Brown, M. C, S. G. Kujawa, and M. C. Liberman. Single olivocochlear neurons in the guinea pig. II. Response plasticity due to noise conditioning.

J. Neurophysiol. 79: 3088–3097, 1998. Previous studies have shown that daily, moderate-level sound exposure, or conditioning, can reduce injury from a subsequent high-level noise exposure. We tested the hypothesis that this conditioning produces an increased activity in the olivocochlear efferent reflex, a reflex known to provide protection to the cochlea. Guinea pigs were conditioned by a 10-day intermittent exposure to 2–4 kHz noise at 85 dB sound pressure level. This conditioning is known to reduce damage from a subsequent high-level exposure to the same noise band. Responses to monaural and binaural sound were recorded from single medial olivocochlear (MOC) efferent neurons, and data from conditioned animals were compared with those obtained from unexposed controls. MOC neurons were classified by their response to noise bursts in the ipsilateral or contralateral ears as ipsi units, contra units, or either-ear units. There were no significant differences in the distributions of these unit types between control and conditioned animals. There were also no differences in other responses to monaural stimuli, including the distribution of characteristic frequencies (CFs), the sharpness of tuning, or thresholds at the CF. For binaural sound at high levels, particularly relevant to sound-evoked activation of the MOC reflex during acoustic overstimulation, the firing rates of MOC neurons with CFs just above the conditioning band showed slight (but statistically significant) elevations relative to control animals. Frequency regions just above the conditioning band also demonstrated maximum conditioning-related protection; thus protection could be due, in part, to long-term changes in MOC discharge rates. For binaural sound at low levels, MOC firing rates in conditioned animals also were increased significantly relative to controls. Again, increases were largest for neurons with CFs just above the conditioning band. For equivalent monaural sound, rates were not significantly increased; thus, conditioning appears to increase binaural facilitation by opposite-ear sound. These data indicate that MOC neurons show long-term plasticity in acoustic responsiveness that is dependent on their acoustic history.

INTRODUCTION

The ear can be protected from acoustic overstimulation by previous exposure to moderate-level sound during a period of days. This protection has been demonstrated via two very different paradigms. In one, the “conditioning” paradigm, animals are exposed to a conditioning noise of moderate level that is presented daily for many days, followed by a high-level traumatic stimulus (Campo et al. 1991; Canlon 1996; Canlon et al. 1985; Kujawa and Liberman 1996; Ryan et al. 1994; reviewed by Canlon 1996). Animals in this condition/trauma group can show up to a 30 dB less permanent threshold shift (PTS) than unconditioned (trauma only) controls. In the second paradigm, “toughening,” animals are exposed daily for a number of hours to a mildly traumatic stimulus. Protection is observed as a progressive reduction in the temporary threshold shifts (TTSs) seen at the termination of each of the daily exposures (Clark et al. 1987; Miller et al. 1963; Subramaniam et al. 1991; reviewed by Henderson et al. 1996; Subramaniam et al. 1996).

Conditioning- and toughening-related protection are of interest because noise-induced hearing loss is common in humans and can be related to daily exposure in the workplace (Sataloff and Sataloff 1993; Schuknacht 1993). Although the biological mechanisms underlying protection are poorly understood, two obvious candidates are the major feedback pathways to the auditory periphery: the middle-ear muscle reflex and the olivocochlear efferent reflex. The middle-ear muscles are activated by loud sounds and can, under certain conditions of stimulation, protect the ear from acoustic overstimulation (Zakrisson et al. 1980). However, studies have shown that elimination of this reflex (by cutting the muscles or by muscle paralysis) does not alter protection afforded by conditioning and toughening paradigms (Daglis and Canlon 1995; Henderson et al. 1994; Ryan et al. 1994). Thus an increase in effectiveness of the middle-ear muscle reflex can be ruled out as the primary mechanism underlying protection.

A second system that can protect the inner ear against acoustic overstimulation is the olivocochlear (OC) efferent system, a feedback system that innervates hair cells and neurons in the cochlea (reviewed by Warr 1992). The protective role of the OC system has been investigated most thoroughly in anesthetized animals in acute experiments (brief overexposures of several minutes duration without any prior noise conditioning). A number of investigators have shown that ears in which the OC system is activated either electrically or acoustically (by addition of contralateral sound) show significantly smaller TTSs in the minutes immediately after the exposure (Cody and Johnstone 1982; Rajan 1995a; Rajan and Johnstone 1988a,b; Reiter and Liberman 1995). The magnitude of this OC-mediated protection can be as great as 25 dB. Such acute protection is eliminated by sectioning the OC bundle (OCB) or by drugs, such as strychnine, that are known to block OC effects on the cochlea (Rajan 1995b; Rajan and Johnstone 1988a; Reiter and Libe-
Current evidence suggests that this protection is mediated by the medial (M) subgroup of OC neurons, which synapse on cochlear outer hair cells. These hair cells are particularly vulnerable to acoustic injury (Johnsson and Hawkins 1976; Liberman and Beil 1979; Robertson 1981).

