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Nicotinic modulation of tone-evoked responses in auditory cortex reflects the strength of prior auditory learning

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Abstract

Nicotinic acetylcholine receptors (nAChRs) contribute to sensory-cognitive function, as demonstrated by evidence that nAChR activation enhances, and nAChR blockade impairs, neural processing of sensory stimuli and sensory-cognitive behavior. To better understand the relationship between nAChR function and behavior, here we compare the strength of nAChR-mediated physiology in individual animals to their prior auditory behavioral performance. Adult rats were trained on an auditory-cued, active avoidance task over 4 days and classified as "good," "intermediate" or "poor" performers based on their initial rate of learning and eventual level of performance. Animals were then anesthetized, and tone-evoked local field potentials (LFPs) recorded in layer 4 of auditory cortex (ACx) before and after a test dose of nicotine (0.7 mg/kg, s.c.) or saline. In "good" performers, nicotine enhanced LFP amplitude and decreased response threshold to characteristic frequency (CF) stimuli, yet had opposite effects (decreased amplitude, increased threshold) on responses to spectrally distant stimuli; i.e., cortical receptive fields became more selective for CF stimuli. In contrast, nicotine had little effect on LFP amplitude in "intermediate" or "poor" performing animals. Nicotine did, however, reduce LFP onset latency in all three groups, indicating that all received an effective dose of the drug. Our findings suggest that nicotinic regulation of cortical receptive fields may be a distinguishing feature of the best-performing animals, and may facilitate sensory-related learning by enhancing receptive field selectivity.

Keywords: Active avoidance; Acetylcholine; Auditory cortex; Behavior; Electrophysiology; Learning; Nicotine

1. Introduction

Cholinergic modulation of sensory cortex is important for sensory–cognitive function (Metherate, 2004; Sarter, Hasselmo, Bruno, & Givens, 2005; Weinberger, 2004). Acetylcholine (ACh) release in sensory cortex is elevated during learning (Butt, Testylier, & Dykes, 1997), and may enhance detection of sensory stimuli and enable cortical plasticity (Hasselmo & McGaughy, 2004; Weinberger, 2004). While many studies have focused on muscarinic actions of ACh, nicotinic ACh receptors (nAChRs) also contribute to cognitive function (Levin, 2002; Levin, McClernon, & Rezvani, 2006; Picciotto, 2003; Sacco, Bannon, & George, 2004). However, the mechanisms by which nAChRs may facilitate sensory-cognitive function are not clear.

One proposed function of nAChRs is to enhance "gating" of sensory information (Hasselmo & McGaughy, 2004; Sarter, Bruno, & Givens, 2003; Sarter et al., 2005). In humans, for example, systemically administered nicotine enhances the perception of, or attention to, important acoustic stimuli, while suppressing the processing of irrelevant signals (Domino & Kishimoto, 2002; Harkrider & Hedrick, 2005). Animal studies have identified in sensory cortex a possible neural contribution to gating: nicotinic activation enhances cortical responses to sensory stimuli, in part by facilitating thalamocortical transmission

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(Clarke, 2004; Gil, Connors, & Amitai, 1997; Kawai, Lazar, & Metherate, 2007; Liang et al., 2006). These findings have led to the proposal that enhancement of sensory cortical processing may mediate, at least partly, the nicotinic contribution to sensory-cognitive function.

To test the prediction of a monotonic relationship between nicotinic enhancement of sensory physiology and cognitive performance, in this study we compared physiological enhancement by nicotine to behavioral performance in the same animal. We first measured performance of adult rats in an auditory-cued active-avoidance task, and then, under anesthesia, determined the effect of systemic nicotine on tone-evoked responses recorded in auditory cortex (ACx). We found that better-performing animals exhibited nicotinic modulation of response magnitude to tones, whereas poorly performing animals did not. Nicotinic effects included enhanced responses to characteristic frequency (CF) stimuli and, surprisingly, suppressed responses to spectrally distant stimuli. The results suggest a neural basis for the nicotinic contribution to auditory-cognitive function, and auditory gating in particular, i.e., that activation of nAChRs improves receptive field selectivity.

2. Materials and methods

All procedures were in accordance with NIH Guidelines and were approved by the University of California, Irvine IACUC. The animals in this study were control subjects in an earlier study (Liang et al., 2006) in which they were injected with saline neonatally (postnatal day 8–12). However all procedures reported here were performed only after the animals reached adulthood (>p60). Rats were maintained on a 12-h light–dark cycle with free access to food and water. The results have not been reported previously except in abstract form (Liang, Poytress, Weinberger, & Metherate, 2007).

