

# Phencyclidine and some of its analogues have distinct effects on NMDA receptors of rat hippocampal neurons

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Phencyclidine (PCP) is a dissociative anesthetic agent which blocks the excitatory effect of *N*-methyl-D-aspartate (NMDA) in the central nervous system. To investigate the role of the PCP reactive site in the control of NMDA activation of hippocampal pyramidal cells, we have examined the action of PCP and some of its analogues on the response properties of single NMDA receptors. Application of NMDA (5–15  $\mu$ M) to outside-out patches of membrane elicited bursts of ion channel openings which were greatly reduced in frequency and duration in the presence of PCP (2.5–10  $\mu$ M) or *m*-amino-PCP (2.5–10  $\mu$ M), a behaviorally active derivative of PCP. These effects of PCP were reversed when the membrane potential was shifted from negative to positive values. Application of the behaviorally inactive agent 1-piperidinocyclohexanecarbonitrile ( $\geq 220$   $\mu$ M) left NMDA-activated currents relatively unaltered. Treatment with another analogue, *m*-nitro-PCP (5–20  $\mu$ M), resulted in an unexpected increase in frequency of openings. At a higher concentration (100–300  $\mu$ M), however, *m*-nitro-PCP acted like PCP in reducing frequency of opening and channel life-time. Like PCP, these effects of *m*-nitro-PCP were reversed at positive potentials. Taken together, these results suggest that PCP and its derivatives block the open state of the NMDA channel. Moreover, the dual effect of *m*-nitro-PCP shows that excitability is not necessarily decreased by PCP analogues but may instead be enhanced depending on modifications of the PCP molecule.

Single channel current; Patch clamp technique; Hippocampal culture; Psychotropic agent

## 1. INTRODUCTION

The psychotropic agent PCP has been shown to cause hallucinations and visual disturbances, enhanced locomotor activity, disorientation, anxiety, and dissociative anesthesia [1–3]. In view of these extensive effects, the question arises as to what mechanisms PCP affects in the central nervous system (CNS). Several hypotheses have been proposed to explain some of the psychopathologi-

cal effects induced by PCP. Initially, it was suggested that the effects of PCP are due to a blockade of potassium channels and release of a variety of neurotransmitters, such as dopamine. Evidence for this hypothesis has been provided by reports that PCP binds to and blocks potassium channels in the rat CNS while its behaviorally inactive analogues do not [4–9]. However, other reports have suggested that the effects of PCP are also related to a blockade of NMDA receptors [10–13]. In the light of such an effect, we have examined the molecular mechanism subserving the action of PCP by direct inspection of its effects on single ion channel currents evoked by NMDA. We were especially interested in the examination of the effects of PCP on frequency and duration of bursts. Outside-out patches of membrane were

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*Abbreviations:* NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; PCC, 1-piperidinocyclohexanecarbonitrile

isolated from neonatal rat hippocampal neurons kept in culture and the NMDA-induced openings were resolved using patch clamp techniques [14]. To correlate the effects of PCP on these currents with the behavioral events, we compared effects of behaviorally active and inactive analogues of PCP [15,16]. It was found that both behaviorally active and some of the inactive analogues of PCP affect the kinetic properties of the NMDA-induced openings.

## 2. MATERIALS AND METHODS

### 2.1. Culture methods

Hippocampal neurons were obtained from Sprague-Dawley rats (Zivic-Miller) 2–7 days after birth. These neurons were selected for study due to their high density of PCP binding sites [17] and because the effects of NMDA on single ion currents have been described in the hippocampus [18]. The methods for tissue culture of neurons have been described elsewhere [19–22].

