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ORIGINAL INVESTIGATION

E.A. Witte · R.T. Marrocco

Alteration of brain noradrenergic activity in rhesus monkeys affects the alerting component of covert orienting

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Abstract Experiments were conducted to elucidate the role of the noradrenergic neurotransmitter system in arousal and the orienting of attention. Rhesus monkeys were trained to perform a peripherally cued, covert orienting task for juice reward, and their manual reaction times (RTs) to visual stimuli were measured. The effects of parenteral injections of the α-2 adrenergic agonists clonidine and guanfacine, and normal saline were compared on the covert task. We assessed 1) overall error rates, 2) the difference in RTs between validly and invalidly cued trials (validity effect), 3) the difference in RTs between neutral and no-cue trials (alerting effect), 4) target location (visual field), and 5) cue-target interval. Changes in noradrenaline levels produced by clonidine (and to a lesser extent guanfacine) significantly decreased the alerting effect, and lowered RTs to stimuli in the left visual field, but did not change the validity effect, suggesting that noradrenaline is involved in maintaining non-spatial, sensory readiness to external cues but not in the shifting of the attentional focus.

Key words Attention · Alerting · Clonidine · Guanfacine · Monkey · Reaction times

Introduction

Attending to complex external and internal environments places contrasting demands on attentional systems. Temporally, the systems must respond to brief and extended events (i.e., be capable of phasic alerting and sustained vigilance); spatially, the systems must be capable of attending to local or global events. There is a variety of evidence that the properties of the noradrenergic neurotransmitter systems may be well suited to mediate these attentional demands (see, e.g., Posner and Petersen 1990; Robbins and Everitt 1995). For example, the axonal projections of locus coeruleus (LC) neurons are widely distributed in the cortex and brainstem and may be suited to the spatial demands of information processing within brain systems (Posner and Petersen 1990). In addition, the ability of the LC system to respond both transiently to novel stimuli (Aston-Jones et al. 1994) and in a maintained fashion during vigilance (Rajkowski et al. 1994) would appear to fit the temporal demands of information processing as well. And finally, lesions of the dorsal noradrenergic bundle impair choice performance primarily during conditions in which distractors are present during stimulus discrimination (Cole and Robbins 1992), substantiating noradrenaline's role in attentional behavior.

Recent studies in humans also support the involvement of the noradrenergic system in spatial and sustained attention. Clonidine, an α_2 adrenoceptor agonist, has been shown to broaden the attentional focus (Coull et al. 1995a), impair performance on tests of sustained attention (Coull et al. 1995b) and increase attention lapses (Smith and Nutt 1996). Clark et al. (1989) assessed the effect of clonidine on reaction times (RTs) to visual targets in a centrally cued, attention shifting task. In this study, targets were cued validly (cue and target at the same location) or invalidly (cue and target at different locations). An index of the costs and benefits of attentional allocation is the *validity effect*, the difference in reaction times between invalid and valid cue conditions. The Clark et al. (1989) study found that clonidine reduced the validity effect. These authors also presented a neutral cue, which cued both spatial locations used, and therefore, was spatially uninformative. Clonidine had no differential effect (compared to valid cue trials) on neutral cue RTs.

Spatially uninformative visual or auditory cues that alert the subject to the impending target have been shown to lower sensory thresholds and prepare the organisms to make a response, thereby reducing RTs but increasing error rates (Posner 1978). The physical characteristics of the cue are relatively unimportant for the

E.A. Witte[1] · R.T. Marrocco (✉)
Institute of Neuroscience, 1254 University of Oregon, Eugene, OR 97403-1254, USA

Present address:
[1] Mental Retardation Center, Neuropsychiatric Institute, University of California, Los Angeles, CA 90024, USA

warning effect (Posner and Cohen 1984), suggesting that the cue may have its main effect on pathways controlling response citerion or preparedness, rather than on the sensory pathways stimulated (Jonides and Yantis 1988). Previous studies have used neutral cues to measure the benefits or costs of spatial orienting (e.g., Clark et al. 1989). Although it is likely that the neutral cue elicits no attentional movement, it is doubtful that it is truly "neutral" (Jonides and Mack 1984), and it will be termed a double cue hereafter. However, determining what information is provided by the double cue is not straightforward. The abrupt onset of the double cue may provide specific temporal information about target appearance, or it may increase the preparedness of the organism to respond to the next stimulus (Jonides and Yantis 1988). Comparing double cue RTs with those from single cue trials may be misleading, because the warning response may be altered by spatial orienting. To understand better the nature of the warning response to double cues, it is necessary to compare double cue RTs with uncued RTs, which lack cue onset and do not cause spatial orienting.

