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Effects of muscarinic and adrenergic agonism on auditory P300 in the macaque

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Abstract

Homologs of human endogenous evoked potentials are known in several species of nonhuman primates, but the neurotransmitter substrates of these potentials remain uncertain. In particular, the role of central cholinergic and adrenergic systems is not yet clearly defined. We recorded cognitive evoked potentials from the scalp in four adult bonnet macaque monkeys during a passive version of the auditory oddball paradigm with unique novel stimuli under saline control conditions. In two subjects each, cognitive evoked potentials were also recorded following intramuscular administration of the m1 muscarinic agonist AF102B or of the α-2A noradrenergic agonist guanfacine. On saline, large positivities resembling the human P300 were recorded over midline sites in response to rare or novel auditory stimuli in all four monkeys. The amplitude of these positivities was sensitive to the delivery of fruit-juice reward in association with rare stimuli in three monkeys tested. At cognition-enhancing doses, AF102B enlarged the amplitude of P300-like positivities in both monkeys tested; guanfacine enlarged the amplitude of P300-like positivities in one of two monkeys tested. These results add to existing evidence of human-like endogenous late positivities in monkeys that are influenced by the cholinergic and adrenergic systems, and suggest a possible role of m1 muscarinic and α-2A noradrenergic receptor subtypes.

Keywords: Primates; Evoked potentials; P300; Muscarinic agonist; Noradrenergic agonist; AF102B; Guanfacine

1. Introduction

Cognitive evoked potentials (EPs) resembling the human P300 (P3) have been identified in many, but not all, monkey species [7,19,29,34,37]. As animal models permit more systematic pharmacological, electrophysiological, and lesioning interventions than human subjects, it is hoped that these models might help reveal the neurofunctional underpinnings of P3 phenomena and thereby increase the utility of the P3 as a clinical sign [33]. For example, P3 amplitude decline and latency retardation exceeding those observed in normal aging are long established in Alzheimer's disease [42], but it is not known how these electrophysiological symptoms relate to the histochemically determined central cholinergic [23,25] and adrenergic [5,9,36,44] deficiencies of the disease. Evidence gathered from primate studies favors both catecholaminergic, especially α-2 noradrenergic [18,38,40,46,47], and cholinergic [1,2] influences on monkey P3 neurogeneration or neuromodulation.

The present exploratory investigation sought to expand the range of nonhuman primate models by testing for the existence of P3-like cognitive evoked potentials in an additional macaque species employed in biomedical research, *Macaca radiata* (the bonnet monkey; [17]), using the auditory oddball paradigm. The oddball P3 is typically elicited by infrequent stimuli that are attended [13,21,41,45]. Stimuli may be attended by instruction, attended through learned association with reinforcement, or attended reflexively, by virtue of stimulus novelty or saliency. In *M. radiata*, it was, therefore, hypothesized that an auditory oddball P3-like positivity could be evoked by rare stimuli, especially if these were linked with reinforcement, and by unique novel stimuli, even if these were not linked with reinforcement.

A second goal was to study the participation of central cholinergic and adrenergic systems in the primate P3 at a

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higher level of receptor specificity by examining the influence of a direct m1 muscarinic agonist, ±-*cis*-ethyl-spiro(1,3-oxathiolane-5,3′)quinuclidine (AF102B), and of an α-2A noradrenergic agonist, N-(aminoiminomethyl)-2,6-dichlorobenzene-acetamide (guanfacine), on putative monkey P3 potentials. These two drugs improve performance on behavioral tasks testing attention and memory in nonhuman primates [3–6,30,31], cognitive functions that are associated with the P3 [13,21,41]. In the auditory oddball, it might thus be expected that these drugs, at appropriate doses, could improve the ability of monkey subjects to recognize and to attend to reinforced stimuli, as well as to ignore unreinforced stimuli. It was, therefore, hypothesized that the amplitude of P3-like responses evoked by rare reinforced stimuli would be increased by both AF102B and guanfacine at doses likely to enhance cognition, while the amplitude of P3-like responses evoked by novel unreinforced stimuli was expected to be unchanged or diminished at the same doses.