### 2.2. Patch-clamp experiments

Electrophysiological recordings were made using patch clamp techniques [14,23]. Single channel currents were studied on 43 outside-out patches of membrane excised from hippocampal neurons. Solutions consisted of (in mM): 165 NaCl, 5 KCl, 2 CaCl<sub>2</sub>, 10 glucose and 5 HEPES for the solution bathing the cells (external solution), and 80 CsCl<sub>2</sub>, 80 CsF, 10 CsEGTA, 10 HEPES for the solution inside the electrode (internal solution). The pH of the external solution was adjusted to 7.3 and the osmolality to 310 mosM while the pH of the internal solution was kept at 7.25 and its osmolality at 320 mosM. The PCP analogues used in these experiments were obtained from the National Institute of Drug Abuse (Rockville, MD).

An LM-EPC-7 patch clamp system (List Electronic, FRG) was used to record single channel currents from outside-out patches of membrane dissociated from pyramidal-shaped hippocampal neurons grown 7–14 days in culture. Pharmacological agents were applied to the external solution only after recording activity for 1–2 min to ensure that spontaneous openings of ion channels were not present. Each membrane patch was usually studied for over 30 min and up to 2 h. In many cases, different pharmacological agents, applied at several concentrations, were tested in the same patch; their effects were reversed after removing PCP and analogues by gentle perfusion with drug-free solution.

Single channel recordings were filtered at 3 kHz (Bessel, –3 db), stored on FM magnetic tape (Racal 4DS), and digitized at 12.5 kHz. Amplitude, duration and frequency of single channel currents were determined using the IPROC-2 program [23] run on an IBM-AT microcomputer. Open times are the durations of valid events that exceed a threshold level of 50% of the single channel amplitude estimate. Since the NMDA-activated openings contain flickers, burst durations are also given – a burst being defined here as any individual open event or series of open events and brief (<5 ms) closed events, separated from neighboring events by >5 ms.

## 3. RESULTS

### 3.1. Effects of PCP, PCC and m-amino-PCP on the NMDA-evoked channel activity

Application of NMDA (1.5–250  $\mu$ M) to the external solution resulted in channel openings of conductance states and properties similar to those previously described in the hippocampus [18] and cerebellum [24]. Samples of the single channel currents recorded during application of NMDA, and

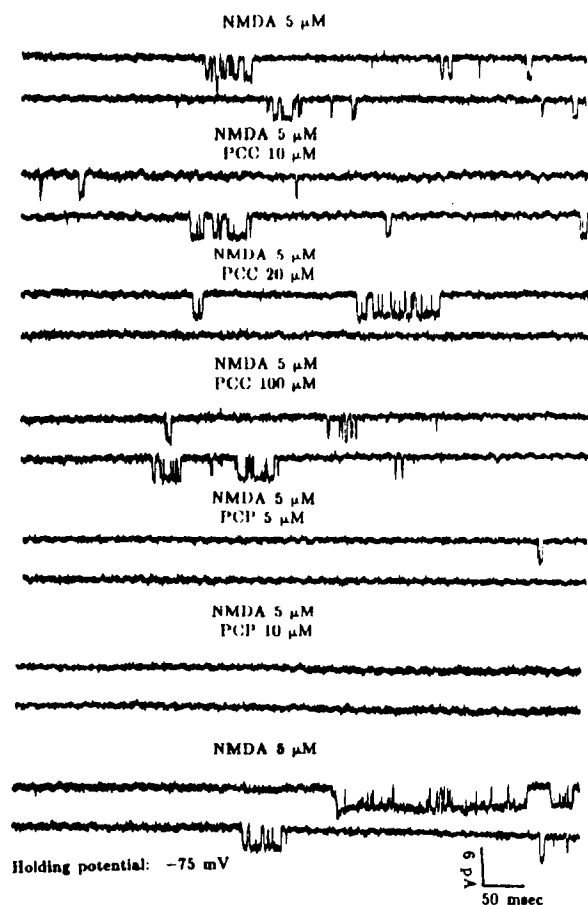


Fig. 1. Single channel currents recorded on an outside-out patch of membrane are shown in the sequential order of testing (from top to bottom) and represent responses to NMDA (5  $\mu$ M), NMDA (5  $\mu$ M) plus PCC (10  $\mu$ M), NMDA (5  $\mu$ M) plus PCC (20  $\mu$ M), NMDA (5  $\mu$ M) plus PCC (100  $\mu$ M), NMDA (5  $\mu$ M) plus PCP (5  $\mu$ M), NMDA (5  $\mu$ M) plus PCP (10  $\mu$ M) and, finally, responses to NMDA (5  $\mu$ M) alone approx. 40 min after perfusion with drug-free solution. While PCC had no discernible effects on the frequency of channel openings or duration of the bursts, PCP markedly decreased frequency of channel openings and burst time.