The purpose of the present set of experiments is to examine the effects of altering the levels of brain noradrenaline in monkeys on the detection of visual targets in a peripherally cued, covert orienting task. We report measurements of the effects of drugs on manual RTs to valid, invalid, double and no-cue trials. Since the monkeys signal target detection with a manual bar press, the tasks is likely to assess parietal and superior frontal attentional function (Corbetta et al. 1993). Because of the demonstrated role of the LC in phasic alerting (Aston-Jones et al. 1994), we postulated that changing the levels of noradrenaline would affect primarily the temporal component of visual cues. A preliminary report of some of these findings has been published (Witte et al. 1992).

Materials and methods

The details of our general procedures have appeared elsewhere (Witte et al. 1996). The procedures used in this research followed those mandated in the Guide for the Care and Use of Laboratory Animals (National Research Council 1996) and were supervised by the university veterinarian.

Subjects

The subjects were two female rhesus macaques (*Macaca mulatta*) between 12 and 14 years of age that weighed between 12 and 15 lb during the study. These animals were trained using a water control reinforcement schedule. During a 2-week period prior to data collection, ad libitum water consumption was measured to determine the animal's daily basaline consumption. During data collection, water and removed from the animal's cage the evening before the experiment and the fluid intake recorded following the next day's experiment. If the animals failed to drink their baseline amount, the difference between intake and baseline was given in the home exercise cage about 2 h after the session. Thus, in any 24-h period, the animals consumed their normal amount of fluid. Body weight, skin turgor, activity levels, and food consumption were also checked on a daily basis during the studies to be sure that weight loss or symptoms of other illnesses did not occur. As we frequently supplemented lab chow with peanuts, raisins, and fresh fruit, weight gains were most frequently observed.

Drug administration

α_2 adrenergic agonists

The α_2 adrenergic agonists clonidine hydrochloride (0.0001, 0.001 mg/kg; Sigma, St. Louis, Mo., USA) and guanfacine hydrochloride (0.00001, 0.0001 mg/kg; Wyeth-Ayerst, Princeton, N.J., USA were diluted in sterile saline and injected IM 15 min (clonidine) or 2 h (guanfacine) prior to testing. Clonidine has been shown to be non-selective for α_2 receptor subtypes, while guanfacine appears selective for the α_{2A} site (Uhlen et al. 1995), is more selective overall at the α_{2A} receptor than clonidine, and has a high affinity for imidazoline (I1) receptors as well (e.g., Hieble and Ruffolo 1995). Clonidine is thought to act preferentially on presynaptic α_2 receptors at low doses and at postsynaptic receptors at higher doses (Arnsten and Cai 1993). At low doses, guanfacine may act preferentially at postsynaptic receptors but at presynaptic receptors at higher doses (Arnsten et al. 1988). In pre-experiment testing, none of the drugs caused sedation, as rated by two observers naive to the drug condition of the animal. Three-to 5-day intervals were allowed between drug sessions.

α-2 adrenergic antagonists

In order to test the specificity of our drugs for action at the α_2 receptor, we co-administered idazoxan hydrochloride (0.006 mg/kg; Sigma) or yohimbine hydrochloride (0.02 mg/kg; Lloyd, Shenandoah, Iowa, USA) with the agonists in an attempt to reverse the effects of the agonists in monkey A. These doses have been shown to be behaviorally effective fot this animal in this task (Davidson et al. 1994). While both these antagonists act on α_2 receptors, they differ in at least two ways: 1) they may have differential selectivities for the α_2 receptor subtypes (Angel et al. 1990; Lanier et al. 1991; Wamsley et al. 1992) and 2) idazoxan, but not yohimbine, has a high affinity for imidazoline receptors (Hieble and Ruffolo 1995). The drugs were administered IM, 15 min prior to the session.

Saline control

As a vehicle control, sterile saline was injected IM 15 min prior to four sessions. As one of our goals was to measure alerting and arousal, we were concerned that the injection itself might change the animal's arousal level and influence the results. We therefore injected saline 2 h prior to three additional saline sessions with monkey A. No differences were observed between the saline data from these two injection intervals, and the data were combined for later analysis. We also used a 15-min delay between saline injections and data collection with monkey B. To minimize between session variance, we randomly intermixed saline trials with drug trials and compared the latter with controls run within the same week(s). Two additional experimental replications using the high doses of guanfacine and clonidine were done with monkey B.

Clonidine and guanfacine were given before three sessions at each dose to each monkey. In the antagonist study with monkey A, there were six session types: clonidine alone, clonidine plus idazoxan, clonidine plus yohimbine, guanfacine alone, guanfacine plus idazoxan, and guanfacine plus yohimbine. Two sessions were devoted to each session type. The order of drug administration was randomized across sessions. Saline was given before eight sessions. A total of 32 sessions (16796 trials) were run with monkey A and 20 sessions (9256 trials) for monkey B.