The role of the OCB in reducing chronic effects of acoustic overstimulation has been less well investigated, but two studies (Handrock and Zeisberg 1982; Zheng et al. 1997a) have reported that lesioning the OCB leads to larger PTSs in awake animals exposed to high-level sounds (without prior noise conditioning). In addition, two recent studies have explicitly attempted to determine if the OC system plays a role in conditioning- and toughening-related protection. One of these studies used the toughening paradigm and compared the TTSs measured in control versus chronically de-efferented animals (Zheng et al. 1997b). In efferent-intact (control) animals, TTSs were reduced from the 1st exposure day to the 10th exposure day at four test frequencies, i.e., protection was evident. In the three successfully de-efferented animals, TTS reductions were eliminated at three test frequencies but remained at a fourth test frequency, making it difficult to draw a firm conclusion regarding the role of the OC system in the toughening phenomenon. In our laboratory, a study was designed to explore the OC role in conditioning-related protection (Kujawa and Liberman 1997). In agreement with earlier studies, OCB sectioning significantly increased vulnerability to acoustic injury; i.e., for all noise-exposure groups, de-efferented animals showed larger PTSs than their efferent-intact cohorts. However, among the surgically de-efferented groups, the condition/trauma animals showed significantly greater PTSs than their trauma-only cohorts: i.e., the same conditioning protocol that reduced PTS in normal animals, further increased PTS in de-efferented animals. It is difficult to tell whether this effect arises because the OCB is mediating conditioning-related protection or simply because an intact OCB provides a large, generalized protective effect against acoustic injury.

Thus it remains an interesting hypothesis that conditioning-related protection is mediated via the OC reflex either because the OC neurons have become more active or because their peripheral effects are amplified due to conditioning-induced changes in the cochlea. To test the hypothesis that OC neurons have become more active, we measured basic response properties of MOC neurons (e.g., discharge-rate-level functions for monaural and binaural sounds, spontaneous rates, and tuning properties) in both conditioned animals and in untreated control animals. We especially were interested in MOC discharge rates for binaural noise at a high level, because this is the relevant evoking stimulus during acoustic overexposure and because binaural noise has been demonstrated to evoke high firing rates in MOC neurons in control guinea pigs (Brown et al. 1998). Our conditioning paradigm is patterned after that of Campo et al. (1991). The noise band (2–4 kHz) contained somewhat higher frequencies than in most previous studies because we wanted to more effectively activate the MOC system, which projects mainly to the mid- and high-frequency regions of the cochlea (Guinan et al. 1984). Our conditioning stimulus level was moderate (85 dB SPL), and after conditioning the sensitivity of the cochlea is normal. Yet this conditioning paradigm affords protection against a subsequent exposure to high-level noise (Kujawa and Liberman 1996), and the magnitude of this protection is similar to that observed with other conditioning paradigms (Campo et al. 1991; Canlon et al. 1988). A preliminary version of the results has been presented (Kujawa et al. 1996).

**Methods**

Albino guinea pigs of either sex were used as experimental animals. This study compares response properties of single MOC neurons in two groups of animals: control (n = 10) and conditioned (n = 10) guinea pigs. Animals in the two groups were matched for age and weight, which ranged from 322 to 542 g on the day of the electrophysiologically experiments. Control animals were a subset of the database used in the companion paper (Brown et al. 1998), but some animals from that study were not included here because they were anesthetized differently or not properly age-matched with the conditioned group. Conditioned guinea pigs were exposed, while awake and unrestrained, to an octave-band noise (2–4 kHz band, 85 dB SPL, 6 h on/18 h off) for 10 consecutive days. The noise was delivered in the free-field to the animals in suspended cages in a small reverberant sound-exposure box. The acoustic stimulus was generated by a white-noise generator, filtered by a Brickwall Filter, amplified by a Crown power amplifier, and delivered using a JBL compression driver through an exponential horn fitted securely to the top of the box. Noise level was calibrated daily by positioning a ¼-in. Bruel and Kjaer condenser microphone at the approximate position of the animal’s head and did not vary by more than 1 dB anywhere within the cage. After the 10-day exposure, a 5-day rest period preceded the acute physiological recordings of single neurons: in our parallel studies of conditioning-mediated protection (Kujawa and Liberman 1997), this is the day on which the traumatic exposure would have been presented.

