

# **Anti-PCP Monoclonal Antibody Binding Capacity is not the Only Determinant of Effectiveness, Disproving the Concept that Antibody Capacity is easily Surmounted**

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Running Title: Anti-PCP mAb Protects against Attempts to Surmount It

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Abbreviations: terminal elimination half-life,  $t_{1/2\lambda Z}$

## Abstract

The effectiveness of a high affinity monoclonal antibody (mAb) antagonist against chronic phencyclidine (PCP) use has been demonstrated in rats. In this study, we tested the hypothesis that intravenous doses of PCP in excess of the binding capacity of an anti-PCP mAb cannot easily surmount the beneficial effects of the mAb, even in the presence of a high body burden of the drug. One day after steady-state PCP concentrations were achieved in male rats by continuous s.c. infusion (18 mg/kg/d), a single i.v. dose of saline or the anti-PCP mAb ( $K_D = 1.3$  nM; at one-third the molar dose of the PCP body burden) treatment was administered. In an attempt to further surmount the effects of the mAb, rats were challenged with a single 1.0 mg/kg i.v. bolus PCP dose (along with a [ $^3$ H]-PCP tracer) three days after the mAb or saline treatment. Total (i.v. bolus + s.c. infusion) PCP concentrations were measured in serum, brain, and testis by RIA before and after the challenge, and [ $^3$ H]-PCP concentrations were measured by liquid scintillation spectrometry. The anti-PCP mAb protected against adverse health effects, significantly increased the serum total and bolus PCP concentrations ( $p < 0.05$ ), and significantly decreased brain total and bolus PCP concentrations ( $p < 0.05$ ) after the i.v. challenge. These results showed the antibody can counteract extreme and potentially fatal PCP challenges and disproved the hypothesis that attempts to surmount the effects of the antibody with extremely high PCP doses would have immediate adverse health effects.

A major goal of treating drug abuse is to provide long-term protection against the harmful effects of uncontrolled, compulsive drug use (Pentel et al., 2000). Passive immunization with anti-drug monoclonal antibodies (mAb, both singular and plural) is one proposed method for protecting individuals in this situation (Kosten and Kranzler, 2004). Advantages of this treatment approach are that IgG mAb have an extremely long half-life of 3 to 4 weeks in humans (Roskos et al., 2004), and thus have the potential to be very long-acting. Also, antibodies are non-addictive pharmacokinetic antagonists that target the drug, not the sites of drug action in the central nervous system (Malin et al., 2001; Pentel, 2004). However, a potential disadvantage is that most mAb would not be expected to have the molar binding capacity to block or attenuate the effects of excessive doses used during uncontrolled, compulsive drug use. Further complicating the clinical utility is the possibility of serious adverse effects or overdose that might result from drug abusers attempting to surmount the protective effects of an anti-drug antibody.

The utility of passive immunization in the reduction of drug- effects has been shown. For instance, a catalytic cocaine monoclonal antibody has been shown to reduce toxic effects of cocaine (Mets, et al., 1998; Briscoe et al., 2001). In this previous study, the cocaine catalytic antibody provided protection against the lethal effects of cocaine. Similarly, the effectiveness of passive immunization against the effects of the stimulant drug phencyclidine (PCP) has been proven in rats (Hardin et al., 2002; Laurenzana et al., 2003). In chronic studies lasting 2-4 weeks, a single dose of a high-affinity anti-PCP mAb ( $K_D = 1.3$  nM) was administered to rats before the rats were given repeated bolus doses of PCP in a binge model of drug abuse (Hardin et al. 2002). In the study by Laurenzana et al. (2003), a high-dose infusion of PCP was given to

rats and allowed to reach steady-state. A single dose of the anti-PCP mAb was then given. The mAb significantly reduced PCP-induced locomotor effects in both of these studies.

The effect of a single dose of the anti-PCP mAb on brain and serum concentrations after institution of a prolonged, high-dose, steady-state PCP infusion has also been studied. In these studies by Proksch et al., (2000a), the mAb-treated groups showed significantly increased serum PCP concentrations and significantly decreased brain PCP concentrations compared with controls. Importantly, these three key studies showed that binding capacity is not a limiting factor for mAb effectiveness because the PCP doses substantially exceeded the numbers of mAb binding sites, and that the protective effects of the mAb were long-lasting (Laurenzana et al., 2003; Proksch et al., 2000a; Hardin et al., 2002).

