Disruption of GABA<sub>A</sub> Receptors on GABAergic Interneurons Leads to Increased Oscillatory Power in the Olfactory Bulb Network

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Disruption of GABA<sub>A</sub> receptors on GABAergic interneurons leads to increased oscillatory power in the olfactory bulb network. J Neurophysiol 86: 2823–2833, 2001. Synchronized neural activity is believed to be essential for many CNS functions, including neuronal development, sensory perception, and memory formation. In several brain areas GABAA receptor–mediated synaptic inhibition is thought to be important for the generation of network activity. We have used GABA<sub>A</sub> receptor β3 subunit deficient mice (β3<sup>−/−</sup>) to study the role of GABAergic inhibition in the generation of network oscillations in the olfactory bulb (OB) and to reveal the role of such oscillations in olfaction. The expression of functional GABA<sub>A</sub> receptors was drastically reduced (>93%) in β3<sup>−/−</sup> granule cells, the local inhibitory interneurons of the OB. This was revealed by a large reduction of muscimol-evoked whole-cell current and the total current mediated by spontaneous, miniature inhibitory postsynaptic currents (mIPSCs). In β3<sup>−/−</sup> mitral/tufted cells (principal cells), there was a two-fold increase in mIPSC amplitudes without any significant change in their kinetics or frequency. In parallel with the altered inhibition, there was a significant increase in the amplitude of theta (80% increase) and gamma (178% increase) frequency oscillations in β3<sup>−/−</sup> OBs recorded in vivo from freely moving mice. In odor discrimination tests, we found β3<sup>−/−</sup> mice to be initially the same as, but better with experience than β3<sup>+/+</sup> mice in distinguishing closely related monomolecular odors. However, β3<sup>−/−</sup> mice were initially better and then worse with practice than control mice in distinguishing closely related mixtures of odors. Our results indicate that the disruption of GABA<sub>A</sub> receptor–mediated synaptic inhibition of GABAergic interneurons and the augmentation of IPSCs in principal cells result in increased network oscillations in the OB with complex effects on olfactory discrimination, which can be explained by an increase in the size or effective power of oscillating neural cell assemblies among the mitral cells of β3<sup>−/−</sup> mice.

INTRODUCTION

Sensory stimulus associated oscillatory synchronization has been described in the olfactory (Adrian 1942, 1950; Bressler and Freeman 1980; Freeman 1975, 1976; Gelperin and Tank 1990; Gray and Skinner 1988; Laurent and Davidowitz 1994; MacLeod and Laurent 1996; Stopfer et al. 1997) and visual (Engel et al. 1997; Gray and Singer 1989) systems of many species. The role of such synchronization is still debated, but several recent studies have provided strong evidence for the essential role of oscillatory synchronization in olfactory information coding in invertebrates (e.g., locust and honeybee). In the locust, information about odor identity is carried not only by the “spatial” component of the active neuronal ensemble, but also by the precise timing of action potential firing (Laurent and Davidowitz 1994; MacLeod and Laurent 1996; Wehr and Laurent 1996). It has been shown in honeybees that odor encoding involves the oscillatory synchronization of an ensemble of projection neurons (PN), and that their desynchronization results in impaired discrimination of molecularly similar odors, but not that of dissimilar odors (Stopfer et al. 1997). In locusts, PN desynchronization also leads to a loss of tuning specificity in neurons found two synapses downstream of the PNs, further implicating neuronal synchronization as being a functionally relevant parameter of neuronal activity (MacLeod et al. 1998). Oscillatory synchronization in the gamma, beta, and theta frequency ranges has been described in the olfactory bulb and piriform cortex of mammals (Adrian 1950; Bressler and Freeman 1980; Freeman 1975; Kay and Freeman 1998), but its role in sensory coding is still unclear. This is mainly due to the lack of experimental tools allowing the selective alteration of oscillatory synchronization in a defined part of the olfactory pathway in vivo without modifying the responsiveness of neurons to naturally occurring stimuli and their spatial arrangements.