Surgery

Using pentobarbitone anesthesia and sterile procedures, a head fixation socket was attached surgically to each animal several

weeks before the start of training. Stainless steel screws coated with dihydroxylapatite were used to anchor the socket to the skull and dental acrylic was applied to cover the screws and exposed skull. In addition, a scleral eye coil (Judge et al. 1980) was placed in one eye. Post-operatively, systemic antibiotics, ophthalmic antibiotic ointment, and pain relieving medication (buprenorphin) were administered prophylactically.

Apparatus

The monkey was placed into a primate chair (Crist Instruments, Damascus, Md., USA) at the start of each session. Its head was immobilized by attaching it to the chair, using a bolt designed to fit into the head socket. Two vertical and two horizontal magnetic field coils were placed around the animal's head and the upper portion of the primate chair. The monkey was then placed into a large Formica chamber with a glass front window. The animal viewed stimuli on a computer monitor placed one meter from its eyes. A Sony video camera allowed the experimentes to monitor the animal's behavior continuously.

A Northgate 386 computer was used to run CORTEX, a program for conducting neurophysiological and behavioral experiments that was provided to our laboratory by Robert Desimone of the National Institutes of Health. Graphics were produced with a Pepper SGT-plus graphics card (Number Nine Computer Corp, Lexington, Mass., USA), and a D/A board (Computer Boards, Cambridge, Mass., USA) was used for measuring eye position, registering bar contact closures and controlling reward solenoids.

Behavioral training

The details of the training protocol are presented elsewhere (Witte et al. 1996). Briefly, after the animal was accustomed to the primate chair, fixation training was begun. The animal learned to maintain fixation within an area of 0.1 are-deg around a small spot on the monitor for about 1 s. Successful fixation was rewarded with water or juice. The animal next learned to press a bar that triggered a microswitch and produced the fixation spot. Successful fixations of criterion duration were rewarded, but failure to make or maintain fixation, or temporally inappropriate presses or releases caused the trial to be arborted. Once this training was complete, the animal began training on a modified version of the CTD developed by Posner (1980).

Cued covert target detection task

In this version of the cued target detection task, each animal learned to initiate the trial by pressing the bar, which displayed a fixation point and two flanking boxes (see Fig. 1A). The fixation point was 0.2 arc-deg in diameter and the boxes were each 1.0 arc-deg on a side and centered 5.7 arc-deg from fixation. The luminance of the boxes was 50 cd/m^2 and the background was 0.1 cd/m^2. After 500–1500 ms (determined randomly), one or both of the boxes increased in luminance to 75 cd/m^2, which served as a cue for the animal to move its attentional focus to the cue without breaking fixation. At 100, 400, or 700 ms after the cue's onset (cue-target interval, CTI), a target (50 cd/m^2) was presented inside one of the boxes (Fig. 1C). Both the cue and target remained on until the bar was released (Posner et al. 1988). The temporally overlapping cue and target were used to facilitate acquisition of the task. Control studies in which the cue and target did not overlap showed no significant RT differences with the overlap results. The animal was rewarded for responding a rapidly as possible to the target and no later than 850 ms of its appearance. Reaction times under 100 ms were discarded as anticipatory. Loss of fixation or incorrect bar performance caused the computer to abort the trial.

Trials in which the target appeared in the same visual hemifield as the cue are termed, *valid* trials and these occurred 57% of the time; those trials in which the target appeared in the opposite

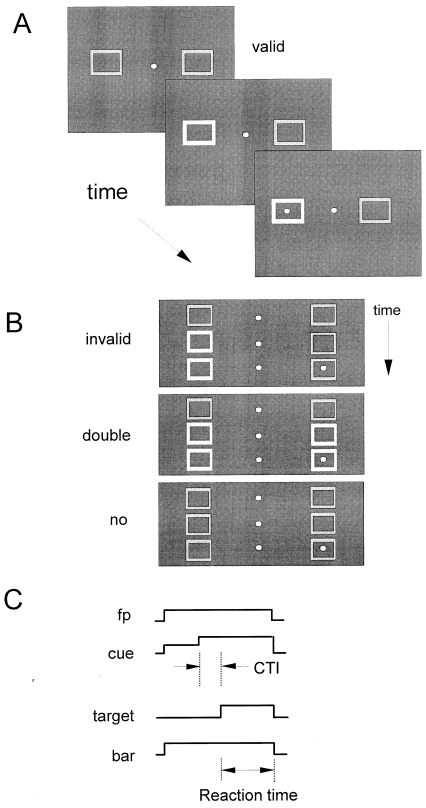

Fig. 1 **A** Schematic of stimulus displays for valid cue trials. **B** Stimulus sequences for invalid, double, and no-cue trials. **C** Timing of events during a correctly executed trial. *FP* fixation point; *CUE* box brightening; *CTI* cue-target interval; *TARGET* small spot inside cue; *BAR* manual bar press and release; *Reaction time* time between target appearance and bar release. See text for details

