state transitions. This conditional matrix lists the probability of entering one state from another. The method has previously been described in detail.[24,29] The open field apparatus was thoroughly wiped with a moist cloth between every animal, to smear out any olfactory cues left by previous mice.

Anxiety levels were assessed using an elevated plus maze, consisting of a cross-shaped platform with two arms without walls (open arms) and two arms with walls (closed arms), elevated 60 cm above the ground. The elevated plus maze provides independent measures of anxiety-like behavior (time spent on open arms) and activity (number of transitions between closed arms).[30] Light intensity in the room was set to 300 lux, and the animal was gently placed in the middle of the platform. Using a video camera mounted in the ceiling, mice's reactions to anxiogenic apparatus were stored on video tapes for off-line analysis. Time spent on the open arms and time spent at the end of the open arms was recorded, as well as the number of transitions between the walled arms. Each mouse remained in the plus maze for 10 min, and the experiment was performed only once, after the second open field test, 24 h after anesthesia.

†† Developed at the US National Institutes of Health and freely available at: http://rsb.info.nih.gov/nih-image/. Accessed June 16, 2008.

Receptor Density

Radioligand binding of high-affinity nAChRs was performed on coronal brain sections (20 μm, cut in cryostat at −20°C) incubated at room temperature with 200 pM [^{125}I]-epibatidine (PerkinElmer Inc., Waltham, MA; specific activity 2,200 Ci/mmol) in 50 mM Tris (pH 7.4) for 60 min. After incubation sections were rinsed 2 × 5 min in the same buffer and briefly in distilled water. Sections were exposed on Kodak Biomax® MS films (Eastman Kodak Company, Rochester, NY) for 24 h. Nonspecific binding was measured in the presence of 10 μM nicotine and was not distinguishable from film background. For labeling of low-affinity nAChRs, [^{125}I]-α-bungarotoxin binding was performed. Brain sections were preincubated with 50 mM Tris (pH 7.4) with 0.1% BSA for 30 min, and then incubated with 2.5 nM [^{125}I]-α-bungarotoxin (PerkinElmer Inc.; specific activity 238 Ci/mmol) in Tris 50 mM (pH 7.4) with 0.1% BSA for 2 h. Nonspecific binding was assessed in presence of 1 mM nonlabeled nicotine. After incubation, sections were rinsed 6 × 30 min in 50 mM Tris (pH 7.4) at 4°C and briefly in distilled water. Sections were then exposed on Kodak Biomax® MS films for 8 days. For labeling of high-affinity choline uptake sites, [^{3}H]-hemicholinium binding was performed. Brain sections (20 μm) were incubated with 8 nM [^{3}H]-hemicholinium (PerkinElmer Inc.; specific activity 140 Ci/mmol) at 4°C for 60 min in 50 mM Tris (pH 7.4) containing 300 mM NaCl. Nonspecific binding was performed in presence of 100 μM nonlabeled hemicholinium-3. After incubation, sections were rinsed 6 × 1 min each in ice-cold 50 mM Tris (pH 7.4) and briefly in distilled water. Sections were then exposed on Kodak Biomax® MS films for 21 days. Optical density in specific brain regions was determined for each radioactive ligand by using the public domain ImageJ program†† and appropriate radioactive standards. All autoradiographic data sets were expressed in arbitrary units ± interquartile range.

Statistics

Statistical analysis was performed using Statistica 7.1 software (StatSoft Inc., Tulsa, OK). For data obtained in the open field, we used Generalized Linear Models with baseline values as covariate for each dependent variable (distance, exploration index, %PA-CA, #PA-CA-PA). Data were normally distributed according to Kolmogorov–Smirnov tests. Planned comparisons were performed to determine significant differences between groups. For the results in the elevated plus maze test, factorial analysis of variance was used to evaluate the effect of genotype (WT *vs.* β_2KO), group (control *vs.* sham *vs.* anesthesia), and age (young *vs.* aged). One-way analysis of variance was used for comparisons between groups, recovery time, body weight, and respiratory rate. *Post hoc* analyses were performed, when appropriate, by Tukey honestly significant difference test. Autoradio-

graphic data were analyzed using the Mann–Whitney U test. Normally distributed data are plotted as mean ± SEM. Results from nonparametric tests are plotted as median ± interquartile range.