In several mammalian and nonmammalian species, oscillatory synchronization of some neural populations requires intact GABA<sub>A</sub> receptor–mediated synaptic inhibition (reviewed by Buzsaki and Chrobak 1995; Singer 1996; Traub et al. 1998). All nerve cells in the mammalian brain express several subunits of the GABA<sub>A</sub> receptor (Fritschy and Mohler 1995; Wisden et al. 1992), which are usually co-assembled into several GABA<sub>A</sub> receptor subtypes. Granule cells in the olfactory bulb express only the β3 variant of the β subunit, whereas mitral and tufted cells express all three known β subunits (β1, β2, and β3) (Laurie et al. 1992; Nusser et al. 1999b). Because β subunits are essential for the formation of functional GABA<sub>A</sub>
receptors, we predicted that after the genetic deletion of the β3 subunit gene (Homanics et al. 1997) functional GABA<sub>A</sub> receptors would be altered in a cell type–specific manner in the olfactory bulb. Namely, we predicted a considerable reduction of functional GABA<sub>A</sub> receptors in granule cells, the local circuit GABAergic interneurons of the bulb, without a large reduction in principal cells (mitral/tufted cells). Previous experimental and modeling studies indicated that disruption of GABA<sub>A</sub> receptor–mediated inhibition between GABAergic local circuit interneurons results in the loss of gamma frequency synchronizations on olfaction.

**METHODS**

**Slice preparation and in vitro electrophysiological recordings**

One 28-day-old and four adult (>3 month old) β3<sup>-/-</sup> mice (DeLorey et al. 1998; Homanics et al. 1997) and four adult β3<sup>+/+</sup> mice were anesthetized with halothane before decapitation in accordance with the guidelines of the UCLA Office for Protection of Research Subjects. The brains were then removed and placed into an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 2.5 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, and 10 d-glucose, pH 7.3 when bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub>. The olfactory bulb was glued to a platform, and 300-μm thick sagittal slices were cut with a Vibratome (Leica VT1000S). The slices were stored submerged at 32°C in ACSF until they were transferred to the recording chamber. During recording, the slices were continuously perfused with 33-36°C ACSF containing 3–5 mM kynurenic acid (Sigma) and 0.7 μM tetrodotoxin (Calbiochem, La Jolla, CA). All recordings were made from the somata of visually identified neurons (Zeiss Axioscope and Leica DMS IR-DIC videomicroscopy, ×40 water immersion objective) with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Patch electrodes were pulled (Narishige PP-83, Tokyo) from thick-walled borosilicate glass (1.5 mm OD, 0.86 mm ID, Sutter Instruments, Novato, CA) and were filled with a solution containing (in mM) 140 CsCl, 4 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, and 10 d-glucose, pH 7.25 and an osmolarity of 280–290 mosmol. The DC resistance of the electrodes was 2–8 MΩ when filled with pipette solution. Series resistance and whole cell capacitance were estimated by compensating for the fast current transients evoked at the onset and offset of 8-ms, 5-nV voltage-command steps and were checked every 2 min during the recording. If the series resistance increased by more than 50%, the recording was discontinued. The series resistance remaining after 75–80% compensation (with 7–8 μs lag values) was 1.4 ± 0.07 and 1.7 ± 0.26 (SE) MΩ for β3<sup>+/+</sup> and β3<sup>-/-</sup> mitral cells, respectively; and 3.9 ± 0.3 and 3.8 ± 0.4 MΩ for β3<sup>+/+</sup> and β3<sup>-/-</sup> granule cells, respectively. Data are expressed as means ± SE and are compared with an unpaired t-test assuming unequal variances unless otherwise stated.

**Analysis of the in vitro electrophysiological data**

All recordings were low-pass filtered at 2 kHz and digitized on-line at 20 kHz, as described earlier (Nusser et al. 1999b). In-house data acquisition and analysis software (written in LabView, National Instruments, Austin, TX) was used to measure the amplitudes, 10–90% rise times, 67% decay times and charge transferred by miniature inhibitory postsynaptic currents (mIPSCs). The decay of the averaged currents was fitted with a single or the sum of two exponential functions. The weighted decay time from the exponential fit [τ<sub>weighted</sub>] was calculated as τ<sub>weighted</sub> = τ<sub>1</sub> * A<sub>1</sub> + τ<sub>2</sub> * (1 − A<sub>1</sub>), where τ<sub>1</sub> and τ<sub>2</sub> are the fast and slow decay time constants, respectively, and A<sub>1</sub> is the contribution of the first exponential to the amplitude. The weighted decay time constant from the area [τ<sub>area</sub>] was calculated by dividing the area of each mIPSC by its peak amplitude.

