PHOTOAFFINITY LABELING OF TWO CLASSES OF PHENCYCLIDINE RECEPTORS IN THE RAT BRAIN. Kloog, Y., R. Haring and M. Sokolovsky. Laboratory of Neurobiochemistry, Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel-Aviv 69978, Israel.

Binding and photoaffinity labeling experiments with the photolabile analog of PCP, [^{3}H]azido-PCP (AZ-PCP) were employed in order to determine the relationship between the apparent heterogeneity of rat brain PCP receptors and the polypeptides which are specifically labeled with [^{3}H]AZ-PCP. Two populations of phencyclidine (PCP) binding sites are shown to exist in the rat brain: a high-affinity monovalent ion sensitive site (K_d of 10–15 nM for [^{3}H]TCP, [^{3}H]N-[1-(2-thienyl) cyclohexyl]piperidine), which exists in both the frontal cortex and the hippocampus, and a lower affinity site (K_d of 80–100 nM for [^{3}H]TCP) which is found in the hippocampus and in lower brain regions but not in the frontal cortex. Photoaffinity labeling of the PCP receptors with [^{3}H]AZ-PCP indicated that five specifically labeled polypeptides of these receptors (Mr 90,000, 62,000, 49,000, 40,000 and 33,000) are unevenly distributed in the rat brain. Two of the stereoselectively labeled polypeptides (Mr 90,000 and 33,000) appear to be associated with the high- and low-affinity [^{3}H]TCP binding sites; the density of the Mr 90,000 polypeptide in various brain regions correlates well with the localization of the high-affinity sites, whereas the density of the Mr 33,000 polypeptide correlates best with the distribution of the low-affinity sites. The high-affinity sites are associated with an Mr 90,000 polypeptide whose labeling by [^{3}H]azido-phencyclidine is selectively inhibited by monovalent ions and by tetraethylammonium. The results suggest that the two apparent classes of PCP receptors in the rat brain each has a distinct polypeptide which carries the ligand recognition site. (Supported in part by NIH Grant DABB IR01 DAO4168-01).

ASSESSING DIRECTLY OBSERVABLE BEHAVIORAL EFFECTS OF PHENCYCLIDINE (PCP)-LIKE DRUGS IN ANIMALS. Koek, W. and J. H. Woods. Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109.

We have recently described (Koek *et al., Psychopharmacology (Berlin),* in press) simple and rapid procedures that provide pharmacologically specific measures of PCP-like behavioral activity. Here we elaborate on these procedures using pigeons, rats and rhesus monkeys. The procedures are simple because they are based on recording the presence or absence of a number of different, directly observable behaviors; they are rapid because cumulative dosing is used to assess entire dose-response curves during a single experimental session; and they provide pharmacologically specific measures because they discriminate behavioral effects of PCP-like drugs from effects of non PCP-like drugs. PCP-induced catalepsy in pigeons, PCP-induced directly observable behavioral effects in rats, and PCP-induced anesthesia in monkeys have been useful in the evaluation of (1) possible antagonist activity of metaphit, a proposed PCP-receptor acylator, and (2) possible PCP-like effects of proposed excitatory amino acid antagonists (e.g., MK 801, PK 26124). Thus, the aforementioned procedures may, by virtue of their rapidity and specificity, be helpful in the evaluation of possible PCP-like activity of compounds and may provide useful tools in the study of the mechanism of action of PCP-like drugs; subsequently, other procedures (e.g., drug discrimination) can be used to provide more sensitive measures of PCP-like behavioral effects.