2.1. Behavioral experiments

Adult male Sprague–Dawley rats were handled 5 min per day for 5 days prior to the beginning of behavioral testing. Rats were tested for avoidance learning using a shuttlebox (83 cm long, 27 cm wide and 33 cm high, model E10-16SC, Coulbourn Instruments, Allentown, PA, USA) placed in an acoustic chamber (IAC, Bronx, New York, USA). There was dim infrared illumination for an overhead video camera that recorded all trials. The shuttlebox was divided in half by a black plastic barrier (2.5 cm high), with each half having a shock grid floor and infrared photobeams to detect the subject's location. A Coulbourn model #E69-20 speaker was located at one end wall and a Coulbourn model #H11-01R 6W house lamp directed upwards was on the opposite end wall. The speaker's frequency response was calibrated with a Bruel and Kjaer ¼″ condenser microphone, pre-amplifier, Hewlett-Packard Sound Wave Analyzer and a Bruel and Kjaer 94 dB 1.0 kHz standard. Animals were trained to cross to the opposite side of the box before the end of a 5 s tone (8 kHz, 70 dB SPL) in order to avoid a shock (60 Hz scrambled bipolar pulses, 0.8–1.0 mA, Coulbourn model #E13-14 shock generator), with inter-trial intervals averaging 45 s (range 30–60 s). "Avoidance" behavior indicates crossing during the tone, prior to the shock, whereas "escape" behavior indicates crossing during the shock. Avoidance training (tone paired with shock) took place over four consecutive days (Days 1–4) preceded by one day of escape training (shock only) for 50 trials. Shock levels were gradually increased during the first 5–10 trials until consistent escape behavior was obtained for each subject. Day 1 of avoidance training began with 10 escape trials, to insure memory for escape learning, followed by 50 avoidance trials where the shock was paired with a preceding tone. Days 2–4 consisted of 50 avoidance trials.

2.2. In vivo electrophysiology

Shortly after the behavioral testing (~1 week), each animal was tested physiologically. The experimenter was blind to the animal's behavioral performance. Rats were anesthetized with urethane (1.5 g/kg i.p., Sigma) and xylazine (10 mg/kg i.p., Phoenix Pharmaceuticals, St. Joseph, MO, USA), placed in a sound-attenuating chamber (IAC), and maintained at 36–37 °C with a temperature controlled heating blanket. A craniotomy was performed over the right temporal cortex and ACx was identified physiologically (Kaur, Rose, Lazar, Liang, & Metherate, 2005). Local field potentials (LFPs) were recorded in layer 4 of ACx with a glass microelectrode (1 MΩ at 1 kHz) positioned at a depth of 600 μm below the pia. Pure tones (100 ms duration, 10 ms rise/fall ramps) were presented to the contralateral ear (open field, ~3 cm from the ear) using an electrostatic speaker (ES-1 with ED-1 driver, Tucker-Davis Technologies, Gainesville, FL). For calibration (SPL in dB re: 20 μPa) a microphone (model 4939 microphone and Nexus amplifier; Bruel and Kjaer, Norcross, GA) was positioned in place of the animal. Frequency receptive fields were determined using tones of 1.25–40 kHz in one-octave steps at intensities from below threshold to 70 dB SPL in 5 dB or 10 dB steps (smaller steps near threshold). Neural activity was filtered, amplified (1 Hz–10 kHz, AI-401, Axon Instruments, Foster City, CA), digitized at 5 kHz (Digidata 1322A, Axon Instruments) and stored on a computer (Macintosh G4, Apple Computer). Data acquisition was triggered 100 ms before acoustic stimulation, responses were averaged and viewed online and analyzed off-line (AxoGraph, Axon Instruments). Response onset latency, initial slope (maximum slope between onset and peak using a 1.2 ms window), peak amplitude, duration (at 50% of the peak) and area were analyzed for LFPs. The criterion for a threshold "response" was determined by averaging baseline fluctuations in a subset of animals over a 100 ms period. We calculated baseline variability (one standard deviation) to be about 40 μV, which we set as the threshold response amplitude provided that the response onset occurred within ~20 ms of stimulus onset and decreased as expected with increasing intensity. CF (the frequency with the lowest threshold response) was determined for each recording site, and intensity functions (from below threshold to 70 dB SPL in 5–10 dB steps) at CF and three octaves below CF ("nonCF") were recorded from each animal. Systemic saline or nicotine (0.7 mg/kg nicotine base, s.c.) was delivered and intensity functions at CF and nonCF were re-recorded. The effects of nicotine were compared to those of saline in the same animal. At the end of each experiment, animals were euthanized with a lethal dose of anesthesia.

Statistics are reported ± standard error of the mean.

3. Results

To examine the relationship between nAChR function and behavior, we first trained 12 animals over 4 days in an auditory-cued active avoidance task and identified sub-groups based on the initial rate of learning and eventual level of performance. Subsequently, under anesthesia, we determined for each animal the effects of a test dose of systemic nicotine on tone-evoked LFPs in ACx. We then averaged physiological results within each behavioral subgroup to relate physiological and behavioral results.

3.1. Avoidance learning

The behavioral task we employed is appropriate for examining the interrelationship of nAChRs, ACx function

and auditory learning, since performance on this task is impaired by ACx lesions (Delay & Rudolph, 1994; Duvel, Smith, Talk, & Gabriel, 2001; Ohl, Wetzel, Wagner, Rech, & Scheich, 1999), and enhanced by systemic administration of nicotine (Erickson, 1971; Sansone, Battaglia, & Castellano, 1994; Sansone, Battaglia, & Pavone, 2000; Sansone, Castellano, Battaglia, & Ammassari-Teule, 1991; Yilmaz, Kanit, Okur, & Pogun, 1997). Rats were trained to avoid a shock by crossing into the safe chamber of a two-chamber shuttle box before the end of a 5 s tone. Most animals learned to avoid the shock over the 4 days of training (50 trials/day; performance measured as percent of trials on which the animals avoided the shock). However, the animals exhibited striking variability in performance as captured by two key measures: the initial rate of learning on Day 1, and eventual degree of success on Day 4. Because cholinergic function could plausibly relate to either or both measures, we used both to identify distinct subgroups of animals.

