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Effects of Lesions in the Medial Prefrontal Cortex on the Activity of Midbrain Dopamine Neurons

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To determine whether lesions in the prefrontal cortex (PFC) alter the activity of midbrain dopamine (DA) neurons, single unit recordings were made from DA neurons in control and lesioned rats. PFC lesions, obtained by local injection of ibotenic acid into the medial PFC, had no effect on either firing rate or bursting activity of DA neurons in the ventral tegmental area (VTA). However, the number of spontaneously active DA neurons in the VTA was significantly decreased. In the substantia nigra (SN), the same lesions increased the firing rate and had no effect on either the bursting activity of the number of active DA cells. These results suggest that PFC lesions alter the activity of DA neurons. However, VTA and SN DA neurons may respond differently to PFC lesions. **© 1996 American College of Neuropsychopharmacology [Neuropsychopharmacology 15:437–441, 1996]**

KEY WORDS: *Prefrontal cortex; Ventral tegmental area; Substantia nigra; Dopamine; Ibotenic acid; Single unit recording*

Lesions in the prefrontal cortex (PFC) have been suggested to lead to an overactivity of dopamine (DA) in subcortical structures. Such an interaction between cortical and subcortical DA systems may play a critical role in the development of psychotic symptoms in schizophrenia (see reviews by Weinberger 1987; Grace 1991; Deutch 1992). Whereas studies, in general, support this suggestion, the mechanism through which the PFC interacts with subcortical DA systems remains unclear. One possibility is that glutamate released from cortical terminals acts directly on DA terminals in DA target areas to cause DA release. Consistent with this suggestion are the findings that glutamate and DA terminals in the striatal complex are often in close proximity to each other (e.g., Bouyer et al. 1984; Sesack and Pickel 1992) and glutamate applied locally in the striatum in vivo or in striatal slices induces a significant increase in DA release (e.g., Marien et al. 1983; Jin and Fredholm 1994; Ohta et al. 1994). However, as PFC projection neurons may also make direct synaptic contacts with DA neurons located in the midbrain (Sesack and Pickel 1992) and regulate the firing activity of DA neurons (Thierry et al 1979; Gariano and Groves 1988; Murase et al 1993), it is possible that PFC lesions lead to an alteration of the activity of midbrain DA neurons, thereby influencing the subcortical DA transmission. Consistent with this suggestion, Taber et al. (1995) recently showed that application of glutamate antagonists to the VTA blocks DA release in the nucleus accumbens induced by activation of the PFC. In this study, we used single unit recording techniques to record directly from DA neurons in the midbrain to determine whether their activity is altered by lesioning the PFC.

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MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 200 and 320 g were divided into three groups: lesioned (n = 12), sham-lesioned (n = 9), and nonlesioned (n = 16). In the first two groups, rats were first anesthetized with chloral hydrate (300 mg/kg, IP). A 30-gauge injection needle was then stereotaxically lowered into the mPFC (from the bregma, AP 3.0 mm, ML 0.8, and DV 4.2 mm), through which ibotenic acid (Sigma, 50 μmol/L, dissolved in saline) or the vehicle was infused with a micropump at the rate of 200 nl/minute for 2 minutes. Recordings were conducted 4 to 14 days after the surgery. In all animals studied, lesions were observed in the infralimbic and ventral prelimbic division of the PFC (Figure 1). All surgical procedures were carried out according to the Guide for the Care and Use of Laboratory Animals by the U.S. Public Health Service and were approved by the Yale Animal Care and Use Committee.

DA neurons were recorded as described previously (Bunney et al. 1973). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, IP with supplemental doses administered via the lateral tail vein) and mounted in a stereotaxic instrument. Body temperature was maintained at 36 to 37°C with a heating pad. Single barrel glass electrodes filled with 1.0 mol/L NaCl and 2% pontamine sky blue dye (5 to 15 MΩ) were lowered through 12 recording tracks in a stereotaxically defined tissue block within the SN (from the lambda, AP 2.7 to 3.3 mm, ML 1.8 to 2.2 mm, DV 6.0 to 8.0 mm) and the VTA (from the lambda, AP 2.7 to 3.3 mm, ML 0.5 to 0.9 mm, DV 6.0 to 8.0 mm), using a hydraulic microdriver (Bunney and Grace 1978). Spontaneously active DA neurons were identified based on previously established criteria (Bunney et al. 1973; Grace and Bunney 1983). Interspike intervals were collected on-line by a PC computer (486 interfaced via a Lab-PC$^+$, National Instrument) using programs written by one of the au-