While these studies have shown that a high-affinity, anti-PCP mAb can prevent PCP effects and reduce ongoing PCP effects, the mechanism of mAb antagonism of these effects is not clear. Specifically, whether this mAb prevents PCP from reaching the brain or removes it from the brain after it has already reached its sites of action is not clear. In the experiment described here, we sought to determine if the beneficial effects of our anti-PCP mAb could be surmounted when PCP concentrations rapidly increase, *i.e.*, immediately after an i.v. PCP dose, and in the presence of an extreme excess of PCP dose that should have effectively saturated the total capacity of the mAb. We hypothesized that anti-PCP mAb can alter the rate and extent of distribution of PCP into and clearance out of the brain, even in the presence of excessive, toxic doses of PCP.

## Methods

**Drugs, chemicals and monoclonal antibody.** PCP HCl (1-[1-phenylcyclohexyl]piperidine hydrochloride) and [<sup>3</sup>H]PCP (1-(1-[phenyl-<sup>3</sup>H](*n*)cyclohexyl)piperidine) were obtained from the National Institute on Drug Abuse (Rockville, MD ). The tritium label on the [<sup>3</sup>H] PCP was at a metabolically stable site on the phenyl ring structure. The [<sup>3</sup>H]PCP (12.68 Ci/mmol) was used as a tracer for the bolus PCP doses and as a standard for determining PCP concentrations in tissue extracts by radioimmunoassay (RIA). All PCP doses were calculated as the free base. Ecoscint A scintillation cocktail was purchased from National Diagnostics, Inc. (Manville, NJ). All other chemicals were obtained from Fisher Scientific (Springfield, NJ), unless otherwise stated.

Monoclonal anti-PCP IgG ( $K_D = 1.3$  nM; IgG1, *k* light chain) was produced from the hybridoma cell line mAb6B5 in a Cell-Pharm System CP2500 hollow fiber bioreactor (Unisyn Technologies, Tustin, CA) as previously described (Proksch et al., 2000a). The anti-PCP mAb was purified by ion exchange chromatography and prepared for i.v. administration as previously described (Laurenzana et al., 2003).

**Animals.** Adult male Sprague-Dawley rats (270-300 g) were purchased from Hilltop Laboratory Animals, Inc. (Scottsdale, PA) with an indwelling external jugular venous cannula (Dow Corning silastic tubing, 0.020" inside diameter; 0.037" outside diameter). The i.v. cannulae were carefully maintained as previously reported (Proksch et al., 2000a). The animals were fed three food pellets per day to maintain their body weights at approximately 300 g (Proksch et al., 2000a; Laurenzana et al., 2003). Rat body weights and the presence or absence of chromodacryorrhea (red lacrimal secretions, a sign of stress; (Harkness, 1980)) were recorded on

a daily basis throughout the experiments. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

**PCP dose selection.** In previous studies we found rats receiving a PCP dose of 18 mg/kg/day s.c. exhibit substantially and statistically significant increases in locomotor activity and/or stereotyped behavior (*e.g.*, head weaving and ataxia) during the first day of dosing (Wessinger and Owens, 1991; Proksch et al., 2000b; Laurenzana et al., 2003). By the second day, the animals become more stressed and ill, as demonstrated by matted fur and chromodacryorrhea. By the third day, this increased locomotor activity and stress begin to decrease as tolerance develops (Wessinger and Owens, 1991). Thus, we chose the fourth day to administer the bolus i.v. dose of PCP, for several reasons. First, we wanted to simulate a clinical situation in which subjects would feel the need to increase the dose as tolerance starts to develop. Second, we wanted the animals to be experiencing significant drug effects, yet be able to survive the bolus PCP injection. Finally, we wanted to know what would happen if PCP was administered at time points when the antibody binding by PCP should be maximal.

**Pharmacokinetic studies.** After being anesthetized with halothane (Proksch et al., 2000a; Laurenzana et al., 2003), osmotic minipumps (Alza Corp., Palo Alto, CA) were prepared and implanted subcutaneously between the scapulae of all rats. The pumps were filled with PCP dissolved in sterile saline at a concentration (approximately 24 mg/ml) that would yield continuous s.c. infusion of PCP at a rate of 18 mg/kg/day (4 µg/min). According to the manufacturer, the pumps were calibrated to deliver approximately 10.0 µl/hr for the duration of the experiment.