the effects of PCP and of its behaviorally inactive analogue PCC on these currents are illustrated in fig.1. As expected, the major component of the NMDA response consisted of high-conductance (approx. 40 pS) channel openings of variable duration which appeared either singly or in bursts of

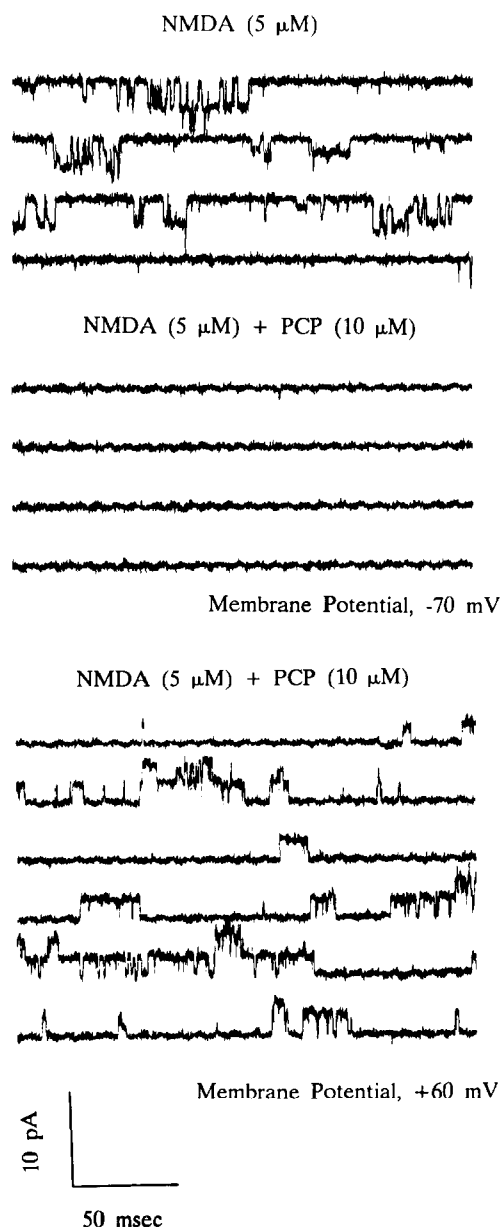


Fig.2. Single channel currents recorded at -70 mV in the presence of NMDA (5  $\mu$ M) and at -70 and +60 mV in the presence of NMDA (5  $\mu$ M) plus PCP (10  $\mu$ M).

several openings in rapid succession. These events were not significantly affected when PCC was applied at concentrations of 10, 20, 100 (fig.1) and up to 220  $\mu$ M (not shown). In contrast, application of PCP (5  $\mu$ M) to the same patch markedly reduced the frequency of open events, and also shortened the burst duration. The few remaining bursts usually consisted of only a single opening. Application of additional PCP (10  $\mu$ M) led to a nearly complete blockade of NMDA-induced openings. These effects of PCP were only reversible several minutes after superfusion with drug-free solution. For instance, as illustrated in fig.1, 40 min were required for a partial recovery of the NMDA response (shown in the lower 2 traces). The partial recovery of channel opening was associated with reappearance of the long-duration bursts, i.e. bursts that last for over 10 ms. Immediate recovery was obtained when the membrane potential was shifted from negative to positive values. For instance, in the example shown in fig.2 the potential was changed from -70 to 60 mV, and the

