GCCCT-3′ and 5′-GATCAGGGCAGGGGTCAA-GGGTTCAGT-3′, and although it lacked the −222 to −215 region that is identical to Sp1 binding site, competed efficiently with site A for binding nuclear proteins. For library screening, oligo A was ^{32}P-labeled at the 5′ end with T4 polynucleotide kinase and self-ligated with T4 DNA ligase. Libraries were plated on *Escherichia coli* Y1090 and isopropylthiogalactoside (IPTG)-induced proteins were transferred to nitrocellulose filters. Filters were denatured in 6 M guanidine hydrochloride in binding buffer [20 mM Hepes, pH 7.9, 40 mM KCl, 3 mM $MgCl_2$, and 1 mM dithiothreitol (DTT)] at 4°C, followed by renaturation in a series of 5-min rinses in five successive 1:1 dilutions of the guanidine solution in binding buffer. After two final 5-min washes in binding buffer, filters were blocked for 30 min in binding buffer containing 5% nonfat dry milk (Carnation). Filters were incubated with probe (2×10^6 cpm/ml) in the presence of denatured, sonicated salmon sperm DNA (5 μg/ml) in binding buffer containing 0.25% nonfat dry milk at 4°C for 12 hours. Filters were washed in binding buffer with 0.25% milk and autoradiographed. Positive clones were purified and subjected to binding-specificity spot tests: ~10^6 plaque-purified phage were spotted on a preformed lawn of Y1090 *E. coli* and the plaques were tested for binding to various oligonucleotide probes as described above. To clone λHP-16, a human genomic DNA library was screened with the λHP-1 insert probe, and a clone containing ~800 bp of the 5′ λHP-1 sequence and extending ~15 kb to the 5′ direction was isolated; a nonrepetitive genomic fragment located upstream to sequences corresponding to λHP-1 was subsequently used as probe to screen the placenta cDNA library. The inserts from both λHP-1 and λHP-16 were subcloned in M13mp18 and sequenced [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977); A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980)]. Protein comparisons were carried out as described [D. Lipman and W. Pearson, *Science* **227**, 1435 (1985)].

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13. A DNA fragment corresponding to ARP-1 DNA binding domain was used as probe to screen cDNA libraries from human placenta, liver, and HepG2 cells. Five different groups of clones were obtained and were termed ARP-1, ARP-2, ARP-3, ARP-4, and ARP-5. Sequence analysis identified ARP-2 as the Ear-3 (*11*), ARP-3 as the Ear-2 (*11*), and ARP-4 as the retinoic acid X receptor hRXRα [D. J. Mangelsdorf, E. S. Ong, J. A. Dyck, R. M. Evans, *Nature* **345**, 224 (1990)]. ARP-5 has not yet been characterized.
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Cortical Computational Maps Control Auditory Perception

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Mustached bats orient and find insects by emitting ultrasonic pulses and analyzing the returning echoes. Neurons in the Doppler-shifted constant-frequency (DSCF) and frequency-modulated (FM-FM) areas of the auditory cortex form maps of echo frequency (target velocity) and echo delay (target range), respectively. Bats were trained to discriminate changes in echo frequency or delay, and then these areas were selectively inactivated with muscimol. Inactivation of the DSCF area disrupted frequency but not delay discriminations; inactivation of the FM-FM area disrupted delay but not frequency discriminations. Thus, focal inactivation of specific cortical maps produces specific disruptions in the perception of biosonar signals.

THE JAMAICAN MUSTACHED BAT (*Pteronotus parnellii parnellii*) uses biosonar pulses to orient and to hunt flying insects. Its auditory cortex (AC) is specialized for processing information contained in the pulse (P) and its echo (E). The AC has at least ten functional areas (Fig. 1A), each containing one or more maps that represent a P-E parameter or combination of parameters important for encoding a particular type or types of biosonar information (*1*).

The P of the mustached bat has four harmonics (H_{1-4}), each consisting of a long constant-frequency (CF_{1-4}) component followed by a short frequency-modulated (FM_{1-4}) component (Fig. 1B). Of these, H_2 is always the most intense. When a bat is not flying, its PCF_2 is about 61.0 kHz (*2*, *3*).