**In vivo recordings from freely moving animals**

Four control and three β3<sup>-/-</sup> mice were anesthetized with 100 mg/kg ketamine and 5 mg/kg xylazine. The skin was opened and small holes (~2 mm) were drilled in the skull. Following the opening of the dura mater, a bipolar electrode (twisted 60-μm tungsten wires with vertical tip separation of ~0.5 mm) was lowered into the dorsal surface of the left olfactory bulb. A stainless steel watch screw was driven into the skull above the cerebellum to serve as a ground electrode. All electrodes were stabilized with dental cement. During surgery and postoperative care, all efforts were made to comfort the animals, in accordance with the guidelines of the UCLA Office for Protection of Research Subjects.

Two days after the surgery, electroencephalograms were low-pass filtered at 200 Hz and digitized at 1 kHz using a data acquisition board (PCI-MIO 16E-4, National Instruments, Austin, TX) and in-house data acquisition software written in LabView (National Instruments). Power spectra and autocorrelograms were computed with LabView. The power spectra were normalized in two ways. 1) The power spectra during both immobility and exploration were normalized to the maximum of the lowest frequency peak during immobility (its value defined as 1). 2) The power spectra were normalized to a mean of 0 and a SD of 1. Almost identical results were obtained with both normalizations, but as the latter method resulted in greater variance within conditions, we have chosen to present our data with the first way of normalization. We discriminated between two behavioral states of the animals during the recordings: immobility, during which the animals did not move and showed no observable sign of sniffing; and exploration, during which the animals moved around in their cage and showed intense sniffing activity.

**Odor discrimination**

All mice (4 adult β3<sup>+/+</sup> and 4 adult β3<sup>-/-</sup> mice) were trained using a protocol developed and modified according to Linster and Hasselmo (1999) and is as presented elsewhere (Kay et al. 2000). Mice were first introduced to the test arena (polycarbonate box similar to the home cage and fitted with a dividing door). They were trained to dig in a small glass dish of sand for a food reward until they reached criterion (initiating digging within 10 s). They were then presented with two dishes, one scented (5 drops of 5% odorant in mineral oil, mixed into the sand) and one unscented (5 drops of mineral oil). The animals learned to dig in the scented dish for a reward.

Odor testing sessions began with 10 training trials, in which the mouse learned to dig in response to the training odor (hexanol or an alcohol mixture) and avoid digging in the control dish. The mouse was then tested on a set of odors in random order, including the learned odor. Each test trial was 20 s long, after which the animal was removed from the arena. In the test trials, there was no reward present in the dish, and the amounts of time spent digging in the odor dish (digging time) and the control dish were measured. To avoid behavioral extinction, the mouse was given one to three reinforcement trials with the trained odor in between unrewarded test trials. In the first experiment, test odors were alcohols of various chain lengths (3-C to 8-C and 10-C) and one nonalcohol, isomyl acetate (IAA). The
training odor was hexanol (6-C). All odors were 5% solutions in mineral oil. The odors were tested twice in each session (round 1 and round 2 in Fig. 7). Generalization was measured as significant digging in an odor other than the training odor.

In the second experiment the four $\beta3^{+/+}$ mice and three of the original four $\beta3^{-/-}$ mice were challenged with a more difficult odor identification task. The training odor in these sessions was a 5% dilution in mineral oil of a mixture of alcohols (OM: butanol, pentanol, heptanol, and decanol). The test odors were the original mixture and four mixtures consisting of one of the four alcohols in the original mixture (M1: pentanol, heptanol, and decanol; M2: butanol, heptanol, and decanol; M3: butanol, pentanol, and decanol; M4: butanol, pentanol, and heptanol). The mice were tested as before, and digging times were recorded for each test mixture and the control dishes. The odors were tested three times (rounds 1–3 in Fig. 8).

Due to the small number of animals and the variability of digging durations across animals, data were normalized by transformation to their Z-scores. Normalized digging times for each test odor were compared in a one-way ANOVA across test odors. The test odor digging times were then compared with each other using a post hoc Newman–Keuls test. Values of $P < 0.05$ were considered significant. Only digging times in the test odors were analyzed, as the mice rarely dug in the control sand.