The relationship between initial learning (percent avoidance on Day 1) and eventual success (percent avoidance on Day 4) is shown for each animal in Fig. 1. Some data clustering is apparent, from which we inferred three groups ($n = 4$ each): "good" performers learned quickly (>50% avoidance on Day 1) and continued to improve over the 4 days of training (i.e., points lie above the diagonal line in Fig. 1). "Intermediate" performers learned slowly on Day 1 (<50% avoidance) but still improved over 4 days to >50% avoidance (points lie above the diagonal line). Animals in these first two groups form two clear clusters in Fig. 1. Performance for the remaining animals, "poor" performers, is more diverse but characterized by the lack of improvement over the 4 days of training (points are near or below the diagonal line), and eventual performance below 50% avoidance.

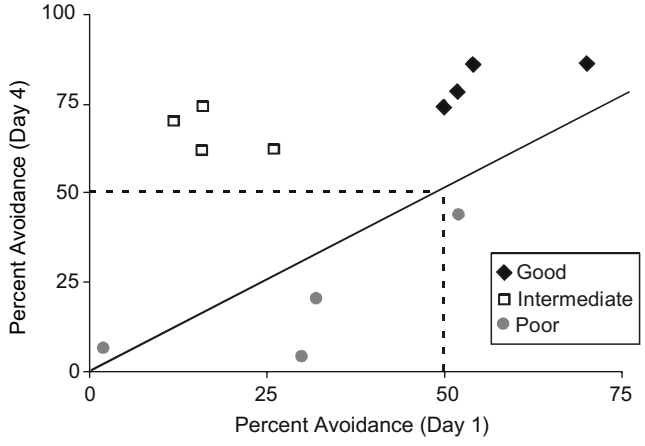

Fig. 1. Relationship between behavioral performance on Day 1 and Day 4 for each animal ($n = 12$). Performance measured as the percent of trials ("percent avoidance") on which the animal avoided the shock by crossing to the safe compartment before the end of the tone cue. Diagonal line indicates equal performance on Day 1 and Day 4; animals above the line improved over the 4 days of training. Dashed lines indicate 50% avoidance. See Results for definitions of "good," "intermediate" and "poor" performers.

For each of the three behavioral groups, Fig. 2 shows an individual example of performance over the training period (left column), and group data for performance on Day 1 (middle column) and over Days 1–4 (right column). Over the 4 days of training, "good" and "intermediate" groups differed from the "poor" group (cf. Fig. 2C, F and I; ANOVA, $F_{1,7} = 8.58$, $p < .05$; Fisher's post hoc, good vs. poor, $p < .05$; intermediate vs. poor, $p < .05$). Since group differences emerged early, we examined Day 1 performance more closely (cf. Fig. 2B, E and H). "Good" performing animals quickly improved over 50 trials, while "intermediate" and "poor" groups avoided on fewer trials (ANOVA, $F_{1,7} = 16.17$, $p < .05$, Fisher's post hoc; good vs. intermediate, $p < .05$; good vs. poor, $p < .05$). Overall, the behavior implies three groups of animals: those that learned well and quickly ("good"), those that learned well but more slowly ("intermediate") and those that did not improve over time ("poor").

The three groups also differed in terms of avoidance latency (latency to avoid after tone onset) on Day 4 of training (Fig. 3B; ANOVA, $F_{1,7} = 76.69$, $p < .001$; *Fisher's post hoc test, $p < .001$). However, prior to any behavioral training there were no differences in escape latency (Fig. 3A; ANOVA, $F_{1,7} = .708$, $p = .52$), implying a lack of sensorimotor differences among groups. Thus, the three behavioral groups are distinguished by both avoidance performance level and latency.

3.2. Nicotinic modulation of physiological responses

In order to relate behavioral performance to cortical physiology, within ~1 week of the training we anesthetized each animal and recorded tone-evoked LFPs in layer 4 of ACx. The experimenter was blind to the animal's prior behavioral performance. We determined CF for the recording site and then determined an intensity function at CF, i.e., responses to CF tones ranging in intensity from 5 to 10 dB below acoustic threshold to 70 dB SPL in 5–10 dB steps. Next, we obtained a second intensity function at a frequency three octaves below CF ("nonCF"). We then re-determined the intensity functions at CF and nonCF after systemic administration of saline (Fig. 4B, gray traces), and again after a test dose of nicotine (0.7 mg/kg) (black traces). This test dose is an effective concentration for behavioral and physiological studies in rats, near levels (e.g., 0.2–0.6 mg/kg) that produce peak enhancement of function (Matta et al., 2006; Picciotto, 2003; Yilmaz et al., 1997), but below levels that produce seizures (e.g., >2 mg/kg (Okamoto, Kita, Okuda, & Nakashima, 1992). From the control (saline) data we determined the threshold response intensity for CF and nonCF stimuli, as well as peak amplitudes and other measures of tone-evoked LFPs (Fig. 4A and Table 1). Mean CF was ~30 kHz, which did not differ among "good," "intermediate" and "poor" performing animals (31 ± 8.8 kHz, 30 ± 11.5 kHz and 28 ± 7.5 kHz, respec-