Each rat received a single i.v. dose of saline (the control group) or anti-PCP mAb (the antibody group) 24 hr after the start of the s.c. PCP infusion. Since the  $t_{1/2\lambda Z}$  (terminal elimination half-life) of PCP in the rat is 3.9 hr (Valentine et al., 1994), the animals were assumed to have achieved steady-state PCP concentrations (i.e., 4–7  $t_{1/2\lambda Z}$ , 15.6–27.3 hr) by the end of day one. The achievement of steady-state concentrations by day one has been previously validated (Wessinger and Owens, 1991; Proksch et al., 2000b; see Fig. 1A).

The dose of mAb binding sites was equivalent on a molar basis to 33% ( $1/3$  mol-equiv) of the amount of PCP in the animal at steady state. The amount of PCP (MW = 243 g/mol) in each animal at steady-state was calculated by multiplying the average concentration of PCP at steady-state from our previous studies ( $C_{SS} = 183$  ng/ml; Proksch et al., 2000b) by the volume of distribution at steady-state ( $V_{dSS} = 27$  L/kg; pharmacokinetic values from Wessinger and Owens, 1991). The corresponding dose of antibody was calculated using a molecular mass of 150 kD for mAb. In addition, the mAb dose was corrected for the presence of two binding sites per mAb molecule. The final  $1/3$  mol-equiv mAb dose was 0.51 g/kg.

The mAb was prepared at a concentration of approximately 60 mg/ml, making the final injection volume about 2.3 ml for each antibody injection. The same volume of saline was administered to the control animals. Anti-PCP mAb or saline was administered via the jugular venous cannula at a rate of approximately 0.5 ml/min.

Four days after the start of the s.c. PCP infusion and (3 days after the anti-PCP mAb or saline control treatment), all animals received a 1.0 mg/kg i.v. dose of PCP, along with a 100  $\mu$ Ci/mg dose of [ $^3$ H]PCP (as a tracer). This i.v. injection was given over 15 sec (the “bolus” dose; Fig. 1B). This i.v. bolus dose of PCP (1 mg/kg) when administered without any other drugs or



treatments produces about  $31 \pm 9$  min of locomotor activity and makes the animal ataxic for  $23 \pm 4$  min (Valentine et al., 1996). The total injection volume was 1.0 ml/kg. The rats were sacrificed at 0.5, 2, 5 or 20 min after the start of the bolus dose of PCP ( $n = 4$  per time point except for the 5 min time point, where  $n = 3$ ). Animals sacrificed at less than 20 min were anesthetized with ethyl ether immediately prior to the i.v. PCP infusion, and anesthesia was maintained until decapitation. Animals in the 20-min group were anesthetized 5 min prior to tissue sampling.

We chose to collect blood and tissue samples for only 20 min for several reasons. First, insignificant amounts of metabolites should have been formed during this time period, and thus we could analyze the [ $^3\text{H}$ ]PCP tracer without worrying about interference from metabolites. In addition, the total body burden of mAb would not be expected to change appreciably over this time period. Because mAb has an elimination half-life of 8.1 days in the rat, the amount of mAb changes very little in a 20 min period. The PCP to mAb ratio at the time of mAb administration should be 3:1, based on administration of a  $1/3^{\text{rd}}$  mol-eq mAb dose. If PCP elimination (elimination half-life of 4 hr) is unchanged by the mAb, the ratio of PCP to mAb before the bolus PCP dose should be 7.7:1 (due to continuous PCP infusion after the single dose of mAb), and 9.3:1 during the time of sampling after the bolus dose. Also, a 1 mg/kg dose of PCP (without the continuous infusion of PCP) only produces about 30 min of locomotor activity (Valentine et al., 1996). Finally, we were most interested in the early events after i.v. PCP dosing and thought 20 min was sufficient to assess these events.

At the predetermined sampling times, animals were anesthetized and blood was collected from the inferior vena cava via a laparotomy. Animals were then quickly sacrificed by decapitation and the brain and right testis were removed, rinsed with water, weighed, and immediately frozen

in liquid nitrogen. Testicular tissue concentrations were studied as a control for the brain because both have membrane barriers to transport of the mAb; however, distribution of PCP into the testis is limited by the organ-blood barrier, whereas PCP distribution into the brain is limited only by cerebral blood flow. The total time required from the start of blood collection until the tissues were frozen was always <2 min. Blood samples were allowed to clot and then centrifuged to obtain serum. All serum and tissue samples were stored at  $-80^{\circ}\text{C}$  until analyzed.