hemifield as the cue are termed *invalid* (Fig. 1B), and made up 14.3% of all trials. The ratio of valid to invalid trials was 4:1. *Double* (sometimes referred to as "neutral") cue conditions made up 14.3% of trials). The double cue condition was compared to the condition in which *no* cue (14.3% of trials) appears before the target. The difference in reaction times between these two conditions is an index of the benefit of the cue onset in the absence of orienting and is termed the *alerting effect.* It is assumed that the information provided by cue onset influences RTs jointly with spatial information in single-cue trials.

Since the double cue is not predictive of target location, no systematic shifts in attentional focus occur (Posner 1978). However, we assume that attention shifts when the target appears, and decreases in double cue RTs are likely to reflect the animal's increased readiness to respond. Increases in double cue RTs would suggest the reverse. Since the double cue is spatially uninformative, its benefit should be unrelated to the proximity to the target, a prediction that has recently been empirically verified (Fernandez-Duque and Posner 1996). RTs for the non-cue condition are indicative of the target's salience only and should be influenced only by changes in physical stimulus parameters, or by general state of arousal or response preparedness (Posner 1978).

Behavioral rating

Fifteen minutes aber the injections, the overt behavioral effect of each drug was rated by two observers naive to the drug condition but familiar with the animal's usual behavior. Sedation was rated on a five-point scale, as per the methods of Arnsten et al. 1988, in

which a score of "0" indicated normal arousal, "1" indicated that the animal was quieter than normal (e.g., lower frequency of skeletal and oculomotor activity), "2" that the animal was sedated (e.g., drooping eyelids), "3" that there was intermittent sleeping, and "4" that the animal was too sedated to test. EEG measures of arousal were not taken because of the physical obstruction to the cortical surface posed by the dental acrylic that surrounded the head socket. Ratings were not made formally during the antagonist experiments, but informal observation suggested no marked differences from drug ratings during the single drug experiments.

Data analysis

RTs for correct trials were analyzed separately from those for incorrect trials. The data for each monkey were analyzed separately as intitial analyses of overall RTs showed that the monkeys reacted in the opposite fashion (increased versus decreased RT) to the same drug. A repeated measures ANOVA was used to examine the data. In separate analyses, but single trial RTs and mean session RTs were used as the dependent variable and drug, cue, cue-target interval were used the as independent variables. No significant differences were found for the two dependent variable analyses for the data considered here and the results from the trial analysis are reported. The significance of drug effects is reported in reference to control saline values. Validity and alerting effects were determined for each session by subtracting the mean RTs for the valid trials from the invalid trials and the mean double cue RTs from the mean no-cue RTs, respectively. The mean session values were then averaged across identical sessions. Post-hoc comprisons were performed with the Tukey HSD statistic on the validity and alerting effects and other comparisons of interest. Response costs were calculated by subtracting double-cue RTs from invalid due RTs; benefits were determined by subtracing valid cue RTs from double-cue RTs.

Results

General sedation

In sessions using clonidine or guanfacine, the average sedation ratings for both subjects were: saline control sessions=0.25, 0.20; high dose of clonidine=0.18, 0.22; low dose of clonidine=0.28, 0.14; high dose of guanfacine=0.36, 0.25; low dose of guanfacine=0.08, 0.19. These data suggest that little if any obvious sedation was produced by noradrenergic agonists with the doses used in this study.

Accuracy

Systemically administered drugs may produce general behavioral effects, including alterations in arousal, motivation level, or accuracy, in addition to specific effects on attention and alerting. These "side effects" may reduce the total number of trials correctly completed under each drug. To assess the effects of the drugs on accuracy, we computed the proportion of trials completed correctly for each drug (averaged across drug dose), and compared these to saline control sessions. The percentage of correct trials for saline, guanfacine, and clonidine for monkey A was 71%, 66%, and 80%, respectively, and for monkey B, 71%, 67%, and 71%, respectively. These non-significant differences may be due partly to intersession differences which, in the present study, were as much as 8%. We concluded that accuracy was not affected in a major way by guanfacine or clonidine in either monkey.

Intersession differences may reflect varying levels of motivation and/or arousal and are difficult to control. If we use the total number of trials generated per session as an index of motivation and/or arousal, there was as much as a twofold variability between sessions. Using overall mean RT as an index of motivation/arousal, the intersession variability was approximately 5%.