During insect pursuit, the mustached bat adjusts its P frequency to stabilize the CF_2 of Doppler-shifted echoes at a reference frequency ~200 Hz above its resting frequency (*4*, *5*). This behavior is known as Doppler shift (DS) compensation. Because the peripheral auditory system of the mustached bat has a disproportionately large number of neurons that are sharply tuned around the reference frequency, the CF_2 of DS-compensated echoes is subjected to fine frequency analysis (*6*). The accuracy of the bat's DS compensation depends on its ability to resolve the frequency of the Doppler-shifted ECF_2.

The cortical area most likely to play a major role in the fine frequency analysis of Doppler-shifted echoes is the Doppler-shifted CF (DSCF) processing area of the primary AC (*7*, *8*). This area is tonotopically organized but only represents frequencies in the range of ECF_2 from 60.6 to 62.3 kHz (*2*). The frequency tuning curves of DSCF neurons are very narrow, even at high stimulus intensities (*8*). Further, bats with bilateral ablation of the DSCF may not be able to detect small echo DSs (*4*).

To determine the range of a target, the mustached bat measures the delay between the emitted P and the returning E. The FM-FM area of the AC probably plays a

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Table 1. Percentage of test blocks within each time window in which discrimination performance was significantly worse than baseline for the frequency discrimination experiments. There were 20 trials per block. The data in this table are based on three bats tested twice each and one bat tested once.

Discrimination level	Δf (kHz)	Time window (hours)		
		0.2–1.7	1.7–3.2	24–25.5
Fine	0.05	67	67	0
	0.1	100	33	0
Intermediate	0.3	50	0	0
	0.5	67	0	0
Coarse	2.0	0	0	0

primary role in this analysis because neurons in this area respond poorly to either P or E alone but show a strong response to the P-E pair at a particular E delay (target range) (*9, 10*). The essential components of the P-E pair are the PFM_1 and the EFM_n, where n = 2, 3, or 4. These neurons are systematically arranged according to the delay at which they respond best, ranging from 0.4 to 18 ms (target ranges of 7 to 310 cm).

To test whether these maps are important for the perception of biosonar signals, we trained bats to make frequency or temporal discriminations involving synthesized P-E pairs. Then we inactivated either the DSCF area or the FM-FM area by surface application of muscimol, a γ-aminobutyric acid (GABA) agonist. GABA is an inhibitory neurotransmitter in the cerebral cortex (*11*), and muscimol has been used to inactivate small regions in the somatosensory cortex (*12*).

Bats were conditioned to discriminate between different 1.2-s trains of P-E pairs. One P-E train (S+) was followed immediately by a train of electric shocks applied to the thigh; a leg flexion during the S+ terminated both the S+ and the programmed shock. The second P-E train (S−) was not followed by shock; leg flexions during the S− had no consequences. A correct response was either a leg flexion during the S+ or the omission of a flexion during the S−. Conversely, an incorrect response was either the omission of a leg flexion during the S+ or the occurrence of a flexion during the S− (Fig. 2) (*13*).

In the frequency discrimination task we used trains of PCF_2-ECF_2. For S+ trials, the PCF_2 and ECF_2 frequencies were equal. For S− trials, the ECF_2 was set at PCF_2 + Δf, where Δf = 0.05, 0.1, 0.3, or 2.0 kHz. In the temporal discrimination task we used trains of PFM_1-EFM_2. For S+ trials the echo delay was always set at 4 ms (P onset to E onset). For S− trials, the echo delay for successive P-E pairs within the train was switched between 4 ms and 4 ms + Δt, where Δt = 1, 2, 4, 6, 16, or 36 ms. Each bat was trained on either the frequency discrimination or the temporal discrimination until it consistently discriminated between the S+ and S− for every Δf or Δt at ≥ 75% (*14*).

After training, baseline data were collected for five test sessions before each application of muscimol. About 2 hours after the last of these sessions, 0.1 to 0.2 μg of muscimol (1 μg per microliter of saline) was applied to each DSCF area or FM-FM area with a 1-μl Hamilton microsyringe (*15*). Then, Gelfoam was placed in the well to minimize leakage of the muscimol from the application site and to increase the time during which the muscimol would be in contact with the cortical surface. The first test session was begun 10 to 15 min later. These sessions were given one after another until the bat's performance had returned to baseline level.