2. Materials and methods

Four healthy adult female bonnet monkeys (*M. radiata*) weighing 4–9 kg served as subjects. Subjects were colony bred, with ages taken from birth records at the UC Davis Regional Primate Facility in Davis, CA (USA). Monkeys were exposed to a passive version of the classic oddball paradigm with unique novel stimuli while they sat alone in primate restraint chairs in a darkened, ventilated, electrically and acoustically insulated recording chamber. A padded head restraint was applied to the chair to reduce gross movement artifacts. Auditory stimuli 50 ms in duration were delivered through a pair of overhead speakers at a sound level of 60 dB at a constant rate of one per 1500 ms. Stimuli comprised frequent tones at 800 Hz (80% of stimuli), rare tones at 1200 Hz (10% of stimuli), and unique novel stimuli (10% of stimuli). Unique novel stimuli consisted of a standard list of sundry startling sounds such as car doors slamming, horns honking, bells ringing, and the like, but no vocal material. Both frequent and rare tones had the same audio envelope, which consisted of an 8-ms rise/fall time and a 34-ms plateau. The audio envelopes of the unique novel stimuli varied, depending on the individual sound. Recordings were carried out under five different drug-administration/reinforcement-delivery conditions as described below. For each condition, monkeys were exposed to the paradigm 3 days a week, with at least 1 day off between sessions in an attempt to forestall possible habituation of EP components [33].

EEG was recorded through platinum needle electrodes inserted into scalp sites "Fz, Cz, Pz" of a simian pseudo-10/20 system. On ketaminized monkeys (5 mg/kg i.m.), the scalp was shaved and carefully measured, and electrode sites were labeled with indelible marker to ensure consistency of placement. Platinum electrodes were placed at the marked sites on awake, head-restrained monkeys just prior to each recording session, and removed immediately thereafter. Electrodes were disinfected with bleach between insertions and scalp sites were washed with betadine just before insertion and just after removal. Animals were examined daily, but no signs of infection or irritation were noted after repeated electrode applications. Behaviorally, neither insertion, presence, nor removal of electrodes resulted in undue overt discomfort on the part of conscious monkey subjects. The recording reference was a Velcro-strap neckring [24] soaked in supranormal saline. A pair of platinum needle electrodes in bipolar configuration inserted at the superior orbital ridge and the external canthus of the right eye was used to monitor eye movements.

EEG and EOG were recorded through Model 7P511J Grass amplifiers with an analog bandpass of 0.1–100 Hz and digitized at 200 Hz. Recording commenced 60 ms before stimulus onset and continued to 550 ms thereafter. Four hundred trials were collected per session. Separate averages of evoked potential trials were computed for the three categories "frequent," "rare," and "novel" under artifact rejection at a threshold of 75 μV. Rejection rates ranged from 5–91% of the trials. Multiple sessions were combined into grand averages to allow sufficient numbers of trials. Based on initial observations, "monkey scalp P3 amplitude" was evaluated at each electrode as the area (negative up) relative to prestimulus baseline in the latency range of 100–500 ms poststimulus onset. Because, however, a possibly independent "P2" component was often visible between 100–200 ms poststimulus, all statistical tests were also conducted on areas in the latency range of 200–500 ms poststimulus. For all tests, criterion for significance was $p < 0.05$.

2.1. Condition 1: Normal saline administration/fruit-juice reward to rare target stimuli

Monkeys #1 (14 years), 2 (15 years), 3 (24 years), and 4 (26 years) participated in recordings under Condition 1. All subjects were already accustomed to the recording chamber, the primate restraint chair, and the head restraint at the time of entering the study. For 4–6 weeks, monkeys learned to associate rare tones only with 0.1-mL fruit-juice reward delivered 1200 ms after tone onset through a nozzle at the monkey's mouth. Training was facilitated by the monkeys' prior experience with drinking from the nozzle. Subjects were freed of head restraint halfway through training sessions to assure that they had learned the association between rare tones and juice reward, as evidenced by motion towards the juice nozzle upon hearing the rare tones, but before efflux of juice from the nozzle, as observed over video monitor. Once such anticipatory orientation could be reliably and repeatedly observed, training concluded, and monkeys then had 3 weeks off without exposure to the paradigm. Following this rest period, oddball-paradigm exposure with concomitant EEG recordings commenced and continued for 2 weeks. Fruit-juice reward was delivered following rare tones only. EEG recording was turned off during the first

trial after each rare tone and then turned back on again for the second and subsequent trials. Approximately 1 cc normal saline vehicle control was given by intramuscular injection 1 h prior to measurements. Effects of stimulus category on amplitude of P3-like potentials were assessed by applying separate one-tailed *t*-tests to the rare-frequent and to the novel-frequent difference areas. One-tailed tests were chosen because the P300 is, in part, defined by the one-way criteria of exhibiting larger amplitudes to rare and to novel than to frequent stimuli. Therefore, positive values were anticipated for each difference area, with zero or negative values representing the null hypothesis. Repeated-measures ANOVA was employed to evaluate the effect of electrode site as a within-subjects factor (Fz versus Cz versus PZ) on amplitude of P3-like potentials. One such ANOVA was carried out for the rare-frequent difference area and one for the novel-frequent difference area.