The methods for surgery and recordings are described in the companion paper (Brown et al. 1998). All surgical procedures were in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. For physiological recordings, guinea pigs were anesthetized with a pentobarbital sodium (Nembutal)/fentanyl/droperidol combination. Single-fiber recordings were made from the spiral ganglion in the basal turn. Responses measured from MOC neurons were 1) rate-level functions for monaural noise in the main ear (noise bursts, usually run from 105 to 15 dB SPL, in 10-dB steps with 10 bursts per level), 2) rate-level functions for binaural noise (main-ear noise-bursts plus simultaneous, continuous, opposite-ear noise at 85 dB SPL), 3) tuning curves, and 4) spontaneous firing rate (10-s sampling period). Firing rates are specified as total rate without subtraction of any spontaneous rate. Noise to the main ear always was presented as bursts. Noise to the opposite ear, which was used as a facilitator, always was presented continuously, at 85 dB SPL, and was presented simultaneously with the main-ear stimulus. Techniques for measurement of noise levels also are described in the companion paper (Brown et al. 1998).

Statistical tests [2-way analyses of variance (ANOVA) or t-tests] were used to compare differences between control and conditioned data. Differences were considered significant at the 5% level of significance (P < 0.05). Sample sizes for the tests are contained in the figures (Figs. 2, 3, and 5A) or in the figure legends (Figs. 4, 5C, and 6).

**Results**

**CAP thresholds**

Thresholds for the compound action potential (CAP) of the auditory nerve were measured in control and conditioned
animals (Fig. 1). Consistent with an earlier study (Kujawa and Liberman 1996), the conditioning protocol did not decrease cochlear sensitivity as measured by the CAP. In fact, conditioned animals showed small increases in sensitivity, especially for test frequencies within the exposure band used for conditioning (2–4 kHz), as previously reported.

Proportions of units in each response class

A total of 273 MOC neurons were recorded (Table 1). Of these, 132 were from control animals and 141 were from conditioned animals. We classified MOC neurons according to their response to monaural noise bursts (65 dB SPL) presented either to the ipsilateral or contralateral ear. Three unit classes have been defined (Brown et al. 1998; Liberman and Brown 1986; Robertson and Gummer 1985): *ipsi units* respond to monaural ipsilateral sound but not to contralateral sound, *contra units* respond to monaural contralateral sound but not to ipsilateral sound, and *either-ear units* respond to either contralateral or ipsilateral sound. The proportions of units in each class in control and conditioned animals (Table 1) are similar to those reported in previous studies (Brown 1989; Liberman and Brown 1986). In conditioned animals, there was a small increase in the percentage of either-ear units. However, none of the differences in the response-class proportions was statistically significant ($X^2$ test, $X^2 = 3.09$, $P_{3.09} = 0.34$).

Sound-evoked firing rates in MOC neurons

To test the hypothesis that conditioning increases the activity of MOC neurons, we measured the sound-evoked firing rates of MOC neurons in conditioned animals and compared them to sound-evoked firing rates in control animals. Our acoustic stimuli included high-level noise because it is under this condition that the OC system would be required to exert a protective influence. The highest level tested was 105 dB SPL, presented to the “main” ear (for *ipsi* units, the main ear was the ipsilateral ear; for *contra* units, the main ear was the contralateral ear; and for *either-ear* units, the main ear was the ear to which the unit responded at the lower SPL). Figure 2 shows MOC firing rates plotted against CF for all recorded neurons in response to 105 dB SPL noise: for a monaural stimulus consisting of monaural noise bursts (Fig. 2A) and for a binaural stimulus (Fig. 2B) consisting of the monaural noise bursts plus continuous noise at 85 dB SPL in the opposite ear. This opposite-ear noise is a particularly effective facilitator of the main-ear response (Brown et al. 1998). That is, although *ipsi* and *contra* units do not respond to monaural sound in the opposite ear, they show binaural facilitation: i.e., an increase in the response to main-ear noise when opposite-ear noise is added (Liberman 1988). Figure 2 shows that units from conditioned and control animals had very similar CF distributions; in both groups, the CF sample extended from ~0.5 to 24 kHz.