Results

Effects on Locomotor Activity and Temporospatial Organization of Trajectories

Exposure to sevoflurane for 2 h caused significant alteration in the locomotor activity 24 h after anesthesia in both young and aged mice lacking the β_2-containing nicotinic receptor subunit. In contrast, young and aged WT mice exposed to sevoflurane did not display changes in locomotor activity after 24 h, when compared with their respective control group.

In young β_2KO mice, spontaneous locomotor activity decreased both in the open field ($P < 0.01$; fig. 1A) and in the plus maze ($P < 0.05$), whereas in the latter, it was observed as a reduction in the number of transitions between the closed arms in the apparatus (fig. 1B). Anesthesia provoked alterations in the characteristics of exploration in young β_2KO mice and caused alterations in three of the patterns used to define temporospatial organization of trajectories in the open field (fig. 1C). As illustrated in figure 1D, anesthesia increased the amount of slow velocity exploration (explo index) in young β_2KO mice compared with controls ($P < 0.01$). Second, the probability to venture into the center of the open field (%PA-CA) was reduced ($P < 0.05$), and third, the total number of large movements across the center (#PA-CA-PA) was reduced ($P < 0.05$) in young anesthetized β_2KO mice compared with controls.

In aged β_2KO mice, the level of locomotor activity in the open field was also reduced by anesthesia ($P < 0.05$; fig. 2A). However, in the plus maze test, we were unable to detect any difference in activity levels between the different groups of aged mice (aged WT control mean = 22 [95% confidence interval (CI), 15–29]; aged WT anesthesia mean = 20 [95% CI, 15–25]; aged KO control mean = 19 [95% CI, 15–24]; aged KO anesthesia mean = 18 [95% CI, 14–24]; fig. 2B).

Nevertheless, in aged β_2KO mice, the amount of slow velocity exploration (explo index) ($P < 0.05$) and the probability to venture into the center (%PA-CA) ($P < 0.05$) were changed 24 h after anesthesia compared with controls (fig. 2C).

Effects on Anxiety-like Behavior

Anesthesia increased anxiety-like behavior in young β_2KO mice, as indicated by a reduction in the time venturing out on the open arms of the plus maze ($P < 0.05$; fig. 3A). In contrast, we were unable to detect effects on anxiety-like behavior induced by sevoflurane anesthesia in young WT (young WT control mean = 78