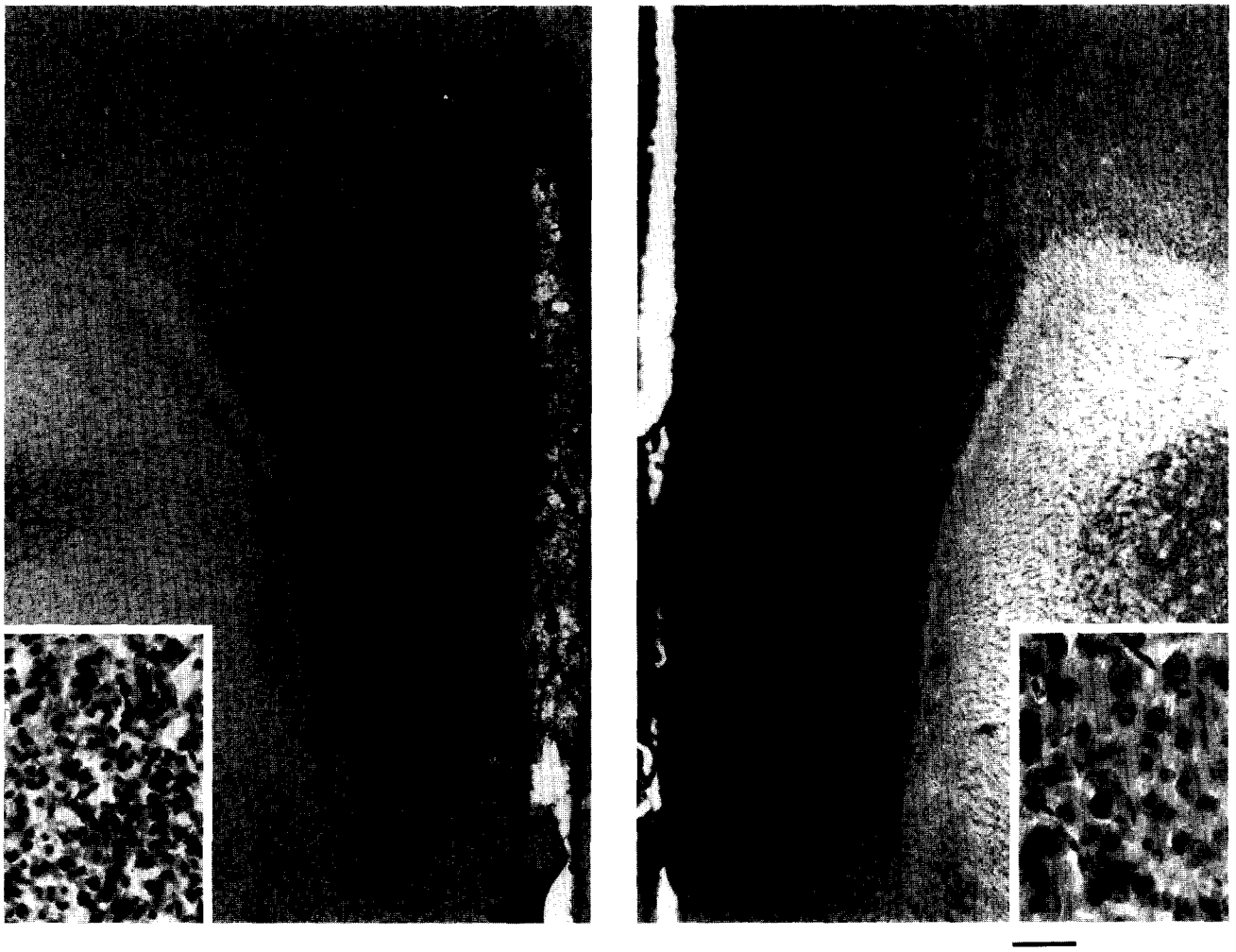

Figure 1. Photomicrographs of Cresyl violet–stained sections through the medial prefrontal cortex with ibotenic acid lesion (*left*, 7 days after the injection) and without (*right*). Inserts are cells in layer V and VI viewed under a high magnification. Note the gliosis with concomitant neuronal loss in the lesioned medial prefrontal cortex. Scale bar = 400 μm and 50 μm for the low and high power, respectively.

thors (W.S.). Off-line data analysis was performed in Microsoft Excel. The onset and offset of burst firing were defined as the interspike intervals of less than 80 milliseconds and greater than 160 milliseconds, respectively (Grace and Bunney 1984). Lesion and recording sites were confirmed in Cresyl violet–stained sections. All values are expressed as mean ± SEM. Differences between groups were evaluated by using ANOVA followed by post-hoc Tukey HSD tests (SYSTAT, Evanston, IL).