2.2. Condition 2: Normal saline administration/no fruit-juice reward to any stimuli

To test for possible extinction of P3-like cognitive evoked potentials, fruit-juice reinforcement was denied to Monkeys #2–4 for recordings under Condition 2. After recordings under Condition 1 were concluded, each monkey went 3 weeks without recordings or exposure to the paradigm. Then, EEG was again recorded for 2 weeks while the monkey was exposed to the paradigm. Normal saline was administered as in Condition 1 above. No fruit-juice reward was delivered at any time. Combining data from Conditions 1 and 2 for the three monkeys who had taken part in both conditions, effects of juice reinforcement were assessed in a repeated-measures ANOVA with reinforcement (no-juice versus juice) as the within-subjects factor. One such ANOVA was done for rare-frequent and one for novel-frequent difference areas.

2.3. Condition 3: AF102B administration/fruit-juice reward to rare target stimuli

Monkeys #1 and 4 participated in recordings under Condition 3. Under Condition 3, monkeys were given three successively rising doses of the partial direct m1 muscarinic agonist AF102B dissolved in approximately 1 cc normal saline by intramuscular injection 1 h prior to beginning the paradigm. For each monkey, one dose likely to enhance cognition ("cognition-enhancing" dose) and two doses unlikely to enhance cognition ("noncognition-enhancing" doses) were tested; 2.1 mg/kg for Monkey #1 and 0.2 mg/kg for Monkey #4 were selected as cognition-enhancing doses. Noncognition-enhancing doses were 3.0 and 4.5 mg/kg for Monkey #1 and 0.1 and 2.0 mg/kg for Monkey #4. Doses were chosen based on our prior published [30,31] and unpublished work with AF102B in this species. Note that doses were lower for the older Monkey #4 than for the younger Monkey #1 in accordance with our prior findings of lower cognition-enhancing (and cholinergic side effect-inducing) doses of AF102B in older than in younger monkeys. Before commencement of evoked potential recordings on AF102B, monkeys experienced a 3-week rest period, as described above. AF102B recordings were then carried out for 2 successive weeks at each dose, with 3 weeks off between doses. For all doses, fruit-juice reward was delivered following rare tones only. Effects of systemic cholinergic agonism were assessed in a repeated-measures ANOVA with drug (AF102B versus saline) as the within-subjects factor. One such ANOVA was done at each dose for rare-frequent and one for novel-frequent difference areas.

2.4. Condition 4: Guanfacine administration/fruit-juice reward to rare target stimuli

Monkeys #1 and 3 participated in recordings under Condition 4. Under Condition 4, monkeys were given two successively rising doses of the α-2A noradrenergic agonist guanfacine dissolved in approximately 1 cc normal saline by intramuscular injection 2 h prior to beginning the paradigm. For each monkey, one "cognition-enhancing" dose and one "noncognition-enhancing" dose, as described for AF102B above, were tested. 0.001 mg/kg was selected as the cognition-enhancing dose and 0.05 mg/kg was selected as the noncognition-enhancing dose for both monkeys. Doses were selected based on the experience of Arnsten et al. [3] in the rhesus macaque. Appreciable side effects were not anticipated at either dose. Monkeys began with a 3-week rest period, as described above. Guanfacine recordings were then carried out for 2 successive weeks at each dose, with 3 weeks off between doses. Effects of systemic noradrenergic agonism on the amplitude of P3-like potentials were determined as for AF102B in Condition 3 above.

2.5. Condition 5: Repeat of normal saline administration/ fruit-juice reward to rare target stimuli

For Monkeys #1, 3, and 4, recordings were again conducted as in Condition 1, 3 weeks after the final drug treatment for each monkey. P3-areas were compared to P3-areas under Condition 1 (predrug saline treatment) using repeated-measures ANOVA as described for AF102B and guanfacine above (Conditions 3 and 4).