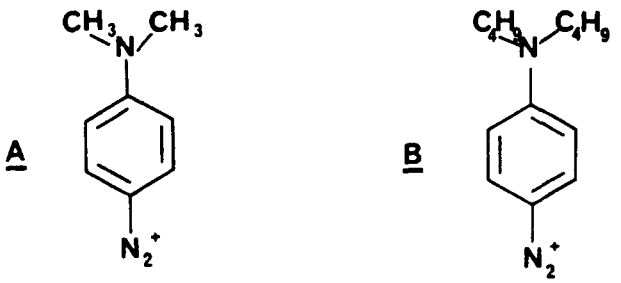
BEHAVIORAL EFFECTS OF NMDA ANTAGONISTS. W. Koek and J. H. Woods. Departments of Psychology and Pharmacology, The University of Michigan, Ann Arbor, MI 48109.

Excitatory amino acid neurotransmission is thought to be mediated through 3 different receptors, characterized in part by the selective agonists N-methyl-D-aspartate (NMDA), quisqualate, and kainate. Phencyclidine (PCP)-like drugs selectively antagonize excitation of neurons by NMDA (e.g., Berry and Lodge, *Biochem Pharmacol* **33,** 3829). A selective antagonist of NMDA, 2-amino-5-phosphonovalerate (AP5), produced PCP-like catalepsy in pigeons following ICV administration (Koek *et al., Behav Brain Res* **19,** 257). We have studied a series of antagonists that vary in potency and selectivity at NMDA, quisqualate and kainate sites. Each of the antagonists produced catalepsy; the potency order was strongly correlated with the potency order to antagonize electrophysiological effects of NMDA and displacement of tritiated AP5. Thus, PCP-like catalepsy induced by these compounds may be initiated by blockade of excitatory neurotransmission at the NMDA site, distinct from, but related to, the PCP site. AP5 administered IM produced PCP-like catalepsy, discriminative stimulus effects, and stereotyped responding in pigeons. In rats, ICV administration of AP5 produced directly observable behavioral effects that were similar to the effects of PCP and different from the effects of pentobarbital- or amphetamine-like drugs. Preliminary observational experiments have compared the effects of AP5, by different routes of administration, in rhesus monkeys to a variety of PCP-like compounds. The PCP-like effects of AP5 show generality across behavioral procedures and species. However, some of the behavioral effects of AP5 may show characteristics that are not shared by PCP. Together, the data support the hypothesis that a reduction of excitatory neurotransmission at NMDA preferring receptors may underly certain behavioral effects of PCP. This hypothesis is strengthened further by results of experiments that were aimed at the evaluation of the possible PCP-like effects of the proposed NMDA antagonists MK-801 and PK 26124.

ARYL DIAZONIUM SALTS AS PHOTOAFFINITY LABELS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR PCP BINDING SITE. F. Kotziba-Hibert, A. Jaganathen, M. Goeldner and C. Hirth. Laboratoire de Chimie Bio-Organique, UA 31 CNRS, Faculté de Pharmacie, Université Louis Pasteur, Strasbourg, France.

Aryl diazonium salts are useful in photoaffinity labelling experiments. These chemicals are highly photosensitive and generate *effective reactive species* namely aryl cation. When suitably substituted on the aromatic ring their chemical stability increases and according to their chromophore their photodecomposition can be induced by an Energy Transfer

process. As such several p.dialkylamino benzene diazonium salts have been synthesized and tested for their reversible binding characteristics on both the α-bungarotoxin and PCP binding sites of the nicotinic acetylcholine receptor from *Torpedo marmorata*. Among these derivatives two have been studied more thoroughly for irreversible inactivation of the PCP binding site (see below).



Reversible binding: Ki (PCP)

A: 1.7×10^{-4} M	desensitized state	B: 1.6×10^{-6} M
2.5×10^{-4} M	resting state	4×10^{-6} M

Irreversible loss of PCP binding capacity (h.).

A: 40% at 2×10^{-4} M	(either state)	B: 65% at 3×10^{-6} M (desens. state)

Tritiated samples.

A and B: all *four subunits* are labelled
protection by excess PCP 50% for A 90% for B

A heterocyclic derivative: 2-diazoimidazole showed a unique feature. For this chemical, which has a low binding affinity for the PCP binding site (over 10^{-3} M) the photoinducted irreversible blocking is only effective when the receptor is in a desensitized state.