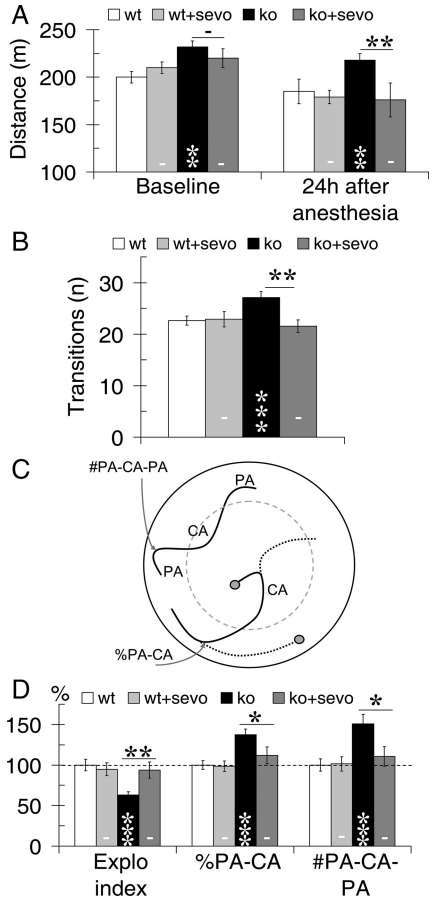

Fig. 1. Locomotor activity and temporospatial organization of trajectories in young mice. (*A*) Distance moved in the open field before (baseline) and 24 h after anesthesia. Anesthesia reduced the distance moved in mice lacking the β_2 subunit of the nicotinic acetylcholine receptor (β_2KO) ($P < 0.05$) but not in wild-type (WT) mice (not significant). (*B*) Activity in the plus maze expressed as number of transitions between the closed arms of the maze. Anesthesia reduced locomotor activity in β_2KO mice ($P < 0.05$) but not in WT mice (not significant). (*C*) Spatiotemporal organization of trajectories in the open field. The number of large movements from the peripheral area (PA) across the central area (CA) of the open field is indicated by #PA-CA-PA. The conditional probability of a transition from a PA to a CA state, *i.e.*, the probability of moving from the periphery to the center, is indicated by % PA-CA. (*D*) Spatiotemporal organization of trajectories in young mice. All values are compared relative to the WT control group, which is set to 100%. Exploration index (explo index) provides information on the repartition of fast *versus* slow movements in the open field and is obtained by dividing time spent at slow movement speed with time spent in fast movement speed. Young control β_2KO mice differed significantly from young control WT mice regarding exploration index ($P < 0.01$) (*D*, *left*), %PA-CA ($P < 0.01$) (*D*, *middle*), and #PA-CA-PA ($P < 0.01$) (*D*, *right*). After sevoflurane anesthesia, exploration index (*D*, *left*), %PA-CA (*D*, *middle*), and #PA-CA-PA (*D*, *right*) in young β_2KO mice were no longer significantly different from those in young WT mice. No effect of anesthesia on these behavioral parameters was demonstrated in young WT mice. Statistical significance for planned comparisons between control and anesthesia groups is indicated *above the corresponding horizontal lines*. *Symbols within vertical bars* indicate statistical significance for planned comparison with WT control. ko = β_2 knockout control group (n = 18); ko + sevo = β_2 knockout anesthesia group (n = 8); wt = wild-type control group (n = 20); wt + sevo = wild-type anesthesia group (n = 11). * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. - $P > 0.05$.

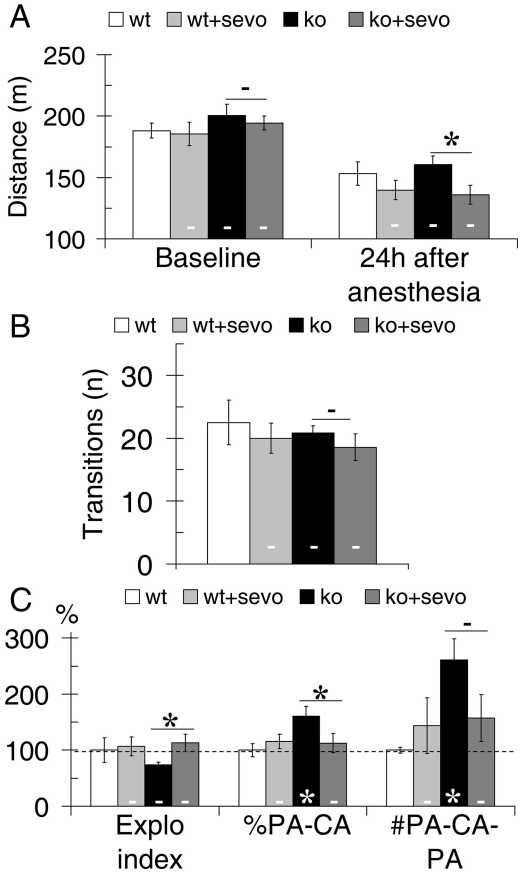

Fig. 2. Locomotor activity and temporospatial organization of trajectories in aged mice. (*A*) Distance moved in the open field before (baseline) and after anesthesia in aged mice. Anesthesia induced a reduction in distance covered in aged mice lacking the β_2 subunit of the nicotinic acetylcholine receptor (β_2KO) ($P < 0.05$) but not in aged wild-type (WT) mice (not significant). (*B*) Activity in the plus maze, expressed as number of transitions between the walled arms, was not significantly affected by genotype or by anesthesia in aged mice. (*C*) Spatiotemporal organization in aged mice. Aged β_2KO mice also increased their exploration index 24 h after anesthesia compared with non-anesthetized controls (*C*, *left*) ($P < 0.05$). Anesthesia affected the probability for venturing into the center of the arena (%PA-CA) ($P < 0.05$) (*C*, *middle*). Statistical significance for planned comparisons between control and anesthesia groups is indicated *above the corresponding horizontal lines*. *Symbols within vertical bars* indicate statistical significance for planned comparison with WT control. ko = β_2 knockout control group (n = 11); ko + sevo = β_2 knockout anesthesia group (n = 11); wt = wild-type control group (n = 7); wt + sevo = wild-type anesthesia group (n = 11). * $P < 0.05$. - $P > 0.05$.