**Analysis of biological samples.** PCP concentrations resulting from the i.v. bolus of PCP (PCP with  $^3\text{H}$ -PCP tracer) and total PCP concentrations (i.v. bolus + s.c. PCP infusion) were determined in serum and tissues by separate analytical methods. For clarity, the combination of the i.v. PCP dose along with the [ $^3\text{H}$ ]PCP tracer dose is referred to as the “bolus PCP dose”, with the resulting concentrations referred to as the bolus PCP concentrations. These concentrations were measured by the quantity of the [ $^3\text{H}$ ]PCP in each serum and tissue sample.

For determination of serum PCP concentrations resulting from the i.v. bolus of PCP, 10  $\mu\text{l}$  aliquots of serum were counted by liquid scintillation spectrometry. The counts per minute in each sample were converted to decays per minute using the external standard correction of the instrument software. The resulting decays per minute value was converted to a PCP concentration in (ng/ml) using the known specific activity of the [ $^3\text{H}$ ] PCP tracer.

For determination of total serum PCP concentrations, the PCP in serum samples was extracted as previously described (Laurenzana et al., 2003). Extraction recoveries were determined based on the amount of radioactivity recovered and were approximately 60–70%. This was easily assessed using the [ $^3\text{H}$ ]PCP already in the sample. The samples were then diluted in PCP-free serum such that the amount of radioactivity in the samples was <20 dpm/ $\mu\text{l}$ . The highly sensitive

RIA with the same [ $^3\text{H}$ ]PCP radioligand (~50,000 dpm/tube) could then be used to determine the amount of unlabeled PCP in the diluted sample. This value was then corrected for the sample dilution factor and extraction recovery. This value represented the non-radioactive serum PCP concentration resulting from the s.c. PCP infusion dose plus the i.v. bolus PCP dose (referred to as “total PCP concentration”). The details of the RIA method have been reported by Proksch et al. (Proksch et al., 2000a).

For determination of PCP concentrations in brain and testis, tissue samples were homogenized in five volumes of ice-cold distilled water using a SDT Tissumizer (Tekmar Company, Cincinnati, OH). For determination of tissue PCP concentrations resulting from the i.v. bolus of PCP, aliquots of tissue homogenate (16  $\mu\text{l}$ ) were counted using liquid scintillation spectrometry. The resulting decays per minute value was corrected for the tissue dilution factor and converted to a PCP concentration in (ng/g) based on the known specific activity of the [ $^3\text{H}$ ] PCP tracer. This value was then corrected for the amount of blood in the tissue (Laurenzana et al., 2003) to assess the true brain tissue concentration, without the contribution of the amount of PCP in the blood stream. This correction was especially important in the mAb tested animals because of the substantial increases in the PCP blood concentrations resulting from mAb treatment. For determination of total (i.v. bolus + s.c. infusion) PCP concentration, aliquots of tissue homogenates (200  $\mu\text{l}$ ) were extracted as previously described (Laurenzana et al., 2003). Extraction recoveries were determined based on the amount of radioactivity recovered and ranged from 40–50% for brain and 55–75% for testis. This was assessed using the [ $^3\text{H}$ ]PCP already in the sample, as described in the previous paragraph. The extracted tissue homogenates were then diluted in PCP-free serum samples analyzed by RIA for determination of PCP concentration, as described for the serum samples. This value was corrected for the dilution

factor, extraction recovery and the amount of blood in the tissue as described in the previous paragraph.

**Statistical analysis.** All values are reported as the mean  $\pm$  the standard deviation of the mean (S.D.). An unpaired t-test was used to compare body weights of the animals on day 4 before the i.v. bolus PCP injections in the saline treated vs. the mAb-treated groups. The percentage of animals demonstrating chromodacryorrhea in the saline vs. the antibody groups was compared with chi-square analysis. Statistical significance was considered to be achieved at a level of  $p < 0.05$ . The Number Crunchers Statistical Software package (NCSS 2000; Kaysville, Utah) was used for statistical analysis of these data sets.