Figure 2 also indicates that discharge rates from control and conditioned animals are largely overlapping both for monaural and binaural noise. To compare the differences with stimulus condition, CF, and exposure history, the rate data were combined into octave CF bands (as delimited on the lowest axis of Fig. 2C), and data within each band were averaged. Average data were plotted in Fig. 2 from control animals (—) and from conditioned animals (——). These data indicate that rates increase with CF. Averages differ little in control versus conditioned animals for monaural stimuli; however, for binaural stimuli somewhat higher rates were seen in conditioned animals in the midfrequency bands (3–6 and 6–12 kHz). Two-way ANOVA tests (1 for monaural and 1 for binaural stimuli) were performed to test the effects of CF and conditioning on rate. These tests reveal a significant effect of CF on firing rates ($P = 0.000$ for both monaural and binaural noise). The effect of conditioning alone was not significant ($P > 0.05$); however, for the binaural noise response, there was a significant interaction of CF and group ($P = 0.036$). An interpretation of this finding is that the higher rates seen in the mid-CF bands in conditioned animals are contributing to a different CF dependence on rate in these animals. Posthoc comparisons using Sheffe’s tests on the binaural rates failed to pinpoint the particular frequency bands that contributed to this effect, consistent with small control/conditioned differences. However, multiple $t$-tests that included experiment-wise correction to minimize type I error (Steel 1961) showed significantly greater rates in the conditioned animals for the 3- to 6-kHz band ($P = 0.02$) and the 6- to 12-kHz band ($P = 0.009$).

<table>
<thead>
<tr>
<th>Response Class</th>
<th>Control</th>
<th>Conditioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsi units, %</td>
<td>64</td>
<td>66</td>
</tr>
<tr>
<td>Contra units, %</td>
<td>28</td>
<td>21</td>
</tr>
<tr>
<td>Either-ear units, %</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

$n = 132$ units for control animals; $n = 141$ units for conditioned animals. MOC, medial olivocochlear.
SOUND CONDITIONING AND OLIVOCOCHLEAR NEURONS

The lowest CF band was not included in the analysis because of the paucity of units there. Two-way ANOVA tests (1 for control animals and 1 for conditioned animals) also were used to test the effect of CF and the effect of stimulus type (monaural vs. binaural). The effect of stimulus type was highly significant for both control and conditioned data ($P = 0.000$ for each), consistent with a significant facilitatory effect of the opposite-ear stimulus. For each neuron, the difference between the discharge rate to the binaural and the monaural stimuli (Fig. 2C) is a measure of the degree of binaural facilitation. Facilitation is small for main-ear stimuli at this high level (105 dB SPL) (Brown et al. 1998). Nevertheless, on average, binaural facilitation is somewhat larger in conditioned animals than in control animals, indicating an increased effectiveness of the opposite ear in conditioned animals. Overall, these results suggest that for binaural sound at high levels, neurons in mid-CF bands showed somewhat higher discharge rates in conditioned animals.

To pursue the possibility that differences between the control and conditioned animals might be level-dependent, we examined average firing rate as a function of level for units from control and conditioned animals. In Fig. 3, the resultant averaged rate-versus-level functions to the monaural (row A) and binaural (row B) noise stimuli are plotted along with a measure of binaural facilitation (row C). In our paradigm, the binaural stimuli consist of a constant opposite-ear noise at a high level (85 dB) and a main-ear noise that is varied in level. Most of these averaged rate-level functions show little evidence of saturation, with wide dynamic ranges often in excess of 80 dB. This behavior reflects, in part, the fact that individual rate-level functions of MOC neurons have wide dynamic ranges and, in part, a kind of recruitment: different neurons have different thresholds and some high-threshold neurons begin to respond as some low-threshold neurons begin to saturate.

We first describe data that are pooled across all CFs (Fig. 3, left-most column). Here, discharge rates to monaural noise are slightly increased in conditioned animals, and rates to binaural noise are more significantly elevated, especially at low stimulus levels. Two-way ANOVA tests (1 for monaural and 1 for binaural stimuli) were performed to test the effect of level and the effect of conditioning on rate (sample sizes are given in Fig. 3). As expected, the effect of level on firing rates was significant ($P = 0.000$ for both monaural and binaural stimuli). The influence of conditioning was significant (Fig. 3, *) for the response to binaural noise ($P = 0.047$) but not monaural noise. For the monaural noise, however, a significant interaction was revealed between level and group ($P = 0.045$), suggesting that conditioning results in a different dependence of rate on level.