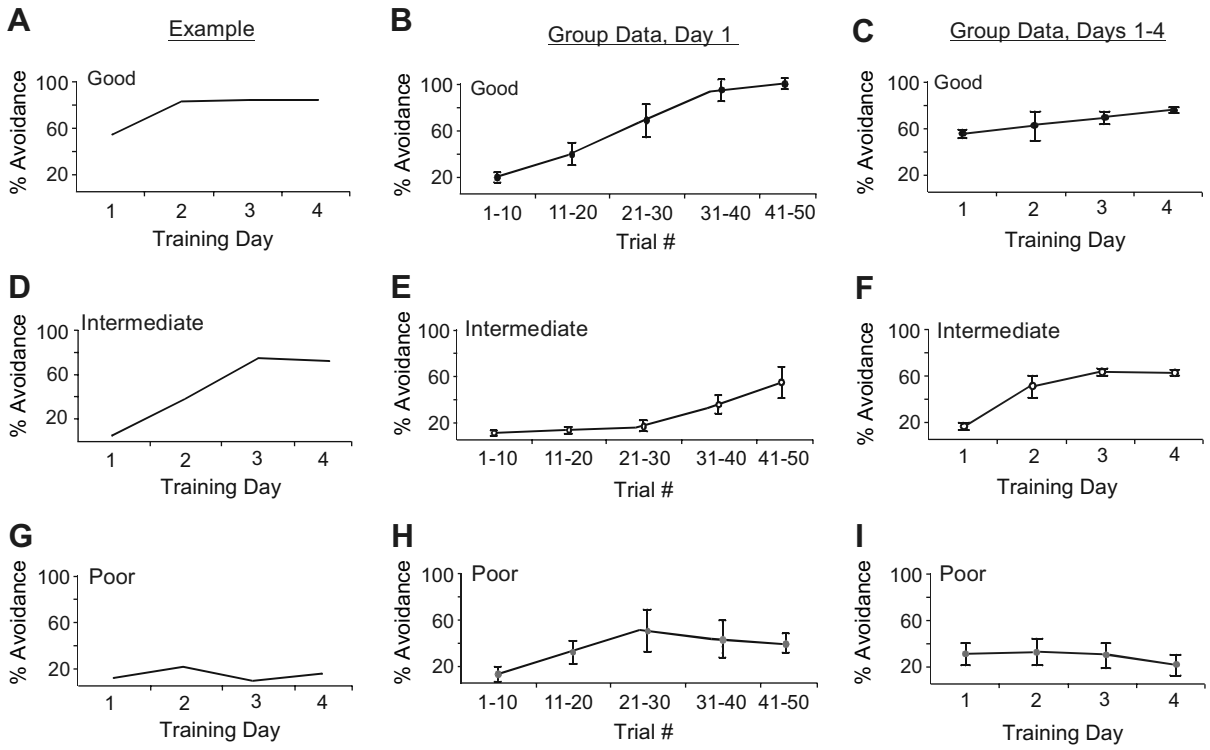

Fig. 2. Behavioral performance of "good," "intermediate" and "poor" performing groups ($n = 4$ each). Left column (A, D and G) shows daily average avoidance (50 trials per day) for an individual example from each group. Middle column (B, E and H) shows group data for performance on the first training day. Right column (C, F, I) shows group data for daily average avoidance across the 4 days of training.

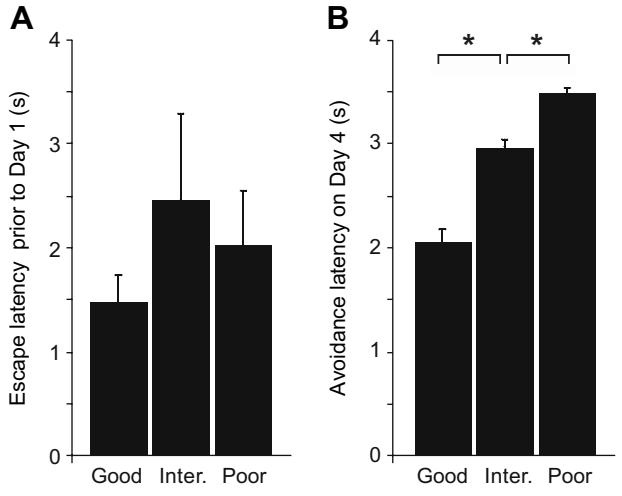

Fig. 3. Behavioral groups ("good," "intermediate" and "poor" performers) showed similar escape latencies prior to training (left; ANOVA, $F_{1,7} = .708$, $p > .05$), but different avoidance latencies after 4 days of training (right; ANOVA, $F_{1,7} = 76.69$, $p < .001$; *Fisher's post hoc test, $p < .001$).

tively; ANOVA, $F_{2,9} = .066$, $p = .94$), nor did the spectral distance from CF to the 8 kHz training frequency differ among groups (ANOVA, $F_{2,9} = .15$, $p = .87$). Thus, the recording sites were similar among the three groups, and similarly far from the representation of the training frequency. Similarly, response amplitudes in "good," "intermediate" and "poor" performing animals did not differ (control intensity functions at CF, 0–60 dB relative to (re.) threshold; ANOVA, $df = 27$, $F = .428$, $p = .96$).