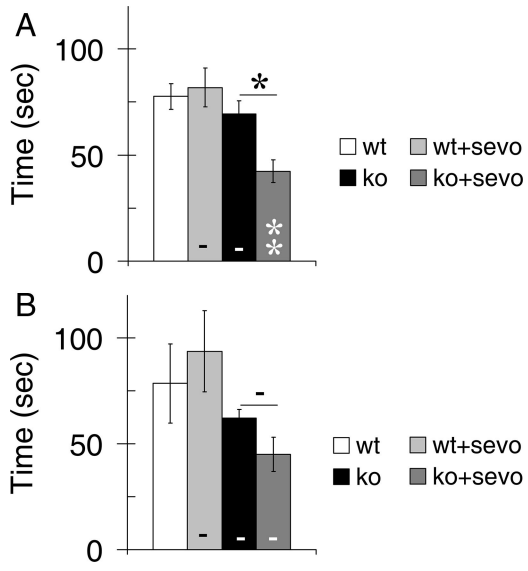

Fig. 3. Time spent on the open arms of the elevated plus maze. Mice with higher anxiety levels spend less time on the open arms. (*A*) Anesthesia reduced time on open arms in young mice lacking the β_2 subunit of the nicotinic acetylcholine receptor (β_2KO), *i.e.*, an increase in anxiety levels ($P < 0.01$). Young wild-type (WT) mice did not display any difference in anxiety levels after anesthesia. (*B*) In aged WT and β_2KO mice, we failed to demonstrate any significant differences in anxiety levels between groups. Statistical significance for planned comparisons between control and anesthesia groups is indicated *above the corresponding horizontal lines*. *Symbols within vertical bars* indicate statistical significance for planned comparison with WT control. ko = β_2 knockout control group (young n = 18, aged n = 11); ko + sevo = β_2 knockout anesthesia group (young n = 8, aged n = 11); wt = wild-type control group (young n = 20, aged n = 7); wt + sevo = wild-type anesthesia group (young n = 11, aged n = 11). * $P < 0.05$. ** $P < 0.01$. - $P > 0.05$.

[95% CI, 65–91]; young WT anesthesia mean = 82 [95% CI, 62–102]; fig. 3A). We did not detect any significant differences in anxiety-like behavior in aged WT mice or in aged β_2KO mice (aged WT control mean = 71 [95% CI, 25–116]; aged WT anesthesia mean = 92 [95% CI, 52–132]; aged KO control mean = 62 [95% CI, 43–81]; aged KO anesthesia mean = 45 [95% CI, 31–58]; fig. 3B).

Time spent on the open arms of the elevated plus maze did not differ significantly between young or aged WT and β_2KO control groups, indicating that neither age nor preexisting nicotinic cholinergic dysfunction *per se* had any effect on anxiety levels.

Effects of Age and Repeated Measurements and Genotype on Locomotor Activity

As opposed to WT, there was a significant effect of age in the β_2KO mice, as illustrated by a reduction of locomotor activity in aged β_2KO mice compared with young β_2KO mice ($P < 0.001$; fig. 4A). This was confirmed in the plus maze test, where aged β_2KO mice displayed lower activity levels than did young β_2KO mice ($P < 0.001$; fig. 4B). We did not detect any significant effect of age on baseline activity levels in WT mice (young WT control mean = 197 [95% CI, 185–210]; aged WT control mean = 188 [95% CI, 162–214]; figs. 4A and B).

Repeating the open field test revealed an effect of habituation, *i.e.*, locomotor distance during the second session was reduced as compared with the first session, in young WT ($P < 0.01$), young β_2KO ($P < 0.05$), aged WT ($P < 0.01$), and aged β_2KO ($P < 0.01$) mice (figs. 4C and D).