To determine the effect of the antibody treatment on tissue concentrations with time, each response (concentration) was modeled using a  $2 \times 4$  or a  $2 \times 5$  factorial analysis of variance. An F-test was used to calculate the factors time, antibody treatment, and the time by treatment interaction. The significance of the interaction (*i.e.*, the failure of the difference in the treatments to be consistent at all observed times) was evaluated first for all responses. If the time by antibody treatment interaction was significant at the  $p < 0.05$  level, 95% confidence intervals and hypotheses tests were estimated for each observed time point. Conversely, if the interaction was not significant, the main effect of the difference in the treatments at each time point was estimated. The MIXED procedure of the SAS/STAT software package (v. 9.1, Cary, NC) was used to analyze these data sets.

## Results

**General experimental observations.** Although we did not attempt to formally quantify all of the behavioral effects during the PCP infusion, we noted that the rats exhibited significant PCP-induced behavioral effects and health changes during the first 24 hr of infusion before saline or mAb treatment. These findings were consistent with our previous observations of PCP effects (Wessinger and Owens, 1991; Proksch et al., 2000b; Laurenzana et al., 2003). These effects included weight loss, increases in chromodacryorrhea, locomotor activity, and an unkempt appearance (*e.g.*, ruffled fur).

After saline or anti-PCP mAb administration (on day 1; see Fig. 1), the severity of the PCP-induced effects over the next three days differed in the two treatment groups. While the average body weight of the groups was not different before antibody treatment at  $284 \pm 13$  g and  $284 \pm 9$  g, the average body weight at the end of the 4-day s.c. PCP infusion (three days after saline or mAb treatment) was significantly lower ( $253 \pm 26$  g) in the saline-treated group than in the mAb-treated group ( $281 \pm 10$  g;  $p < 0.05$ ). In addition, a significantly greater percentage of the rats in the saline-treated group (87%) exhibited chromodacryorrhea than the animals in the mAb-treated group (33%;  $p < 0.05$ ).

We also observed the behavior in the rats after the 1 mg/kg PCP i.v. dosing in the 5 and 20 min tissue collection groups before they were anesthetized. In general, the mAb treated animals were in good condition and showed some signs of low dose PCP-induced locomotor activity, whereas all of the animals in the saline-treated group were profoundly sedated and two experienced severe respiratory depression and apnea. These rats were excluded from the analysis and replaced because of the uncertainty of their hemodynamic status. These observations indicated

the near lethality of the PCP bolus dose when added to an ongoing PCP infusion dose. In previous studies, we have determined that the LD<sub>50</sub> for an i.v. bolus dose of PCP is about 10 mg/kg (Hardin et al., 1998). We have also shown that the s.c. infusion dose used in these studies (18 mg/kg/day) can kill 25% of the animals between days 4 and 7 of the infusion (Laurenzana et al., 2003). We purposely chose this near lethal s.c. dose to test an extreme model of our preclinical scenario for our hypothesis.

**Serum PCP concentrations.** There was a significant time by mAb treatment interaction for total and bolus PCP concentrations ( $p < 0.05$ ). This meant that the differences between the mAb and saline concentrations were not consistent over time, reflecting a difference in rate of PCP tissue distribution over time. Therefore, confidence intervals for the differences between saline and mAb group concentrations at each time point were calculated. These calculations indicated the anti-PCP mAb significantly increased both total and bolus serum PCP concentrations at all time points. (Fig. 2). The differences between saline ( $232 \pm 74$  ng/ml) and mAb ( $2608 \pm 798$  ng/ml) groups were already present in the baseline steady-state PCP infusions just prior to the i.v. bolus PCP dosing, and they persisted after the i.v. bolus PCP dose.

We also calculated the ratio of average antibody group concentration to average saline group concentration at each time point for the total and bolus PCP measurements to better understand the antibody effect on drug partitioning between tissues (Table 1). The total PCP serum concentrations increased immediately after the bolus PCP dose in the mAb and saline groups, but they increased more in the saline group, resulting in a large decrease in the mAb:saline concentration ratio from 11.2 (at baseline) to 2.9 (at 0.5 min). Thereafter, PCP concentrations decreased more slowly in the mAb group than in the saline group, resulting in an increase in the antibody to saline concentration ratio to 5.2. This also occurred in the bolus PCP dose group.

The ratio of bolus PCP serum concentrations in the mAb group compared with the saline group doubled from 1.8 at 30 sec to 3.7 at 20 min.