3. Results

3.1. P3-like components of the auditory scalp evoked potential: General

The evoked potential in all four monkeys began with a "P1–N1–P2" sequence of early components (Fig. 1), whereby the "P2" was not always visible. This sequence varied little across the three scalp sites and was evoked by all three auditory stimulus categories in all five conditions. It was followed by a slow, high-amplitude late positivity that varied strongly with stimulus category and drug/reinforcement condition (Figs. 1 and 2). These observations were similar to simian auditory cognitive evoked potentials

in previous reports [7,19,29,34,35,47]. Based on the resemblance to literature findings, the late slow positivity was referred to as the "P300-like positivity" or simply as the "P3." Attempts to divide the "P3" into "P3a" and "P3b" subcomponents were unsuccessful.

3.2. P3-like components of the auditory scalp evoked potential: Conditions 1 and 2

Figure 1 shows scalp potentials evoked by the passive auditory oddball paradigm for two sample monkeys subjected to Condition 1: normal saline administration with juice reinforcement to rare stimuli. Under Condition 1, large P3-like positivities were evoked both by novel (except in

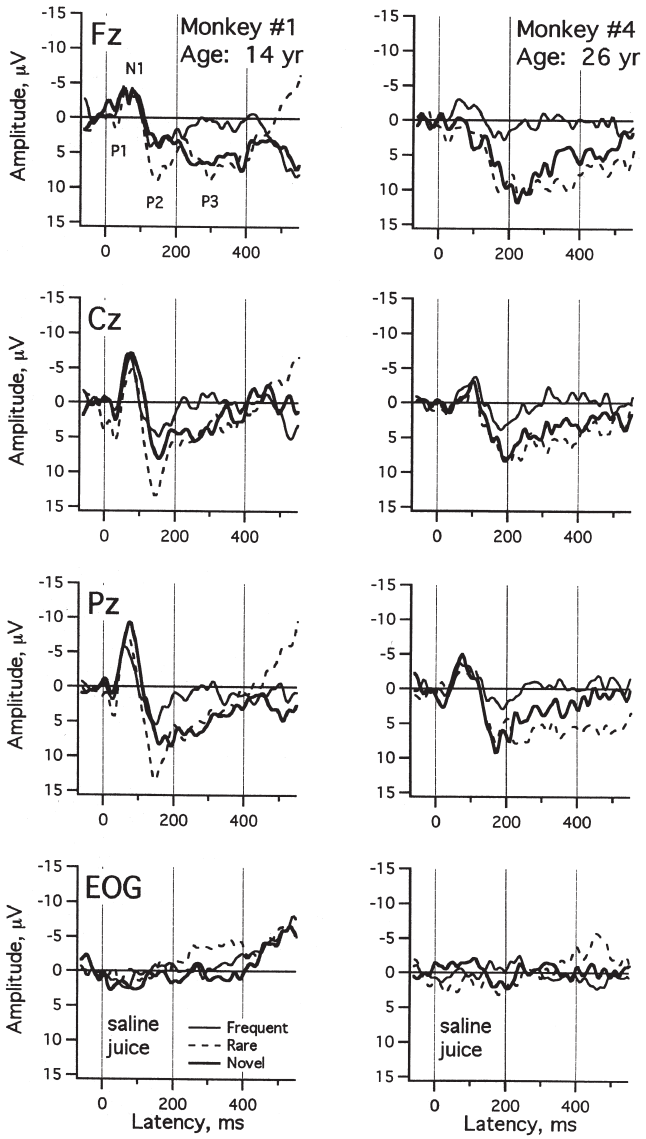

Fig. 1. Scalp-evoked potentials recorded in a passive auditory oddball paradigm with unique novel stimuli under Condition 1 (normal saline administration/fruit-juice reward to rare target stimuli) for one younger (left) and one aged (right) macaque. Greater positivity to rare and to novel stimuli (P300-like component) was observed for both monkeys in the latency range 100–500 ms poststimulus.

