Recovery From GABA-Mediated Hemiplegia in Young and Aged Rats: Effects of Catecholaminergic Manipulations

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Received 9 September 1985

BRAILOWSKY, S. AND R. T. KNIGHT. Recovery from GABA-mediated hemiplegia in young and aged rats: Effects of catecholaminergic manipulations. NEUROBiol Aging 8(5) 441-447, 1987.—We investigated the participation of catecholaminergic mechanisms in the functional recovery from motor cortex lesions in young (9 months) and aged (26 months) rats. The animals were studied during the recovery period from an hemiplegic syndrome secondary to small motor cortex lesions potentiated by the localized, chronic (7 days) infusion of GABA into the lesion site. Acute administration of haloperidol (0.1 mg/kg IP) to these recovered animals induced a re-emergence of the contralateral motor syndrome in both groups. In the young group, the haloperidol-induced hemiplegia lasted one day whereas in the aged animals the deficit was significantly prolonged lasting three days. Apomorphine administration (0.5 mg/kg IP) prior to or immediately after haloperidol injection failed to prevent or reverse the reappearance of the motor deficit. Adult animals recovered from motor cortex aspirations performed 7 to 12 months prior were refractory to haloperidol effects. Amphetamine administration to young rats treated chronically with saline or GABA infusion into the somatomotor region also failed to alter the clinical evolution of the motor deficit. The evidence suggests that dopaminergic mechanisms are involved in the functional recovery from brain lesions and that these mechanisms are most susceptible to neuroleptic blockade during the early post-lesional period. The deleterious effects of dopaminergic blockage are heightened in aged populations. The use of dopaminergic antagonists in brain-lesioned subjects, and particularly in geriatric populations, is considered potentially harmful, particularly in the early stages of the recovery process.

Aged rats Motor cortex Hemiplegia GABA Dopamine Functional recovery Brain plasticity

AGING is accompanied by changes in several neurotransmitter systems with catecholamines (CA) being particularly affected [15, 16, 26, 31]. The senescent organism also develops deficits in motor performance [26, 41] which correlate with impairment of dopaminergic function, as evidenced by diminution in cell number in the substantia nigra [30], decreased activity of tyrosine hydroxylase [27, 28], decreased concentration of homovanillic acid (HVA) in the CSF [35, 38] and a decreased number of binding sites for various dopaminergic ligands [8, 16, 26, 32].

Studies in aged rats also report an increased sensitivity to the activity-inhibiting and cataleptic effects of haloperidol [4], to the depressive effects of butyrophenones on the rate of lateral hypothalamic self-stimulation [40] and to the dopamine depleting effect of intracerebral injections of the neurotoxin 6-hydroxydopamine (6-OHDA) [30]. An increased behavioral response to dopaminergic agonists [39] has also been reported. This progressive decline in dopaminergic and motor function is partially reversed by L-DOPA administration in both man and rats [29] or by intracerebral dopaminergic grafts in rats [13].

Age of the organism at the time of injury is also a major determinant of the extent of recovery from acute brain lesions with younger individuals in general having a better prognosis. However, the mechanisms underlying this age-determined neural plasticity are not well defined [1, 12]. Since lesioned brain is more susceptible to dopaminergic manipulation [14, 18, 22, 39], age related changes in dopaminergic function may be involved in the differential recovery from brain lesions in young versus aged populations.

Dopaminergic mechanisms are also involved in the recovery process from motor deficits due to cortical damage. Animals with motor cortex lesions manifest a slowed recovery from hemiplegia after haloperidol administration and an enhanced recovery after amphetamine administration when this drug is given 24 hours after motor [11] or frontal [21] cortex ablations. These studies did not investigate the effects of aging on the degree of dopaminergic sensitivity. We have shown that chronic (7 days), local infusion of the inhibitory neurotransmitter GABA to the hindlimb cortical representation via osmotic minipumps results in the poten-
Surgical Procedures

Animals were housed in groups of 5, in Plexiglas cages (70x45x25 cm) with free access to food and water, and maintained on a 12 hours light-dark cycle. The animals were exposed and ablated by aspiration (unilaterally) of the somatomotor cortex of nine young adult (9-12 months) rats was exposed and ablated by aspiration with a modified Pasteur pipette, bent to an adequate angle (approximately 45°) and provided with a small hole through which the aspiration pressure could be controlled. The cavity left was filled with gelfoam, the bone and skin replaced, and systemic antibiotic treatment instituted.

