

# Noradrenergic Modulation of Space Exploration in Visual Neglect

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**Visual neglect after stroke is often associated with a failure to explore contralesional space. Here, we show that guanfacine, a noradrenergic agonist that modulates dorsolateral prefrontal cortex, improves leftward space exploration in selected right-hemisphere patients with neglect. The positive effects of guanfacine were associated with extended ability to maintain attention on task. The results suggest that neuropharmacological targeting of intact frontal areas might be one way to enhance cognitive function after damage to posterior brain regions in selected individuals.**

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The neglect syndrome is a common and disabling disorder after right-hemisphere stroke that is often associated with impaired leftward exploration of space.<sup>1,2</sup> It has been hypothesized that spatial<sup>2</sup> as well as nonspatial mechanisms<sup>3,4</sup> might contribute to neglect, with impaired vigilance being one possible nonspatial component.<sup>5,6</sup> Such a nonspatial deficit may interact with spatial components of neglect.<sup>3,4</sup> Consistent with this view, nonspatial phasic alerting has been reported to transiently ameliorate some aspects of the spatial deficit in awareness in right-hemisphere patients with neglect.<sup>7</sup> Moreover, the right parietal and frontal regions commonly damaged or hypoperfused in neglect<sup>2,3,8</sup> are also considered to be critical for maintaining generalized vigilance.<sup>9–13</sup>

Because vigilance during task performance has been closely linked with central noradrenergic pathways,<sup>11,12,14</sup> we hypothesized that noradrenergic modulation might provide a means to improve leftward neglect, by enhancing the ability to maintain attention

when exploring space. To test our hypothesis, we used guanfacine, a noradrenergic agonist that has been shown to modulate cognitive function,<sup>15–17</sup> most likely via post-synaptic  $\alpha_2A$  receptors in dorsolateral prefrontal cortex (DLPFC).<sup>16,17</sup> If guanfacine can improve neglect through its actions on DLPFC, it would be predicted that the individuals most likely to benefit are those whose strokes do not involve this region. We therefore related behavioral performance, with and without guanfacine, to lesion anatomy in our experimental proof-of-principle study.

## Patients and Methods

Three right-hemisphere patients (>23 months after stroke) who had given informed consent participated in the study. All had chronic neglect, thereby minimizing concerns about improvements in baseline neglect severity or spontaneous recovery during the course of investigation. Patient N1 had involvement of parietal and temporal cortex (lesion volume, 94.5cm<sup>3</sup>), whereas Patient N2's lesion involved parts of parietal and temporal cortex, as well as the insula and subcortical frontal regions (lesion volume, 29.1cm<sup>3</sup>). In both cases, DLPFC was spared. In contrast, the third patient (Patient N3) had extensive prefrontal damage involving DLPFC and underlying subcortical white matter, as well as the insula (lesion volume, 38.5cm<sup>3</sup>) (Fig 1A). Thus this patient, unlike the other two, had damage to the region that guanfacine is considered to modulate in healthy individuals.<sup>16,17</sup>

Each patient was tested six times using the same battery of tests. On day 0, they were assessed in two sessions, 90 minutes apart, without any intervention. A week later, on day 7, patients were tested again with the same battery and then given either a single dose of oral guanfacine (29 $\mu$ g/kg body weight) or placebo. Both patients and investigators were blind to whether guanfacine or placebo was administered. Patients were re-tested on the behavioral battery 90 minutes after drug/placebo (peak time of action of guanfacine). A washout period of a week was used (much greater than the mean half-life of guanfacine, approximately 17 hours). Then on day 14, patients underwent the same procedures as on day 7, but this time were given the alternative to the drug/placebo they had received previously. Thus, critically, each patient acted as their own control.