Immunohistochemistry

Light microscopic immunostaining for GABA$_A$ receptor subunits was performed as described previously (Nusser et al. 1995). Olfactory bulbs from a control and a $\beta3^{-/-}$ mouse were removed and placed into ice-cold fixative containing 4% paraformaldehyde, 0.05% glutaraldehyde, and ~0.2% picric acid made up in 0.1 M phosphate buffer (PB, pH 7.4) for 50 min. Vibratome sections (sagittal, 70 μm in thickness) were cut and collected in PB. Normal goat serum (20%) was used in 50 mM Tris-HCl (pH 7.4) containing 0.9% NaCl (TBS) as the blocking solution for 0.5 h followed by the incubation with purified primary antibodies diluted in TBS containing 1% normal goat serum and 0.05% Triton X-100 overnight. The primary antibodies were used at the following final concentrations: β1 [code No. β1(350–404)R16/2] (Jechlinger et al. 1998), 1.25 μg protein/ml; β2 [code No. β2(351–405)R20] (Jechlinger et al. 1998), 1.9 μg/ml; and β3 [code No. β3(345–408)R21] (Slany et al. 1995), 1 μg/ml. After washing, the sections were incubated for 90 min in biotinylated secondary antibodies (diluted 1:50 in TBS; Vector Lab., Burlingame, CA), followed by further washings and incubation in avidin biotinylated horseradish peroxidase complex (1:100 dilution in TBS) for 2 h. Peroxidase enzyme reaction was carried out with 3,3′-diaminobenzidine tetrahydrochloride as chromogen and H$_2$O$_2$ as oxidant. The sections were then routinely processed for light microscopic examination (Somogyi et al. 1989).

RESULTS

Alteration of GABAergic synaptic neurotransmission in the olfactory bulb

First, we recorded mIPSCs from granule cells of the olfactory bulb in the presence of the non-NMDA glutamate receptor blocker kynurenic acid (3–5 mM) and the sodium channel blocker tetrodotoxin (0.7 μM) under whole-cell voltage-clamp configuration in acute brain slices. Because granule cells were held at −70 mV and because symmetrical Cl$^-$ concentrations were used, GABA$_A$ receptor–mediated mIPSCs were inward. In agreement with our previous studies (Hajós et al. 2000; Nusser et al. 1999b) in control mice, mIPSCs occurred relatively infrequently (1.16 ± 0.30 Hz, $n = 7$), had an average amplitude of 74.8 ± 11.9 pA, a weighted decay time constant of 8.3 ± 0.6 ms and the charge carried by each mIPSC was 0.68 ± 0.12 pC (Fig. 1). In $\beta3^{-/-}$ mice, there was an ~80% reduction in mIPSC frequency (from 1.16 ± 0.30 Hz to 0.24 ± 0.04 Hz, $n = 7$, $P = 0.01$, unpaired t-test) with an accompanying decrease in the amplitude (43% reduction, from 74.8 ± 11.9 pA to 42.9 ± 8.2 pA, $P = 0.02$) and decay time (42% reduction, 8.3 ± 0.6 ms in control and 4.8 ± 0.4 ms in $\beta3^{-/-}$, $P < 0.001$). As a consequence, the total current entering through synaptic GABA$_A$ receptors was reduced by 93% ($P < 0.01$) in $\beta3^{-/-}$ mice compared with the controls (Fig. 1). To test whether the observed reduction in GABAergic synaptic currents was due to a decrease in surface GABA$_A$ receptors, we bath applied ~100 μM muscimol, a GABA$_A$ receptor agonist.
and recorded the drug-evoked whole-cell currents. As shown in two representative cells in Fig. 1C, muscimol evoked a much smaller inward current in β3−/− granule cells compared with controls. As we did not use rapid agonist application, we did not attempt to quantify the results of these experiments.

Taken together, these results suggest that there was a great reduction in the expression of functional GABA_A receptors on the surface of β3−/− granule cells. However, it is clear that there is no complete loss of functional GABA_A receptors from the surface of granule cells. Using immunocytochemistry with subunit-specific antibodies, we tested whether a compensatory up-regulation of other β subunits could explain the incomplete loss of synaptic currents. In control granule cells, similar to our previously published data (Nusser et al. 1999b), no immunoreactivity for the β1 and β2 subunits could be detected, but very strong staining for the β3 subunit was observed (Fig. 2). In the external plexiform layer, all three β subunit variants were strongly expressed. There was no detectable staining for the β3 subunit in the whole brain of β3−/− mice, including the olfactory bulb, in agreement with the results of previous studies showing a complete loss of the β3 subunit (DeLorey et al. 1998). We could not detect any significant labeling for the β1 or β2 subunits in β3−/− granule cells, whereas these subunits were strongly expressed in mitral/tufted cells.