We observed a significant effect of genotype on baseline locomotor activity in young mice ($P < 0.01$), whereas this was not found in aged animals (aged WT

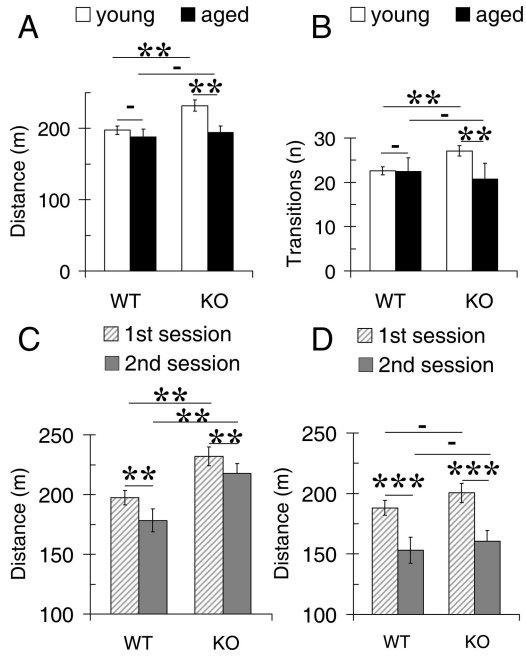

Fig. 4. Effects of age, repeated measurements, and genotype. (*A*) Comparison of locomotor activity in the open field between young and aged control mice of both genotypes. Age was a significant factor only in knockout (KO) mice, where aged mice moved a significantly shorter distance than young mice did ($P < 0.01$). Among wild-type (WT) mice, no effect of age was observed. Genotype was a significant factor only in young mice, where KO mice moved a significantly longer distance than WT mice did ($P < 0.01$). In aged mice, no such effect of genotype was observed. (*B*) Effects of age on locomotor activity in the plus maze, expressed as number of transitions between the walled arms of the apparatus. Aged KO mice made fewer transitions than young KO mice did ($P < 0.01$), whereas no such age difference was observed in WT mice. (*C*) Comparison of locomotor activity in the open field between the first and second test session, *i.e.*, effect of habituation, in young control mice of both genotypes. Locomotor activity is significantly higher in young KO mice than in young WT mice on both the first and second test occasion ($P < 0.01$ for both). The reduction in activity from the first to the second session is significant for all groups ($P < 0.01$ for all groups). (*D*) Comparison of locomotor activity in the open field between the first and second test session, *i.e.*, effect of habituation, in aged control mice of both genotypes. In aged mice, there is no longer any difference between KO and WT in activity on the first and second sessions. However, within each genotype, the difference from the first to the second session is significant ($P < 0.001$ for both). Statistical significance for planned comparisons between groups is indicated *above the corresponding horizontal lines*. ko = β_2 knockout control group (young n = 18, aged n = 11); wt = wild-type control group (young n = 20, aged n = 7). ** $P < 0.01$. *** $P < 0.001$. - $P > 0.05$.