The effects of nicotine differed dramatically among the three behavioral groups (Figs. 4 and 5 and Table 1; all effects vs. response after saline injection). In "good" performers, nicotine increased response amplitudes at CF (Fig. 4B, left) and, notably, produced responses to stimuli that previously were subthreshold (Fig. 4B, peri-threshold response; group data in Fig. 5A, left, $n = 4$, dotted line indicates baseline noise, see Section 2). As a result, mean response threshold at CF decreased by 8.8 ± 1.25 dB (i.e., threshold responses were elicited by stimuli 5 or 10 dB below control threshold; intensities below −10 dB were not tested). Surprisingly, for the same animals nicotine *decreased* response amplitudes at nonCF (Fig. 4B, right; Fig. 5A, right), and as a result *increased* threshold at nonCF by 20 ± 7.64 dB (range 5–30 dB). In contrast to these effects in "good" performers, nicotine had little effect on responses in "intermediate" and "poor" performing animals (Fig. 5C and E). To quantify the effect of nicotine on peri-threshold responses, we first expressed nicotine's effect as a percent change in amplitude at the lowest intensities for which there was a response in either condition, control or nicotine (i.e., we averaged across −10 to 0 dB for CF, and 0 to +10 dB for nonCF). In "good" performers nicotine altered peri-threshold responses to CF and nonCF stimuli differently (ANOVA, $F_{1,15} = 5.65$, $p < .05$).

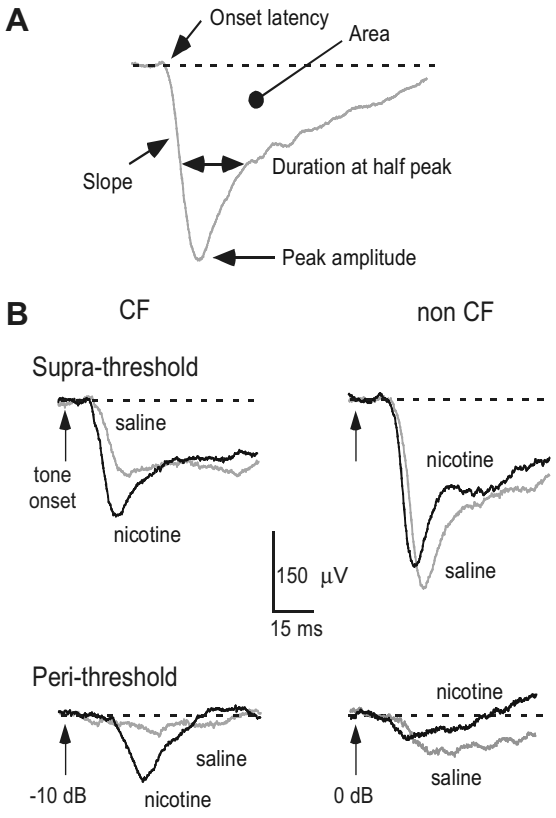

Fig. 4. Physiological measurements (A) and example (B) of nicotine's differential effect on CF vs. nonCF tone-evoked responses in a "good" performing animal. (A) Five LFP measures were obtained from tone-evoked responses in each animal, and group data are reported in Table 1 for each behavioral group. (B) Representative tone-evoked LFPs, from the same animal as in Fig. 2A, following systemic injection of saline (gray traces) and nicotine (0.7 mg/kg, s.c., black traces). At a supra-threshold intensity (top traces) nicotine enhanced the peak response to the CF stimulus (40 kHz, 60 dB SPL), but reduced the peak response to a spectrally distant stimulus (nonCF, 5 kHz, 70 dB SPL). Similar effects on near-threshold responses (lower traces) produced opposite changes in threshold for CF and nonCF: a CF stimulus that initially was below threshold (gray trace; 0 dB SPL, −10 dB re. threshold) after nicotine produced a clear response (black trace), indicating a lower threshold at CF. For nonCF, the illustrated response was initially at threshold (gray trace; 10 dB SPL, 0 dB re. threshold) and nicotine reduced its amplitude (black trace) to below the criterion for a response (see Section 2).

However, nicotine did not affect peri-threshold responses in "intermediate" or "poor" performers ($p > .05$).

A similar analysis was repeated at suprathreshold intensities (20–60 dB re. threshold) with similar results (Fig. 5B, D, F and Table 1). Intensity functions at CF and nonCF were expressed as the percent change in peak amplitude from the response in saline; these functions were then averaged across suprathreshold intensities for each animal and then averaged for each group (Fig. 5, right and Table 1). In "good" performers, nicotine enhanced the response to CF stimuli, and reduced the response to nonCF stimuli (Fig. 5B), thereby increasing the contrast between responses to CF and nonCF stimuli (paired t-test, $n = 4$, $t = 4.07$, $p < .05$). However, in "intermediate" and "poor"

Table 1
Measures of cortical LFPs elicited by CF (top) and nonCF (bottom) stimuli after systemic saline or nicotine in "good", "intermediate" and "poor" performing animals

