Loss of asymmetric spine synapses in dorsolateral prefrontal cortex of cognitively impaired phencyclidine-treated monkeys

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Abstract

Schizophrenia patients, long-term abusers of phencyclidine (PCP), and monkeys treated with PCP all exhibit enduring cognitive deficits. Evidence indicates that loss of prefrontal cortex spine synapses results in cognitive dysfunction, suggesting the presence of synaptic pathology in the monkey PCP model; however, there is no direct evidence of such changes. In this study we use the monkey PCP model of schizophrenia to investigate at the ultrastructural level whether remodelling of dorsolateral prefrontal cortex (DLPFC) asymmetric spine synapses occurs following PCP. Subchronic PCP treatment resulted in a decrease in the number of asymmetric spine synapses, which was greater in layer II/III than layer V of DLPFC, compared to vehicle-treated controls. This decrease may contribute to PCP-induced cognitive dysfunction in the non-human primate model and perhaps in schizophrenia. Thus, the synapse loss in the PCP model provides a novel target for the development of potential treatments of cognitive dysfunction in this model and in schizophrenia.

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Introduction

It has been observed in both rodents and primates that withdrawal from subchronic phencyclidine (PCP) treatment leads to lasting deficits in frontal lobe-associated cognitive functions and to decreased prefrontal dopamine (DA) utilization (Jentsch et al. 1997). At the same time, there are no signs of DA system lesions or any impairment of motor, motivational or associative learning processes. Damage to the prefrontal cortex in humans and monkeys leads to signs that resemble the negative symptoms of schizophrenia (Park & Holzman, 1992; Ridley et al. 1993), particularly cognitive disturbance (Tammenga et al. 1998). Because cognitive performance and memory processes appear to be associated with remodelling of pyramidal dendritic spine synapses in prefrontal cortex (Hof & Morrison, 2004; Nimchinsky et al. 2002), loss of prefrontal spine synapses may contribute to cognitive dysfunction. Supporting this view, recent studies have demonstrated a strong correlation between the loss of asymmetric spine synapses in monkey prefrontal cortex and the impairment of cognitive functions during ageing (Dumitriu et al. 2010; Peters et al. 2008). Based on these data, it is hypothesized that loss of prefrontal spine synapses underlies cognitive dysfunction and decreased prefrontal cellular activity both in schizophrenia patients and in the PCP schizophrenia model. Indeed, dendritic spine density of prefrontal pyramidal neurons is decreased in schizophrenia (Glantz & Lewis, 2000; Kolluri et al. 2005), suggestive of spine synapse loss. More recently, we have described an extensive reduction in the number of prefrontal asymmetric (excitatory) spine synapses in the rat PCP model (Hajszan et al. 2006), at a time when animals exhibit cognitive deficits and decreased prefrontal DA turnover. However, electron microscopic demonstration of synaptic alterations in either schizophrenia patients or primate models of schizophrenia is so far lacking. Because schizophrenia is a disease of higher
brain functions, non-human primate models are critically important in schizophrenia research. Non-human primates can be assessed with relative ease for cognitive functions using tasks with both face and construct validity (Jentsch et al. 2000). In addition, important aspects of biochemistry and morphology of the monkey frontal cortex are similar to those of humans, and different from rodents (Berger et al. 1991; Williams & Goldman-Rakic, 1998). We use electron microscopic stereology to investigate the remodelling of prefrontal asymmetric spine synapses in response to a subchronic PCP treatment paradigm in monkeys (Jentsch et al. 1997), providing significantly more relevance to the neurobiology of schizophrenia than rodent models.

Method

Young adult (age 6 yr) male green (vervet) monkeys (Chlorocebus sabaeus, n = 8) used in this study were born and treated at the St Kitts Biomedical Research Foundation animal facility (AAALAC accredited; St Kitts, West Indies).

Experiments were approved by the institutional animal care and use committees of Yale University and St Kitts Biomedical Research Foundation. Monkeys were injected intramuscularly either with 0.3 mg/kg phencyclidine hydrochloride (Sigma-Aldrich, USA) or saline vehicle (0.1 ml/kg) twice daily for 14 d as described previously (Jentsch et al. 1997). The two groups each comprised four animals, matched for mean weight (PCP 6.5 ± 0.4 kg, saline 6.6 ± 0.3 kg) and mean age (PCP 6.0 ± 0.1 yr, saline 6.2 ± 0.2 yr). Seven days after completion of treatment, at a time when PCP-treated monkeys have been shown to exhibit cognitive deficits in the object retrieval detour task (Jentsch et al. 1997, 1999), saline-treated and PCP-treated monkeys were euthanized with an overdose of sodium pentobarbital. Brains were perfused with heparinized saline (1.0 l) followed by a fixative (1.5–2 l) containing 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). Subsequently, brains were post-fixed overnight in glutaraldehyde-free fixative and transported to Yale University in phosphate buffer containing 0.1% sodium azide for analysis.