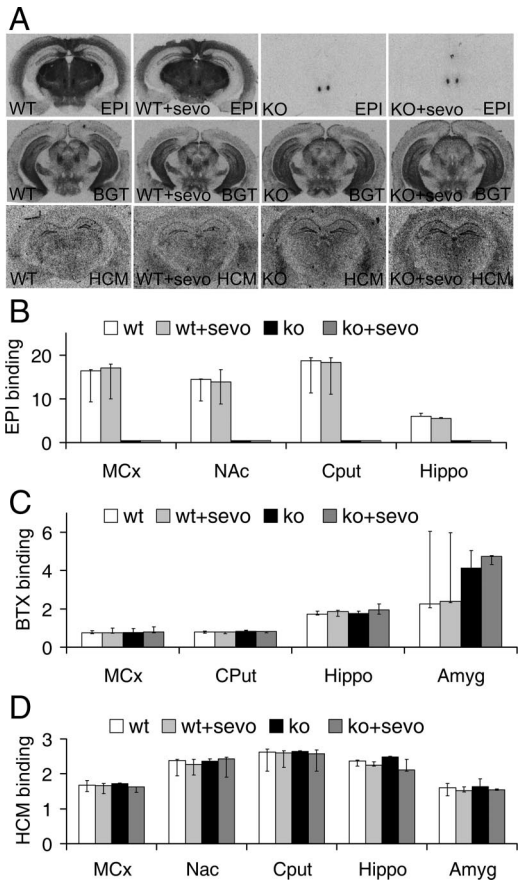

Fig. 5. (*A*) Representative film autoradiograms of [^{125}I]-epibatidine (EPI) binding at bregma level −2.7 mm (*top*), [^{125}I]-α-bungarotoxin (BGT) binding at bregma level −3.4 mm (*middle*), and [^{3}H]-hemicholinium (HCM) binding at bregma level −2.3 mm (*bottom*) of adult wild-type mice in control group (WT), wild-type mice in anesthesia group (WT + sevo), mutant mice in control group (knockout [KO]), and mutant mice in anesthesia group (KO + sevo). Residual binding of [^{125}I]-epibatidine to non–β_2-containing nicotinic acetylcholine receptors in certain nuclei, such as the fasciculus retroflexus, is observed in mice lacking the β_2 subunit of the nicotinic acetylcholine receptor. (*B*) Quantification of [^{125}I]-epibatidine (EPI) binding, (*C*) [^{125}I]-α-bungarotoxin (BTX) binding, and (*D*) [^{3}H]-hemicholinium (HCM) binding, expressed in arbitrary units as median ± interquartile range. Exposure to sevoflurane did not alter the density of either high- or low-affinity binding sites in any region, nor did it alter the regional cholinergic transmission indexed by the choline transporter. Amyg = amygdala; CPut = caudate putamen; Hippo = hippocampus; ko = β_2 knockout control group (n = 5); ko + sevo = β_2 knockout anesthesia group (n = 5); MCx = motor cortex; NAc = nucleus accumbens; wt = wild-type control group (n = 5); wt + sevo = wild-type anesthesia group (n = 5).

control mean = 197 [95% CI, 185–210]; aged KO control mean = 200 [95% CI, 181–221]). That is, young β_2KO mice had higher locomotor activity in the open field (fig. 4A) and in the plus maze (fig. 4B) compared with young WT mice.

Receptor Density

Anesthesia was not associated with changes in nicotinic receptor density (fig. 5A) either in [^{125}I]-epibatidine–labeled, high-affinity receptors (fig. 5B) or in [^{125}I]-α-bungarotoxin–labeled, low-affinity receptors (fig. 5C). Nor was any effect of anesthesia observed in [^{3}H]-hemicholinium–labeled, high-affinity choline uptake sites (fig. 5D).

Anesthesia

The recovery time, expressed as time to return of the righting reflex, was significantly longer ($P < 0.05$) in young β_2KO mice (178 ± 130 s) compared with young WT mice (78 ± 78 s). Among elderly mice, no significant

difference in recovery time was observed between the genotypes. During anesthesia, no differences regarding animal body temperature, sevoflurane concentration, or respiratory rate were observed between genotypes or age groups (table 1).

Two young β_2KO mice and one young WT mouse were excluded because of behavioral abnormalities before experiments. Among old mice, one β_2KO mouse was excluded because of unexpected disturbing sounds in the animal facility during experiments.

Discussion

Our study is the first to demonstrate that cognitive processes are affected by a single sevoflurane anesthesia in mutant mice lacking the β_2 subunit–containing high-affinity nAChR, whereas these processes remain unaffected in WT mice.

The β_2-containing nAChR is the most widespread cholinergic receptor in the central nervous system and is implicated in modulation of cognitive function.[1] β_2KO mice have been proposed as a suitable animal model for studies related to human behavior pathology, such as attention deficit hyperactivity disorder, physiologic ageing, nicotine addiction, and Alzheimer disease.[22,24,26] In this context, β_2KO mice have undergone extensive behavioral testing and have been used for the study of pharmacologically induced behavioral alterations.[22–24,26] The β_2KO mice used in our experiment carried the gene deletion since fertilization. It is therefore possible that compensatory phenomena in the expression of various nAChR subunit genes together with possible reorganization of neuronal circuits might have taken place. However, β_2 subunit gene re-expression experiments have shown that the neuronal, physiologic, and behavioral phenotypes are not caused by the actual absence of β_2-containing nAChRs, but rather by their deficit in defined circuits involving, in particular, dopaminergic neurons in the ventral tegmental area.[24]