We also divided the pooled level functions (Fig. 3) into the five CF bands used previously in Fig. 2. These functions show many of the features described for the pooled functions; however, differences between control and conditioned animals are less robust because of the smaller sample sizes. For the five frequency bands and the monaural and binaural stimulations, differences were significant for four panels in Fig. 3: the 1.5- to 3-kHz CF band for monaural stimuli (demonstrating a control/conditioned difference at $P = 0.006$), the 3- to 6-kHz band for monaural stimuli...
FIG. 3. Average firing rate and facilitation (±SE), as a function of main-ear noise-burst level, for MOC neurons, grouped according to CF as indicated at the top of each column. A: monaural noise bursts to main ear. B: binaural noise (main ear noise bursts plus opposite-ear, continuous noise at 85 dB SPL). * Significant effect of conditioning or an interaction of level and conditioning (P < 0.05, according to a 2-way ANOVA). C: average facilitation from opposite-ear noise, the difference in firing rate between the binaural and monaural responses. Numbers of neurons are given in each panel: control sample size above conditioned. First column includes all MOC neurons from which level series were obtained (including 165 of known CF and 84 of unknown CF).

(demonstrating a differing interaction of control/conditioned groups with sound levels at P = 0.001), and the 6- to 12-kHz CF band for monaural and binaural stimuli (interaction at P = 0.001, and control/conditioned difference at P = 0.022). For all of the differences except the first, the conditioned rates are clearly higher than the control rates. Binaural facilitation (Fig. 3, row C) is usually larger in conditioned than in control animals, especially at low sound levels. Again, these results indicate an increased effectiveness of the opposite ear in facilitating rates in MOC neurons and show that this increase is most obvious at low sound levels.

We confirmed that both ipsi and contra units from conditioned animals showed higher discharge rates to binaural stimuli. In data pooled across CF, both ipsi and contra units showed higher firing to binaural noise and increased facilitation in conditioned animals. The facilitation for ipsi and contra units is plotted in Fig. 4 as a function of level. Ipsi and contra units both had increased facilitation in conditioned animals, especially at low main-ear levels. Two-way ANOVA tests were used to test the effect of level and the effect of conditioning (see Fig. 4 legend for sample sizes). There were statistically significant control/conditioned differences both for ipsi units (P = 0.026) and contra units (P = 0.045). Contra units had greater facilitation than ipsi units in both control and conditioned animals. In Fig. 4, facilitation is defined as an absolute increase in firing rate. If facilitation is calculated as a percentage increase in firing rate, the percent increase for control animals (averaged across unit classes) is 71% at 25 dB, 48%

FIG. 4. Average facilitation (±SE) vs. main-ear noise level for ipsi units (A) and contra units (B). As denoted by the asterisks, both ipsi and contra units showed statistically significant increases in rate in conditioned animals (2-way ANOVA tests). Sample size for control animals was 47 ipsi and 27 contra units and for conditioned animals was 33 ipsi and 13 contra units.
at 65 dB, and 13% at 105 dB SPL. In conditioned animals, the percent increases are 162, 53, and 18%. Thus facilitation is relatively more important at low main-ear levels, and furthermore the increase in conditioned animals is most obvious at low main-ear levels.

Tuning-curve measurements and spontaneous rates

Tuning curves of MOC neurons were measured using monaural stimuli presented to the neuron’s main ear. Tuning-curve thresholds at CF ranged from 0 to 90 dB and were widely scattered even within one CF band (Fig. 5A). There was complete overlap in the thresholds for control and conditioned animals. When the thresholds were combined into octave bands and averaged (Fig. 5B), thresholds of control and conditioned animals were not significantly different ($P > 0.05$ by 2-way ANOVA). This lack of threshold difference is consistent with the finding that responses to low-level monaural sound were similar across groups (Fig. 3A).

MOC neurons are highly frequency selective with tuning curves that, in guinea pigs especially, are comparable in sharpness with those of auditory-nerve afferents (Brown 1989; Liberman and Brown 1986; Robertson and Gummer 1985). A measure of tuning sharpness is the $Q_{10}$ (the CF of the tuning curve divided by the tuning-curve bandwidth at 10 dB above threshold). In both groups of animals, $Q_{10}$ of MOC neurons increases with increasing CF (Fig. 5C). Control/conditioned differences were seen mainly for low CFs; however, these differences were not statistically significant ($P > 0.05$).

MOC neurons discharge spontaneously at low rates (<20 spikes/s) in the absence of applied sounds. Average spontaneous rates (SRs) (Fig. 6) were decreased by conditioning in two CF bands: 1.5–3 kHz and 3–6 kHz. Note that these frequency bands span the bandwidth of the conditioning noise. These decreases, however, were not statistically significant ($P > 0.05$).