Application of muscimol to the DSCF area disrupted frequency discriminations, but its effect varied as a function of the Δf used (Fig. 3D and Table 1) (*16*). The frequency discriminations could be divided into three clusters: coarse (Δf = 2 kHz), intermediate (Δf = 0.5 and 0.3 kHz), and fine (Δf = 0.1 and 0.05 kHz). There was no significant disruption in the coarse discrimination. Shortly after muscimol application (0.2 to 1.7 hours), the intermediate discrimination was significantly disrupted on more than half of all test blocks, and the fine discrimination was significantly disrupted on almost all test blocks. By 1.7 to 3.2 hours after muscimol application, performance on the intermediate discriminations was virtually normal while performance on the fine discriminations was still significantly disrupted on about half of the test blocks. Performance on the latter discriminations returned to normal after approximately 6 to 7 hours. In contrast, application of muscimol to the FM-FM area had no significant effect on any of the frequency discriminations (Fig. 3B).

Application of muscimol to the FM-FM area disrupted temporal discriminations, but its effect varied as a function of the Δt used (Fig. 3A, Table 2). The temporal discriminations could be divided into three clusters: coarse (Δt = 36 ms), intermediate (Δt = 4, 6, or 16 ms), and fine (Δt = 1 or 2 ms). There was no significant disruption in the coarse discrimination. Shortly after muscimol application (0.2 to 2.2 hours), performance on both the intermediate and the fine discriminations was significantly disrupted. At 2.2 to 4.2 hours after muscimol application, performance was still significantly disrupted on the fine discriminations but not on the intermediate discriminations. Performance on the fine discriminations returned to normal by 7 to 8 hours after muscimol. Application of muscimol to the DSCF area had no significant effect on any of the temporal discriminations (Fig. 3C).

Our results indicate that the DSCF area is important for fine frequency discriminations involving Doppler-shifted echoes, and the FM-FM area is important for fine temporal discriminations involving E delays. The limits of the frequency and temporal discrimination deficits are predictable from the electrophysiologically determined ranges of

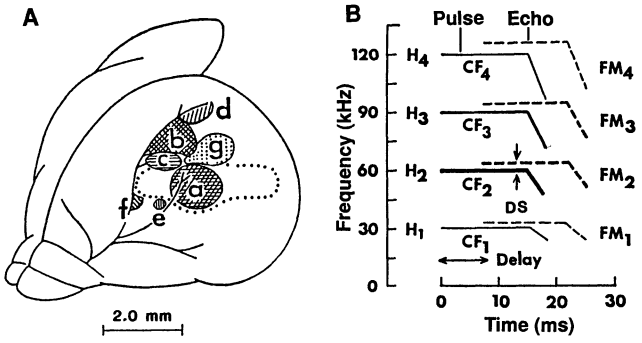

Fig. 1. (**A**) Functional organization of the auditory cortex of the mustached bat (dorsolateral view of the left cerebral hemisphere). The DSCF area (a) has axes representing both target velocity (echo frequency) and subtended angle (echo amplitude). The FM-FM area (b) consists of three types of FM-FM combination-sensitive neurons (FM_1-FM_2, FM_1-FM_3, and FM_1-FM_4), which segregate into three subareas. Each subarea has an axis representing target range (echo delay). The CF/CF area (c) consists of two types of combination-sensitive neurons (CF_1/CF_2 and CF_1/CF_3), which segregate into two subareas, each having two frequency axes that represents target velocity (echo Doppler shifts). The dorsal fringe area (d) has an axis for the shorter half of the target ranges represented in the FM-FM area, and the ventral fringe area (f) has an axis for the shorter half of the target ranges represented in the dorsal fringe area. The ventral anterior (e) contains only combination-sensitive neurons that respond to the first harmonic of the pulse and the second harmonic of the echo. The dorsal medial area (g) appears to have an azimuthal axis representing the azimuthal location of a target. The dotted line indicates the boundary of the primary auditory cortex. (**B**) Schematized sonogram of a biosonar pulse and its echo. There are four harmonics (H_1 through H_4), each consisting of CF and FM components (CF_{1-4} and FM_{1-4}); H_2 is always the most intense. The echo is higher in frequency than the pulse as a result of the Doppler shift (DS). There is a delay between the onset of the pulse and the onset of the echo due to the distance between the bat and its target.