We wanted to study the effects of anesthesia also in aged WT and β_2KO mice because, in humans, the risk of developing postoperative cognitive dysfunction increases with age.[8] Our assumption before the experiment was that aging would further accentuate the characteristic hyperactive phenotype of β_2KO mice. Instead, hyperactivity became attenuated with increasing age. It has previously been demonstrated that β_2KO mice display accelerated neurodegeneration upon aging, unrelated to the amount of other cholinergic receptor subtypes.[22] We therefore speculate that age-dependent neurodegenerative processes counteract the hyperactive phenotype in mutant mice, making their exploratory and anxiety-like behavior more similar to that of their WT counterparts.

Because all of the observed behavioral changes after sevoflurane anesthesia have been restricted to β_2KO mice, normally functioning cholinergic neurotransmission seems to play a protective role on cognitive function after anesthesia, as illustrated by the lack of behavioral changes after sevoflurane anesthesia in WT mice. Alternatively, the lack of β_2-containing nAChRs might facilitate or permit sevoflurane-induced disturbances in neurotransmission.

One distinct characteristic phenotype in β_2KO mice is increased and disorganized locomotion in the open field.[23,24,29] In our study, exposure to sevoflurane attenuated the hyperactive phenotype of young β_2KO mice, which seemingly restored their normal behavior. Not only did sevoflurane anesthesia reduce their overall exploratory activity to levels similar to those of WT mice, but it also affected some patterns of sequential organization of trajectories, robustly modified in β_2KO animals as compared with WT mice. A similar, although not necessarily identical, compensatory process has previously been proposed in β_2KO mice upon chronic nicotine exposure and was interpreted as resulting from the enhancement of an opposing process mobilizing α_7 nAChR–mediated cholinergic transmission.[29]

On the surface, it might seem paradoxical that an agonist of the nicotinic system and a nonselective antagonist, acting also on several other receptor systems, would induce similar behavioral changes. However, nicotinic agonists primarily act indirectly *via* nAChRs located on or near nerve terminals where they mediate calcium-dependent release of neurotransmitters including dopamine, norepinephrine, glutamate, γ-aminobutyric acid, and acetylcholine.[2] Furthermore, nicotine administration has been shown to increase or decrease locomotor activity depending on dose, species, and strain.[31] A nonselective antagonist, such as sevoflurane, can act by directly suppressing the same systems,[13] and we therefore consider it possible for a nonselective antagonist to cause behavioral changes similar to those of a nicotinic agonist.

However, at this stage, and still as a working hypothesis, we suggest that the long-term effect of sevoflurane anesthesia may be due to action on the balance between nicotinic subtypes in cholinergic neurotransmission. Such intrinsic cholinergic balance is important to the proper functioning of the entire nervous system, and the absence of the β_2 subunit in β_2KO mice has created a cholinergic balance different from that of WT mice.[29] In many aspects, the β_2KO mice compensate for this aberrant receptor content, but nevertheless display a distinct behavioral phenotype.[20,21,23] Others have proposed that the role of β_2-containing nAChRs in the modulation of cognitive processes might be compensated in the normal adult brain, and that the contribution of β_2-containing nAChRs would become apparent only in the presence of other deficits[22] or in challenging cognitive situations.[20,21,23] We suggest that sevoflurane anesthesia elicits changes in neurotransmission that are unveiled

only in β_2KO mice already having a preexisting nicotinic cholinergic dysfunction. That is, sevoflurane might possibly act, among other mechanisms, as a modulator of α_7 nicotinic receptor transmission, as has previously been suggested for isoflurane.[15] On the other hand, mice with normal nicotinic cholinergic neurotransmission seem to be able to compensate for the effects of sevoflurane anesthesia and therefore do not display detectable behavioral changes in our model.