Responses of afferent neurons in control and conditioned animals

Although our primary focus was to record from MOC efferent neurons, some recordings also were obtained from afferent fibers of the auditory nerve as identified by irregular interspike intervals and short latency responses to tone and noise bursts (Brown 1989; Robertson 1984). Tuning curves and spontaneous rates were obtained from 220 afferent fibers: 112 from control and 108 from conditioned animals. Given the basal location of the recording site in the cochlea, only a limited range of afferent CFs was sampled: roughly 12–21 kHz.

Tuning curves, thresholds, and spontaneous rates of afferent fibers were not significantly different in control versus conditioned animals. Afferent fibers have been classified into subgroups on the basis of their SR, because SR is systematically related to threshold sensitivity (Liberman 1978; Schmiedt 1989; Tsuji and Liberman 1997; Yates 1991). Control and conditioned animals had SR distributions that were almost identical, as shown in Table 2: low SR (<1 spike/s), medium SR (1 < SR < 20 spikes/s), and high SR (>20 spikes/s). Consistent with earlier studies, our sample of fibers showed threshold differences between the unit classes in both control and conditioned animals with no significant difference between control and conditioned animals in this regard.
**MOC firing rates and conditioning-related protection**

The major purpose of the present study was to assess whether the OC system contributes to conditioning-related protection. The hypothesis tested was that conditioning exposures increase the sound-evoked activity in the MOC system. Because sound-evoked MOC activity in nonconditioned animals appears to have significant protective effects, it was tempting to speculate that, in conditioned animals, sound-evoked activity in the MOC pathway might be elevated at the time of the acoustic overexposure, leading to the observed decreases in PTS. In this context, the most relevant data from the present study are the MOC firing rates to the high-level binaural stimulus because high-level stimuli are used for overexposures. Figure 7A replots the average firing rates to binaural noise (main ear: 105 dB SPL noise bursts, opposite ear: 85 dB noise) from Fig. 2B. There were significant increases in firing rates to this high-level stimulus for the two CF bands spanning the range from 3 to 12 kHz. We know from intracellular labeling studies (Brown 1989; Liberman and Brown 1986; Robertson and Guummer 1985) that there is rough agreement between the tonotopic maps of afferent and efferent fibers; thus the MOC neurons with increased firing (3- to 12-kHz CFs) send the majority of their peripheral terminals to the 3- to 12-kHz cochlear region. Conversely, MOC neurons without increased firing send their terminals to other cochlear regions.

We now compare the frequency dependence of MOC firing increases after conditioning to the frequency dependence of protection after conditioning. As shown elsewhere (Kujawa and Liberman 1996), our conditioning protocol produces substantial levels of protection against acoustic injury, and the amount of this protection depends on frequency and thus cochlear location. A measure of protection, the difference in PTS between condition/trauma and trauma only animals, is plotted versus frequency in Fig. 7C. The postexposure threshold shifts used to compute protection are shown in Fig. 7B. Although none of the animals in the present study were exposed to the traumatic stimulus, the day of the physiological tests was the same as the day on which the exposure would have taken place. Protection extends throughout all frequencies tested but is largest in the 6.5 kHz region where it has an average magnitude of 20 dB (Fig. 7C). This amount of protection is generally equivalent to the amount of protection seen in earlier studies (Campo et al. 1991; Canlon et al. 1988). The frequency dependence of the protection is likely to depend on the spectrum of the noise used for the overexposure as well as that used for the conditioning (Subramaniam et al. 1991, 1993). For the 2- to 4-kHz noise band we used for both conditioning and overexposure (Kujawa and Liberman 1996), PTS is maximal in trauma only animals between ~2.5 and 7 kHz. This frequency range corresponds well to the frequency range over which maximum conditioning-related protection is seen. Thus maximum PTS and maximum protection are both found above the exposure band, consistent with a previous report (Subramaniam et al. 1996). We now show that this frequency range corresponds fairly well to the frequency range over which maximum conditioning-related protection is seen. On the other hand, there was no significant change in firing rates for the CF group at the lower side of the exposure band (CFs 1.5–3 kHz) and for the highest CF group (CFs > 12 kHz). Yet there is some conditioning-related protection for these cochlear regions. These observations suggest that, although increases in sound-evoked MOC firing rates may contribute to conditioning-related protection, they probably cannot explain the entire effect. A similar conclusion can be drawn from a recent study of the effects of chronic de-efferentation on the protection seen in a toughening paradigm: the daily reduction in TTS seen in control animals at four test frequencies persisted at one test frequency in the de-efferented group (Zheng et al. 1997b).

