Research report

Infusion of apomorphine into the dorsocentral striatum produces acute drug-induced recovery from neglect produced by unilateral medial agranular cortex lesions in rats

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Abstract

Previous studies have shown that systemic administration of apomorphine is effective in producing acute drug-induced recovery from neglect induced by unilateral medial agranular cortex (AGm) lesions. More recent studies have demonstrated that recovery from neglect may be due to plastic changes occurring in the dorsal central striatum (DCS). Further, lesions of the DCS produce neglect that does not respond to systemic administration of apomorphine, suggesting that this area may be crucial for the therapeutic effects of apomorphine. In the present study, the behavioral effects of apomorphine infused into the DCS of animals with AGm lesion-induced neglect were examined to determine whether the DCS is a site of drug action. An infusion of 0.375 μg of apomorphine into the DCS, but not a lateral striatal control area, was effective in producing acute recovery from neglect. The results of this study support the crucial role of the DCS in recovery from neglect induced by unilateral AGm lesions and suggest that the DCS may be an important site of action for the therapeutic effects of apomorphine. Because dopamine agonist therapy has been shown to be effective in humans with neglect, the results of the current study may represent an important step in the development of future pharmacotherapies.

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1. Introduction

In humans, approximately 40% of all cases of right hemisphere brain damage result in a complex array of neurological deficits known as the neglect syndrome [1]. Neglect is characterized by a failure to report, respond, or orient to novel or meaningful stimuli presented to the side of the body opposite a brain lesion in the absence of an elementary sensory or motor deficit [14,18,19]. Denes et al. [12] found that the presence of neglect following a stroke was the only significant indicator of poor recovery 1 year later. Unfortunately, behavioral interventions for the treatment of neglect have demonstrated limited utility and generality outside of the clinical setting [17,21].

A rat model of the neglect syndrome has been developed to examine the mechanisms of behavioral recovery from neglect induced by cortical lesions [10,11,23]. These studies have demonstrated that severe multimodal neglect can be produced by unilateral destruction of the ventral lateral orbital cortex [24], the medial agranular cortex (AGm; the rodent analog of the frontal eye fields in primates) [7,10,11,31], the posterior parietal cortex [6], or their disconnection [3]. The results of these studies in the rat model have demonstrated that recovery from neglect occurs in three contexts: (1) as in humans, spontaneous, although incomplete, recovery may occur over a period of weeks to months [10,11,22,39], (2) virtually complete recovery can be produced by an environmental manipulation, 48 h of light deprivation [2,4,9,11], and (3) as in humans, acute drug-induced recovery can be produced by systemic administration of dopamine receptor agonists [6,7,22]. A recent series of studies have indicated that the crucial site for the mechanisms of recovery in all three contexts may be the AGm projection zone in the dorso central striatum (DCS) [5,29,30].

Vargo and Marshall [41] found that changes in striatal glutamatergic receptors were temporally correlated with neglect and spontaneous recovery. In subjects with AGm lesion-induced neglect, they found that binding to the glu-
Humans. The results of these studies indicate by cortical or subcortical lesions in rats and drug-induced recovery of function from neglect produced the finding that the striatum has also been implicated in acute recovery was made strictly on logical grounds. A more recent study has provided more direct evidence for the role of the striatum, specifically the DCS. In the study by Van Vleet et al. [35], apomorphine did not produce a therapeutic effect in subjects with severe neglect produced by unilateral DCS lesions. The finding that apomorphine does not produce acute recovery in DCS operates suggests that the DCS may be a crucial site for acute apomorphine-induced recovery as has been found for spontaneous, and light deprivation-induced recovery [34,35].

Despite the enormous potential of pharmacotherapy in treating patients with neglect, drug therapies are rarely used because there has been no rational framework for understanding the site of drug action or the mechanism(s) which lead to a therapeutic effect. As a result, drug therapies have only been used in chronic neglect patients with stable behavioral baselines [13,15,20]. As mentioned above, studies utilizing the rat model of neglect have consistently indicated that recovery may be due to plastic changes occurring in the DCS [8,40–43].

In order to more clearly define the site of action for dopamine agonists, the present study directly examined whether the DCS is the site of action for the therapeutic effects of apomorphine by studying the effects of direct cannulation of apomorphine into the DCS in subjects with severe AGm lesion-induced neglect.