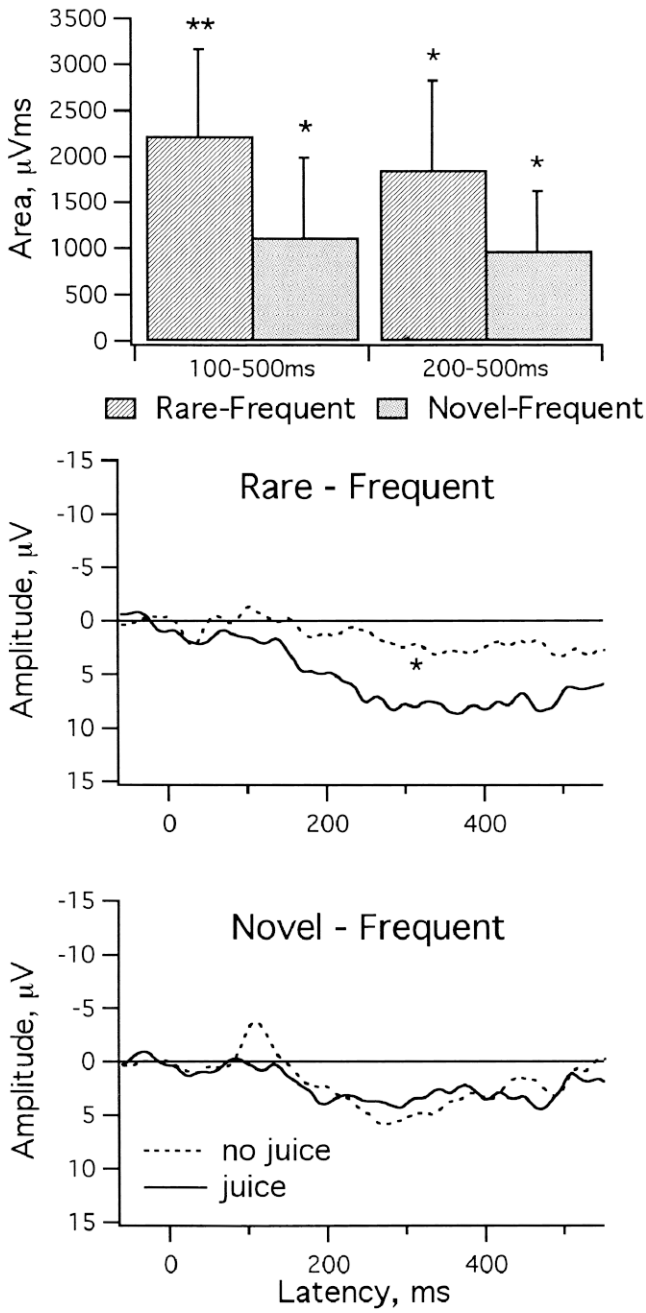

Fig. 2. Effects of stimulus category and fruit juice reinforcement on P300-like monkey auditory cognitive evoked potentials. Top: areas of potentials evoked by rare and novel auditory stimuli (relative to areas evoked by frequent auditory stimuli) in the 100–500-ms and 200–500-ms poststimulus latency ranges. Areas are for Condition 1 (normal saline administration/fruit-juice reward to rare target stimuli) averaged across three midline electrode sites. (Mean of monkeys #1–4. Error bars indicate ± 1 SD.) Areas were significantly larger for rare and for novel than for frequent stimuli in both latency ranges (*$p < 0.05$, **$p < 0.01$).Middle: rare-frequent difference potentials under Conditions 1 ("juice") and 2 (normal saline administration/fruit-juice reward to rare target stimuli withdrawn; "no juice"). Grand average across sites for the three monkeys (#2–4) participating in both conditions. Bottom: the same for novel-frequent difference potentials. Significant reduction of the P300-like positivity was seen for rare (*$p <$ 0.05 in both latency ranges), but not for novel, stimuli when juice was withdrawn.

Monkey #3) and by rare (in all monkeys) stimuli. Repeated-measures ANOVA failed to find an effect of electrode site on either the rare-frequent [100–500 ms, $F(2, 6) = 3.18$, NS; 200–500 ms, $F(2, 6) = 2.93$, NS] or the novel-frequent [100–500 ms, $F(2, 6) = 2.56$, NS; 200–500 ms, $F(2, 6) = 2.88$, NS] difference areas. Therefore, means across the three electrode sites were employed in all subsequent analyses. Rare-frequent and novel-frequent difference areas for Condition 1 averaged across all four monkeys are shown in Fig. 2 for both the 100–500-ms and the 200–500-ms post-stimulus latency ranges. In one-tailed *t*-tests, mean "P3" area was significantly larger to rare [100–500 ms: $t(3) = 4.70$, $p < 0.01$; 200–500 ms: $t(3) = 3.85$, $p < 0.05$] and to novel [100–500 ms: $t(3) = 2.57$, $p < 0.05$; 200–500 ms: $t(3) = 2.98$, $p < 0.05$] than to frequent stimuli.