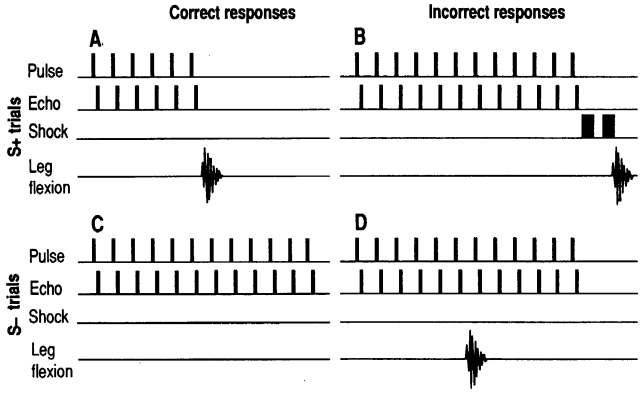

Fig. 2. Schematic of the discrimination paradigm used. The number of P-E pairs within the 1.2-s stimulus trains is 12 in the frequency discrimination task and 24 in the temporal discrimination task. A train of electric shocks is programmed to follow the train of P-E pairs on S+ trials but not on S− trials. A leg flexion by the bat during the S+ turns off the S+ and avoids the shock (**A**). A failure to make a leg flexion during the S+ results in shock delivery, but a subsequent leg flexion enables the animal to escape further shock (**B**). On S− trials, whether the bat withholds its leg flexion (**C**) or makes a leg flexion (**D**), the S− is presented in its entirety, and no shock follows. A correct response consists of either making a leg flexion during the S+ (A) or withholding a leg flexion during the S− (C).

Table 2. Percentage of test blocks within each time window in which discrimination performance was significantly worse than baseline for the temporal discrimination experiments. There were 20 trials per block. The data in this table are based on one bat tested four times and one bat tested twice.

Discrimination level	Δt (ms)	Time window (hours)			
		0.2–2.2	2.2–4.2	7.5–9.5	24–26
Fine	1	100	75	0	0
	2	100	75	0	0
Intermediate	4	75	0	0	0
	6	100	0	0	0
	16	100	0	0	0
Coarse	36	0	0	0	0

these parameters mapped in the DSCF and FM-FM areas. The deficits cannot be attributed to a general confounding effect such as a decrease in sensitivity to shock but must have been caused by the specific inactivations. Our data also indicate that the inactivations were focal. The muscimol applied near the center of the FM-FM area did not spread significantly into the DSCF area, or vice versa (the distance from the center of each area to the border of the other area was only 1 mm). Consistent with this finding, applications of larger amounts of muscimol (1.5 μg) to portions of the monkey somatosensory cortex less than 2 mm apart produced readily distinguishable behavioral effects lasting more than 5 but less than 20 hours (*12*).

Neural response properties necessary for the computation of E frequency and delay are largely created in subcortical nuclei. These nuclei project to the DSCF and FM-FM areas where E frequency and delay are systematically represented (*7–10*). Combined with the present results, this suggests that the DSCF and FM-FM areas are the sites at which the perception of these target properties is mediated.

Earlier AC ablation experiments often have failed to show a deficit in frequency discrimination despite the use of much larger ablations, usually including all of the primary, as well as most or all of the surrounding, AC (*17–19*). We suggest four explanations for this discrepancy. (i) Earlier studies focused on whether animals with permanent ablations could relearn frequency discriminations. An immediate test more directly addresses whether a cortical area is normally involved in a particular function, whereas a relearning test more directly addresses whether the area is indispensable for that function (*20*). (ii) Many of the earlier studies used coarse discriminations. Our results also suggest that coarse frequency discriminations may not require the AC. (iii) The stimuli used in the earlier studies had no direct biological significance to the animals tested, whereas those we used are information-bearing elements for mustached bats. (iv) The species differed.

Much is known about the biosonar behavior of the mustached bat (*21*) and about the cortical computational maps that encode particular types of biosonar information, such as a target's relative size and range and the bat's relative velocity (*1*). The present selective inactivation studies forge a crucial link between these maps and the biosonar behavior.