2. Materials and methods

2.1. Subjects

Subjects consisted of 38 male Long–Evans hooded rats bred from stock purchased from Harlan Sprague–Dawley (Indianapolis, IN). Throughout the duration of the study, the subjects were housed individually with food and water available ad libitum in a room that maintained a 12 h/12 h light/dark cycle. Prior to the surgical procedures, the subjects were handled daily for 2–4 weeks in order to reduce struggling or “freezing” responses during behavioral testing. After sufficient handling, the subjects were randomly assigned into one of the surgical and dosage groups.

2.2. Surgical procedures

In order to evaluate the effects of direct administration of apomorphine into the DCS of animals with neglect, an AGm/DCS group received a unilateral AGm lesion and a simultaneous implantation of a cannula into the DCS (n = 24). AGm/DCS subjects were then randomly assigned to one of the following apomorphine dosage groups: (1) 0.125 µg group, (2) 0.25 µg group, (3) 0.375 µg group, and (4) vehicle control group.

In order to examine the location specificity of the effects of apomorphine, an AGm/lateral striatal control (LSC) group received an AGm lesion combined with an implantation of a cannula into the striatum laterally adjacent to the DCS (n = 14). This is a very conservative control group because the injection site is also located in the dorsolateral...
striatum. As with the AGm/DCS groups, AGm/LSC ani-
mals were randomly assigned to one of four apomorphine
dosage groups: (1) 0.125 μg group, (2) 0.25 μg group, (3) 0.375 μg group, and (4) vehicle control group.

All of the surgical procedures were approved by the
Northern Illinois University Institutional Animal Care and
Use Committee. All surgical procedures were performed
under aseptic conditions using sterile gloves and instru-
ments. A ketamine-xylazine (Rompun) mixture (87 mg/ml:
13 mg/ml) was administered at a dosage of 0.80 ml/kg,
t.i.p. After the animal was sufficiently anesthetized as indi-
cated by the absence of a corneal reflex and a lack of respon-
siveness to a tail pinch, the head was shaved and the animal
was placed in a stereotaxic equipped with blunt-tipped ear
bars to prevent damage to the tympanic membrane.

A skull window matching the dimensions of the AGm
was drilled in the skull at 5 mm rostral, 2.5 mm caudal, and
2.5 mm lateral with respect to bregma. In addition, a small
notch at 3.4 mm lateral to bregma for the AGm/DCS group
and 4.4 mm lateral to bregma for the AGm/LSC group was
also drilled to accommodate the placement of the cannula.
Following the removal of the skull window, the dura was
incised and the underlying cortex was removed using gen-
tle subpial aspiration through a fine gauge pipette. After
hemostasis was achieved, gelfoam was gently placed in
the cavity (Upjohn, Kalamazoo, MI) and the skull win-
dow was covered with a thin sheet of transparent plastic.

Three securing screws were then placed in a triangular
fashion around the skull window. The guide cannula for the
AGm/DCS groups was placed at 0.5 mm rostral to bregma,
3.4 mm lateral with respect to bregma, and 4.2 mm ventral
to the surface of the dura matter. The guide cannula for the
AGm/LSC groups was placed at 0.5 mm rostral to bregma,
4.4 mm lateral to bregma, and 5.5 mm ventral to the surface
of the dura matter. The guide cannula was held in place by
the stereotaxic instrument while dental epoxy (Archer Vi-
tone) is built up around the guide cannula and the securing
screws to allow for permanent placement. After the enamel
dried, an obturator was screwed into the guide cannula to
ensure that the opening remained unobstructed.

Following surgery, the animals were treated with
Neosporin ointment topically, kept warm, and monitored
prior to being returned to their home cage. Animals were
monitored twice a day for 7 days for any qualitative changes
in activity level, eating and drinking behavior, weight fluc-
tuation, and possible signs of distress.

2.3. Behavioral testing

All subjects were tested for the presence of neglect on
days 3 and 7 post-surgery. Testing was conducted at approx-
imately the same time during the light phase of the light/dark
cycle 11:00-14:00 h. All testing was done in a room with
standard overhead fluorescent lighting.