The battery of tests we used included pen-and-paper tests for neglect (18cm line bisection and Bells cancellation test) as well as several computerized paradigms. For our measure of space exploration, we used a self-ordered search test that required participants to find as many targets as they could on a large touchscreen display (see Fig 1B). In total, there were 64 targets embedded among 128 distractors. Patients indicated they found a target by touching it but, unlike standard cancellation tasks, no visible mark was left. Furthermore, no time limit was imposed. Both these features of the space exploration paradigm mean that it more closely resembles search in naturalistic settings where no visible marks are left on locations that have been visited. In addition to our space exploration task, we used computerized tests of single target visual search and naming objects in a large projected array (both time-limited or "speeded"), as well as simple measures

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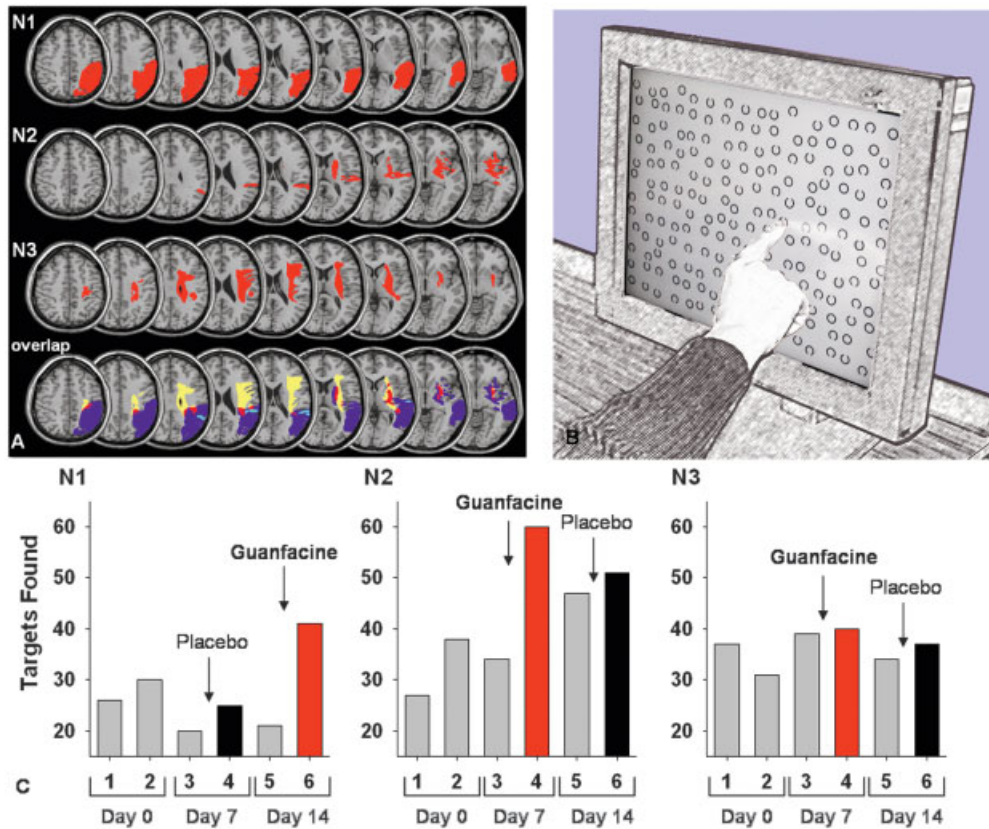


Fig 1. (A) Lesion reconstructions for Patients N1 to N3 plotted from magnetic resonance imaging acquisitions. The overlap (bottom panel) shows in yellow the frontal regions involved in Patient N3 that were not affected in Patients N1 and N2. Light blue regions are those parietal areas common to N1 and N2, whereas the red zone indicates the insula and subcortical lesion areas common to Patients N2 and N3. (B) Touchscreen system used to measure visual space exploration. In this case, the participant is searching for targets that are circles with gaps at the top. Distractors are whole circles and those with gaps at the bottom. Screen dimensions:  $40 \times 32$ cm. (C) Number of targets found by Patients N1-N3 on space exploration task. Patient N1 was randomized to receive guanfacine between the first and second testing session on day 14, whereas Patients N2 and N3 were given the drug on day 7. After guanfacine, both Patients N1 and N2 found more targets on the space exploration task but Patient N3 showed no such change.  $213 \times 190$ mm ( $300 \times 300$  DPI).

of sustained attention and spatial working memory<sup>18</sup> (see Table for further details).