Drug Administration

Haloperidol studies. At 3 weeks post-minipump implantation, or when the animals had returned to pre-GABA motor performance or had reached stable motor scores, 0.1 mg/kg of haloperidol (donated by McNeil Pharmaceutical, Spring House, PA) was administered IP to GABA and saline minipump implanted young and aged rats and to trained, non-operated young and aged control groups. This dose was chosen after pilot experiments showing that higher doses interfered with normal motor performance on the beam due to the intensity of the neuroleptic syndrome. The group of 9 animals recovered from the hemiplegic syndrome due to motor cortex ablation (performed 7 to 12 months prior) were also assessed for sensitivity to the neuroleptic. This group was given the haloperidol after retraining on the beam until stable scores were reached (7 to 10 days).

Apomorphine studies. The dopaminergic agonist apomorphine (Sigma) was administered systemically both to animals recovered or stabilized for at least 3 days from the GABA-mediated motor deficit, and to animals recovered from the motor cortex aspiration (see above, Haloperidol studies). In the young group, this was from 5 to 7 days after the beginning of the GABA infusion. In aged rats, this was from 10 to 15 days after minipump implantation since aged animals did not reach stable motor performance after GABA application until this period [2]. Subcutaneous doses of 0.5 and 1.0 mg/kg were employed since higher doses reproduced stereotyped behavior ("freezing") that interfered with the motor task.

Amphetamine studies. These experiments were conducted only in young animals. After training, either saline- or GABA-filled minipumps were implanted (n=20/group).

### TABLE 1

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Scale × Percentage = Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unable to run</td>
<td>6</td>
</tr>
<tr>
<td>Dragging limb</td>
<td>5</td>
</tr>
<tr>
<td>Falls or &gt;3 slips</td>
<td>4</td>
</tr>
<tr>
<td>&lt;3 slips and/or 4 toes off beam</td>
<td>3</td>
</tr>
<tr>
<td>(unilaterally)</td>
<td></td>
</tr>
<tr>
<td>Hypotonus (limping)</td>
<td>2</td>
</tr>
<tr>
<td>Wider sustentation</td>
<td>1</td>
</tr>
<tr>
<td>base (4 toes off)</td>
<td></td>
</tr>
<tr>
<td>(beam, bilaterally)</td>
<td></td>
</tr>
<tr>
<td>No apparent deficit</td>
<td>0</td>
</tr>
</tbody>
</table>

Behavior (range)

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Surgical Procedures

Minipump groups. Under ketamine-pentobarbital anes-
Behavioral Effects of Minipump Implantation

RESULTS.

Behavioral Effects of Minipump Implantation

The motor deficits observed in the GABA-treated animals have been previously reported [2]. Briefly, these deficits ranged from a severe contralateral hemiplegia on the first two days after surgery, to more discrete abnormalities in paw placing. As in prior studies, the GABA-treated group had a motor deficit that was significantly greater than the saline group over the seven days post-implantation. Similarly, the aged GABA-treated group had significantly greater motor impairments than aged saline-treated animals for at least 25 days after surgery. Comparison between the young and aged GABA-treated animals revealed that the aged group had significantly greater deficits, $F(1,11)=13.97$, $p=0.004$, largely due to unilateral motor abnormalities subsisting in the late post-infusion period (i.e., after day 7). Both groups reached stable motor performance 10 to 15 days after surgery.

The deficits were predominantly motor since sensory abnormalities became rare by the third post-implantation day in both young and aged GABA and saline-treated animals. The sensory deficits consisted of an initial decreased or absent orientation to the contralateral stimuli, with withdrawal apparent by the second day and no lateralized deficits apparent by day 3 or 4 in any group.