Testing for neglect was identical to that used in previous
studies [7,34], which is a modification of the testing proce-
dures developed by Crowne and Pathria [10] to mirror bed-
side testing for neglect. Briefly, the subject was transferred
to the testing room in its own cage which was placed on the
testing platform for a 2-min adaptation period during which
any signs of circling behavior were noted in order to acco-
cunt for any rotational bias that may affect the results of
the neglect testing. The experimenter recorded the number
of rotations, if any, and their direction: right or left to the
nearest half turn. A turn was constituted by a turning of the
total body including the hindpaws. After the 2-min adapta-
tion period, the animal was removed from its home cage and
placed directly onto the testing platform while being gen-
tly restrained by hand from behind without restricting head
movement. The subject was placed on the testing platform so
that its body was aligned with the center line of the platform.

Stimuli were presented only when there was no evidence
of struggling, no asymmetry of body posture, and the head was
oriented in direct line with the body (i.e. with the center line).

As in previous studies [7,33], the stimuli were presented
as follows. First, the visual stimulus consisted of the pre-
sentation of a light-reflecting, silver metallic rod (5 mm in
diameter and 10 cm in length) which was waved in five
small circles approximately 5 cm in diameter, at a distance
of approximately 7.5–10 cm from the animal, and at a rate
of approximately 1 ips. The auditory stimulus consisted of
a single, approximately 114 dB (SPL) click generated by a
hand-held clicking device held caudal to the animal’s vi-
sual field at midbody, approximately 5 cm from the animal.
The tactile stimulus consisted of a single caudal-to-rostral
stroke through the vibrissa using a 1.5 cm Puritan applicator
(Harkwood Products Co., No. 807).

Three cycles of stimuli presentation comprised one test
session. The order of stimulus presentation has been found
to have no effect on the results of behavioral testing [39].
Stimulus presentations were separated by approximately 5 s.

For all behavioral testing, the experimenter was blind
with respect to the subject’s group membership. The exper-
imerter rated the degree of head turning either toward (ap-
propriate responding) or away from (alliesthetic/allokinetic
responding) the stimulus. The magnitude of orientation
was measured by the position of the tip of the snout over
the test platform’s markings. Head turns of less than 30°
were scored as 0, turns between 30 and 45° scored as 1.0,
those between 45 and 60° as 1.5, and those greater than
60° as 2.0. Orientation responses made more than 2 s af-
ter either the auditory or tactile stimulation were scored as
a 0. Orientation responses that occurred after the third
revolution (approximately 3 s) of the visual stimulation
were given a maximal score of 1.5. Appropriate and alles-
thetic/allokinetic responses were scored separately. Using
this scoring method, the maximal score for appropriate
responding was 6.0 for each of the three different stimuli,
or 18.0 total for each body side. Subjects that achieved
a score of at least 5.5 on one body side, and responded
in two stimulus modalities were included in the statistical
analyses [7].
As in previous studies [7,33], total neglect ratios were calculated using the following formula: (total contralesional responses + total ipsilesional responses)/total contralesional responses + total ipsilesional responses) [36]. The use of the neglect ratio allows for an indication of severity of neglect by taking into account responsiveness on both body sides. Similarly, individual modality neglect ratios were calculated for each test session by the following formula: (contralesional responding within each modality + ipsilesional responding within each modality)/(contralesional responding within each modality + ipsilesional responding within each modality) [37].

2.4. Drug infusion

Subjects within each group were infused with apomorphine or vehicle 1–2 h after the initial neglect test on post-surgical day 3, or 1–2 h after the neglect test on post-surgical day 7. Subjects from each group were tested for neglect on both days, but were randomly assigned for infusion on only one of the two days.

Prior to infusion, the subject’s obturator was removed from the guide cannula and replaced with an injector cannula. The 0.1 μl solution of either apomorphine or vehicle was infused at a constant rate of 0.1 μl/min, and allowed to diffuse for an additional 2 min before the injector cannula was removed from the guide cannula. The volume of the infusion was constant regardless of the dosage level group. Following infusion, the animal was returned to its home cage for exactly 10 min, after which time neglect testing, as previously described, was resumed. Subjects were also tested for neglect 2–3 days after the post-infusion neglect test in order to control for signs of spontaneous recovery as a possible confound.