## Results

Figure 1C shows the number of targets found by each subject on our space exploration task in all six sessions, with performance after guanfacine shown in red. To take into account random baseline variation in neglect severity, as well as any possible systematic effects over time (eg, practice), we compared for each patient the effects of guanfacine against all the other five test sessions. Both Patients N1 and N2 found significantly more targets on the space exploration task after guanfacine than in the control sessions (Figs 1C and 2A–E). Patient N1 found 41 targets after guanfacine compared with a mean of 24.4 (standard error [SE], 1.8) in the five control sessions ( $z = 4.11$ ,  $p < 0.0001$ ). Patient N2 found 60 targets after guanfacine compared with a

mean of 39.4 (SE = 4.3) in control sessions ( $z = 2.12$ ,  $p = 0.017$ ).

Figure 2A to C show how space exploration in Patient N2 was limited to the right on three control sessions before taking guanfacine. However, after administration of the drug, there was a marked shift into the previously neglected left space (see Fig 2D). The positive effect of guanfacine on leftward search is shown formally in plots of individual targets found across space for Patients N1 and N2 (see Fig 2F, G). In contrast, Patient N3, who had DLPFC involvement, showed no significant increase in the total number of targets found after guanfacine (see Fig 2E).

Interestingly, Patient N2 performed better on the visual exploration task on day 14 (1 week after guanfacine) than before receiving the drug (see Fig 1C). Whether this is attributable to natural random varia-

Table. Performance on Tests Other Than Space Exploration Task

Patient	Sustained Attention <sup>a</sup> Omissions (max = 100)	Sustained Attention <sup>a</sup> Reaction Time (msec)	Line Bisection Right Deviation (cm)	Bells Cancellation (max = 35)	Single Target Visual Search <sup>b</sup> (max = 24)	Projected Objects Named <sup>c</sup> (max = 20)	Spatial Working Memory <sup>d</sup> (max = 50)
<b>Patient N1</b>							
Mean of 5 control sessions (SEM)	13.6 (3.5)	630 (10.8)	2.0 (0.3)	30.2 (1.4)	16.2 (1.1)	11.1 (0.5)	32.4 (1.0)
After guanfacine	1 <sup>e</sup>	520 <sup>e</sup>	2.1	26	19	11	32
<b>Patient N2</b>							
Mean of 5 control sessions (SEM)	2.6 (1.9)	429 (27.6)	2.0 (0.4)	25.6 (1.7)	13.6 (1.4)	14.3 (1.0)	44.8 (1.0)
After guanfacine	3	406	2.4	29	12	13.5	46
<b>Patient N3</b>							
Mean of 5 control Sessions (SEM)	27.2 (7.4)	630 (8.9)	-0.6 (0.3)	27 (1.5)	12.8 (1.5)	8.9 (1.0)	33.6 (0.9)
After guanfacine	42	639	0.1	31	13	9	31

<sup>a</sup>Sustained attention test required subjects to respond rapidly with a button press (within 1 second) to the infrequent (1–7 seconds) appearance of a small (8mm diameter) black central circle on a laptop display (28.5 × 21.5cm). 100 stimuli were presented over 8 minutes.

<sup>b</sup>Single target visual search involved finding a target (letter “L”) among distractors (rotated “T”s) on a computer touchscreen within 15 seconds (n = 24 trials per testing session).

<sup>c</sup>Naming projected objects required patients to name within 15 seconds all the objects (10 either side of the midline) projected onto a 1.6 × 2.2m screen. Images were of everyday objects or animals, each measuring 20 × 20cm when projected. Two projected displays were shown per testing session.

<sup>d</sup>1-5 locations on a vertical array were displayed and patients asked to judge whether a probe location had been shown in the previous sequence (see Malhotra and colleagues<sup>18</sup>).

<sup>e</sup>Asterisks indicate where there was a significant difference between control sessions and after guanfacine.