The amount of protection obtained by the observed increases in MOC discharge is difficult to predict from existing literature. When MOC discharge is evoked by shocks in control animals, a doubling of shock rate (from 50 to 100 shocks/s) increases protection by only ~5 dB for overexposures at high frequencies (10 kHz) where the MOC system should exert large protective effects (Rajan 1995a; Rajan and Johnstone 1988a; Reiter and Liberman 1995). The sound-evoked rate increases we observed were much less than a doubling, and the protective effects were significantly larger.

**TABLE 2. Data for afferent units in spontaneous-rate classes**

<table>
<thead>
<tr>
<th>Unit Percentages</th>
<th>SR Class</th>
<th>n*</th>
<th>Low-SR units</th>
<th>Medium-SR units</th>
<th>High-SR units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, %</td>
<td></td>
<td>112</td>
<td>11</td>
<td>24</td>
<td>65</td>
</tr>
<tr>
<td>Conditioned, %</td>
<td></td>
<td>108</td>
<td>13</td>
<td>24</td>
<td>63</td>
</tr>
<tr>
<td>Unit Thresholds†</td>
<td></td>
<td></td>
<td>35.8 ± 1.9</td>
<td>30.6 ± 2.6</td>
<td>23.8 ± 1.5</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>40.4 ± 3.3</td>
<td>30.4 ± 1.8</td>
<td>28 ± 1.3</td>
</tr>
</tbody>
</table>

SR, spontaneous rate. * Number of total units. † Values are means ± SE of the thresholds in dB SPL.
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than 5 dB. Taken in isolation, this result suggests that only a small amount of the observed protection is mediated by the observed increase in MOC discharge rate. However, the electric stimulation data of Rajan also showed that the decibel reduction in TTS caused by a particular shock rate increased as the overall magnitude of the TTS increased (with increasing exposure level). In the trauma paradigm on which the present study is modeled, the TTSs are almost certainly much larger than those in Rajan’s study. Additional effects of interstudy differences are also unknown: the proportion of MOC neurons actually activated by electrical stimulation may be different from the proportion activated by acoustic stimulation, and the highly synchronized ensemble firing pattern evoked by electric shocks may have different effects than the more randomized pattern evoked by acoustic stimulation. Finally, we also do not know if MOC firing rates in awake animals (used for overexposures in some experiments) are significantly higher than in anesthetized preparations (used for Rajan’s experiments and for our MOC recordings).

Even if the MOC rates are only somewhat elevated by conditioning, it is possible that conditioning changes aspects of peripheral response to the MOC action such that the protective effects of a given MOC discharge rate are amplified. Recent studies suggest that OC reduction of temporary threshold shifts are mediated by a “slow” effect of the interaction between acetylcholine (the MOC transmitter) and the MOC synapse on the outer hair cell (OHC). This effect ultimately causes an increase in intracellular calcium concentration in OHCs, possibly via a calcium-induced calcium release process within the profuse system of the OHC subsurface cisternae (Reiter and Liberman 1995; Sridhar et al. 1995, 1997). In normal (nonconditioned) animals, this slow effect is largest in basal cochlear regions. It is possible that conditioning amplifies this putative calcium release process or induces its upregulation in more apical cochlear regions resulting in a greater extent of protection.

Acoustic history and plasticity in the MOC reflex

Present results clearly show significant changes in the responsiveness of the MOC reflex according to sound-exposure history. In addition to small changes in discharge rates at high sound levels, conditioning also increased MOC discharge rates for binaural stimuli at low levels of the main ear stimulus (Figs. 3B and 4), usually without increases in the responses to monaural stimuli (Fig. 3A). This increase in binaural facilitation was present mainly for neurons with CFs above the conditioning band (>3 kHz, Fig. 3C). In the present study, we also saw a small, albeit insignificant (P > 0.05), increase in the number of binaurally responsive, i.e., either-ear, units (Table 1). Conceivably, these may rep-

![Diagram](http://jn.physiology.org/)