2.5. Histological procedures

Following behavioral testing on post-surgical day 7, the subjects were euthanized by an overdose (65 mg) of sodium pentobarbital (Nembutal). When the animal was unresponsive to a tail pinch, it did not demonstrate a corneal reflex and respiration had ceased. The subject was intracardially perfused with a 0.9% saline solution followed by 10% formalin. The brain was removed from the skull, placed into 10% formalin for at least 3 days, and then frozen and sectioned at 50 μm in the coronal plane. Every fifth section throughout the extent of the lesion was saved, mounted, and stained with cresyl violet.

All lesions were examined to determine the completeness of A9m destruction, extent of damage to adjacent areas, and location of cannula placement. Lesion size was measured by tracing the extent of lesion and areas of gliosis through an image analysis software program (Optimus, BioScan, Inc., Edmonds, WA). Lesion extent was traced onto standard brain diagrams [27]. Statistical analyses were performed to ensure equivalent lesions sizes among groups. All histology was done with the experimenter blind with respect to the behavioral performance and group membership of the subjects.

3. Results

3.1. Histology

As indicated in Fig. 1, and as found in prior studies [35], removal of the A9m typically resulted in some minor damage to the adjacent lateral A9m, the dorsal cingulate cortex, and the cingulum bundle. This minor damage has been found not to be related to the behavioral deficits [35]. Total extent of damage was quantified for each subject utilizing an image analysis software program (Optimus, BioScan, Inc.). An analysis of the lesion extent for the four A9m/DCS groups and the two A9m/LSC groups revealed no significant differences in lesion size among the groups (F(5, 32) = 0.294, P < 0.91; Fig. 1). Further, the histological analysis revealed that the unilateral A9m lesions in all the groups were virtually identical to those in prior studies [4,7,23,40]. An analysis of cannula location revealed consistent placements in each subject that are consistent with placement in the DCS as defined in previous studies [33,35].

3.2. Effectiveness of apomorphine on neglect

3.2.1. Total neglect

An analysis of the pre-test data for days 3 and 7 revealed that subjects in the DCS dosage groups (high, 0.375 μg; medium, 0.25 μg; low, 0.125 μg; vehicle) infused with apomorphine or vehicle on either post-surgical day 3 or 7 demonstrated no difference in pre-test severity of neglect (F(3, 23) = 1.222, P < 0.32). As subjects did not differ in the baseline level of neglect prior to drug administration, the remainder of the analyses were conducted collapsing across day of infusion.

A Group × Test repeated measures ANOVA was conducted comparing all four apomorphine dosage level groups across pre-test, drug test, and post-test. The results indicated a main effect of test (F(2, 12) = 5.398, P < 0.01) and a significant group by test interaction (F(6, 12) = 3.753, P < 0.01). To explore the significant group by test interaction, one-way ANOVAs comparing the groups on each test were conducted. The ANOVA comparing the A9m/DCS groups at pre-test revealed no significant differences in the severity of baseline level of neglect (F(3, 23) = 1.319, P < 0.29). A Group × Test ANOVA comparing the total neglect ratios of the groups at the time of drug administration (test 2) revealed a significant difference in level of neglect among the dosage groups (F(3, 23) = 5.427, P < 0.007). A post hoc Fischer’s LSD analysis revealed that the 0.375 μg (high) dosage A9m/DCS group exhibited a significant reduction in total neglect ratio as compared to both the low dosage and vehicle groups (P’s < 0.006), but not compared to the medium dosage group (P < 0.075). Further, Fischer’s LSD analysis revealed
that the level of neglect in the medium dosage group at the
time of drug administration (test 2) was not significantly dif-
ferent from any of the other groups. These findings indicate
that, while the 0.375 µg dosage of apomorphine appeared to
be producing the most beneficial and therapeutic effect, the
0.25 µg (medium) dosage group may also have experienced
a degree of amelioration from neglect as well (Fig. 2). To fur-
ther examine the therapeutic effect of apomorphine, pre-
and post-drug administration total neglect ratios were com-
pared within each of the groups via *t*-tests with Bonferroni
adjusted *P*-values. Similar to the findings of Corwin et al.
[7] with systemic injections of apomorphine, the results re-
vealed a significant pre- versus post-drug infusion reduction
in total neglect ratios for the apomorphine 0.375 µg (high)
dosage AGm/DCS group (*t*(5) = −8.51, *P* < 0.001) but
not for the 0.25 µg (medium) dosage group (*t*(5) = −1.499,
*P* < 0.19), the 0.125 µg (low) dosage group (*t*(5) = −0.854,
*P* < 0.43), or the vehicle group (*t*(5) = 0.160, *P* < 0.87).
This finding indicates that only the apomorphine 0.375 µg
(high) dosage AGm/DCS group exhibited a significant acute
therapeutic effect. On a subsequent post-apomorphine test,
the subjects in the 0.375 µg group exhibited severe neglect
virtually identical to the to the pre-apomorphine baseline
level, and significantly different from the apomorphine test
(*t*(5) = 4.209, *P* < 0.008). Overall, these results are consis-
tent with previous studies [6,7] that demonstrated the ther-
apeutic effectiveness of apomorphine on neglect and which
indicated that the striatum was the site of action [25].