For the three monkeys subjected to Condition 2: normal saline administration and no juice reinforcement, Fig. 2 also compares grand average difference evoked potentials (rare-frequent and novel-frequent) for Conditions 1 and 2. The removal of fruit juice reward to rare stimuli resulted in a significant drop in the rare-frequent [repeated-measures ANOVA: 100–500 ms: $F(1, 2) = 28.03$, $p < 0.05$; 200–500 ms: $F(1, 2) = 25.60$, $p < 0.05$] but not in the novel-frequent difference area.

3.3. P3-like components of the auditory scalp evoked potential: Conditions 3 and 5

Figure 3 shows the effects of the m1 muscarinic agonist AF102B at cognition-enhancing dose on rare-frequent and novel-frequent difference areas recorded with juice reinforcement to rare stimuli (Condition 3) versus predrug (Condition 1) and postdrug (Condition 5) normal saline control administrations. Results are means of all three electrode sites averaged across Monkeys #1 and 4. Results were similar in both monkeys. Postdrug responses to normal saline did not differ significantly from predrug responses for rare-frequent or for novel-frequent difference areas. The rare-frequent difference area was significantly larger following administration of AF102B at cognition-enhancing dose than following normal saline administration [repeated-measures ANOVA: 100-500 ms: $F(1, 1) = 271.00$, $p < 0.05$; 200–500 ms: $F(1, 1) = 186.07$, $p < 0.05$]. This was not the case for the novel-frequent difference area. The two noncognition-enhancing doses of AF102B (not shown) had no significant effect on either difference area.

3.4. P3-like components of the auditory scalp evoked potential: Conditions 4 and 5

Figure 3 also shows the effects of the α-2A noradrenergic agonist guanfacine at cognition-enhancing dose on rare-frequent and novel-frequent difference areas recorded with juice reinforcement to rare stimuli (Condition 4) versus predrug (Condition 1) and postdrug (Condition 5) normal saline control administrations. Results are means of all three electrode sites averaged across Monkeys #1 and 3. Monkey #1 exhibited a large increase in rare-frequent difference area, as well as a large decrease in novel-frequent difference area, in response to guanfacine, but these changes were not seen for Monkey #3. Consequently, mean rare-frequent and novel-frequent difference areas for guanfacine at cognition-enhancing dose did not differ significantly from normal saline areas. The noncognition-enhancing dose of guanfacine (not shown) had no significant effect on either difference area. Postdrug responses to normal saline did not differ significantly from predrug responses for rare-frequent or for novel-frequent difference areas.

4. Discussion

The major findings of this study were: (1) The passive auditory oddball paradigm with unique novel stimuli elicits an evoked potential positivity at midline scalp sites in the 100–500-ms latency range in the bonnet monkey (*Macaca radiata*) that resembles the human P300 as well as P300-like components observed in other monkey species; (2) the amplitude of the monkey "P3" to reinforced rare tones was significantly enhanced following systemic administration of the m1 muscarinic agonist AF102B at cognition-enhancing dose; (3) a nonsignificant increase in "P3" amplitude to reinforced rare tones was associated with systemic administration of the α-2A noradrenergic agonist guanfacine at a cognition-enhancing dose. Taken together, these findings strengthen the view that macaques possess late positive evoked potential components similar to the human P300, and suggest that these components may be influenced by m1 cholinergic and α-2A noradrenergic agonism.

The first major finding was that *M. radiata* exhibits a P3-like auditory evoked potential. As mentioned above, both the "P3" positivity of the present study [7,19,47], and its various preceding auditory evoked potential components [7,19,34,35,47] resembled the waves recorded in other macaque species. As all macaques are similar in brain anatomy and behavior, similarity in their cognitive evoked potentials is to be expected. The homology of these monkey P3-like potentials to the human P3 has been discussed in detail [33]. Briefly, behavioral paradigms such as auditory oddball elicit a similar sequence of extracranial evoked potential components in monkeys and humans. The sequence includes a late positivity (P300) that is larger to infrequently presented than to frequently presented stimuli.