**FIG. 7.** Comparison of the frequency distribution of conditioning-related changes in MOC firing rates (**A**) to conditioning-related protective effects (**B and C**). **A:** from the present study, average firing rate for MOC neurons, averaged within CF bands as was plotted in Fig. 2B. Sound stimulus was binaural (main ear: 105 dB SPL noise bursts, opposite ear: 85 dB SPL noise). * Conditioned vs. control differences that are statistically significant at the P = 0.05 level of significance according to t-tests in each of the bands except the lowest, which was omitted because of the small number of units there (the multiple t-tests included experiment-wise correction to minimize type I error) (Steel 1961). Compared with control values, these conditioned rates were 21.2% higher (left asterisk) and 13.1% higher (right asterisk). **B** and **C:** data from another study (Kujawa and Liberman 1996) using the same conditioning protocol as in the present study. CAP threshold shifts are shown (**B**) for 2 groups of animals: animals conditioned and then overexposed to the octave band noise at 109 dB SPL for 4 h (condition/trauma) and animals overexposed to the high-level noise without prior conditioning (trauma only). Conditioning-related protection (**C**) is the difference between the trauma only and condition/trauma curves (downward arrows in **B**).
resent ipsi and contra units with opposite-ear excitatory inputs, which must exist given the normal phenomenon of binaural facilitation, that were amplified enough to classify them as either-ear units.

That responses of some MOC neurons are changed by sound conditioning demonstrates that some characteristics of these neurons are determined by acoustic history and therefore are “plastic.” This is the first such demonstration of long-term plasticity in MOC neurons. Plasticity has been demonstrated in auditory cortical neurons after peripheral hearing loss (Robertson and Irvine 1989; Willott et al. 1993) or in classical conditioning paradigms in which the acoustic stimulus is paired with a painful stimulus (Bakin and Weinberger 1990; Weinberger et al. 1993). Plasticity at lower levels has not been as well documented, although there are some indications of some brain stem plasticity after large hearing losses (Willott et al. 1991). Shortly after exposure to high-level noise, MOC neuron firing rates to main-ear stimuli are increased: a phenomenon called sensitization (Liberman 1988). However, this type of sensitization wanes within a few minutes. The changes seen in the present study were observed days after the end of the noise exposure and thus are much longer lasting; however, further study will be necessary to determine the exact time course of these changes. Another difference between sensitization and the long-term plasticity from conditioning is that sensitization produces large changes in response for monaural stimuli, whereas conditioning produces small changes in response for such stimuli. Such differences, along with the observations that both conditioning and toughening can be elicited in monaural animals (Campo et al. 1991; Subramaniam et al. 1991), indicate that the effects of acoustic history are complex and depend on time as well as the initial state of the animal.

Conceivably, changes anywhere in the MOC reflex pathway could produce the changes in MOC activity that were observed. The cochlea is likely to be affected in many ways by conditioning and toughening paradigms, especially those paradigms that create damage (Boettcher et al. 1992; Sinex et al. 1987). Our conditioning paradigm, however, uses moderate-level noise and does not compromise cochlear sensitivity. Thus damage to the cochlea is not likely to be the mechanism of changes in MOC activity. A mechanism that may contribute to changes in MOC activity, however, may be a conditioning-mediated increase in responsiveness of afferent neurons in the MOC reflex pathway. We have shown previously that both threshold and suprathreshold CAPs were enhanced in a frequency-sensitive manner by conditioning (Kujawa and Liberman 1996). Such changes in afferent sensitivity could not be verified at the single neuron level in the present study, most likely because our recording location in the spiral ganglion gave us access only to afferent neurons of high CF (>12 kHz). In that high-frequency region, our conditioning paradigm is not likely to produce changes in afferent responsiveness. Another mechanism for increased responsiveness might be central changes mediated via adrenergic or peptidergic pathways: many MOC neurons in tissue slices are depolarized by noradrenaline (Wang and Robertson 1997), and noradrenaline-containing fibers are known to project into the superior olivary complex (Wynne and Robertson 1996). MOC cell bodies also are contacted by terminals containing the neuromodulator, substance P (Adams 1996). Perhaps these inputs mediate increased facilitation in MOC neurons. Facilitation has different properties than main-ear responses and has been suggested to result from different reflex pathways (Brown et al. 1998). Present results demonstrate a further difference: that noise conditioning increases facilitation while leaving main-ear response relatively unchanged. Although this increase in facilitation may not explain all of the protection afforded by conditioning, the changes in MOC reflex suggest that plasticity of the reflex is a characteristic of the MOC system.

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Present address for S. G. Kujawa: Virginia Merrill Bloedel Hearing Research Center and the Department of Otolaryngology-Head and Neck Surgery, University of Washington, Seattle, WA 98195.

Address for reprint requests: M. C. Brown, Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, 243 Charles St., Boston, MA 02114.

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