3.2.2. Raw scores

Because neglect ratios give no indication of whether an
asymmetry in orientation results from lower contralesional
(non-neglect side) or higher ipsilesional (neglect side)
scores, separate analyses of raw scores for ipsi and con-
tralesional responding within the AGm/DCS groups were conducted. A Group × Test repeated measures ANOVA comparing ipsilesional raw scores across the tests for each group revealed a significant Group × Test interaction (F(3, 20) = 6.237, P < 0.004). In order to evaluate the significant interaction, one-way ANOVAs comparing ipsilesional raw scores between groups at pre- and drug infusion tests were conducted. The analysis revealed no significant difference in level of ipsilesional responding between groups at pre-test (F(3, 23) = 1.335, P = 0.29) or at drug test (F(3, 23) = 2.366, P < 0.1). These results are consistent with previous studies [6,7].

A Group × Test repeated measures ANOVA comparing the contralesional raw scores across tests for each group revealed a significant main effect of group (F(3, 20) = 4.331, P < 0.01), test (F(1, 8) = 7.231, P < 0.01), and group interaction (F(10, 18) = 4.134, P < 0.02). In order to evaluate the significant interaction, individual Group × Test ANOVAs were conducted. The results indicate that only at the drug infusion test was there a significant difference in the amount of contralesional responding between groups (F(3, 23) = 6.799, P < 0.002). A post-hoc Fischer’s LSD analysis indicated that the apomorphine 0.375 μg (high) dosage AGm/DCS group exhibited significantly greater contralesional responding than any other group (P’s < 0.02). Paired samples t-tests comparing contralesional responding at pre-test versus drug test within each group indicated that only the 0.375 μg (high) dosage AGm/DCS group exhibited a significant increase in contralesional responding in response to apomorphine administration (t(5) = −4.928, P < 0.004). These findings reveal that, on the drug infusion test, only the 0.375 μg (high) dosage group is exhibiting a significant increase in contralesional responding indicative of an acute drug-induced behavioral recovery. Overall, the analysis of ipsilesional and contralesional raw scores support the findings from the neglect ratio analyses, and those from previous studies [6,7].

3.2.3. Modalities

In order to evaluate the effects of apomorphine on individual modalities within the AGm/DCS groups, Group × Test repeated measures ANOVAs were run comparing the level of visual, tactile, and auditory neglect across groups. As illustrated in Fig. 3, in general, the results obtained in the individual modalities support the total neglect findings and those found in prior studies of neglect produced by unilateral AGm destruction [4,7] and unilateral lesions of the DCS [34].

3.2.3.1. Visual. The analyses of the visual neglect ratios neither reveal any significant main effects for group (F(3, 20) = 2.153, P < 0.12) nor any significant group by test interaction (F(3, 8) = 0.424, P < 0.73) suggesting that there was no change in the level of visual neglect across tests or between groups. Despite the considerable, but insignificant, reduction in the level of visual neglect in the 0.375 μg (high) dosage apomorphine group at the time of drug infusion, the failure of these subjects to demonstrate recovery from visual neglect is consistent with previous studies examining behavioral recovery from neglect [4,7,34]. Specifically, the inherent variability in the presentation of neglect symptoms following manipulations intended to produce recovery often results in subjects that may not exhibit recovery across all modalities [4,7,34].