**Brain PCP concentrations.** The single dose of anti-PCP mAb resulted in a significant time by mAb treatment interaction for the total and bolus brain PCP concentrations, reflecting a difference in rate of PCP tissue distribution over time ( $p < 0.05$ ; Fig. 3). The confidence intervals demonstrated significant differences, but not at all time points. At baseline (pre-i.v. PCP dose), total brain PCP concentrations in the mAb group ( $489 \pm 108$  ng/g) were significantly lower than in the saline group ( $1173 \pm 637$  ng/g;  $p < 0.05$ ). At the 30 sec time point, the differences were not significant. By the 2 min sample, total PCP brain concentrations were significantly decreased in the mAb group; and at 5 min, bolus PCP concentrations were significantly less in the mAb group. Brain total and bolus PCP concentrations remained significantly decreased at the subsequent time points in the mAb group.

The average antibody concentration to average saline concentration ratios at each time point for the total and bolus groups in the brain after the 30 sec measurement showed the brain PCP concentrations in the antibody group were 60–70% of that in the saline group for total PCP concentrations and 70–90% for bolus PCP concentrations (Table 1). Thus, concentrations decreased at similar rates in the antibody and serum groups in the brain after the 2-min time point.

**Testis PCP concentrations.** The single dose of anti-PCP mAb did not produce a significant time by antibody treatment interaction for the total testis PCP concentrations. Thus, the differences between the antibody and saline groups for total PCP concentrations were statistically consistent over time. Therefore, these data were re-analyzed for main effects. At baseline (pre-i.v. PCP

dose), total testis PCP concentrations were not different in the antibody group compared to the saline group, but total testis PCP concentrations were significantly reduced at all other time points ( $p < 0.05$ ). There was a significant time by mAb treatment interaction for the bolus testis PCP concentrations ( $p < 0.05$ ; Fig. 4). However, the only time point at which a significant reduction in bolus testis PCP concentrations occurred was at the 20 min time point. At this time point, concentrations were reduced by 32% in the antibody group.

The ratio of PCP concentrations in the mAb-treated to the saline-treated animals was remarkably consistent over time at 60–70%. This ratio varied more with bolus PCP concentrations, but in general, the antibody had little effect on bolus testis PCP concentrations, except at the 20 min time point (Table 1).

**Brain to Serum and Testis to Serum PCP concentration ratios.** The anti-PCP mAb further altered the relationship of the organ (brain or testis) to serum PCP transfer. This was more clearly reflected by studying the time-dependent brain to serum PCP concentration ratios (Fig. 5). The brain to serum ratios increased rapidly in the first 5 min after bolus PCP administration in the saline-treated group, while they remained essentially constant in the mAb-treated groups (less than 1) at all time points after bolus PCP administration (Fig. 5 upper panel). This change with antibody treatment likely resulted from a more rapid clearance of PCP from the brain, a slower decrease in serum concentrations, or some combination of the two. The testis to serum total PCP concentration ratio in the saline treated animals increased in a relatively linear fashion up to a maximum ratio of about 6:1 during the 20 min tissue collection period. The rate of increase in bolus PCP concentration ratios increased at a very slow rate and only achieved a ratio of 1.7:1 at 20 min. As with the brain concentrations, mAb treatment kept the testis to serum concentration ratios low for total and for bolus PCP doses (Fig. 5 lower panel).



## Discussion

We tested our hypothesis in an extreme model of drug abuse by attempting to surmount the beneficial effects of the anti-PCP mAb with PCP doses in excess of the binding capacity of the mAb. At the time of mAb administration, the mole ratio of PCP to mAb binding sites was 3:1. Allowing for elimination of mAb over a three day period (IgG elimination half-life in the rat is 8.1 d (Bazin-Redureau et al., 1997), the ratio of PCP (bolus plus infusion) to mAb binding sites when the bolus PCP dose was given was calculated to be 7.7:1. These ratios could underestimate the degree of excess PCP dose over mAb binding sites, however, because they do not account for the amount of the mAb that is bound by PCP, or the continuous PCP infusion that is replacing the body burden of PCP at a rate of approximately 15% of the ongoing infusion dose per hour. While useful, it is difficult to calculate a ratio of available mAb binding sites to PCP ratio because the experiments are not conducted under equilibrium conditions. Thus, there is ongoing elimination of mAb, continuous PCP infusion, and continually changing PCP volume of distribution and clearance due to the changing mAb concentrations and PCP:mAb binding. Nevertheless, this model represents a real-life scenario and an extreme test of the functional capabilities of the mAb.

