Enduring Cognitive Deficits and Cortical Dopamine Dysfunction in Monkeys After Long-Term Administration of Phencyclidine

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The effects of the psychotomimetic drug phencyclidine on the neurochemistry and function of the prefrontal cortex in vervet monkeys were investigated. Monkeys treated with phencyclidine twice a day for 14 days displayed performance deficits on a task that was sensitive to prefrontal cortex function; the deficits were ameliorated by the atypical antipsychotic drug clozapine. Repeated exposure to phencyclidine caused a reduction in both basal and evoked dopamine utilization in the dorsolateral prefrontal cortex, a brain region that has long been associated with cognitive function. Behavioral deficits and decreased dopamine utilization remained after phencyclidine treatment was stopped, an indication that these effects were not simply due to direct drug effects. The data suggest that repeated administration of phencyclidine in monkeys may be useful for studying psychiatric disorders associated with cognitive dysfunction and dopamine hypofunction in the prefrontal cortex, particularly schizophrenia.

Phencyclidine (PCP) is a noncompetitive N-methyl-D-aspartate receptor antagonist with psychotomimetic effects. A single ingestion of PCP can induce transient clinical symptoms of schizophrenia in humans (*1*); repeated ingestion can result in a long-lasting syndrome marked by neuropsychological deficits, social withdrawal, and affective blunting as well as hallucinations, formal thought disorder, paranoia, and delusions (*2*). In particular, cognitive dysfunction, a prominent and stable symptom of schizophrenia (*3*, *4*) and frontal lobe damage (*5*, *6*), has been observed in long-term abusers of PCP (*2*), and decreased utilization of glucose in the frontal lobes (hypofrontality) of PCP abusers has been reported (*7*). These data suggest that frontal lobe dysfunction occurs after long-term intake of PCP, but the neurochemical mechanism underlying PCP-induced behavioral changes is not clear. The antiglutamatergic effects and psychotomimetic properties of PCP contributed to development of a hypoglutamatergic hypothesis of schizophrenia (*8*).

The dopaminergic innervation of the frontal lobe and the prefrontal cortex in particular has a critical neuromodulatory role in the regulation of cognition in the primate (*5*). Increased perseveration (inappropriate repetition of a previously rewarded response) and abnormalities in working memory, behavioral inhibition, affect, attention, planning, drive, and social interactions have been observed in schizophrenic individuals (*4*) and in PCP-abusing humans (*1*, *2*), or as a result of lesions of the prefrontal cortex in humans and monkeys (*7*). In monkeys, many of these behavioral deficits can be induced by manipulations that specifically or preferentially disrupt dopamine transmission in the prefrontal cortex (*9*, *10*).

Because long-term abuse of PCP in humans may represent a pharmacological model of cognitive deficits of the frontal cortex that are associated with schizophrenia, we explored the behavioral and neurochemical effects of long-term exposure to PCP (twice a day for 14 days) in the vervet monkey (*Cercopithecus aethiops sabaeus*), an Old World primate. We designed experiments to test whether repeated treatment with PCP induces enduring cognitive dysfunction. Cognitive functions of the frontal lobe depend on the integrity of dopaminergic innervation; therefore, to better understand the neurochemical pathology underlying observed behavioral deficits, we measured dopamine utilization in the forebrains of PCP-treated monkeys.

Monkeys were trained on the "object retrieval with a detour" task (*11*); performance of this task is sensitive to disruptions in function of the prefrontal cortex (*12*) and to reductions in mesotelencephalic dopaminergic tone (*10*) but not to hippocampal lesions (*12*). We had demonstrated earlier that this task is an appropriate measure of behavioral disinhibition and of perseveration in monkeys (*10*), both symptoms of dysfunction of the frontal cortex (*3*, *4*, *6*). Compared with saline-treated controls, PCP-treated monkeys displayed significant performance deficits ($P < 0.001$) (Fig. 1A) 7 days after cessation of drug administration. PCP-treated monkeys displayed deficits in response inhibition ($P < 0.001$) (Fig. 1B) and exhibited a greater propensity to persevere than control animals ($P < 0.001$) (Fig. 1B). These deficits were still present in PCP-treated animals at least 4 weeks after cessation of drug treatment. Thus, repeated exposure to PCP resulted in prolonged behavioral deficits. These impairments (perseveration and poor response inhibition) are similar to those observed in schizophrenic individuals (*3*, *4*) and in