Interestingly, simultaneously to the reduced activity observed in anesthetized β_2KO mice, an increase in anxiety-like behavior was found. Although β_2KO mice show higher levels of circulating stress hormones, they display normal anxiety levels.[22,23] The current study shows that exposure to sevoflurane anesthesia increases anxiety-like behavior selectively in β_2KO mice but not in WT mice. One plausible hypothesis is that the observed increase in anxiety-like behavior seen in β_2KO mice after anesthesia is due to sevoflurane-induced changes in nicotinic cholinergic transmission of neuronal circuits regulating anxiety. Indeed, nicotinic cholinergic receptors modulate γ-aminobutyric acid–mediated interneurons in the hippocampus and nucleus accumbens,[32] and in dorsal hippocampal regions, anxiety is modulated by interaction between serotonergic and cholinergic neurons.[33] Cholinergic tone in these regions mediates anxiolytic effects,[34] and both α_7 and α_4 β_2 nAChRs are proposed to participate in the maintenance of this balance.[35] The observed increase in anxiety-like behavior seen in β_2KO mice after anesthesia may be due to sevoflurane-induced changes in α_7 nAChR–mediated cholinergic transmission and/or muscarinic receptors of neuronal circuits regulating anxiety. Such hypothesis would require further pharmacologic manipulation to be tested.

Exposure to sevoflurane or desflurane for 3 h can induce long-lasting alterations in protein level expressions.[36] Such changes are proposed to contribute both to short- and long-term effects such as memory alteration and neurocognitive reactions.[37] In our study, we did not observe any changes in density of either β_2-containing nAChRs, α_7-containing nAChRs, or choline uptake sites. Whereas the direct effects of volatile anesthetics on nAChRs are well described,[38–40] the downstream intracellular effects are more obscure. We propose that the behavioral effects noted in our study are most likely due to sevoflurane-induced changes in signaling pathways involving this receptor subtype.[41,42]

Anesthetic sensitivity of β_2KO mice has been shown to be similar to that of WT mice.[43] In our study, we maintained constant sevoflurane concentration at 2.6%, corresponding to 1 minimal alveolar concentration in mice,[44] which provided adequate anesthesia without severe respiratory depression. We also maintained normal animal body temperature during the 2-h anesthesia. Respiratory rate during anesthesia was not different between the genotypes, also indicating similar levels of anesthetic depth. We observed that after anesthesia, the time to return of the righting reflex was significantly longer in young β_2KO mice. However, young β_2KO animals weighed slightly more than WT mice did, which could account for some of the prolonged recovery time, but there is still a possibility that β_2-containing nAChRs might be involved in the recovery after anesthesia. It also suggests a role for nAChR agonists for enhanced recovery after general anesthesia. Because behavioral testing was performed 24 h after recovery and the difference in recovery time was measured in seconds, we consider it unlikely that a slower recovery from anesthesia would explain the behavioral differences noted.

We chose to evaluate locomotor activity and anxiety-like behavior 24 h after anesthesia, to ascertain that the effects were not due to lingering concentrations of sevoflurane. Hence, behavioral changes observed by this time could not be attributed to an acute sevoflurane effect but were regarded as long-term effects of anesthesia. Furthermore, to address the effects of anesthesia *per se*, we avoided all surgical manipulation or painful stimuli to the animals during anesthesia. The observed behavioral effects are thus likely to be due solely to interaction between sevoflurane anesthesia and the particular β_2KO phenotype, because WT animals did not display behavioral changes related to sevoflurane anesthesia exposure. Behavioral effects of repeated open field testing in young and aged WT mice is a phenomenon previously described.[45] By comparing anesthetized mice with control mice, we were able to control for this effect.

In conclusion, sevoflurane anesthesia altered exploratory and anxiety-like behavior in mice lacking the β_2 nAChR subunit. The duration of this effect, its cellular origin, and possible modulation by pharmacologic intervention remain to be determined. The current behavioral results suggest that brain circuits involved in organization of locomotor behavior or anxiety should be further investigated.

The authors thank Per Lindestam, M.Sc. Phys. (Artema Medical AB, Stockholm, Sweden), for lending the gas analyzer and Anne Cormier, Ph.D. (Research Assistant, Département de Neuroscience, Institut Pasteur, Paris, France), for excellent technical assistance.

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