In addition, the PCP infusion dose (18 mg/kg/day) was purposely chosen to be near the maximally tolerated dose. Correcting for species differences in body weight, this is the human equivalent of 1.26 g/day of PCP in an average size human male of 70-kg. This is clinically relevant because there are reports of phencyclidine (PCP) users self administering up to 1.2 g/day of PCP (Burns and Lerner, 1976). Indeed, Laurenzana et al. (2003) showed one out four rats die from this dose between 4 and 7 days if they do not achieve tolerance or if they are not

treated with the anti-PCP mAb. This extreme model allowed us to address a major criticism of passive immunization for drug abuse, and provided proof of the mechanism of action of the anti-PCP mAb.

The major criticism of passive immunization that was addressed here is that attempts to surmount the effects of an anti-drug antibody can have disastrous health effects. Indeed, the presence of the antibody seemed to offer protection against the potentially devastating effects of the additional bolus PCP dose. The animals in the mAb-treatment group maintained their body weight, and had a lower incidence of chromodacryorrhea prior to administration of the bolus PCP dose. Our previous studies have shown the same beneficial health effects of this mAb (Laurenzana et al., 2003). The animals in the mAb-treated group were healthier and better able to tolerate the additional PCP bolus, as shown by the lack of acute life-threatening respiratory depression, in addition to beneficial alterations in brain PCP concentrations. Other previous studies have shown that an active vaccine against nicotine can reduce the incidence of seizures in rats caused by an acute intraperitoneal nicotine dose given during a chronic s.c. nicotine infusion (Tuncok et al., 2001); however, the current studies are the first to show that passive immunization can also protect against attempts to surmount antibody effects with acute drug dose given during a chronic drug infusion.

The results of the current studies showed the anti-PCP mAb favorably and substantially altered the amount of drug distributed to the tissues. These findings are important because they show that the mechanism of antibody action involves more than simple stoichiometric binding of its ligand. It appears that the antibody works by altering the processes responsible for PCP distribution into and out of the brain. Because the PCP is given in such excess in these studies, it appears that the antibody does not completely block its entry into the brain (see 30 sec time

point, Fig. 3); however, once in the blood stream, and even in a situation of extreme PCP excess, the antibody is effective in quickly redistributing the PCP from the brain, and significantly reducing brain PCP concentrations.

At least part of the reason the mAb is successful in reducing brain concentrations so rapidly has to do with the flux characteristics of PCP across the blood-brain barrier. PCP is a small, lipid soluble molecule that moves rapidly across the blood-brain barrier as a function of blood flow – *i.e.*, it is flow-limited in its distribution. Conversely, in the testis, concentrations rise more slowly and do not peak as rapidly as brain concentrations. Indeed, we have previously described distribution of PCP into the testis as diffusion-limited (Proksch et al., 2000b). These physiologic differences in the tissues are likely one reason why there was less distribution of PCP into the testis after the bolus, and why testis PCP concentrations did not drop as rapidly in the presence of antibody after the bolus as they did with the brain. Thus, the antibody reduces PCP concentrations more quickly in rapidly equilibrating organs like the brain than in more slowly equilibrating organs like the testis. While the overall reduction in brain concentrations in association with the mAb treatment is not multifold, it appears to be adequate to reduce the profound effects of the high PCP doses used here.

The findings from the current study provide proof of the mechanisms of antibody action. We have proposed previously that the presence of the antibody changes the brain PCP clearance from non-restrictive to restrictive (Valentine and Owens, 1996; Proksch et al., 2000a). That is, instead of all PCP in the cerebral circulation reaching the brain parenchyma, brain clearance becomes dependent on antibody binding, and only the free PCP fraction is able to cross the blood-brain barrier. Previous studies in our lab have shown that PCP given by continuous infusion is 95% protein bound in the presence of the anti-PCP mAb, even in the presence of a

large excess of PCP (Proksch et al., 2000a). In these previous studies, the unbound anti-PCP mAb fraction was thought to be sufficient to slow input of PCP into the brain given by continuous infusion, and as redistribution continues, to reduce brain PCP concentrations (Proksch et al., 2000a). In the current studies, the bolus dose of PCP was adequate to overwhelm the small amount of available free antibody, temporarily allowing PCP distribution into the brain. However, as PCP redistribution occurred in the body, a small, albeit significant, free antibody fraction became available again, reducing brain concentrations.