In the present study, significantly larger "P3" positivities were evoked by rare tones associated with fruit-juice reinforcement and by nonreinforced unique novel stimuli than by nonreinforced frequent tones. Classically, human P3b generation is associated with stimuli that are infrequent and attended [13,21,41], criteria that also seem to apply in the monkey. Several investigators [7,18,47] have also found macaque late positivities that were larger to rare than to frequent auditory stimuli in oddball paradigms. There is also evidence that the amplitude of the macaque P3 is greater to attended than to nonattended stimuli [33]. Linkage to rein-

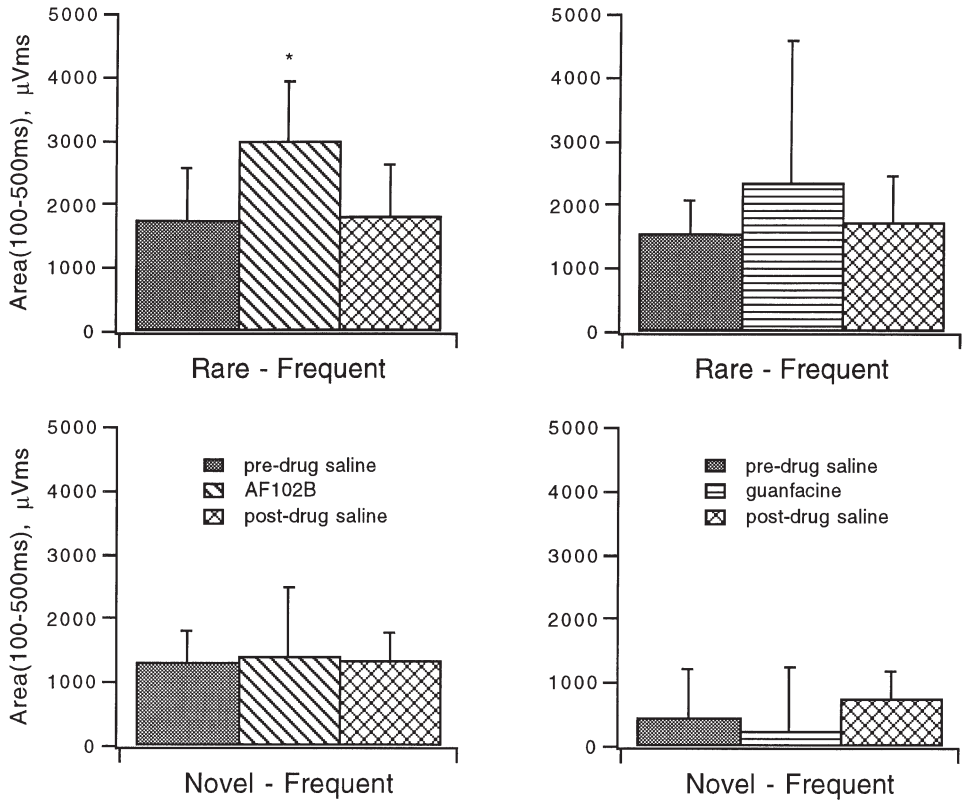

Fig. 3. Effects of cognition-enhancing intramuscular doses of a central m1 muscarinic agonist (AF102B) and of a central α-2A noradrenergic agonist (guanfacine) versus normal saline control on P300-like auditory-evoked potentials in monkeys. Plotted are rare-frequent (upper) and novel-frequent (lower) difference areas in the 100–500-ms poststimulus latency range averaged across electrodes. Left: Conditions 1 (predrug saline), 3 (cognition-enhancing dose of AF102B), and 5 (postdrug saline). Mean of monkeys #1 and 4. Right: Conditions 1, 4 (cognition-enhancing dose of guanfacine), and 5. Mean of monkeys #1 and 3. (Error bars indicate ± 1 SD in all plots.) Fruit-juice reward to rare target stimuli was delivered under each condition. Pre- and postdrug saline responses did not differ significantly for any comparison. The P300-like response to rare stimuli was significantly increased (*$p < 0.05$) at the cognition-enhancing dose of AF102B (2.1 mg/kg for monkey #1, 0.2 mg/kg for monkey #4). The cognition-enhancing dose of guanfacine (0.001 mg/kg for both monkeys) also increased the rare-frequent difference area, but not significantly. The P300-like response to novel stimuli was not affected significantly by either drug. Similar results were found in the 200–500-ms poststimulus latency range.

forcement raises the probability that an animal will attend a stimulus. Thus, the rare, reinforced stimuli in the present study might be expected to evoke large P3-like positivities analogous to the human P3b. Unique novel stimuli that evoke the human P3a are infrequent, are not linked to instructions or to reward, and are attended more reflexively than by intent [45]. Such potentials have also been seen in macaques [1,35]. Paller et al. [35] hypothesized that rare complex sounds may elicit large P3s because such stimuli attract more attention than do simpler, more frequent tones. Thus, the unreinforced novel stimuli in the present study might be expected to evoke large P3-like positivities analogous to the human P3a. However, due to component overlap, it is difficult to judge the extent to which the P3-like responses recorded to rare and to novel stimuli in the present study may reflect P3a, P3b, or a superposition of both late positive components.