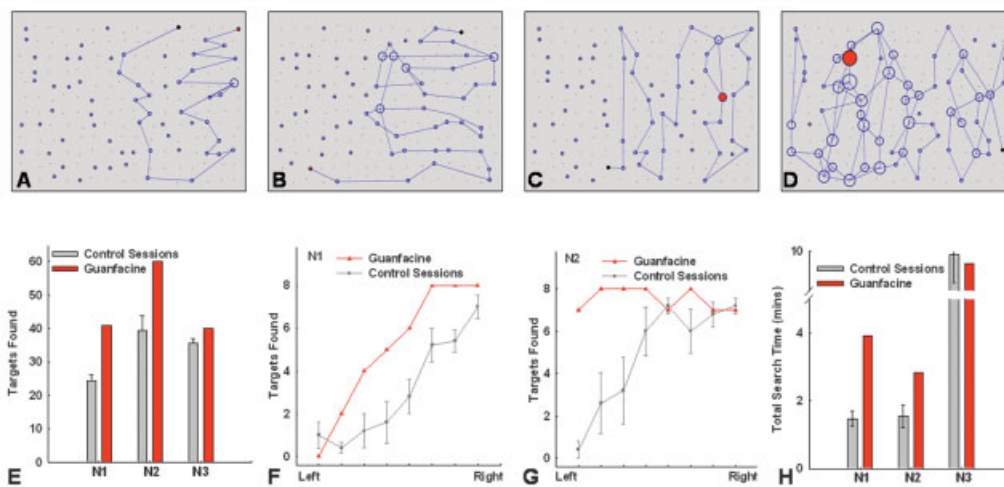


Fig 2. (A–C) Examples of space exploration in Patient N2 in three test sessions before guanfacine. (D) Space exploration after guanfacine in Patient N2. Note neglect of the left half of these arrays before guanfacine and marked leftward shift after the drug. Blue lines show the search paths, with black dots indicating start and red dots showing end locations. In this task, no visible marks are left on targets that are found. In the figures, the size of the dots on touched targets indicates number of visits, with the largest circle denoting four touches on a target. Untouched targets are shown as blue circles through which the blue line does not pass. (E) Total number of targets found after guanfacine. Both N1 and N2 showed a significant increase in number of targets (maximum, 64) found compared with the five control sessions. (F, G) Number of individual targets found across space for N1 and N2. Space across the array has been binned into eight columns, each with a maximum of eight targets (error bars = SEM). There was a leftward shift in the search functions for both these patients. (H) Total search time on space exploration task. Both N1 and N2 also showed a significant increase in time spent on the space exploration task after guanfacine compared to the five control sessions. 254 × 137mm (300 × 300 DPI)

tions in the performance baseline, systematic changes due to practice effects or a long-lasting effect of guanfacine, as has been reported in one study with monkeys,<sup>19</sup> is uncertain. What remains clear is that for Patient N2 the change in performance between first and second testing sessions on each experimental day was maximal immediately after guanfacine, compared with day 0 or after placebo (see Fig 1C). Furthermore, by taking into account the variation across all five non-guanfacine sessions when testing for significance we adopted the most conservative criteria for such comparisons, which assumed that Patient N2's performance on day 14 was independent of the effects of guanfacine.

The beneficial effects of guanfacine on visual exploration in N1 and N2 were associated with significant increases in *total* search time (see Fig 2H). In Patient N1, the total search time went up to 235 seconds from a mean of 89 seconds (SE, 14) in the control sessions ( $z = 4.79$ ,  $p < 0.0001$ ), whereas in Patient N2 it increased to 170 seconds from 93 seconds (SE, 20) ( $z = 1.7$ ,  $p < 0.05$ ). There was no alteration in the mean time required to find individual targets (ie, rate of target finding). For Patient N3, there was no significant change in either total search time or mean time to find targets.

Patient N1 also showed a significant improvement on our test of sustained attention after guanfacine, with a reduction in both reaction time and error rate after the drug (after guanfacine reaction time, 520 vs 630 milliseconds [SE = 10.8]) in control tests ( $z = 4.56$ ,  $p < 0.0001$ ), and error rate decreased to 1 omission from 13 (SE = 3.5) in control trials ( $p = 0.05$ , one-tailed). However, there was no such significant improvement in either of the other two patients.

None of the participants showed a benefit after guanfacine on line bisection, pen-and-paper cancellation, single target search, naming objects in a projected array or on our test of spatial working memory<sup>18</sup> (see Table). There were no adverse effects of guanfacine reported by participants.