To assess the effect of the β3 subunit gene deletion on GABAergic synaptic currents recorded in the principal cells of the olfactory bulb (mitral and tufted cells), mIPSCs were pharmacologically isolated and were recorded under whole-cell voltage-clamp. In control mitral cells, mIPSCs occurred with a frequency of 2.3 ± 0.8 Hz (n = 9) and had amplitudes of 42.9 ± 4.9 pA at −70 mV. The decay of the currents could be described either with a single exponential (τ = 3.7 ms, n = 6 cells) or with the sum of two exponentials [τ_1 = 2.5 ms (80%), τ_2 = 9.9 ms, n = 3 cells]. In β3−/− mitral cells, there was no significant change in the frequency of the synaptic currents (2.4 ± 0.7 Hz, P > 0.05 compared with controls; Fig. 3). The most parsimonious explanation of this result is that there is no change in the number of GABAergic synapses on mitral/tufted cells, consistent with the expression of additional β subunits (β1 and β2) in these cells. However, we observed a significant increase (118%) in the amplitude of mIPSCs (from 42.9 ± 4.9 pA to 93.4 ± 18.9 pA, n = 9, P = 0.01, Fig. 3) in β3−/− mitral/tufted cells without any significant change in their kinetics [τ_w(t) = 3.7 ± 0.2 ms in control and τ_w(t) = 4.3 ± 0.4 ms in β3−/−, P > 0.05].

**Effect of altered synaptic inhibition on network oscillations**

To assess the role of GABAergic synaptic inhibition in the generation of synchronous network activity of the olfactory bulb, we recorded electroencephalograms (EEG) from the dorsal surface of the olfactory bulb of freely moving control and β3−/− mice (Kay and Freeman 1998). We discriminated two behavioral states: 1) immobility, periods during which the animals did not move, and no sign of sniffing was observed; and 2) exploration, during which the animals explored their cage, and prominent sniffing activity was apparent. In control mice, during immobility, a prominent breathing-associated theta band (2–12 Hz) oscillation was apparent as described in several other species (Freeman 1976; Kay and Freeman 1998). By examining the power spectra of EEG recorded during immobility, we observed two peaks in the theta frequency band with frequencies of 4.2 ± 0.9 Hz and 9.7 ± 1.8 Hz, respectively (Figs. 4 and 6). During exploration, the lower frequency (2.5 ± 0.3 Hz) theta oscillation had smaller power (41 ± 8% of control) than that during immobility, but the power of the higher frequency theta oscillation (6.7 ± 0.5 Hz) was almost identical to that in immobility (normalized power 0.51 ± 0.2 during immobility and 0.50 ± 0.11 during exploration). In control mice, gamma frequency oscillations were readily observed in both behavioral states with only slightly different
frequencies and power (Figs. 4 and 6; immobility: frequency = 43 ± 5 Hz, normalized power = 0.33 ± 0.17; exploration: frequency = 52 ± 5 Hz, normalized power = 0.36 ± 0.13), similar to that seen in rats (Kay and Freeman 1998). The most striking difference in the EEG patterns between control and β3−/− mice was the very pronounced power increase in the gamma frequency band in β3−/− animals (Figs. 5 and 6). During exploration, the normalized power (see METHODS) in gamma frequency band was increased almost threefold (normalized power: 0.36 ± 0.13 in control, n = 4; and 1.00 ± 0.10 in β3−/−, n = 3; P < 0.01, unpaired t-test), whereas the oscillation frequency remained unchanged (52 ± 5 Hz in control and 52 ± 3 Hz in β3−/−). We also compared the area under the nonnormalized power spectra between 40 and 80 Hz and found a very similar increase (320%) in β3−/− mice. During immobility, the power of the gamma frequency band was also greater in β3−/− mice, but this increase did not reach significance (normal power: 0.33 ± 0.17 in control, n = 4; and 0.60 ± 0.15 in β3−/−, n = 3; P = 0.15, unpaired t-test). During immobility, there was no significant change in either the frequency or the normalized power of the two theta frequency bands in β3−/− mice compared with controls (frequency: 4.3 ± 0.9 Hz, n = 4 vs. 3.0 ± 0.0 Hz, n = 3, P = 0.14 and 9.7 ± 1.8 Hz, n = 4 vs. 7.0 ± 1.0 Hz, n = 3, P = 0.14; normalized power for the higher frequency band: 0.51 ± 0.20, n = 4 vs. 0.78 ± 0.24, n = 3, P = 0.22 unpaired t-test). During exploration, however, the lower frequency theta oscillation had a significantly higher power (0.41 ± 0.08, n = 4 vs. 0.75 ± 0.09, n = 3, P = 0.02) with similar frequencies (2.5 ± 0.3 Hz, n = 4 vs. 4.3 ± 0.9 Hz, n = 3, P > 0.05) in β3−/− mice compared with controls. The higher frequency theta oscillation had a higher frequency (6.8 ± 0.5, n = 4 vs. 8.7 ± 0.3, n = 3, P = 0.01) and power (0.5 ± 0.1, n = 4 vs. 0.9 ± 0.16, n =
Taken together, these results show that the almost complete disruption of GABAergic synaptic inhibition of granule cells, and the increased mIPSC amplitude in mitral/tufted cells, results in an enhanced oscillatory power in $\beta_3^{-/-}$ olfactory bulb (OB). We next tested the effect of such altered neuronal network oscillations on odor discrimination.