Monkey "P3" to rare tones in the present study exhibited apparent amplitude extinction when reinforcement to that stimulus type was removed. Monkey "P3" to nonreinforced novel stimuli was not significantly affected by the presence or absence of reinforcement to rare tones. This essentially reproduces the finding of Glover et al. [19] for rare stimuli in *M. fascicularis*, and extends it to positive, in addition to negative, reinforcement. The smaller, remaining "P3" to unreinforced rare tones in both studies may be interpreted as an incompletely extinguished residue of the earlier response to the reinforced tones or, alternatively, as a modest response that even unreinforced rare tones evoke by virtue of their rarity.

The second major finding was that "P3" amplitude to rare, reinforced tones was enhanced by administration of a systemic cholinergic agonist at "cognition-enhancing" dose in two monkeys. Others have reported effects of the central muscarinic cholinomimetic levo-acetyl-carnitine on the macaque P3 [1,2,32], as well as opposing effects of the muscarinic blocker scopolamine [2]. Similar findings have been noted in humans [10,11,20,26–28,43,48], although some workers found no effect of central cholinesterase inhibitors on the P3 of Alzheimer's patients [8,49]. The

present study suggests that central m1 muscarinic mechanisms may participate in cholinergic amplitude-building effects on the primate auditory P3. Significant "P3" amplitude increase was observed for AF102B only in the cognition-enhancing dose range, which suggests that the P3 may represent an electrophysiological concomitant of cognitive actions of m1 cholinergic agonism in primates. Significant effects of AF102B on "P3" amplitude were not seen for unreinforced novel sounds, suggesting that the brain response to these stimuli reflected in the P3 may have a distinct neuropharmacological profile.

The third major finding was that "P3" amplitude to rare, reinforced tones was enhanced by a systemic noradrenergic agonist in only one of two monkeys. Several reports suggest that central α-2 noradrenergic inhibition reduces the amplitude of human and monkey P3 [14,22,38–40,46,47]. Guanfacine is believed to excite the primate prefrontal cortex via α-2A noradrenergic agonism [6,12] and thereby to improve focused attention and to reduce distractibility to paradigm-irrelevant stimuli [4]. These properties, however, are strongly dose dependent and the dose–effect curve varies from subject to subject [3]. In the present study, it is conceivable that guanfacine-induced sharpening of attention and lowered distractibility led to increased "P3" amplitude to reward-associated rare stimuli and to reduced "P3" amplitude to reward-irrelevant novel stimuli in Monkey #1. Monkey #3, in contrast, may have experienced no such effects under guanfacine because neither of the two doses tested were within the effective portion of the dose–effect curve for that subject.

The present study has several limitations. The overall investigation involved only four monkeys, and the two drug interventions involved only two subjects each, implying that our findings need to be reproduced in larger numbers of subjects. One subject (Monkey #3) was atypical in that she did not exhibit an appreciable "P3" to unique novel auditory stimuli under any test condition. Neither did this monkey show the anticipated enhancement of "P3" amplitude to rare tones in response to guanfacine exhibited by Monkey #1. These findings point to heterogeneities in intersubject P3 behavioral physiology and drug response that need to be mapped out in larger simian subject populations. Another limitation was that drug agents were given in ascending dose series, introducing a potential bias from order effects. The return of postdrug "P3" amplitudes to predrug levels (Fig. 3) suggests that the study measures pharmacologically induced short-term changes in stable drug-free responses; however, this should be verified systematically in future experiments. A further limitation was that drug agonist effects on the "P3" were not confirmed through administration of corresponding antagonists. It is, however, fairly well accepted that guanfacine works via an α-2A mechanism in the primate neocortex [6], and that AF102B, at least in rodents, acts selectively at central m1 receptors [15,16]. Notwithstanding these limitations, the present study further supports the existence of P300-like late positive cognitive-evoked potentials in nonhuman primates and suggests that m1 and α-2A receptor subtypes may play a role in central muscarinic and noradrenergic influences on these potentials. Amplitude augmentation of the primate P3 may accompany attention- and memory-improving actions of m1 and α-2A agonists.

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