## Discussion

The results presented here show for the first time to our knowledge that noradrenergic modulation can lead to improvements in leftward space exploration in some patients with neglect. The two patients (N1 and N2) who showed a benefit after a single dose of guanfacine both had DLPFC spared, whereas the patient who did not show any improvement (N3) had this region damaged, consistent with the proposal that guanfacine exerts its beneficial effects via its actions on DLPFC.<sup>16,17</sup> The different effects observed in the three patients could not be accounted for by lesion volume because both the patient with the largest and smallest lesion

showed improvement, whereas Patient N3, with intermediate size lesion, did not.

The positive effects observed in Patients N1 and N2 are likely to be caused, at least in part, by enhanced vigilance. Guanfacine extended the duration of search, and this was associated with an increase in the number of targets found, without any change in the rate of target finding. Moreover, one patient also showed improvements on our test of sustained attention. Guanfacine may produce its beneficial effects via prefrontal modulation, or top-down control, over attention.<sup>12</sup> Right prefrontal cortex plays a critical role in maintaining attention on task goals,<sup>9,10,12,13</sup> and guanfacine reduces distraction from task goals, most likely through its actions on DLPFC.<sup>16,17</sup>

Although guanfacine's mean half-life in humans is approximately 17 hours, beneficial effects have been reported in one monkey study more than 7 days after a single dose of the drug, perhaps as a consequence of actions on second-messenger systems.<sup>19</sup> There is a suggestion that there may have been a long-lasting effect of guanfacine in Patient N2, who was randomized to receive the drug on day 7 and therefore was re-tested subsequently when he received placebo on day 14. Future studies may need to extend the time of testing after guanfacine to evaluate whether it has positive long-term effects, in addition to the immediate effects observed in Patients N1 and N2 in this study.

None of the participants showed a significant benefit after guanfacine on single target search or naming objects in a projected array, demonstrating that spatial bias on speeded visual search tasks was not modulated by the drug. Thus, the benefits were most pronounced on a self-ordered space exploration task which has no time limits. Patients also did not perform significantly better on pen-and-paper tasks for neglect after guanfacine. However, these tasks may not be as sensitive as the touchscreen task we used. This has a much larger array and gives no visible feedback to remind subjects of locations they have already searched. Even pen-and-paper versions of such "invisible" cancellation tests are more sensitive than traditional cancellation where visible marks are made.<sup>20</sup>

The findings presented here demonstrate the potential for guanfacine in ameliorating aspects of the neglect syndrome. They suggest that modulation of vigilance may indeed lead to improvements of space exploration in neglect. In addition, they point the way toward exploring the effects of possible cognitive treatments on an *individual* basis using the single case, crossover design we used, which combines magnetic resonance lesion mapping with detailed behavioral assessment. More generally, the results suggest that it may be possible to compensate for posterior cortical dysfunction by neuropharmacological targeting of prefrontal cortical areas.



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# COL6A1 Genomic Deletions in Bethlem Myopathy and Ullrich Muscular Dystrophy

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**We have identified highly similar heterozygous COL6A1 genomic deletions, spanning from intron 8 to exon 13 or intron 13, in two patients with Ullrich congenital muscular dystrophy and the milder Bethlem myopathy. The 5' breakpoints of both deletions are located within a minisatellite in intron 8. The mutations cause in-frame deletions of 66 and 84 amino acids in the amino terminus of the triple-helical domain, leading to intracellular accumulation of mutant polypeptides and reduced extracellular collagen VI microfibrils. Our studies identify a deletion-prone region in COL6A1 and suggest that similar mutations can lead to congenital muscle disorders of different clinical severity.**

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Mutations in collagen VI genes, *COL6A1*, *COL6A2*, and *COL6A3*, cause Bethlem myopathy (BM) and Ullrich congenital muscular dystrophy (UCMD).<sup>1</sup> Collagen VI is a ubiquitous connective tissue component comprising three subunits, the  $\alpha 1(\text{VI})$ ,  $\alpha 2(\text{VI})$ , and  $\alpha 3(\text{VI})$  collagen chains, which are folded into a monomer through their central triple-helical domains.<sup>2–4</sup> The monomers undergo intracellular assembly into dimers and tetramers, which then are secreted extracel-

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