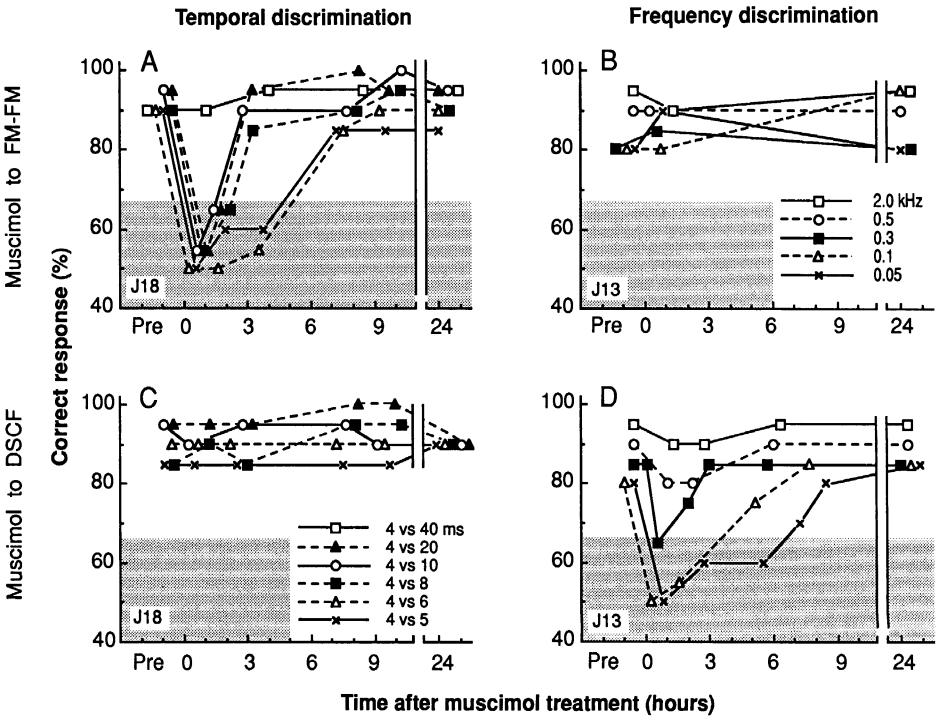

Fig. 3. Percentage of correct responses in each 20-trial block as a function of the number of hours before (baseline) or after muscimol application. Four bats were tested on the frequency discrimination task: three bats were tested twice each, and one bat a single time. Two bats were tested on the temporal discrimination task: one bat was tested four times, and the second bat two times. All the bats on all the replications showed the same basic patterns displayed here. The shaded areas indicate percentages that do not significantly ($P > 0.05$) differ from chance. (**A** and **B**) Muscimol application to the FM-FM area produces a deficit in temporal discrimination performance that is related to the magnitude of the Δt (A) but has no effect on frequency discrimination performance (B). (**C** and **D**) Muscimol application to the DSCF area has no effect on temporal discrimination performance (C) but produces a temporary deficit in frequency discrimination performance that is related to the magnitude of the Δf (D).

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14. The bat was encased in a body mold with its head and legs protruding. A sling was looped around the bat's right leg just above the ankle and was attached to a strain gauge. Electric shocks were delivered to the bat's right thigh through two silver plates located above and below the thigh. The voltage was adjusted to the minimum level needed to elicit a consistent leg flexion. Conditioning sessions consisted of one 20-trial block (10 S+ and 10 S− trials, randomly ordered) at each frequency or delay. Trials were 30 s apart. In the frequency discrimination tests, each stimulus train consisted of 12 pairs of PCF_2-ECF_2 delivered at 80-dB sound pressure level (SPL). The PCF_2 and the ECF_2 each were 20 ms long, with 0.5-ms rise and fall times, and were separated by a 10-ms silent period. The PCF_2 was 500 Hz above the bat's resting frequency, which was measured at the start of training from a sample of 100 pulses. In the temporal discrimination tests, each stimulus train consisted of 24 pairs of PFM_1-EFM_2. We used EFM_2 rather than EFM_3 or EFM_4 because (i) H_2 (Fig. 1B) is the most intense, and (ii) the population of FM-FM neurons responding to PFM_1-EFM_2 is the largest. Both the PFM_1 and the EFM_2 were 3.5 ms long, with 0.5-ms rise and fall times, and were repeated at 20 pairs per second. The PFM_1 linearly swept from 31 to 21 kHz, and the EFM_2 linearly swept from 62 to 46 kHz. The PFM_1 and the EFM_2 were delivered at 80 and 75 dB SPL, respectively. For the 36-ms Δt, the presentation rate was decreased to 10 pairs per second.
15. Two to four weeks before the first muscimol application, the bat was given an intramuscular injection of a neuroleptic analgesic, Innovar-Vet [0.08 mg of Fentanyl per kilogram of body weight and Droperidol (4 mg/kg)], the dorsal surface of its skull was exposed, holes 200 by 200 μm^2 were made over the DSCF or FM-FM area, and a small well was placed over each hole.
16. Statistical analyses were based on the proportion of correct responses within selected time windows. These windows were chosen post hoc with the constraints that within a given experiment (i) all time windows were the same width and (ii) the same windows were used for every bat. Within each time window there was at least one 20-trial block at each Δf or Δt on each test day. We compared (i) baseline performance to performance after muscimol treatment (Tables 1 and 2); (ii) performance during each time window relative to chance (50%) (Fig. 3); and (iii) performance among the different Δf or Δt test blocks within each time window (not shown). There were no inconsistencies among these analyses.
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ACh Receptor–Rich Membrane Domains Organized in Fibroblasts by Recombinant 43-Kildalton Protein