	Good			Intermediate			Poor		
	Saline	Nicotine	% change; stats.	Saline	Nicotine	% change; stats.	Saline	Nicotine	% change; stats.
Response to CF stimulus									
Peak ampl. (μV)	274 ± 30	356 ± 32	35.6 ± 8.9[a,b]	379 ± 22	370 ± 21	11.5 ± 5.2	240 ± 8	218 ± 19	−4.9 ± 8.6[b]
Area (μV ms)	9730 ± 1556	15845 ± 1395	113.4 ± 28.9[b]	10527 ± 783	13482 ± 1130	35.5 ± 8.5	9507 ± 8194	8194 ± 1300	−8.3 ± 11.0[b]
Initial slope (μV/ms)	34 ± 4	39 ± 5	26.1 ± 8.1[a,b]	48 ± 7	46 ± 7	13.4 ± 6.6	32 ± 2	30 ± 3	−7.8 ± 5.9[b]
Onset latency (ms)	13 ± 1	11 ± 1	−12.4 ± 1.0[c]	17 ± 1	16 ± 2	−7.1 ± 3.3[c]	15 ± 1	13 ± 1	−12.6 ± 1.6[c]
Duration (ms)	27 ± 2	36 ± 2	42.0 ± 5.4[a]	37 ± 1	35 ± 3	9.8 ± 5.5[b]	32 ± 3	31 ± 2	8.6 ± 13.6
Response to nonCF stimulus									
Peak ampl. (μV)	136 ± 41	75 ± 27	−42.8 ± 8.0[a,b]	273 ± 118	249 ± 114	−11.6 ± 10.9	212 ± 58	210 ± 68	−8.3 ± 4.5[b]
Area (μV ms)	3841 ± 1195	2981 ± 1702	−24.2 ± 27.2	5641 ± 1556	10062 ± 5037	13.6 ± 28.2	11485 ± 3319	12525 ± 4899	−4.3 ± 11.4
Initial slope (μV/ms)	20 ± 5	13 ± 5	−44.6 ± 7.9[a]	34 ± 13	32 ± 13	−7.6 ± 6.1	23 ± 4	24 ± 2	10.9 ± 8.7
Onset latency (ms)	15 ± 1	14 ± 0.4	−7.4 ± 9.5[c]	15 ± 1	14 ± 1	−2.8 ± 2.8[c]	16 ± 1	14 ± 0.3	−11.8 ± 3.6[c]
Duration (ms)	21 ± 2	17 ± 3	−32.3 ± 17.3[a]	23 ± 2	24 ± 2	11.6 ± 15.0	50 ± 5	50 ± 8	−5.1 ± 6.4

Each value is the average (±SE) response in four animals, and the value for each animal is an average of responses to stimuli 20–60 dB above threshold.

[a] Change at CF different from change at nonCF (paired t-test, $p < .05$).

[b] Change in "good" animals different from corresponding value in "poor" animals (unpaired t-test, $p < .05$).

[c] Significant change re. saline control (paired t-test, $p < .05$).

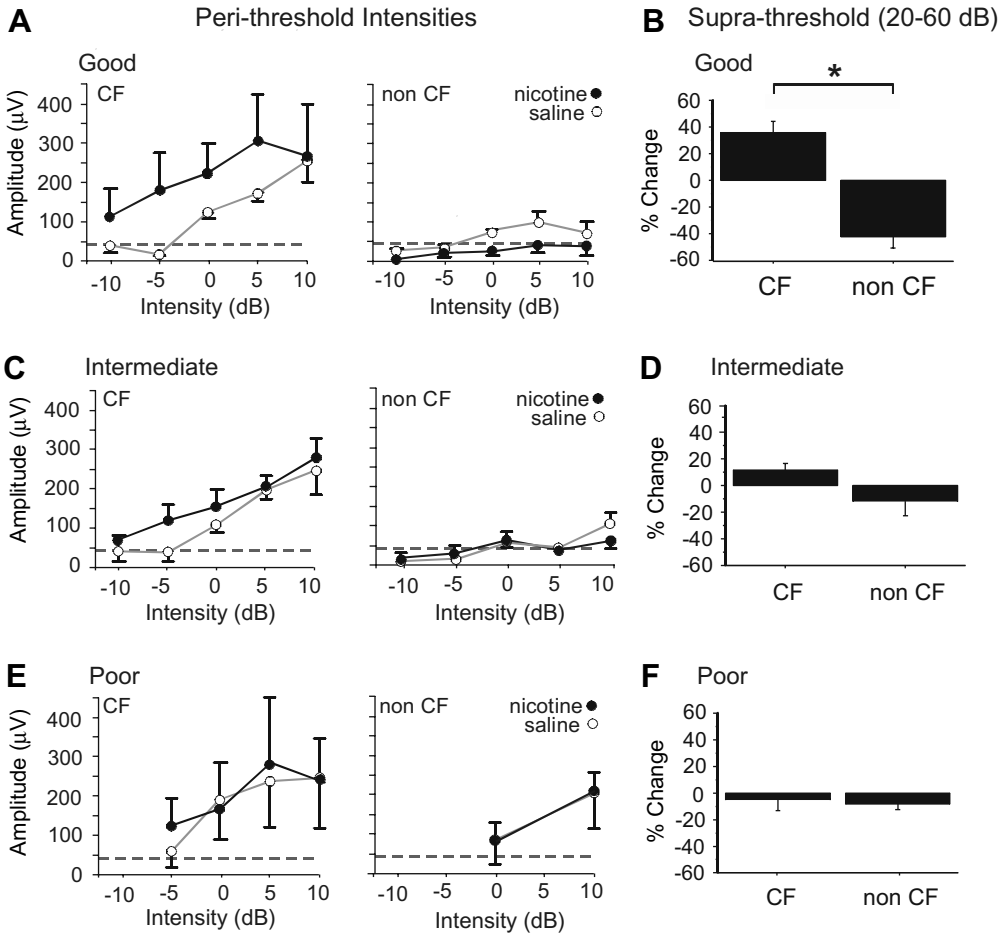

Fig. 5. Nicotine alters tone-evoked response amplitude and threshold in "good," but not "intermediate" or "poor,"—performing animals ($n = 4$ in each group). Graphs (A, C and E) show response amplitudes to peri-threshold stimuli (±10 dB; dotted lines indicate baseline noise, see Section 2), and histograms (B, D and F) show responses to supra-threshold stimuli (20–60 dB re. threshold) expressed as percent change from the control response in saline and averaged across intensity for each animal. (A) In "good" performers, nicotine enhanced responses to CF stimuli (left) and decreased responses to nonCF stimuli (right) at peri-threshold intensities. Nicotine decreased CF response thresholds by increasing response amplitudes to CF stimuli above noise levels, and increased nonCF threshold by decreasing response amplitudes to nonCF stimuli below noise levels. (B) Similar differential effects of nicotine on CF vs. nonCF -evoked responses occur at supra-threshold intensities. *Paired t-test, $p < .05$. (C–F) Nicotine produced little effect on peri-threshold and supra-threshold responses in "intermediate" and "poor" performers. Fewer intensity levels were tested in a few animals, by coincidence these were mostly "poor" performers (E).