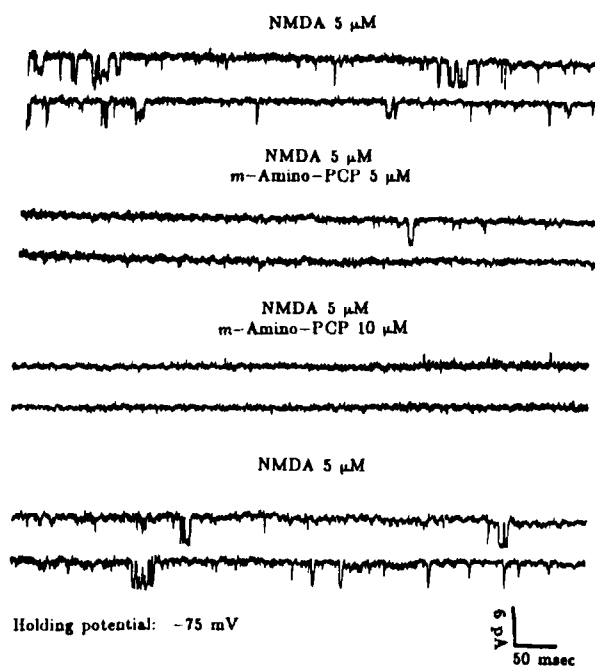


Fig.3. Single channel currents recorded in the presence of (from top to bottom): NMDA (5  $\mu$ M), NMDA (5  $\mu$ M) plus *m*-amino-PCP (5  $\mu$ M), NMDA (5  $\mu$ M) plus *m*-amino-PCP (10  $\mu$ M) and NMDA (5  $\mu$ M) 30 min after wash.