Altered odor discrimination in $\beta_3^{-/-}$ mice

A first observation was that the $\beta_3^{-/-}$ mice were more active than the $\beta_3^{+/+}$ mice, as has been reported elsewhere (Homanics et al. 1997). All animals learned to dig in an odorized dish (geraniol) versus control (mineral oil), which indicates that the $\beta_3^{-/-}$ mice can smell.

Odor identification/discrimination was tested with two tasks. The first task was a simple identification of a learned alcohol (hexanol) in a randomly presented series of chemically similar alcohols and a chemically unrelated odorant IAA. The mice were tested two times in the same session on the randomized series of odorants. Time spent digging in a scented dish was used to assess identification and generalization. In the first round of tests, none of the mice showed significant generalization to odorants other than the training odor (Figs. 7, A and B). In the second round of tests, the $\beta_3^{+/+}$ mice generalized to heptanol and dug very little in the other odorants (Fig. 7C). The $\beta_3^{-/-}$ mice dug significantly only in hexanol, showing no generalization (Fig. 7D). Thus with practice, the $\beta_3^{-/-}$ mice performed better than the $\beta_3^{+/+}$ mice in distinguishing this monomolecular alcohol from closely related alcohols.

The second odor identification test was a more complex mixture identification task. The mice were trained on a mixture of four alcohols and then tested on the original mixture (OM: butanol, pentanol, heptanol, and decanol) and four close mixtures (those consisting of 3 of the original 4 components; M1–M4, see METHODS). They were each tested three times on randomized series of the five mixtures in a single session. In the first round the $\beta_3^{+/+}$ mice made no distinction among the odors (Fig. 8A), whereas the $\beta_3^{-/-}$ mice generalized to those mixtures lacking the long chain components (M3 and M4) and discriminated those mixtures lacking the short chain components (M1 and M2; Fig. 8B). In the second round the $\beta_3^{+/+}$ mice generalized to one mixture (M4 in Fig. 8C), and the $\beta_3^{-/-}$ mice generalized across all odor mixtures (Fig. 8D). In the third and final round, the $\beta_3^{+/+}$ mice correctly distinguished the learned odor from the other mixtures (Fig. 8E), and the $\beta_3^{-/-}$ mice did as well as the $\beta_3^{+/+}$ mice had done on the second round (Fig. 8F). While the $\beta_3^{-/-}$ mice initially performed better than the $\beta_3^{+/+}$ mice on this task, with subsequent exposure to the panel of test odors, they confused the mixtures (round 2) and then began to relearn the discrimination (round 3). The generalization patterns seen in the mixture identification test suggest that effective concentration may also play a role in performance in both sets of animals. The mixture most readily confused with the training mixture (OM) was that lacking the longest chain, and thus less volatile, alcohol (M4). This alcohol may be less noticed in a mixture of more volatile alcohols and so may participate less in the representation of the OM.

![Figure 5](image-url)
D I S C U S S I O N

We have demonstrated a dramatic reduction of synaptic GABA_3 receptor-mediated inhibition in GABAergic interneurons (granule cells) of the OB caused by the targeted disruption of the GABA_3 receptor β3 subunit gene. Because there was an increase, rather than a decrease, in the mIPSC amplitudes in β3−/− principal cells (mitral and tufted), a cell-type-selective abolition of synaptic inhibition was achieved in the olfactory bulb of β3−/− mice. In parallel with these altered patterns of synaptic inhibition, we observed a large increase in the amplitude of olfactory bulb theta and gamma frequency oscillations in vivo during exploration, i.e., while the mice showed intense sniffing activity. In two olfactory discrimination tasks, β3−/− mice showed both an increased ability to discriminate monomolecular alcohols and a decreased ability to discriminate closely related mixtures of alcohols, relative to wild type littermates.