3.2.3.2. Tactile. The Group × Test analysis of the ratios for tactile neglect revealed a significant main effect for test (F(1, 2) = 19.845, P < 0.001). In order to examine the significant effect of test, tactile neglect ratios were collapsed...
Fig. 3. Individual modality neglect ratios in the four apomorphine dosage AGm/DCS groups (high, 0.375 μg; medium, 0.25 μg; low, 0.125 μg; vehicle) as a function of test. A ratio of 0.0 represents symmetrical responding. The symbol (*) indicates that the high dosage (0.375 μg) group was significantly different from all other groups in the auditory modality and all groups except the medium dosage group in the tactile modality at drug test ($P < 0.05$). The symbol (●) indicates a significant difference between the medium dosage (0.25 μg) group and the low dosage and vehicle groups at drug test. Error bars represent standard errors.

The analysis revealed significant differences among the groups at pre-test versus drug test ($t(23) = -4.117$, $P < 0.001$). In order to investigate the possibility of individual group differences in level of tactile neglect pre- versus drug test, individual pairwise comparisons were conducted. The results indicate that both the 0.375 μg (high) dosage ($t(5) = -8.592$, $P < 0.001$) and 0.25 μg (medium) dosage groups ($t(5) = -3.078$, $P < 0.028$) exhibited a significant reduction in the level of tactile neglect at drug test compared to pre-test. This finding is consistent with the outcome of the total neglect ratio analyses which demonstrated that the 0.375 μg (high) dosage of apomorphine infused into the DCS produced a significant therapeutic effect, while the medium dosage produced a modest effect (across modalities) (Fig. 4). Significant reduction of tactile neglect in the medium dosage group suggests that the modest effects on neglect at this dosage are due, in large part, to its effectiveness within the tactile modality. Further, these results suggest that only a medium dosage of apomorphine is required to alleviate tactile neglect.

3.2.3.3. Auditory. The Group × Test analysis for the auditory modality revealed a significant effect for test ($F(3, 8) = 7.092$, $P < 0.01$). Auditory neglect ratios were collapsed across groups and compared pre- versus drug test via a paired samples t-test with Bonferroni adjusted $P$-values. In order to investigate individual group differences in level of auditory neglect pre- versus drug test, individual pairwise comparisons were conducted. The results indicate that only the apomorphine 0.375 μg (high) dosage AGm/DCS group exhibited a significant reduction in the level of auditory neglect at drug test compared to pre-test ($t(5) = -14.015$, $P < 0.001$). This finding is consistent with the outcome of the total neglect ratio analyses which demonstrated that only the 0.375 μg (high) dosage of apomorphine produced a significant therapeutic effect. Overall, the results obtained...
3.2.4. Location specificity of drug action

An analysis of pilot data revealed a failure of apomorphine to produce a therapeutic effect in LSC subjects. The absence of an effect was demonstrated in the 0.375 µg (high) dosage group (n = 6), a dosage with demonstrated therapeutic effectiveness in the DCS group (Fig. 2), 0.25 µg (medium) dosage group (n = 2), 0.125 µg (low) dosage group (n = 1), and vehicle group (n = 2). As a result of the ineffectiveness of either the high, medium, low, or vehicle dosages of apomorphine to produce a therapeutic effect on neglect when infused into the LSC (see analysis below), the authors decided to explore the possibility that the AGm/LSC group could simply be exhibiting a different dose-response reaction in the AGm/LSC group, a Group × Test repeated measures ANOVA comparing the total neglect ratios in the apomorphine high dosage AGm/DCS and AGm/LSC groups and the apomorphine extra high dosage AGm/LSC group across tests was conducted. The analysis revealed a significant main effect of group (F(2, 11) = 8.423, P < 0.001), and a significant test by group interaction (F(4, 11) = 6.086, P < 0.001). To explore the significant group by test interaction, one-way ANOVAs comparing the groups on each test were conducted. The ANOVA comparing the groups at pre-test revealed no significant difference in severity of neglect prior to drug administration (F(2, 13) = 0.239, P < 0.791). This finding indicates that all groups demonstrated comparable baseline levels of neglect prior to exposure to the drug. The ANOVA comparing the total neglect ratios of the groups at the drug infusion test revealed a significant difference in level of neglect between the groups (F(1, 12) = 20.886, P < 0.001). A post hoc Fischer’s LSD analysis revealed that the apomorphine high dosage AGm/DCS group exhibited a significant reduction in total neglect ratio as compared to both the high and extra high dosage AGm/LSC groups (P’s < 0.001); the AGm/LSC groups did not differ from one another (P’s < 0.106). To further examine this effect, pre- versus post-drug administration total neglect ratios were compared within each of the groups via t-tests with Bonferroni adjusted P-values. The results revealed a significant pre- versus post-drug infusion reduction in total neglect ratio for the apomorphine high dosage AGm/DCS group (t(5) = −8.51, P < 0.001) but not the high or extra high dosage AGm/LSC groups (t(5) = −0.136, P < 0.89) (t(2) = 3.303, P < 0.081), respectively. This finding indicates that even when a substantially higher dosage of apomorphine (i.e. extra high, 0.5 µg) was infused into the LSC, no therapeutic effect was demonstrated. The lack of an effect in the AGm/LSC groups supports the notion that the effects of apomorphine are specific to the DCS and not simply the striatum. Further these results are not explainable by a differential dose-response reaction in the AGm/LSC group. These results are also consistent with a recent study [35] that demonstrated the importance of the DCS, and not the LSC, for accelerated behavioral recovery. Specifically, subjects that received a combined lesion of the AGm and LSC exhibited an accelerated behavioral recovery from neglect while those subjects that received a combined lesion of the AGm and DCS did not [35].