The number of spine synapses in layer II/III and layer V of Walker's area 46 of DLPFC was calculated as published previously (Hajszan et al. 2006; Leranth et al. 2008). Serial sections (200 μm) were cut in the coronal plane throughout the entire DLPFC on a vibratome, and systematically sorted into ten groups. One randomly selected group of sections was post-fixed in 1% osmium tetroxide (40 min), dehydrated in ethanol (the 70% ethanol contained 1% uranyl acetate, 40 min) and flat-embedded in Durcupan (Electron Microscopy Sciences, USA) between slides and coverslips. The volume of sampling areas was estimated using the Cavalieri Estimator module of the Stereo InvestigatorTM system (MicroBrightField Inc., USA). The boundaries of Walker’s area 46 were determined according to the description of Leranth et al. (2008).

Thereafter, 20 sampling sites for electron microscopic analysis were localized in both sampling areas using a systematic-random approach, as described previously (Leranth et al. 2008). Blocks were assembled for ultracutting, trimmed, and four ~75-nm thick consecutive ultrasections were cut at each identified sampling site using a Reichert Ultracut E ultratome. At each sampling site, digitized electron micrographs were taken for the physical dissector in a Tecnai-12 transmission electron microscope (FEI Company, USA) furnished with a Hamamatsu HR/HR-B CCD camera system (Hamamatsu Photonics, Japan), at a final magnification of ×11000. The dissector technique requires picture pairs depicting identical regions in adjacent ultrasections, these identical regions being identified by landmarks, such as myelinated fibres, which do not change significantly between adjacent ultrasections due to their size. Prior to synapse counting, the pictures were coded for blind analysis. This sampling technique provided 20 dissectors for each examined layer in DLPFC, i.e. 40 dissectors per brain in total.

Asymmetric spine synapses were counted according to the rules of the dissector technique (Leranth et al. 2008) within an unbiased counting frame superimposed onto each electron micrograph. Synapsing spines were identified by the presence of post-synaptic densities, as well as by the absence of mitochondria, microtubules, and synaptic vesicles. The average volumetric density (synapse/μm³) of spine synapses for both sampling areas was then determined by dividing the sum of spine synapses counted in all samples taken from that particular sampling area by the dissector volume. The dissector volume was calculated by multiplying the area of the unbiased counting frame (79 μm²) by ultrasection thickness (average 75 nm) and by the number of dissectors (n = 20). Thus, the average dissector volume, uniformly for each sampling area, was 237.6 μm³. Finally, the volumetric density of spine synapses was multiplied by the volume of the sampling area, determined earlier, to arrive at the total number of spine synapses. The number of spine synapses was calculated independently by two different investigators (C.L. and T.H.); and the results were cross-checked to preclude
systematic analytical errors. The number of asymmetric spine synapses was compared between vehicle- and PCP-treated monkeys for each analysed region using repeated-measures ANOVA.

Results

In the electron microscope, no obvious qualitative ultrastructural changes were revealed in DLPFC of PCP-treated monkeys (Fig. 1). By contrast, electron microscopic stereology demonstrated that PCP treatment elicits a significant decline in the number of asymmetric spine synapses which was greater in layer II/III (Fig. 2a) than in layer V (Fig. 2b) of DLPFC. The PCP-induced loss in layer II/III (42%) was also greater than in layer V (33%) when the asymmetric synapses were compared as percent losses from the appropriate saline-treated controls ($t_1 = 5.2$, $p < 0.02$, two-tailed paired Student’s $t$ test).