RESULTS

The activity of DA neurons was not altered by the sham operation as there was no difference in any of the parameters measured (number of active cells, firing rate or spikes in burst) between nonlesioned and sham-lesioned animals. However, DA neurons in both the VTA and SN were affected by the mPFC lesion (Figure 2). In the VTA, the number of active DA neurons was significantly reduced in PFC lesioned rats when compared with either nonoperated or sham-lesioned rats (nonlesion: 1.36 ± 0.09 cells/tract, $n = 10$; sham lesion: 1.40 ± 0.14, $n = 4$; lesion: 0.56 ± 0.11, $n = 7$). The average firing rate of the cells was, however, not significantly different between groups (nonlesion: 3.64 ± 0.14 spikes/second, $n = 169$, sham lesion: 3.91 ± 0.26, $n = 47$; lesion: 4.21 ± 0.30, $n = 37$). VTA DA neurons in PFC-lesioned rats also showed more bursting activity. However, the change was statistically not significant (nonlesion: 26.75 ± 2.00% of spikes in burst, $n = 169$; sham lesion: 22.90 ± 3.70%, $n = 47$; lesion: 34.83 ± 4.17%, $n = 37$).

Unlike in the VTA, the number of active DA cells in the SN was not significantly altered by PFC lesioning, although there was a tendency toward a decrease in the lesioned group (nonlesion: 1.15 ± 0.1, $n = 6$; sham lesion: 1.02 ± 0.11, $n = 5$; lesion: 0.83 ± 1.1, $n = 5$). The basal firing rate, however, was significantly increased in PFC lesioned rats (4.89 ± 0.26 spikes/second, $n = 46$) when compared with either nonlesioned (3.85 ± 0.21, $n = 73$) or sham-lesioned rats (3.71 ± 0.25, $n = 50$). As observed in the VTA, a statistically nonsignificant increase in bursting activity was observed in the SN (nonlesion: 15.75 ± 2.60% of spikes in burst, $n = 73$; sham lesion: 15.49 ± 3.15%, $n = 50$; lesion: 20.11 ± 3.28%, $n = 46$).

DISCUSSION

These results are consistent with the hypothesis that PFC lesions alter the activity of DA neurons located in the midbrain. The data suggest, however, that DA neurons in the VTA and the SN respond in different ways to the lesion. In the VTA, the overall activity of DA neurons was decreased, whereas that in the SN was increased by the same lesions in the PFC.

A decrease in VTA DA neuron activity in PFC-lesioned rats is consistent with previous studies showing that the PFC has an excitatory effect on DA neurons.