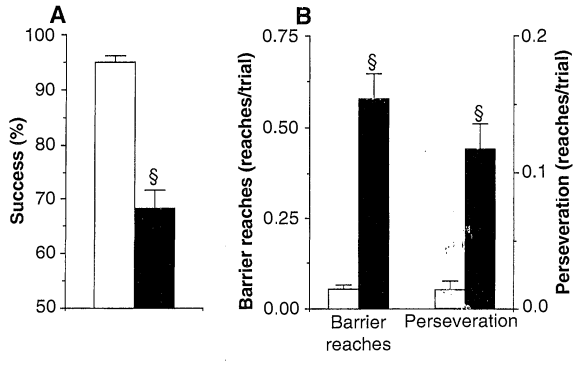

Fig. 1. (**A**) Monkeys treated with PCP (solid bars) (0.3 mg/kg twice a day, intramuscularly) for 14 days ($n = 6$) were significantly less successful ($^{\S}P < 0.001$) on performance of the object retrieval with a detour task than were saline-treated monkeys (open bars) ($n = 5$). In this task, subjects retrieve a reward from a transparent box affixed to a tray. The box has one open side, which is directed to the front, left, or right of the monkey. When the open side is directed to the right or left, the monkey has to inhibit a prepotent response to reach directly at the reward. Success was scored on a trial if a monkey obtained the reward in one reach response without touching a closed, transparent face of the box. (**B**) Monkeys treated with PCP (solid bars) ($n = 6$) produced significantly more barrier reaches (defined as a reach into a closed, transparent side of the box) ($^{\S}P < 0.001$) and perseverative responses (defined as a reach at a closed, transparent side of the box that is identical to the previous successful, rewarded response) ($^{\S}P < 0.001$) than saline-treated animals (open bars) ($n = 5$). Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) with repeated measures and Scheffe's F-test were used to determine significance.

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humans and monkeys with damage to the prefrontal cortex (*7*).

We next investigated the effects of 2 weeks of exposure to PCP on regional dopaminergic utilization in the primate brain. Although a single dose of PCP increases cortical dopamine utilization (*13*), monkeys treated repeatedly with PCP (0.3 mg per kilogram of body weight twice a day for 14 days) displayed a measurable decrease in dopamine utilization in the dorsolateral prefrontal cortex (to 60% of control; $P < 0.01$) (Fig. 2A). A profound decrease in dopamine utilization was also observed in the prelimbic cortex (to 32% of control; $P < 0.001$) (Fig. 2A). No significant effect was observed in other regions of the frontal cortex such as the dorsomedial prefrontal cortex, lateral orbital cortex, and anterior cingulate cortex or other dopamine-rich subcortical structures such as the caudate nucleus, putamen, and nucleus accumbens core or shell (Fig. 2A). The changes in dopamine utilization in the dorsolateral prefrontal and prelimbic cortices were due to decreases in concentrations of the dopamine metabolite homovanillic acid, because we did not observe any differences in absolute dopamine concentrations of saline- and PCP-treated animals. This suggests that PCP-induced changes in dopamine function are due not to neurotoxicity (that is, a decrease in cortical dopamine innervation), but rather to functional hypoactivity of mesocortical dopamine neurons. No changes in serotonin utilization in the dorsolateral prefrontal cortex were observed in PCP-treated animals.

Next, we compared drug-evoked increases in dopamine utilization in the prefrontal cortices of PCP-treated monkeys with saline-treated control animals. The increase in dopamine utilization in the dorsolateral prefrontal and prelimbic cortices in monkeys given a single injection of PCP was not observed in monkeys after long-term exposure to PCP (Fig. 2B), similar to what has been observed in rodents (*14*). Thus, repeated treatment with PCP leads to a drug-induced inhibition of basal and PCP-evoked dopamine utilization in the prefrontal cortex.