Cell type-selective reduction of synaptic inhibition in the olfactory bulb of β3−/− mice

Examination of the expression of GABA_3 receptor subunits in the mammalian brain revealed that most nerve cells express a large variety of subunits (Fritschy and Mohler 1995; Persohn et al. 1992; Wisden et al. 1992), which are co-assembled into several GABA_3 receptor subtypes. Even within a single subunit class, most nerve cells express several subunit variants. For example, cortical and hippocampal pyramidal cells, like olfactory bulb mitral cells, express at least three α and three β subunit variants. Thus after genetic deletion of a single subunit, the total elimination of functional GABA_3 receptors is not predicted. Less frequently, some neurons express only a single subunit of a given subunit class (Fritschy and Mohler 1995; Persohn et al. 1992; Wisden et al. 1992). For example, granule cells of the olfactory bulb express strongly only the β3 as the β subunit (Nusser et al. 1999b). Because it is impossible to form functional GABA_3 receptors without β subunits, we expected to observe a total disappearance of functional GABA_3 receptors from granule cells in the β3 subunit’s absence. In good agreement with this prediction, we found a dramatic reduction of the muscimol-evoked whole-cell current and the total current mediated by mIPSCs in β3−/− granule cells, without an accompanying decrease of mIPSCs in the principal cells. These results are in excellent agreement with those of a previous study using the β3−/− mice to study the alteration of synaptic inhibition in the thalamus (Huntsman et al. 1999), where neurons of the reticular thalamic nucleus express only β3 as the β subunit, whereas relay neurons in the ventrobasal complex express other β subunits. The amplitude, duration, and frequency of the spontaneous IPSCs were greatly reduced in neurons of the reticular thalamic nucleus of β3−/− mice, but those recorded from the ventrobasal nucleus were unaltered. Our work and that of Huntsman et al. (1999) showed an incomplete loss of functional GABA_3 receptors in β3 subunit-expressing cells in β3−/− animals. To test whether a compensatory up-regulation of the β1 or β2 subunits is responsible for the incomplete loss of GABA_3 receptors in β3−/− olfactory granule cells, we performed light microscopic immu-
show SE of the M1, pentanol, heptanol, and decanol; M2, butanol, heptanol, and decanol; M3, F0.01. It is possible that an as yet unidentified subunit is expressed in granule cells or that its expression is turned on in Fmixtures [2830 NUSSER, KAY, LAURENT, HOMANICS, AND MODY].

Although the β3 subunit was not present in mitral/tufted cells in β3−/− mice, we found an increase rather than a decrease in mIPSC amplitudes recorded from these neurons. Because a compensatory up-regulation of the β1 or β2 subunits was not observed by light microscopic immunocytochemistry in β3−/− mitral/tufted cells, the reason for this observation is unknown. An increased synaptic concentration of GABA could explain the observed increase in mIPSC amplitudes. Such increased concentration may be achieved by an increase in the GABA content of synaptic vesicles or a change in the geometry of the neurolip surrounding the synapse with altered GABA diffusion/uptake. Furthermore, the conductance of the GABA A receptors in β3−/− mitral cells could also be increased as a consequence of the altered subunit composition. With our experimental approach, we cannot exclude the possibility that the large mIPSCs in β3−/− mitral cells are glycinegic synaptic currents. However, this possibility would require that the glycinegic synaptic currents had the same decay kinetics compared with the GABAergic mIPSCs in control (under control conditions, mIPSCs are bicuculline sensitive). Furthermore, as we did not detect a change in the mIPSC frequency, a complex regulation would be required to decrease the GABAergic IPSC frequency in proportion to the appearance of the glycinegic synaptic currents in β3−/− mitral cells. Irrespective of the mechanism of the increased mIPSCs in mitral cells, our data show that a cell type–selective reduction of synaptic GABA A receptor–mediated inhibition could be achieved in the β3−/− olfactory bulb. However, it is important to point out that in the β3−/− mice, we did not find a complete loss of functional GABA A receptors in granule cells, and we did observe an increase in mIPSC amplitudes in mitral/tufted cells, which could be the consequence of some compensatory mechanisms, as observed in other GABA A receptor subunit–deleted mice (Brickley et al. 2001; Jones et al. 1997; Nusser et al. 1999a). Future experiments with cell type–specific and inducible knock-out animals will be required to achieve selective elimination of GABAergic inhibition without possible secondary, compensatory effects in the olfactory bulb network.