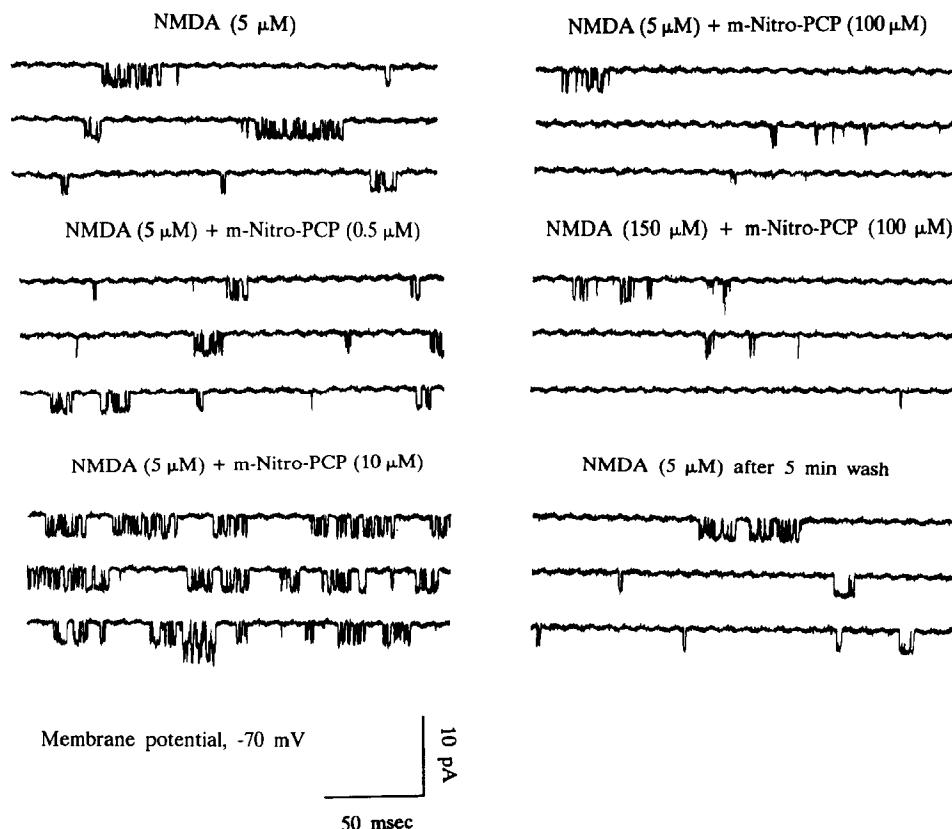


Fig.4. Single channel currents obtained in the presence of (from top to bottom): NMDA (5  $\mu$ M), NMDA (5  $\mu$ M) plus *m*-nitro-PCP (0.5  $\mu$ M), NMDA (5  $\mu$ M) plus *m*-nitro-PCP (10  $\mu$ M), NMDA (5  $\mu$ M) plus *m*-nitro-PCP (100  $\mu$ M), NMDA (150  $\mu$ M) plus *m*-nitro-PCP (100  $\mu$ M) and finally NMDA (5  $\mu$ M) again 5 min after wash. At a concentration of 10  $\mu$ M, *m*-nitro-PCP strongly facilitated the NMDA response by increasing frequency of openings. In contrast, a blocking effect was present at 100  $\mu$ M *m*-nitro-PCP.

frequency of channel opening and the channel lifetime reversed to a condition similar to control.

Similar results were obtained with *m*-amino-PCP, a behaviorally active analogue of PCP. Fig.3 shows traces of single channel openings evoked by NMDA which were markedly reduced in frequency after the application of *m*-amino-PCP (5  $\mu$ M). At a relatively low concentration, *m*-amino-PCP (5  $\mu$ M) reduced the number of channel openings and decreased burst duration (compare these results with the effects of PCP shown in fig.1). Administration of *m*-amino-PCP to a final concentration of 10  $\mu$ M led to a complete blockade of channel activity. Nevertheless, some recovery of channel function was observed approximately 30 min after washing the membrane with external solution free of *m*-amino-PCP (fig.3).

### 3.2. Effects of *m*-nitro-PCP on the NMDA evoked channel activity

In contrast to PCP and *m*-amino-PCP, the effects obtained with the behaviorally inactive analogues were more variable: while PCC did not alter the NMDA responses (see above), *m*-nitro-PCP markedly facilitated the NMDA responses by increasing the frequency of NMDA-activated openings. As shown in fig.4, single channel openings occurred at a higher rate in the presence of NMDA (5  $\mu$ M) plus *m*-nitro-PCP (10  $\mu$ M) than in the presence of NMDA (5  $\mu$ M) alone. Otherwise, the kinetic properties of these openings remained relatively unaffected by *m*-nitro-PCP and they cannot be distinguished from those present under control condition. The facilitatory effects induced by *m*-nitro-PCP reached a peak at 10  $\mu$ M, and

subsequently decreased at higher concentrations. At a concentration of  $100\text{ }\mu\text{M}$  *m*-nitro-PCP, the frequency of openings decreased drastically and kinetic properties were substantially affected as revealed by the appearance of longer intraburst closures and the reduced burst durations (fig.3). These effects of *m*-nitro-PCP were quickly reversed upon superfusion with drug-free solution, such that control conditions were reached in less than 5 min, in contrast to over 30 min in all cases when PCP and *m*-amino-PCP were tested.

The effects of PCP and *m*-nitro-PCP on NMDA responses were not only restricted to the frequency of openings, but included the duration of the single channel currents. To provide a quantitative analysis of these findings, we have computed the duration of NMDA-evoked single channel currents. As illustrated in the histograms of fig.5, which plot the normalized number of NMDA-

induced events (openings or bursts) as a function of event duration, PCP at a concentration of over  $2.5\text{ }\mu\text{M}$ , reduced both the open time and the burst duration. Burst duration was particularly affected, with most bursts lasting less than 10 ms in the presence of PCP. The same figure also shows that both the open time and the burst duration were apparently unaltered by  $10\text{ }\mu\text{M}$  *m*-nitro-PCP.