performers, nicotine had little effect on responses to either CF or nonCF stimuli (Fig. 5D and F; percent change in "poor" performers differed from that in "good" performers: unpaired t-test, $n = 4$; for CF, $t = 2.34$, $p < .05$; for nonCF, $t = -3.75$, $p < .05$). The effects of nicotine in "good" performers were reflected in several LFP measures, including response area, initial slope and duration (Fig. 4 and Table 1).

The lack of nicotine effect in "intermediate" and "poor" performers did not result from a failure of the drug to reach the brain in sufficient concentrations. Nicotine consistently reduced LFP onset latency across all groups (Table 1), for both CF (ANOVA, $df = 22$, $F = 4.51$, $p < .05$) and nonCF (ANOVA, $df = 14$, $F = 4.90$, $p < .05$) stimuli. Thus, all animals received physiologically effective doses of nicotine, yet the drug affected cortical receptive fields (e.g., response amplitude and threshold) only in "good" performers.

3.3. Nature of the relationship between behavioral performance and nicotinic modulation

Since the three behavioral sub-groups were created based on differences in initial rate of learning on Day 1 and eventual level of performance on Day 4—as reflected in the clustering of data in Fig. 1—we attempted to determine if nicotine sensitivity was correlated with either of the two behavioral indices used to distinguish the groups. For each index we conducted a linear regression analysis to examine the relationship between physiology and behavioral performance. The physiological index used was the nicotine-induced percent change in LFP amplitude elicited by CF stimuli. For initial learning on Day 1, the behavioral index was percent avoidance over the last 10 trials (Fig. 2, middle column, trials 41–50). A linear regression analysis showed that nicotinic modulation of LFPs was not correlated with Day 1 performance ($n = 12$, $r = .302$, $p = .34$).

For eventual level of performance the behavioral index was percent avoidance on Day 4 (Fig. 2, right column). Using the same physiological data, the regression analysis showed a trend that did not reach statistical significance ($n = 12$, $r = .551$, $p = .06$). Given the clear effect of nicotine on "good" performers (Fig. 5), the regression analysis suggests that the relationship between nAChR physiology and behavior is nonlinear, e.g., nicotine's effects on response magnitude occurred mainly in the best performers.

For the analyses performed thus far, we have grouped animals according to behavioral performance and then compared physiological effects of nicotine among the groups. In a final analysis, we did the reverse, grouping animals by physiological effects and examining behavioral performance in the groups thus formed. Our rationale was that if strong physiological effects and behavioral performance were linked, then either metric could be used for grouping animals. We first ranked all 12 animals according to the strength of nicotinic enhancement (mean "percent enhancement" of response to CF stimulus, averaging across suprathreshold intensities for each animal). Nicotine enhancement for the top half of animals ("strong-physiology" animals, $n = 6$) was greater than for the bottom half ("weak-physiology" animals; $40 \pm 14.4\%$ vs. $-22 \pm 10.1\%$; ANOVA, $F_{1,10} = 12.4$, $p < .01$). Moreover, for "strong-physiology" animals, nicotine enhanced the response to CF stimuli (by definition) but suppressed the response to nonCF stimuli (CF: $40 \pm 14.4\%$, nonCF: $-26 \pm 9.4\%$; ANOVA, $F_{1,9} = 13.1$, $p < .01$). However, there was no differential effect of nicotine in "weak-physiology" animals (ANOVA, $F_{1,9} = 1.4$, $p > .05$). We then compared behavioral performance in the "strong-physiology" vs. "weak-physiology" animals. Both groups avoided similarly on Day 1 of training ("strong-physiology" animals, $38 \pm 9.8\%$ avoidance; "weak-physiology" animals, $30 \pm 8.3\%$ avoidance; ANOVA, $F_{1,10} = .42$, $p = .53$). However, by Day 4, "strong-physiology" animals avoided on more trials than did "weak-physiology" animals ($75 \pm 4.4\%$ avoidance vs. $36 \pm 12.7\%$ avoidance; ANOVA, $F_{1,10} = 8.1$, $p < .05$). These results reinforce the notion that the best-performing animals are those in which nicotine produces its strongest physiological effects.

4. Discussion

This study investigated the interrelationship of nAChRs, cortical function and auditory learning. We measured responsiveness to nicotine during physiological experiments to infer the strength of nAChR function, and related the findings to the animals' prior performance on an auditory-cued active avoidance task. Nicotine had clear physiological effects only in the best performing group of animals, in which it simultaneously enhanced responses (and decreased threshold) to CF stimuli, but reduced responses (and increased threshold) to spectrally distant nonCF stimuli, indicating that receptive fields became more selective for CF stimuli. Since endogenous ACh may similarly activate nAChRs during behavior, increased selectivity of cortical receptive fields is a possible mechanism by which nAChRs could enhance cognitive function.