Data from schizophrenic subjects support the idea that a decrease in cortical dopamine function may contribute to the cognitive dysfunction in schizophrenia. Augmentation of mesotelencephalic dopaminergic function by administration of amphetamine or apomorphine lessens the cognitive dysfunction or the failure of metabolic activation of frontal cortical regions during performance of a cognitive task in schizophrenic individuals (*15*). Moreover, many of the typical neuroleptic drugs (which share D2 receptor antagonist actions) have minimal efficacy on, and may in fact exacerbate, cognitive dysfunction in schizophrenia (*16*). These data suggest that this subset of behavioral deficits in schizophrenia may be due to hypoactivity of dopamine systems that innervate the frontal cortices.

If the behavioral and biological abnormalities induced by repeated PCP administration resemble those observed in schizophrenia, then PCP-induced behavioral impairments may respond to drug treatment in a manner that is qualitatively similar to that of the deficits of schizophrenia. Although the effects of clozapine (a so-called atypical neuroleptic agent) on schizophrenic cognitive deficits, in particular, are controversial (*17*), this drug has been reported to alleviate the symptoms of treatment-refractory schizophrenic patients without inducing measurable extrapyramidal effects (*18*). We treated monkeys trained to perform the object retrieval with a detour task with clozapine (*19*). There was a definitive improvement in those animals previously treated with PCP; that is, the performance deficits were partially ameliorated ($P < 0.05$) (Fig. 3). In contrast, clozapine treatment impaired the performance of control animals ($P < 0.05$) (Fig. 3); these results are similar to the effects of clozapine that have been observed in normal humans and monkeys (*20*).

The beneficial effects of clozapine on behavioral impairments may be related to its ability to increase extracellular dopa-

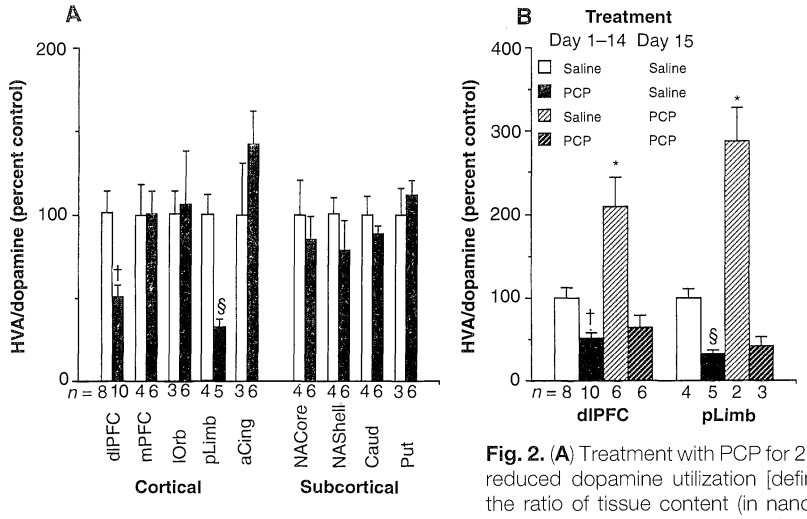

Fig. 2. (**A**) Treatment with PCP for 2 weeks reduced dopamine utilization [defined as the ratio of tissue content (in nanograms per milligram of protein) of homovanillic acid (HVA) to dopamine] in the dorsolateral prefrontal ($^{\dagger}P < 0.01$) and prelimbic cortices ($^{\S}P < 0.001$), but not other regions, of the monkey brain. PCP (solid bars) (0.3 mg/kg) or saline (open bars) (0.1 ml/kg) was administered twice daily for 14 days to young adult (2.0 to 3.5 kg) male and female monkeys. Twenty-four hours after the final treatment, the animals were killed and saline-perfused, the brain was removed, and the tissue was dissected, stored, and analyzed as described (*13*). (**B**) A single dose of PCP increases dopamine utilization in the dorsolateral prefrontal and prelimbic cortices of control monkeys ($^{*}P < 0.05$) but not monkeys that were treated with PCP for 2 weeks. Monkeys were treated with either saline or PCP for 14 days (0.3 mg/kg twice a day), and on the 15th day, a single dose of PCP (0.3 mg/kg) or saline was administered, and the monkeys were killed. Tissue was harvested and processed as in (A). dlPFC, dorsolateral prefrontal cortex; mPFC, medial prefrontal cortex; lOrb, lateral orbital cortex; pLimb, prelimbic cortex; aCing, anterior cingulate cortex; NACore, core of nucleus accumbens; NAShell, shell of nucleus accumbens; Caud, caudate nucleus; Put, putamen. All values are means ± SEM, and data are expressed as percentage of control. One-way analysis of variance and Scheffe's *F*-test were used to compare group responses.