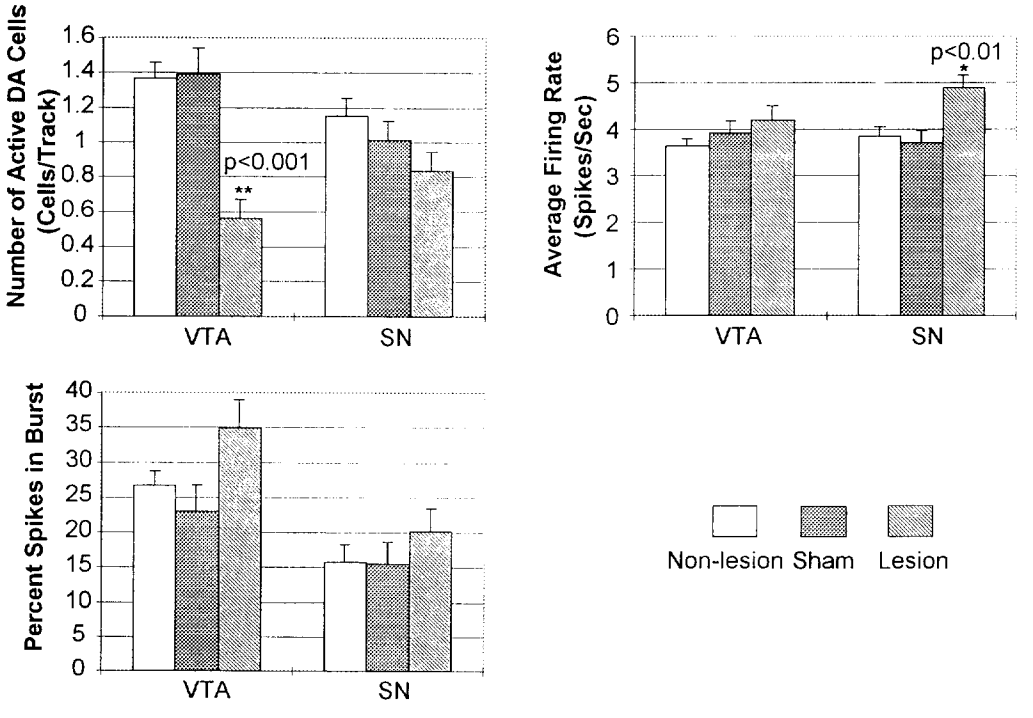

Figure 2. Effects of lesion of the mPFC on the activity of midbrain DA neurons. *Upper left*: The number of active DA cells in the VTA was significantly decreased in mPFC-lesioned rats. The same lesion, however, had no effect in the SN. *Upper right:* mPFC lesion significantly increased the firing rate of SN DA neurons but had no effect on VTA DA neuron activity. *Lower left*: A tendency toward an increased bursting was observed for both VTA and SN DA neurons in mPFC-lesioned rats; however, the change was statistically not significant.

Electrical and pharmacologic stimulation of the mPFC has been shown to increase the burst firing of spontaneously active DA neurons (Thierry et al. 1979; Gariano and Groves 1988; Murase et al. 1993), whereas cooling the PFC decreases burst firing (Svensson and Tung 1989). Recently, Taber et al. (1995) showed that application of glutamate antagonists to the VTA blocks PFC stimulation-induced DA release in the nucleus accumbens. The present study, showing a reduction in the number of spontaneously active VTA DA cells in PFC-lesioned rats, suggests that a tonic input from the mPFC may be necessary for some VTA DA neurons to fire spontaneously.

However, in addition to the direct PFC–VTA pathway, the PFC may affect DA neurons indirectly through other areas such as the nucleus accumbens, which receives massive input from the PFC (Sesack et al. 1989) and, in turn, projects to the VTA. Because accumbens projections to the VTA are GABAergic, PFC lesions may, via PFC–accumbens–VTA pathway, disinhibit VTA DA neurons leading to a depolarization inactivation of VTA DA neurons. Another possible explanation for our findings is that ibotenic acid injection in the PFC caused a degeneration of VTA DA neurons projecting to the PFC, resulting in a reduced number of active VTA DA neurons. Further investigation will be required to determine whether and how each of these possible mechanisms may contribute to the observed effect of PFC lesion on VTA DA neurons.

A direct PFC projection to the SN may also exist (Christie et al. 1985; Fiedler and Bustos 1991; Goswell and Sedgwick 1973; Kornhuber et al. 1984; Naito and Kita 1994). However, this projection seems unlikely to be responsible for the observed increase in SN DA neuron activity in PFC-lesioned rats. PFC projection neurons are believed to be excitatory, using either glutamate or aspartate. By lesioning this PFC-SN excitatory pathway, one would expect, instead of an increase, a decrease in DA neuron activity in the SN. Thus, other non-DA neurons that are affected by the PFC may be involved. One possibility is that PFC terminals synapse on primarily SN non-DA neurons (GABAergic), which, in turn, inhibit DA neurons (Fujimoto and Kita 1992). Removal of the PFC afferent may thus lead to a decrease in the activity of these inhibitory neurons and indirectly cause an increase in SN DA cell firing. The corticostriatal pathway may also play a role, as PFC lesions may decrease the activity of striatal neurons involved in the inhibitory feedback pathway of SN DA neurons.

Whereas the exact mechanism through which midbrain DA neurons are affected by PFC lesions remains to be determined, changes in the activity of DA neurons would predict corresponding changes in DA terminal release. However, variable effects of PFC lesions on DA levels have been reported. For example, Pasinetti et al. (1991) noticed a significant decrease in DA levels in the striatum after unilateral ablation of the frontal cortex. Jaskiw et al. (1990a), however, reported a transient increase in striatal DA levels. In the nucleus accumbens, PFC lesion produced either no effect or a transient increase in DA levels (Christie et al. 1986; Jaskiw et al. 1990b). When the animal is subjected to mild stress, a prolonged increase in DA release in the nucleus accumbens is observed in PFC-lesioned rats (Jaskiw et al. 1990b). Taken together, these observations suggest that a PFC lesion may affect DA neurons in different ways under different circumstances. As the experiments of the present study were performed in anesthetized animals, whether and how PFC lesions affect the activity of DA neurons in awake animals, particularly under stress, remains to be determined.

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