GABAergic inhibition of granule cells plays a role in oscillatory synchronization in the OB

Oscillatory synchronization in the theta and gamma frequency ranges has been described in several brain regions, including the hippocampus, thalamus, visual cortex, olfactory cortex, and the olfactory bulb. Several studies using experimental and/or modeling approaches pointed to the importance of GABAergic interneurons in the generation of network oscillations (Cobb et al. 1995; Lytton and Sejnowski 1991; Rall...
increased specifying rate of the relatively hyperactive $\beta^3-/-$ mice. The mechanisms underlying the increased power of gamma frequency oscillation are unclear, but may include the following: 1) increased synaptic conductances in mitral/tufted cells; 2) higher excitability of mitral/tufted cells; 3) larger numbers of mitral/tufted cells participating in the oscillation; 4) increased oscillatory coherence between the active principal cells; 5) altered centrifugal input to the granule cells, resulting in increased synchrony of mitral cells (Gray and Skinner 1988); or 6) combinations of the above. Future studies on inducible GABA_A receptor knock-out animals with multunit recordings will be required to elucidate some of the above hypotheses.

An unresolved issue about the circuitry of the mammalian olfactory bulb is the source of GABAergic synapses on granule cells. Previously, we identified two distinct populations of mIPSCs in granule cells and suggested that they may originate from different sources (Nusser et al. 1999b). One obvious source is the input from the GABAergic short axon cells present in the granule cell layer (Schneider and Macrides 1978). The second source may be interconnection of granule cells through dendritic synapses. Finally, the basal forebrain (diagonal band nuclei) and, to a lesser extent, the ventral pallidum, anterior amygdala, and the nucleus of the lateral olfactory tract could also provide a GABAergic innervation of the granule cells (Zaborsky et al. 1986). It remains to be determined whether the reduced synaptic inhibition in $\beta^3-/-$ granule cells affects all inputs or just some of them.

Finally, our results showed that with the changes in OB oscillatory synchrony, on behavioral tests the $\beta^3-/-$ mice performed better than their control littermates in identifying a monomolecular alcohol but worse in discriminating–highly overlapping mixtures of alcohols. These differences were dependent on experience with the odors, as in initial tests the $\beta^3-/-$ mice performed the same as the control mice on the single alcohol discrimination test and better than the control mice on the mixture discrimination test. These results indicate that increased network synchrony has a complex effect on odor learning, representation, and discrimination.
animals specialists for that odor. Experience would then enhance the separation of NCAs more in β3−/− than control mice. Training on a mixture of closely related alcohols produces an initial behavioral response that is better for β3−/− than control mice, due to the increased size of the NCA. Subsequent experience with the test mixtures produces competing, highly overlapping NCAs, since the test mixtures are very similar in chemical composition and the NCAs representing monomolecular odors in β3−/− mice are presumed to be larger and more separated than those of control mice. This could explain the decrease in performance of the β3−/− mice in the second round of the mixture task. However, the β3−/− mice are not so impaired that they cannot eventually learn to discriminate the mixtures, as is indicated by their performance in the third round of tests. It is probable that with further training they could have done as well as their control littersmates. It is also possible that prior learning of the monomolecular discrimination task affected subsequent performance in the mixture task differently in the two groups of mice. Because of the small numbers of animals and the difficulty of breeding the β3−/− mice, we were unable to test this possibility.

To prove the role of network oscillations in odor coding and discrimination, the animals should be first trained in one state (e.g., in control state) and consequently tested in another (e.g., discrimination, the animals should be first trained in one state and dr. Tamas Freund for help with the in vivo recordings. We are grateful to Dr. Werner Sieghart for providing the GABA A receptor β subunit-specific antibodies. We also thank C. Fergusson and J. Steinmiller for expert technical assistance with the β3−/− animals and Dr. Tamás Freund for comments on the manuscript. This work was supported by a Wellcome International Travelling Fellowship, a Boehringer Ingelheim Award, and a Hungarian Science Foundation fellowship, a Boehringer Ingelheim Award, and a Hungarian Science Foundation grant to Z. Nusser; a Burroughs-Wellcome fellowship in Computational Molecular Biology to L. M. Kay; National Institutes of Health (NIH) Grants GM-52035 and NS-30549 and NS-35985 and the Coelho Endowment to I. Mody.

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