The main, novel results of this study are that (i) nicotinic modulation of cortical responses occurs only in better-performing animals, and (ii) in those animals nicotine simultaneously enhances responses to CF stimuli and reduces responses to spectrally distant stimuli. The physiological results are consistent with proposed nicotinic mechanisms for regulation of cortical processing during periods of ACh release (see below), and the general importance of nAChRs for cortical and cognitive function (Clarke, 2004; Domino & Kishimoto, 2002; Levin, 1992; Levin & Simon, 1998; Liang et al., 2006; Parkinson, Kratz, & Daw, 1988). However, the group of "good" performers comprised only a third of the overall population, and nicotinic effects were absent, on average, in the other groups (except for effects on response latency, see below). [However, note that more subtle actions of nicotine may be revealed with a larger sample size—e.g., intermediate-magnitude effects of nicotine may be observed in the "intermediate" performing animals (Fig. 5C and D)] Thus, nicotinic regulation of cortical processing may distinguish better-performing from lesser-performing animals, rather than enhancing performance in all animals. Performance variability among groups in our study does not appear to result from simple sensory or motor differences, given no differences in baseline acoustic physiology or initial escape latencies. A recent preliminary report suggests an anatomical correlate to our result, in that "good learners" of a classical conditioning task have higher levels of nAChRs ([^{3}H]epibatidine binding) in hippocampus and temporal-parietal cortex than "poor learners" (Woodruff-Pak, Lehr, Li, & Chen, 2007). Although nAChR binding levels do not always reflect function (due to, for example, receptor internalization, inactivation or desensitization), higher levels of cortical nAChRs could underlie our results in "good" performers. If future studies confirm a link between nAChR expression and cognitive function, it will be important to determine if poor learning is associated with nonfunctional nAChRs, or whether nicotine doses higher than that used in the current study would be effective. An additional, perhaps more important, goal will be to determine whether strong learning results from—or, instead, produces—enhanced nAChR expression.

Although the location of the nAChRs regulating cortical responses in our study is unknown (nicotine was administered systemically), indirect evidence points to the involvement of cortical receptors. Nicotine consistently decreased response onset latency in all animals, not just "good" performers, but in the latter nicotine also altered response magnitude (and threshold). While the effect on response latency indicates that all animals received effective drug doses, the differential effects on response latency vs. magnitude also provide insight to the locus of nicotinic action. That is, nicotinic regulation of onset latency has been attributed to sub-cortical receptors, since it is not affected

by intracortical manipulations that do alter response magnitude (Liang et al., 2006). Thus, nicotinic regulation of cortical response latency in all animals may involve subcortical nAChRs (Morley & Happe, 2000), whereas regulation of cortical response magnitude in "good" performers may involve cortical nAChRs. However, possible alternative mechanistic explanations of the present results must include nicotinic effects on other brain structures and neurotransmitter systems that regulate auditory cortex. Additional studies using direct manipulations of cortical nAChR function will be needed to resolve these issues.

The present study is also the first to demonstrate nicotinic regulation of cortical receptive field selectivity (increased response to CF stimuli and reduced response to nonCF stimuli). The effect of nicotine to enhance receptive field selectivity may involve differential regulation of neural pathways mediating cortical responses to CF vs. nonCF stimuli. Recent studies suggest that information about CF stimuli is relayed to a particular cortical location by direct thalamocortical inputs, whereas long distance intracortical ("horizontal") pathways may preferentially convey information about spectrally distant (nonCF) stimuli (Happel, Jeschke, Deliano, & Ohl, 2007; Kaur, Lazar, & Metherate, 2004; Kaur et al., 2005; Metherate et al., 2005; Miller, Escabi, Read, & Schreiner, 2001; Winer, Sally, Larue, & Kelly, 1999). Nicotinic ACh receptors facilitate thalamocortical transmission (Clarke, 2004; Gil et al., 1997; Kawai et al., 2007) and thereby enhance cortical responses to CF stimuli (Kawai et al., 2007). Potential mechanisms by which nAChRs could suppress responses to nonCF stimuli are less clear. Suppression of responses to nonCF stimuli may involve excitation of cortical inhibitory interneurons (Christophe et al., 2002; Porter et al., 1999) that regulate intracortical horizontal pathways, or other, including subcortical, mechanisms. Other models of cholinergic regulation of cortex suggest that muscarinic suppression of intracortical processing may achieve a similar end, i.e., leading to narrower tuning of receptive fields (Hasselmo, 2006; Metherate et al., 2005; Soto, Kopell, & Sen, 2006). Regardless of the mechanism, enhanced receptive field selectivity in auditory cortex could contribute to improved cognitive function, including gating of relevant auditory stimuli and suppression of distracters (see Section 1).

An important point to emphasize regarding nicotine's effect is that receptive field selectivity was likely enhanced throughout auditory cortex, and not just at the representation of the behavioral training frequency (since recording sites were distant from sites representing the training stimulus). How this might improve sensory-cognitive behavior is a crucial, unanswered question, but higher-order processing of the training stimulus may benefit from sharper receptive fields regardless of whether the effect also occurs elsewhere in auditory cortex. This notion is highly speculative, of course, and must be tested in future experiments.

Finally, although we did not record neural activity during behavioral training, it is likely that training produced changes in receptive field selectivity to favor detection of the tone signaling the shock (Fritz, Shamma, Elhilali, & Klein, 2003; Polley, Steinberg, & Merzenich, 2006; Weinberger, 2004). Future experiments should investigate the involvement of nAChRs in training-induced receptive field plasticity.

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