Main effects: drug, cue type, cue-target interval

The main effect of cue type was significant [monkey A, $F(3, 16795)=12.27$, $P<0.0001$; monkey B, $F(2, 9255)=16.55$, $P<0.0001$). As in previous work (Witte et al. 1996), valid cue RTs were faster than invalid cue RTs, and double cue RTs were faster than no-cue RTs, while RTs for valid cue trials were faster than those for double cue trials. Both monkeys also showed a significant main effect of drug [monkey A, $F(3, 16795)=44,46$, $P<0.0001$; monkey B, $F(3, 9255)$, $P<0.0001$] (Fig. 2); clonidine increased RTs, and guanfacine decreased RTs.

With increasing CTI, the temporal uncertainty about target presentation was reduced [monkey A, $F(2, 16795)=142.17$, $P<0.0001$; monkey B, $F(2, 9255)=60.56$, $P<0.0001$], indicating that the monkeys benefitted from the increased predictability of target appearance at longer intervals. RTs declined on average by about 60 ms in saline trials. No evidence for a slowing of RTs was seen at the longest intervals.

Hemispheric anatomical asymmetries are not commonly reported in studies of monkey brains. However, functional differences established by patterns of usage may be present. Therefore, we asked whether stimuli presented to the left or right visual fields produced the same behavioral reaction times. Overall, the visual fields were essentially equivalent in their perceptual salience and no significant differences in RTs were seen in either monkey in control conditions.

Drug by cue interaction

One of the central questions of this study was whether the drugs affected alerting and validity effects. Our results showed a significant two-way interaction of cue and drug for both monkeys [monkey A, $F(6, 16795)=1.91$, $P=0.0014$; monkey B, $F(6, 9255)=3.44$, $P=0.0001$]. Clonidine and guanfacine did not affect the orientation of attention, as judged by the lack of significant change in the magnitude of the validity effect (see Fig. 3A and Table 1). In contrast, clonidine significantly reduced the size of the alerting effect in a dose-dependent fashion (Tukey, $P<0.01$, Fig. 3B). On average, the high dose of clonidine changed the alerting effect for monkey A by 25 ms (from +15 to −10 ms), and for monkey B by 30 ms (from +45 to

Table 1 Effects of cue type and drug dose on RT (SEM)

	Monkey A					Monkey B				
	Saline	Clonidine		Guanfacine		Saline	Clonidine		Guanfacine	
		Low	High	Low	High		Low	High	Low	High
Valid	433 (2.3)	440 (4.1)	440 (3.7)	390 (3.8)	422 (3.6)	389 (2.8)	415 (4.0)	412 (3.7)	374 (4.1)	394 (5.1)
Invalid	455 (4.9)	474 (8.3)	474 (7.0)	412 (5.4)	444 (7.8)	455 (4.7)	473 (7.5)	480 (7.9)	425 (9.2)	440 (10.5)
Double	431 (4.8)	453 (8.5)	466 (7.8)	382 (5.3)	429 (5.9)	415 (5.1)	464 (8.8)	448 (7.9)	411 (8.4)	428 (9.7)
No	446 (4.9)	458 (8.2)	456 (7.9)	403 (5.5)	412 (7.4)	460 (5.0)	483 (7.8)	462 (7.2)	453 (7.8)	472 (11.2)

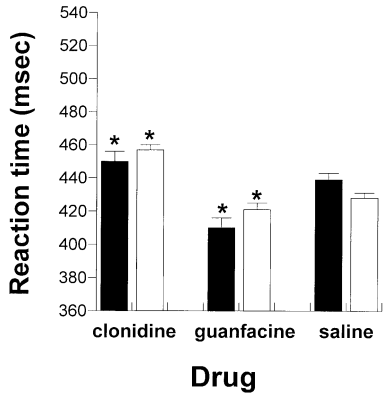

Fig. 2 Overall main effect of drugs for monkey A (*black bars*) and monkey B (*unfilled bars*). Reaction time (*RT*) is averaged across all trials. Error bars=1 SEM. *Asterisks* indicate significant difference between drug and control of P=0.0001