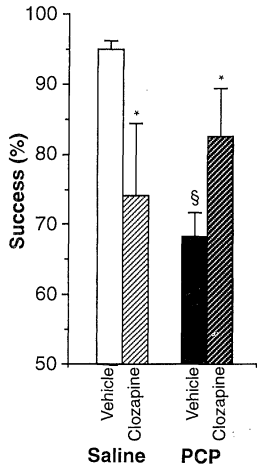

Fig. 3. Clozapine (1 mg/kg per day for 3 days) improves successful performance of the object retrieval with a detour task in monkeys previously treated for 2 weeks with PCP ($n = 6$) and tested 2 hours after the last injection of clozapine. In contrast, clozapine impairs normal, control animals ($n = 5$). Paired *t* -tests were used to compare performance on vehicle with performance on clozapine. Data represent mean ± SEM. *Different from vehicle, $P < 0.05$; §Impaired relative to saline controls, $P < 0.001$.

mine. A single dose of clozapine increases dopamine release in the primate prefrontal cortex, and long-term administration increases basal extracellular dopamine concentration in the prefrontal cortex (*21*). Although this may not be the only mechanism by which clozapine elicits its effects on PCP-induced cognitive dysfunction, this activation of the dopamine system of the prefrontal cortex may contribute to the ability of clozapine to ameliorate the impairments in our model and, perhaps, in schizophrenia.

Our data show that repeated administration of PCP inhibits basal and stimulated dopaminergic function in the prefrontal cortex of the monkey brain. The deficiency of dopamine in the prefrontal cortex that is induced by repeated administration of PCP is associated with a long-lasting cognitive deficit, which is ameliorated by the atypical therapeutic drug clozapine. These effects are observed long after PCP administration is stopped and thus cannot be attributed to direct effects of the drug. This primate model of dopamine dysfunction in the cortex may provide a paradigm for investigating the pathophysiology underlying neuropsychiatric disorders associated with a primary cognitive dysfunction in the cortex and a dopaminergic deficit in the prefrontal cortex, as is hypothesized in schizophrenia (*22*). It also may provide a means for evaluating therapeutic agents that are selectively targeted toward alleviating cortical dopamine hypofunction.

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Telomerase Catalytic Subunit Homologs from Fission Yeast and Human

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Catalytic protein subunits of telomerase from the ciliate *Euplotes aediculatus* and the yeast *Saccharomyces cerevisiae* contain reverse transcriptase motifs. Here the homologous genes from the fission yeast *Schizosaccharomyces pombe* and human are identified. Disruption of the *S. pombe* gene resulted in telomere shortening and senescence, and expression of mRNA from the human gene correlated with telomerase activity in cell lines. Sequence comparisons placed the telomerase proteins in the reverse transcriptase family but revealed hallmarks that distinguish them from retroviral and retrotransposon relatives. Thus, the proposed telomerase catalytic subunits are phylogenetically conserved and represent a deep branch in the evolution of reverse transcriptases.

Telomerase is a ribonucleoprotein enzyme responsible in most eukaryotes for the complete replication of chromosome ends, or telomeres (*1*). Its RNA subunit provides the template for addition of short sequence repeats [typically 6 to 26 nucleotides (nts) to the chromosome 3′ end (*2*). In ciliated protozoa and yeast, telomerase is regulated and the average telomere length is maintained (*3*). In most human somatic cells, however, telomerase activity cannot be detected and telomeres shorten with successive cell divisions (*4*). Telomerase activity reappears in immortalized cell lines and in about 85% of human tumors, which has led to studies of the usefulness of telomerase for cancer diagnostics and therapeutics (*5, 6*).

Telomerase RNA subunits have been identified and analyzed in ciliates, yeast, and mammals (*2, 7*), but the protein subunits have been elusive. In *Tetrahymena*, two telomerase-associated proteins (p80, p95) have been described (*8*), and p80 homologs have been found in humans and rodents (*9*); the presence of catalytic active site residues in these proteins has not been