#### 4. DISCUSSION

The present results demonstrate that the behaviorally active and inactive analogues of PCP can be distinguished by their effects on single channel currents evoked by NMDA. Both PCP and its behaviorally active derivative *m*-amino-PCP at concentrations of  $2\text{--}10\text{ }\mu\text{M}$  blocked NMDA currents by drastically reducing frequency and duration of channel openings. In contrast, the

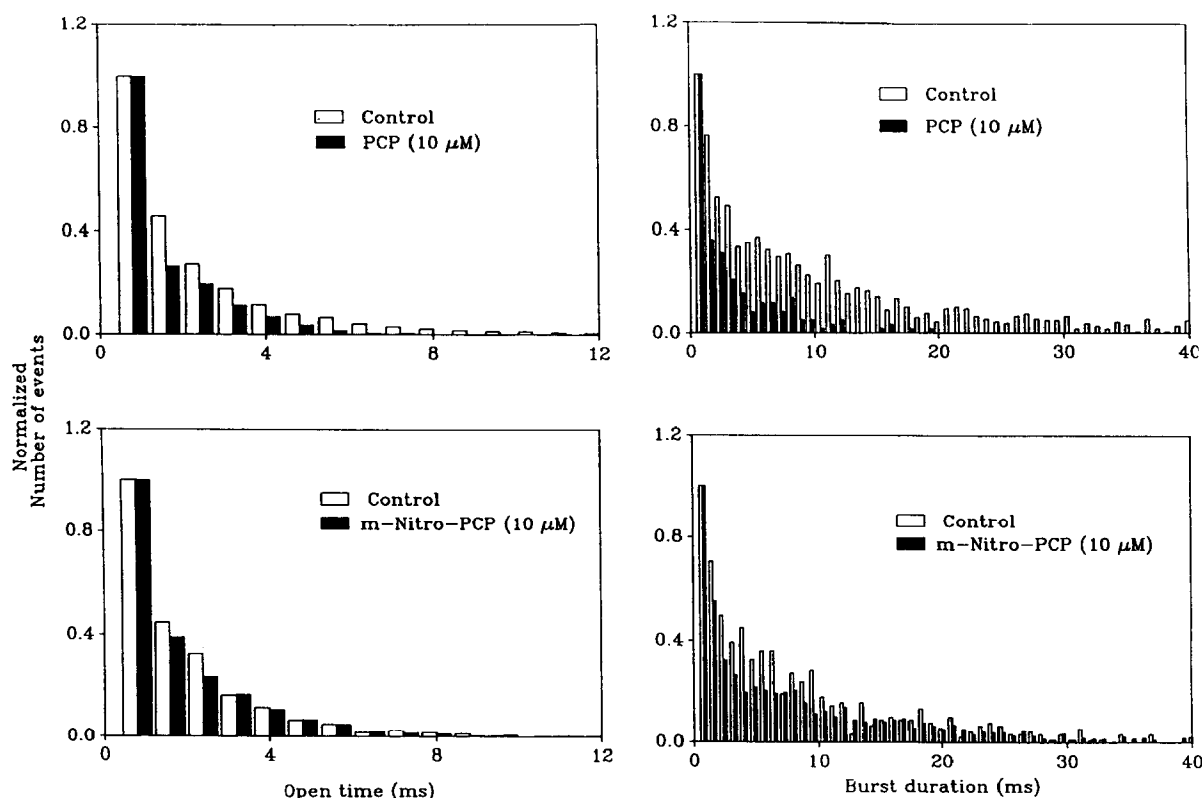


Fig.5. Histograms representing number of events plotted as a function of open time duration or burst duration. Recordings were conducted in the presence of  $5\text{ }\mu\text{M}$  NMDA (control), NMDA ( $5\text{ }\mu\text{M}$ ) and PCP ( $10\text{ }\mu\text{M}$ , upper panels) or NMDA ( $5\text{ }\mu\text{M}$ ) and *m*-nitro-PCP ( $10\text{ }\mu\text{M}$ , lower panels). Number of events was normalized so that the largest bar is equal to 1.0. Actual number of events was between 340 and 1856. Holding potential =  $-75\text{ mV}$ .

behaviorally inactive analogue *m*-nitro-PCP displayed two distinct effects on NMDA openings. At low concentration (5–20  $\mu$ M), *m*-nitro-PCP markedly increased the frequency of NMDA-induced openings but had no effect on channel lifetime. At a higher concentration (greater or equal to 100  $\mu$ M) *m*-nitro-PCP reduced both channel lifetime and frequency.