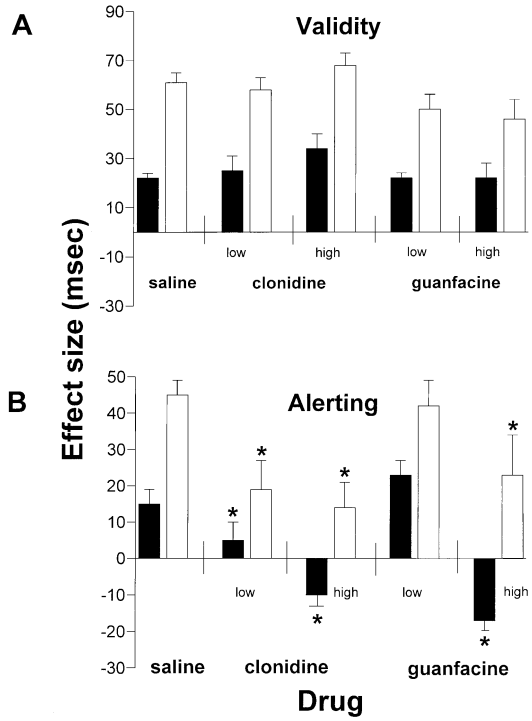

Fig. 3A, **B** Effects of clonidine and guanfacine on validity and alerting effects. Validity effect size determined by subtracting valid trial RTs from invalid trial RTs; alerting effect size computed by subtracting double cue trial RTs from no-cue trial RTs. Numbers beneath x-axis represent drug dosages in mg/kg. Error bars=1 SEM. *Asterisks* represent significant differences between drug and saline control trials. * P<0.01. See text for details

+15 ms). For monkey A, the high dose of guanfacine reduced the alerting effect by 33 ms (+15 to –18 ms); for monkey B, the reduction was only 2 ms. To determine whether this small effect in monkey B was due to inadequate dosage or some other variable, we ran a second, identical series of trials with the same dose of guanfacine. In this series, the overall reduction in RT was similar to that in the first guanfacine session (415 versus 420 ms). In addition, the behavioral sedation ratings, the total number of trials completed (1818 versus 1745), and the accuracy (67 versus 69%) were similar. However, a reduction in the alerting effect of 45 ms was found (+18 to –27 ms). We therefore averaged the data from the two sessions and the result is shown in Fig. 3B. In contrast, replications of the high clonidine dose in the same monkey showed comparable negative alerting effects in both data sets. What factors contributed to the differences in monkey B's guanfacine data are as yet unclear. In summary, the reduction of the alerting effect by alteration in the levels of noradrenaline with clonidine and to a lesser extent guanfacine was similar in both monkeys.

Alteration of the alerting effect can occur by two means, a change in either double-cue or no-cue RTs. Post-hoc comparisons of the data in Fig. 3 (see Table 1) show clearly that the change in alerting effect for both drugs is a result of a significant increase in RTs for double cues (Tukey, monkey A, P=0.02; monkey B, P=0.03). Change in no-cue trial RTs, if present, were usually smaller than those seen for the double cue trials.

Drug by CTI interaction

To determine whether the drugs altered the rate at which temporal uncertainty of target presentation was de-

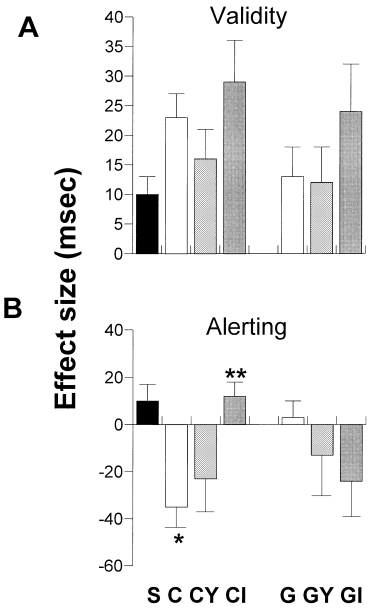

Fig. 4A, B Effects of antagonists of clonidine and guanfacine on validity (**A**) and alerting (**B**) effects for monkey A. *S* (*black bars*)=saline control; *C* (*unfilled bars*)=clonidine (0.001 mg/kg); *CY* (*light grey bars*)=clonidine+yohimbine (0.02 mg/kg); *CI* (*dark grey bars*)=clonidine+idazoxan (0.006 mg/kg); *G* (*unfilled bars*)=guanfacine (0.0001 mg/kg); *GY* (*light grey bars*)=guanfacine+yohimbine; *GI* (*dark grey bars*)=guanfacine+idazoxan. *Single asterisk* indicates significant reduction (P=0.001) of alerting effect by clonidine. *Double asterisk* indicates significant difference (P=0.009) between clonidine trials and clonidine+idazoxan

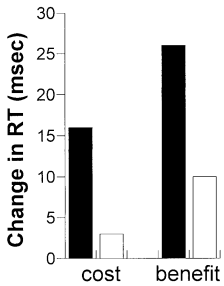

Fig. 5 Cost-benefit analysis for high dose of clonidine. Cost is computed by subtracting double trial RTs from invalid trial RTs. Benefit is computed by subtracting valid trial RTs from double trial RTs

creased, we compared the effects of drugs on RTs at different CTIs. Each animal showed a statistically significant decline in RTs over the range of CTIs tested for the saline condition [monkey A, $F(6, 16795)=5.11$, $P<0.0001$; monkey B, $F(6, 9255)=3.99$, $P<0.0001$]. The same overall pattern was seen for each drug and there was no difference between saline and drug conditions. In summary, our data provide little support for hypothesis that the effects of noradrenergic drugs are time-dependent across the intervals used in this study.