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Neurotransmitter receptors are generally clustered in the postsynaptic membrane. The mechanism of clustering was analyzed with fibroblast cell lines that were stably transfected with the four subunits for fetal (α, β, γ, δ) or adult (α, β, ε, δ) type mouse muscle nicotinic acetylcholine receptors (AChRs). Immunofluorescent staining indicated that AChRs were dispersed on the surface of these cells. When transiently transfected with an expression construct encoding a 43-kilodalton protein that is normally concentrated under the postsynaptic membrane, AChRs expressed in these cells became aggregated in large cell-surface clusters, colocalized with the 43-kilodalton protein. This suggests that 43-kilodalton protein can induce AChR clustering and that cluster induction involves direct contact between AChR and 43-kilodalton protein.

A CRUCIAL STEP IN SYNAPSE FORMATION is the aggregation of neurotransmitter receptors in the postsynaptic membrane. In mammalian muscle and electric organ of the marine ray Torpedo, for example, the postsynaptic membranes contain about 10^4 AChR molecules per square micrometer (*1*), whereas nonsynaptic portions of the membrane are virtually devoid of AChRs. Although the precise colocalization of pre- and postsynaptic specializations at a synapse clearly involves interactions between nerve and muscle, muscle fibers are capable of aggregating AChRs even in the absence of nerves (*2*). A synapse-associated, intracellular, 43-kD peripheral membrane protein isolated from AChR-rich membranes of Torpedo electroplaque (*3*) has been indirectly implicated in this latter process. Exposure of electroplaque membranes to pH 11 releases this 43-kD protein and other peripheral proteins from the membrane and also causes an increase in lateral (*4*) and rotational (*5*) mobility of AChR molecules. The 43-kD protein is concentrated in AChR-rich membranes where it is present in equimolar amounts with AChR (*6*). Finally, chemical cross-linking experiments reveal a close association between 43-kD protein and the β-subunit of the AChR (*7*).

The availability of cDNA clones for the 43-kD protein (*8, 9*) permitted us to study directly its role in AChR cluster formation by coexpression with cDNAs for AChR subunits in fibroblast cell lines. Because the development of the neuromuscular junction is accompanied by a transition from fetal (α, β, γ, δ) to adult (α, β, ε, δ) AChR (*10, 11*), and by major changes in the morphology of the junctional AChR clusters (*12*), we determined the effects of 43-kD protein on both receptor types. The quail fibroblast line, QT-6 (*13*), which expresses no detectable endogenous AChR (*14*), was transfected with expression constructs for each of the four subunit cDNAs of the fetal or adult muscle AChR (*15*). Fibroblast clones selected on the basis of the cotransfected neomycin resistance gene were screened for cell-surface α-bungarotoxin binding sites and clones expressing fetal AChR (Q-F18) and adult AChR (Q-A33) were isolated. The functional integrity of AChR in the clones was determined by voltage-clamp studies on isolated membrane patches (*16*). Membrane patches excised from transfected cells showed currents elicited by ACh that were not blocked by 100 nM atropine (Fig. 1). The channels expressed in clone Q-F18 (*15*) showed a slope conductance of about 40 pS for inward current and a burst duration of about 5 to 10 ms. Q-A33 exhibited channels of larger conductance and briefer burst duration. Similar properties have been reported for fetal- and adult-type bovine AChRs

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