Discussion

Our results demonstrate that subchronic PCP treatment of adult male monkeys, using a paradigm that causes enduring cognitive deficits and decreased DA utilization in the DLPFC, leads to significant reduction in the number of asymmetric spine synapses in layers II/III and V of the DLPFC. To our knowledge, this is the first electron microscopic demonstration of synaptic alterations in a primate model of schizophrenia and parallels our findings in PCP-treated rodents (Hajszan et al. 2006). Electron microscopic measures of spine synapse numbers provide valuable insights into the existence and magnitude of trophic or atrophic effects, as well as into the excitability and activity of pyramidal neurons. Thus, our finding of reduced prefrontal spine synapse numbers in this monkey model of schizophrenia is in line with clinical observations that long-term abusers of PCP develop hypofrontality, i.e. decreased glucose utilization and cellular activity in the frontal lobe (Hertzmann et al. 2006).
Our data are also in line with the results of previous studies in schizophrenia. A reduction in pyramidal cell dendritic spine density in schizophrenia has been observed in layer II/III of DLPFC of subjects with schizophrenia (Glantz & Lewis, 2000); this synaptic pathology appears to be most marked in layer III, and either more modest or absent in layer V (Kolluri et al. 2005). These results contribute to the growing body of evidence that synaptic alterations in prefrontal cortex may be critical components in the pathophysiology of schizophrenia (Lewis, 2004; Owen et al. 2005).

Dendritic spines and their asymmetric synapses are the primary mediators of glutamatergic excitation. Reducing the number of these glutamate-responsive structures is a logical defensive step for pyramidal cells to protect themselves against the danger of glutamate excitotoxicity, exerted when the calcium-buffering capacity of spines is exceeded. Alterations in glutamate signalling are known to induce dendritic spine remodelling in prefrontal cortex (McKinney, 2010). Via this cellular defence mechanism, prolonged PCP-elicited glutamate release may act to reduce the number of prefrontal spine synapses in our monkey PCP model.

In addition to glutamatergic mechanisms, dopaminergic tone may also be critical for the maintenance of asymmetric spine synapses. In the primate prefrontal cortex, dopaminergic synapses form part of a three-way synaptic complex in which the dendritic spine of a pyramidal neuron is innervated by both a dopaminergic symmetric synapse and a glutamatergic asymmetric synapse (Goldman-Rakic et al. 1989). This arrangement allows for direct strategic DA modulation of the overall excitability of cortical projection neurons by altering local spine responses to excitatory inputs. An earlier study has shown that there is a specific loss of dopaminergic innervation in the prefrontal cortex of schizophrenia patients (Akil et al. 1999). Similarly, subchronic administration of PCP produces a sustained decrease in prefrontal dopaminergic tone in both rats and monkeys by reducing DA release and turnover (Jentsch et al. 1997, 1998). It is known that selective disruption of DA neurotransmission in the prefrontal cortex leads to significant cognitive deficits (Brozoski et al. 1979). Particularly relevant to our present morphological findings is the demonstration that selective damage to the prefrontal cortex dopaminergic innervation results in a loss of prefrontal dendritic spines in the rat, while atypical antipsychotic drugs, which restore DA tone, can reverse this effect (Wang & Deutch, 2008). This role of DA in regulating synapse numbers in the prefrontal cortex is reminiscent of the finding that striatal DA depletion leads to a rapid and profound loss of spines and glutamatergic synapses on striatopallidal medium spiny neurons (Day et al. 2006). Thus, altered prefrontal dopaminergic neurotransmission may be a critical factor in primate PCP models of schizophrenia, provoking not only cognitive deficits, but also contributing to changes in the number of prefrontal spine synapses. Because our studies using the rat and monkey PCP models have shown that clozapine can attenuate both the cognitive and dopaminergic dysfunctions (Elsworth et al. 2008; Jentsch et al. 1997), it will be of interest to see if clozapine or atypical antipsychotic drugs that normalize PFC DA will attenuate the spine synapse loss, or indices of this change, in these PCP-induced models of schizophrenia, as well as in schizophrenia. In fact, recent findings show that olanzapine, which increases release of DA in PFC, can restore asymmetric spine synapse numbers in the rodent PCP model (Elsworth et al. 2011), so these new data support the idea that atypical antipsychotics may be able to normalize dystrophic changes in the PFC in schizophrenia by a DA-dependent mechanism.

In summary, the finding of a loss of asymmetric spine synapses in the DLPFC of this primate PCP model, which is greater in layer II/III than layer V, is consistent with indirect imaging and post-mortem observations in schizophrenia, and suggest that synapse loss may be responsible in part for the cognitive deficits following PCP treatment in human and non-human primates, as well as in schizophrenia. Thus, the current data support the use of the PCP model to understand the mechanism for the synapse loss, and to investigate treatments that reverse this remodelling.

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Statement of Interest

None.

References


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