Receptor specificity of noradrenergic results

Clonidine could exert its actions at receptors other than the α_2 adrenoceptors. In addition, the adrenergic agonists can bind to α_2 adrenoceptors in several different locations, including those on the LC cell body, presynaptic nerve terminal, or postsynaptic cell body (Dennis et al. 1987). Activation of postsynaptic adrenoceptors might lead to different behavioral effects than activation of the presynaptic autoreceptors (Arnsten and Cai 1993). In order to test the specificity of clonidine and guanfacine, we co-administered these drugs with two α_2 adrenergic antagonists with different degrees of specificity: idazoxan, which binds preferentially to presynaptic receptors (Dennis et al. 1987), and yohimbine, effective at both pre- and postsynaptic receptors (Goldberg and Robertson 1983).

The antagonists differed in their ability to reverse the effects of clonidine and guanfacine (see Fig. 4). Like the main clonidine experiments, no significant alterations of validity scores were seen. No antagonism of validity scores was evident for either antagonist (Fig. 4A). However, clonidine (C, Fig. 4B) produced a significant reduction in alerting ($P<0.001$) that was not antagonized by yohimbine (CY; $P=0.41$) at the dose used but completely reversed by idazoxan (CI; $P=0.009$). Guanfacine produced a small reduction in alerting (G; $P=0.09$) which neither yohimbine (GY) nor idazoxan (GI) was able to antagonize ($P=0.72$, $P=0.85$, respectively). Thus, our results provide some evidence that clonidine's effect on the alerting effect is mediated by action at presynaptic α_2 adrenoceptors.

Cost-benefit analysis

Another method used widely in the literature to calculate the effect of attentional orienting is to determine whether noradrenergic drugs alter response costs and/or benefits (but see Jonides and Mack 1984). In calculating costs and benefits (see Materials and methods), saline drug sessions were subtracted first from those for drug sessions to normalize the data (see Table 1). The results for clonidine are shown in Fig. 5. The pattern of results was the same for both monkeys. Monkey A showed a larger cost and benefit (16 and 28 ms, respectively) (than monkey B (3 and 10 ms, respectively). Guanfacine data were comparable to clonidine but the costs and benefits were smaller (data not shown). More will be said about the validity of cost and benefit analyses in the Discussion.

Discussion

The main findings of this study were that the noradrenergic agonists clonidine and to a lesser extent guanfacine reduced the alerting produced by peripheral cues and changed overall RTs. No evidence was found that these agonists affected the expectancy of stimulus appearance or attentional orienting. The use of the antagonist idazoxan indicated that the reduction in alerting produced by clonidine may have been mediated by presynaptic α_2 adrenoceptors. Thus, our hypothesis on the role of noradrenaline in alerting was supported.

Clonidine and guanfacine produced different changes in overall RT. This presumably means that they affected

general arousal in opposing ways. The reason for this is not clear. Perhaps the drugs produce different patterns of stimulation of pre- and postsynaptic receptors at the sites controlling general arousal level. Alternatively, the drugs may have produced different behavioral effects depending on the animal's baseline arousal level. Further work will be necessary to clarify these issues.

As the drugs produced no significant alteration of RTs with increasing CTI, we suggest that the drugs did not affect impair the animal's ability to predict the target appearance. Rather, we speculate that the impairment is probably due to a decrease in the animal's general preparedness that is reflexively evoked by the sensory event. If this interpretation is correct, then it might lead one to ask why the drugs produced no change in the validity effect, calculated from trials whose cues provided spatial information and presumably increased general response preparedness as well. One possibility is that spatial information in this task is processed rapidly and gains a processing advantage over any facilitation of response preparedness. Therefore, a drug-induced reduction of preparedness would be minimal or absent in valid or invalid trials. Another possibility is that the drugs reduced preparedness equally for valid and invalid trials, producing no net change in validity. The guanfacine data do show support for the second possibility, but the clonidine data produced the reverse effect, despite the observation that both drugs have comparable effects on alerting. Thus, our data favor the processing advantage explanation over the equal reduction alternative.