Drug Effects

Haloperidol and apomorphine studies. Acute administration of the neuroleptic to recovered aged and young GABA-treated animals produced a re-emergence of the contralateral motor deficit. The motor syndrome was similar to that developed by the animals during the initial 3 days post-minipump implantation, again consisting of initial tilting off the beam contralateral to the implantation site followed by dragging and placing abnormalities in the hindlimb contralateral to the GABA-infusion site. The young group had largely recovered from this motor deficit by 24 hours. In contrast, the aged group had a significant prolongation of the contralateral motor deficit, for young vs. aged, $F(1,15)=100.80$, $p<0.001$, which lasted for 72 hours post-haloperidol administration (see Fig. 1). Haloperidol given to non-operated control animals produced non-specific deficits, consisting mainly of a wider hindlimb base without any evidence of a lateralized motor deficit as seen in both the young and aged GABA-treated groups. This gait instability cleared in the first 24 hours after drug administration.

Acute administration of haloperidol to animals recovered from motor cortex lesions performed 7 to 12 months prior failed to produce any consistent lateralized motor deficit. All these animals, however, showed a characteristic neuroleptic syndrome (freezing, squeaking, and some degree of catalepsia), which was similar to the non-operated, control group.

We attempted to modify the effects of haloperidol by administering apomorphine before or after the dopaminergic antagonist. In both young and aged animals, we found that a 1 mg/kg dose induced the well-described syndrome of gnawing, sniffing, licking and stereotypies [7]. This stereotyped behavior interfered with motor performance on the beam. Apomorphine administration at a lower dose (0.5 mg/kg) did not interfere with beam walking, but did not reverse or prevent the deleterious effect of haloperidol on motor function in either young or aged GABA-treated groups.

Amphetamine studies. Acute administration of amphetamine in young GABA and saline-treated young adult animals failed to alter the progression of the motor deficit produced by the intracortical infusions. Both amphetamine- and saline-treated groups showed comparable motor per-
Behavioral Effects of Motor Cortex Aspiration

This group had transient (less than 3 days) motor impairments which did not differ significantly from the ones observed in the saline-minipump group (see Fig. 2). In the first day post-surgery, the motor cortex aspirated animals were unable to walk on the beam and remained immobile, grasping the bar with their body tilted contralateral to the operated side. By the second day, most of the rats were able to walk on the beam, although lateralized motor deficits consisting in abnormalities in paw placing were readily apparent. After the third post-aspiration day, the animals appeared more active and their motor deficit scores approached those of the pre-surgery period.
The sensory deficits detected were comparable to those seen in the minipump groups being apparent only in the first two or three days post-surgery and negligible thereafter. These animals were followed daily for 2 to 4 weeks and intermittently thereafter. When tested after more than two weeks of no-training, the motor performance was similar to the one presented by the animal at the initiation of motor training on the beam: clumsy and slow, but without any signs of lateralization. After 7 to 10 days of retraining, the animals again reached stable motor scores on the beam.

**Histology**

All lesions in the minipump implanted group were localized to the hindlimb representation of the motor cortex [9, 17, 37]. In these animals, the extent and distribution of the pathology was comparable to that observed in the initial studies [2,3] and consisted of a 2.2×1.5×1.8 mm (in length, width, and depth, respectively) lesion in the young, GABA-treated animals, and a 1.74×0.78×1.9 mm lesion in the aged group. No statistically significant differences in lesion size were found between groups. Figure 3 shows the location and extent of the lesions in the motor cortex aspiration group.

**DISCUSSION**

Localized, chronic administration of GABA to the motor cortex of young and aged rats potentiates a contralateral hemiplegia due to small cortical lesions. Both young and aged animals recovered or stabilized from the initial motor deficit by the end of the second post-surgical week. However, the aged animals took a significantly longer time to reach stable motor performance and never attained pre-GABA infusion motor scores [2]. After recovery or stabilization from the GABA-mediated hemiplegia, a systemic low dose of haloperidol induced the re-emergence of a severe contralateral motor deficit in both young and aged animals which was significantly prolonged in the aged population. As illustrated in Fig. 1, the young animals showed, both in control and GABA-infused groups, greater responsivity and faster recovery (by the second post-injection day) from the neuroleptic effects than the aged rats.