Wilcoxon Signed-Ranks tests revealed that unlike previous studies [4,7], none of the groups demonstrated a significant difference in ipsi versus contralesional allosiessia/akinesia (P’s > 0.05). However, as demonstrated in a previous study [9], the absence of significant allosiessia/akinesia in the AGm operates demonstrate that significant neglect can be obtained without allosiessia/akinesia. Wilcoxon Signed-Ranks tests were conducted within each group revealed a significant difference in ipsi versus contralesional circling for the apomorphine low dosage AGm/DCS group only at pre-test (z = −2.226, P < 0.02). Thus, for this and all other AGm groups, the severe neglect exhibited is not explainable by a circling bias.

![Fig 4. Mean total neglect ratios in the high apomorphine dosage AGm/DCS and AGm/LSC groups (high, 0.375 µg) and the extra high dosage AGm/LSC group (E-high, 0.5 µg) as a function of test. A ratio of 0 represents symmetrical responding. The symbol (●) indicates that the high dosage (0.375 µg) group was significantly different from all other groups at the drug test (P < 0.05). Error bars represent standard errors.](image-url)
4. Discussion

The results of the present study indicate that significant acute drug-induced behavioral recovery from neglect can be produced by direct infusion of apomorphine into the DCS, the striatal projection zone of the AGm. Infusion into a laterally adjacent region, the LSC, did not produce recovery. The results of the current study also address the possibility that animals infused with apomorphine into the LSC may simply exhibit a different dose response than DCS animals. At the most effective dose of apomorphine in DCS animals (0.375 \( \mu \)g), and at a significantly higher dose (0.5 \( \mu \)g), animals experiencing drug infusion into the LSC failed to exhibit a therapeutic effect. The lack of an effect at the 0.375 \( \mu \)g or the 0.5 \( \mu \)g dosage further supports the notion that the DCS is the site for the therapeutic effects of apomorphine.

The positive effects of direct infusion of apomorphine into the DCS are consistent with and expand upon an existing conceptual framework for understanding the mechanism(s) which lead to acute drug-induced behavioral recovery. The recovery exhibited by subjects infused with the best therapeutic dosage of apomorphine (0.375 \( \mu \)g) was virtually identical to that produced in previous studies examining the effects of systemic injections of apomorphine in animals with neglect induced by either cortical or subcortical lesions [7,26]. In these studies, it was thought that reduction in cortical or subcortical input to the striatum reduces medium spiny cell output, resulting in compromised contralateral motor activity as seen in neglect [7,26]. Accordingly, activation of these neurons by the dopamine agonist apomorphine, may compensate for lost excitatory input from AGm [7]. The results of the current study support this notion, and provide a specific site of action for the therapeutic effects of apomorphine. Direct infusion of apomorphine to the DCS, as verified by a tracer injection, and not the laterally adjacent LSC, produced acute behavioral recovery from neglect.

The present results are also consistent with previous studies that have examined behavioral recovery of function from neglect [7,42,43]. Vargo et al. [40] found that dynamic changes in glutamatergic receptors in the dorsolateral striatum correlated with neglect and spontaneous recovery. Vargo and Marshall [41] also demonstrated that changes in IEG (c-fos, zif/268, junB) in the dorsolateral striatum correlate with spontaneous behavioral recovery from neglect. Recent studies support these findings by demonstrating that DCS operates do not exhibit spontaneous recovery throughout a prolonged period of neglect testing (15 weeks), and that systemic injections of apomorphine did not produce acute recovery in DCS operates [34,36].