Comparison with previous work

The present work and that of Clark et al. (1989) found comparable reductions in the cost of invalid cueing following injections. However, Clark et al. interpreted their reduction in cost as an indication that clonidine facilitated attentional disengagement. Facilitated attentional disengagement would imply decreased RTs in the invalid condition, that is, attention repositioning is more rapid. While clonidine slowed RTs in the double cue conditions in our study, it did not alter valid or invalid cue RTs. Thus, if one uses the valid and double due condition RTs as a baseline (as did Clark et al. 1989), slowing of double cue RTs would lead to the conclusion that clonidine is speeding invalid cue RTs; if, on the other hand, one uses saline controls as the baseline, one would conclude (as does the present work) that clonidine does not affect invalid cue RTs. Based on these considerations, we believe that Clark et al's. conclusion about the role of the noradrenergic neurotransmitter system in the disengage operation may be premature.

However, close comparisons between the studies are limited by several methodological differences. First, the cost-benefit method assumes a truly "neutral" double cue, which we believe is unlikely, and any deviation from true neutrality will undesirably influence the results (Jonides and Mack 1984). Second, Clark et al. used a centrally cued version of the covert target detection task whereas we used a peripherally cued version and the two may be mediated by different neurochemical systems (Witte and Marrocco, in preparation). Third, results were obtained from different species. While Witte et al. (1996) have shown that humans and monkeys perform nearly identically in the CTD task in the undrugged state, catecholaminergic drugs might produce different behavioral alterations in human brains whose noradrenergic systems are at least partly anatomically asymmetric.

Synaptic site of action of adrenergic drugs

The main result of this study showed that clonidine affected alerting by increasing RTs to double cue trials, which we interpreted as a reduction in response preparedness. We have assumed that clonidine increased RTs by reducing synaptic noradrenaline, which could have occurred either by a direct presynaptic or indirect postsynaptic action. Direct action on presynaptic autoreceptors could have limited the release of noradrenaline, which directly reduced a facilitatory effect on downstream neurons and slowed RTs. Alternatively, an indirect effect could have occurred if clonidine excited neurons that inhibited downstream cells and slowed RTs. While it is difficult to decide between these alternatives, previous work has suggested that, at the doses we used, clonidine acts primarily at presynaptic receptors (Arnsten et al. 1988). The high dose of guanfacine had less impact on alerting than that obtained with clonidine in both monkeys. If one may generalize from previous work using a different task (Arnsten et al. 1988), the doses of guanfacine we used acted postsynaptically. However, the lack of a significant reduction in the alerting effect could suggest both a pre- and postsynaptic action.

Unfortunately, evidence from other areas of research relevant to the site of action is also mixed. Receptor binding studies following destruction of noradrenergic cell bodies support the interpretation that most of the α_2 receptors are postsynaptic (Heal et al. 1993). In contrast, the anatomical localization of α_2 receptors in human tissue with selective ligands parallels the previously reported distribution of presynaptic receptors (Pascual et al. 1992). Studies using in vivo microdialysis, HPLC, in vivo electrochemistry, and single-cell recording suggest that noradrenaline levels decline in a dose-dependent manner following administration of clonidine (e.g., Svensson et al. 1975; Mermet and Quitin 1991; Collazo and Marrocco 1992; Heyn and Marrocco, unpublished observations), also consistent with a presynaptic site of action.

In a working memory task, however, clonidine improved the performance of aged monkeys (Arnsten and Contant 1992; Arnsten and Cai 1993). The authors argued that clonidine increased noradrenaline by action at postsynaptic sites. The dosages of the drugs needed to produce pre- or postsynaptic effects appear to be critical, but com-

parable dosages of the adrenergic agonists were used in the present task and the working memory task. Why the same nominal dose should be facilitatory in one task and inhibitory in another is open to speculation. The most obvious possibility is that the critical dosages for pre- and postsynaptic effects are task dependent. Another possibility is that clonidine always stimulates the same brain site, but activates a process that aids working memory but interferes with attentional orienting. A third possibility is that a different distribution of adrenoceptors may exist in the frontal lobe, which is active during mnemonic tasks (Arnsten et al. 1988) and in the parietal lobe which is active during covert orienting to peripheral targets (Corbetta et al. 1993). And lastly, clonidine but not guanfacine may alter alerting by action on imidazoline receptors.

Precisely where in the brain clonidine's presynaptic action alters the alerting effect is a matter of conjecture. The site could be directly onto LC neurons, which are likely to respond to cues in the CTD or in the terminal fields of LC axons, perhaps by prejunctional modulation. A likely candidate for noradrenergic modulation of the alerting effect is the parietal cortex. We are currently exploring this possibility using single-cell recording (Davidson, in preparation).

The present results complement those of the following paper (Witte et al. 1997), in which we demonstrate that nicotine, a cholinergic agonist, mainly affects the orienting of attention. Taken together, our data imply that covert orienting to peripheral visual stimuli is mediated, at least in part, by noradrenergic and cholinergic influences on attentional centers of the brain.

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