Our results are in accord with previous reports in non-aged rats in which haloperidol administration 24 hours after motor cortex ablation slowed recovery of locomotor activity and blocked the facilitation of recovery produced by amphetamine [11]. Haloperidol-induced reappearance of postural deficits or contralateral inattention has been reported in rats previously given either cortical ablations [18] or neurochemical lesions [29]. In humans, worsening of neurological deficits has also been reported in stroke patients receiving adrenergic blockers such as phenoxybenzamine or propranolol [33].

The prolonged effects of haloperidol in aged rats may relate to increases in dopaminergic receptor sensitivity [20,30], to a reduced biosynthetic rate of dopaminergic receptors [19] or to slower rates of dopaminergic receptor turnover occurring with age [25]. Systemic pharmacokinetic differences may also contribute to the slower recovery reported here since aged rats have a reduced plasma clearance of haloperidol [24].

Of interest are observations that administration of dopaminergic blockers prior to central lesions has been reported to be beneficial to recovery of function [22,42]. Chronic administration of neuroleptics has been shown to induce denervation supersensitivity [14], probably mediated by increases in receptor population. This enhancement of dopaminergic receptor function might provide an extended security margin in cases of lesions affecting structures with dopaminergic influence. The beneficial effects on motor performance of dopaminergic agonists [6,29] or nigral grafts [13] in aged animals with or without lesions would support this idea. Conversely, an aged brain with diminished dopaminergic function would be more susceptible to both the effects of brain lesions and to the effects of dopaminergic antagonists.

The reported beneficial effects of amphetamine administration in the initial stages of a motor [11] or visual [10] cortex lesions were not found in this study. The method of repeated motor training on the beam in the 24 hours post-drug administration employed by these authors (one trial on the beam every hour for 6 hours) appears to be one basic difference. Previous reports [6,34] have also failed to demonstrate a significant effect of amphetamine on recovery after cortical or nigral lesions in either cats or rats. The reported beneficial effects of amphetamine appear then to be contingent on reinstalling intensive motor training in the immediate 24 hours post-lesion, and perhaps on the use of higher (>2 mg/kg) doses of the drug.

Systemic apomorphine administration (0.5 mg/kg) failed to protect the GABA-treated animals from the deleterious effects of haloperidol on motor function. The dose used is well above the ED50 for stereotypies reported [5], for both young (0.14 mg/kg) and aged (0.05 mg/kg) rats. The animals were evaluated 15 to 30 minutes after drug administration at a time which the drug has been reported to show maximal effects. However, pharmacokinetic differences in the stereotyped behavioral response between young and aged rats have been reported to occur 40 to 60 minutes after administration [5] indicating that subsequent studies will need to evaluate the effects of apomorphine given at longer intervals and with different doses. Focal delivery of apomorphine might also be expected to provide protection against the deleterious effects of haloperidol.

The findings of a lack of haloperidol effect on motor performance in 7–12 months post-lesioned animals is comparable to the reported lack of effects of haloperidol administration on animals recovered from somatosensory cortex lesions for long periods of time (3–8 months) [10]. This suggests that the increased susceptibility to neuroleptics in brain-lesioned individuals is transitory.

It is of particular interest to note that the debilitating effect of haloperidol is not observed when phenytoin, a drug claimed to interact with GABA receptors, is given to animals recovered from an intracortical, chronic GABA infusions following identical procedures as those used in this study. In contrast, the anticonvulsant increased the severity of the motor deficit when administered during the GABA treatment [3]. This would indicate the specificity of haloperidol effects.

Our findings of an increased susceptibility to haloperidol in aged animals may have important clinical implications. A study on the use of psychoactive agents in elderly populations has shown that there is a significant increase in sensitivity to these drugs with age, with the greatest effect occurring with antipsychotics [36]. Our results suggest caution in prescribing dopaminergic antagonists to brain-lesioned patients in the initial stages of the recovery process, since dopaminergic blockade enhances the accompanying deficits, particularly so in aged organisms.