Functional changes within the dorsolateral striatum have also been correlated with the behavioral recovery produced by an environmental manipulation; specifically, 48 h of light deprivation. Vargo et al. [40] found that light deprivation-induced recovery was correlated with symmetrical IEG or c-fos expression in the dorsolateral striatum. The authors concluded that light deprivation may produce accelerated behavioral recovery by reducing asymmetries in dorsolateral striatal function [40]. A more recent investigation [35] has demonstrated the importance of the AGm projections to the dorsolateral striatum (i.e. the DCS), and not the laterally adjacent LSC, for light deprivation-induced behavioral recovery. Subjects that received a lesion of the DCS did not exhibit light deprivation-induced behavioral recovery from AGm lesion-induced neglect while those subjects that received an LSC lesion recovered [35]. Thus, a specific subregion of the dorsolateral striatum, the DCS, appears to be necessary for light deprivation-induced recovery [35,40]. Overall, results from the current study are consistent with data from previous studies, which indicate that recovery from neglect may be due to plastic changes occurring in the DCS [8,32,40,42,43].

In human pharmacotherapy studies, the dopamine receptor agonist bromocriptine [13,20] or apomorphine [15] was administered to patients with chronic neglect following right hemisphere damage. Following drug administration, the patients improved on all measures examined including orientation to stimuli, extinction to bilateral simultaneous stimulation, motor impairment, and line bisection and figure cancellation tasks [13]. In addition, trauma to the neglect side decreased, and in one patient, there was an increase in libido. The condition of patients worsened after withdrawal of the drug. The results of the present study would suggest that the basis of the therapeutic effects of these dopamine agonists may be due to their effects on the striatum. Recent anatomical findings have indicated that in rats there is overlap in the projections from the AGm and the posterior parietal cortex to the DCS [5,29]. This result is of some interest because apomorphine has been found to produce a therapeutic effect on neglect in posterior parietal cortex operates [6]. These findings may also explain why dopamine agonists have been found to produce a therapeutic effect in patients with neglect produced by damage to differing cortical areas [13,15,20].

One possible consideration is that apomorphine may have produced a general change in arousal or slight head deviations which may have led to the therapeutic effects obtained in the DCS infusion group. This outcome is extremely unlikely. The behavioral methods precluded testing if there was any evidence of a postural asymmetry, and this could be readily determined by the use of the center line on the testing board. Further, the failure to find a therapeutic effect in the lateral infuson controls indicates that apomorphine was not inducing a general change in arousal which led to the therapeutic effects. Finally, there was no asymmetry or change in circling behavior associated with apomorphine infusion as might be expected if motor asymmetries were responsible for the acute drug-induced recovery. The site-specificity of the therapeutic effects of apomorphine point to the crucial role of the DCS apomorphine-induced recovery.

Although the effects of direct infusion of apomorphine into the DCS were only examined on neglect and alesthesia, recent evidence from our lab indicates that apomorphine is ineffective in producing a therapeutic effect on extinction
to bilateral simultaneous stimulation [28]. Pyter et al. [28] found that rats with AGm lesion-induced neglect failed to exhibit any therapeutic effect when administered apomorphine systemically. Recent evidences [37] suggest that neglect and extinction may have separate, but related, neural mechanisms. Van Vleet et al. [36] found that rats with DCS lesions, while exhibiting severe multimodal neglect, did not demonstrate extinction. AGm lesioned rats, as in previous studies [23], demonstrated both neglect and extinction. These findings are particularly important because in many "recovered" neglect patients, extinction deficits remain [21]. The results suggest that different pharmacological interventions may be required for the constellation of deficits that comprise the neglect syndrome [7,13,15,20,22].

Despite the enormous potential of pharmacotherapy in treating patients with neglect, drug therapies are rarely used because there has been no rational framework for understanding the mechanism(s) that produce a therapeutic effect or site of drug action [16]. While the results of the present study provide potentially important information in establishing a rational model for the development of dopamine agonist therapy in the treatment of humans with neglect, it is unknown whether the DCS is the only site of action for the therapeutic drug effects. Currently, investigations are underway to examine this issue.

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References