In the light of these effects, the question arises whether *m*-nitro-PCP acts at the binding site for PCP. An alternative possibility is that *m*-nitro-PCP acts at the binding site for glycine, which has also been reported to facilitate NMDA receptor activity [25]. The answer to this question may be obtained by a comparison of the effects of these agents on the kinetic properties of the NMDA openings. For instance, in contrast to *m*-nitro-PCP, glycine has been reported to increase channel lifetime [25]. Moreover, we have observed that glycine even at high concentration (up to 100  $\mu$ M) did not reduce channel lifetime or frequency, as occurred with *m*-nitro-PCP (see fig.2 and Ramoa et al., in preparation). Therefore, it seems unlikely that *m*-nitro-PCP and glycine bind at a similar site. On the other hand, the effects of *m*-nitro-PCP administered at a high concentration are similar to those elicited by PCP. In addition, the blocking effects of PCP and *m*-nitro-PCP were relieved at a positive potential (Ramoa et al., in preparation), thus suggesting that both agents interact within the ionic channel component of the NMDA receptor. Such a reversal of PCP effects at positive potentials was also observed at the nicotinic acetylcholine receptor (AChR) ion channel at the neuromuscular junction [26], where actions of PCP on both closed and open conformations of the AChR were reported. The present results lend support to the notion that PCP affects the open conformation of the NMDA receptor. However, and additional action of PCP on the closed channel state, as suggested for the nicotinic AChR [26], cannot be ruled out.

These findings have implications for understanding the functional organization of the NMDA receptor. It is generally thought that PCP blocks ion currents from flowing by binding inside the open channel macromolecule and impeding the transmembrane ion fluxes [27–29]. It is unclear, however, whether PCP regulates channel permeability by allosteric mechanisms or by occluding

the channel and preventing ionic conductance [27–31]. However, an open-channel model in which the presence of the ligand molecule within the channel creates a physical impediment to the ion fluxes cannot readily explain the facilitatory effects observed with application of *m*-nitro-PCP. Instead this agent, which differs from PCP by a nitro group, may bind inside the ion channel and lead to enhancement of activity through a conformational modification of the NMDA receptor-ion channel complex. Perhaps, *m*-nitro-PCP can increase the affinity of the receptor macromolecule to NMDA.

The results obtained with PCP and its analogues help us to understand not only the functional modulation of the NMDA receptor but also how changes in this modulation may contribute to behavioral effects. The correlation found here between the blockade of NMDA response and the behavioral effects of PCP analogues is consistent with the notion that interference with the function of the NMDA receptor contributes to some of the behavioral disturbances observed with PCP. Nevertheless, the correlation between blockade of NMDA receptors and the central effects of PCP should be interpreted with great caution. It may be difficult to explain the wide spectrum of effects of PCP, which in humans ranges from perceptual disturbances to dissociative anesthesia [2], by a single underlying electrophysiological mechanism. It is more likely that PCP alters synaptic transmission mediated by a variety of neurotransmitter systems. PCP has been shown to interact with nicotinic and muscarinic receptors [32,33] and to block potassium channels [4–9]. Furthermore, as previously suggested [4,6,9], the blockade of presynaptic potassium channels could lead to an increased release of several neurotransmitters, including excitatory amino acids (which can still activate the kainate and quisqualate receptors in the presence of PCP [10]). Thus, an increased release of synaptic transmitters combined with the antagonism of a variety of post-synaptic receptors may provide the substrate for most of the effects of PCP.

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