INTERACTIONS BETWEEN THE NMDA-TYPE RECEPTOR COMPLEX AND PCP RECOGNITION SITES. Lehmann, J. and P. L. Wood. Neuroscience/Cardiovascular Research, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

While there are a number of different sites of action of phencyclidine (PCP), one common site of action of PCP and a number of its analogs seems to have emerged. This PCP recognition site may be defined by the rank-order of potency of compounds at binding sites labeled by [3H]PCP, [3H]TCP, or [3H]MK-801. The same rank order of potency of PCP analogs is found in the inhibition of NMDA-type receptor-mediated responses: NMDA-elicited increases in firing of neurons measured extracellularly or their depolarization measured intracellularly, NMDA-elicited [3H]ACh release, and, in vivo, cerebellar cGMP levels. The mechanism by which PCP and its analogs inhibit the function of the NMDA-type receptor/effector complex is not known. Several observations indicate that PCP analogs are not competitive antagonists of NMDA-type receptors, for example: (1) Lack of activity of PCP analogs at binding sites labeled by the competitive NMDA-type receptor antagonist, [3H]CPP; (2) Non-competitive kinetics with respect to inhibition of NMDA-induced [3H]ACh release; and (3) Hill coefficients less than one in decreasing cerebellar cGMP (in contrast to competitive NMDA antagonists, which have Hill coefficients of one). Several different models have been proposed to describe the interaction between PCP recognition sites and NMDA-type receptors: (1) A strict analogy to the GABA receptor/benzodiazepine/chloride channel complex, in which the PCP site mediates allosteric regulation of the affinity of glutamate for its receptor; (2) A direct modulation by the PCP recognition site of the ion channel associated with NMDA-type receptors, but which is apparent only when the NMDA-type receptor is occupied by agonist, and opens the ion channel; (3) A binding site for PCP and its analogs within the NMDA-activated ion channel itself, where they block cation flow by steric hindrance. The currently available biochemical and electrophysiological data cannot conclusively reject or prove any of these three models.

STEREOSELECTIVE METABOLISM OF NORKETAMINE, THE *N*-DEMETHYLATED METABOLITE OF KETAMINE, IN RAT LIVER MICROSOMES. Leung, L. Y. and T. A. Baillie. Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195.

The metabolism of the dissociative anesthetic agent, ketamine, ((±)-2-o-chlorophenyl-2-methylaminocyclohexanone) has been studied by a number of groups both *in vitro* and *in vivo*. However, the origin of the isomeric 4-, 5- and 6-hydroxynorketamine metabolites remains unclear. These products could arise from norketamine, the *N*-demethylated metabolite of ketamine, or alternatively, they could be formed from the corresponding hydroxylated metabolites of ketamine. Thus, the intermediacy of norketamine as the precursor to these hydroxylated norketamine derivatives was investigated *in vitro* using both racemic norketamine and the individual enantiomers as substrates for incubations. Norketamine was found to yield the corresponding hydroxylated metabolites in rat liver microsomal preparations. Incubations using the separate enantiomers revealed a pronounced substrate enantioselectivity and product regioselectivity in the formation of these hydroxylated products. Thus, S-norketamine was hydroxylated at the C-6 position to yield exclusively 6-OH norketamine, whereas 4-OH norketamine was produced almost solely from the R-enantiomer. Experiments using pseudoracemic mixtures of norketamine as substrates suggest the presence of enantiomeric interaction on the formation of these hydroxylated products. A previous study on the metabolism of the enantiomers of ketamine itself showed analogous stereoselectivity in the formation of these hydroxylated norketamine metabolites. Our findings thus indicate that norketamine is the intermediate in the metabolic transformation of ketamine to hydroxynorketamine isomers in liver tissues, and lend support to the view that the formation of active metabolites via stereoselective processes may contribute in part to the observed CNS effects of ketamine. (Support by NIH Grant NS 17956.)