While the anti-PCP mAb did not prevent PCP from entering the brain, it altered the rate of PCP distribution into and out of the brain. We have previously shown that brain PCP concentrations do not decrease as rapidly as serum concentrations during the pharmacological effect period after i.v. PCP administration in absence of the antibody (Proksch et al., 2000b). This dynamic disequilibrium between brain and serum concentrations is the likely cause of the rapid rise in brain to serum concentration ratios, and we think this is a significant contributor to the euphoric ‘rush’ and profound effects sought by drug abusers (Proksch et al., 2000b). The anti-PCP mAb reduced the brain to serum PCP ratio to less than 1 for the total and bolus PCP concentrations for the entire time course of the study whereas brain concentrations rose to nearly 5 times higher than serum in the saline-treated group (Fig. 5). While elevations in serum PCP concentrations contributed to the reduction in this ratio, the rapid reduction in brain concentrations caused by the antibody maintained this ratio at its low level. The therapeutic consequence of the reduction in brain to serum PCP concentration ratio is a reduction in PCP central nervous system effects. This aspect of the mAb’s pharmacokinetic antagonism is critical to understanding the mechanism of action of the antibody, and is proof that the mechanism of antibody action is not only its

ability to bind the drug, but also due to its ability to alter PCP distribution into and out of the brain.

The results of this study showed that the anti-PCP mAb favorably and substantially altered the amount of drug distributed to the tissues, and it altered the rate of PCP distribution despite the extreme excess of PCP administered. Thus, the evidence presented indicates that this antibody will be safe even when the user takes extreme measures (i.e., potentially fatal doses) to use drugs.

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## Footnotes

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S. Michael Owens and W. Brooks Gentry have a financial interest in and serve as Chief Scientific Officer and Chief Medical Officer, respectively, of InterveXion Therapeutics, LLC, a pharmaceutical biotechnology company, whose main interest is in developing new monoclonal antibodies for treatment of human diseases, including drug abuse.

## Figure Legends

**Figure 1: Basis for experimental design for pharmacokinetic studies.** **A.** Graph showing predicted brain PCP concentrations when a single 0.33 mol-equiv mAb6B5 dose is given 17 hr after starting a continuous 18mg/kg/d sc infusion in rats (based on data from Laurenzana et al., 2003). **B.** Rats (n = 4 per time point except for the 5 min time point, where n = 3) were implanted with a subcutaneous osmotic minipump prepared to deliver an 18 mg/kg/day infusion of PCP (day 0). The following day (day 1), rats received anti-PCP mAb (0.33 mol-equiv) or saline. Three days after the mAb or saline dose (on day 4), rats received an i.v. PCP bolus containing [<sup>3</sup>H]PCP as a tracer. Rats were then sacrificed at 0.5, 2, 5, and 20 min after the i.v. PCP dose to determine PCP levels (total and bolus PCP) in serum and tissue.

**Figure 2: Anti-PCP mAb increases PCP serum concentrations.** The effects of a single dose of mAb6B5 on serum concentrations before and after a 1.0 mg/kg i.v. dose of PCP and a 100  $\mu$ Ci/mg PCP dose of [<sup>3</sup>H] PCP (as a tracer) given over 15 sec during a steady-state s.c. infusion of 18 mg/kg/d of PCP are shown as average total PCP concentrations (squares) and bolus PCP concentrations (circles) in serum. The saline group data are shown as white symbols, and the antibody group data are shown as black symbols. Steady-state (pre-bolus PCP i.v.) values are also shown. These values were determined 4 days after the start of the PCP infusion. The zero time point on this graph represents the start of the 1.0 mg/kg i.v. dose of PCP (with tracer). Serum concentrations in the antibody groups were significantly higher at all time points for bolus (# sign signifies p < 0.05) and total (\* signifies p < 0.05) PCP measurements.

**Figure 3: Anti-PCP mAb lowers brain concentrations of PCP.** The effects of a single 0.33 mol-equiv dose of mAb6B5 on bolus and total PCP brain concentrations are shown (see legend

for Figure 2 for experimental details). The brain total PCP concentrations were significantly reduced in the antibody group at all time points except 0.5 min (\* signifies  $p < 0.05$ ), and the brain bolus PCP concentrations were significantly reduced at the 5 and 20 min time points (# sign signifies  $p < 0.05$ ).

**Figure 4: Anti-PCP mAb lowers testis concentration of PCP.** The effects of a single 0.33 mol-equiv dose of mAb6B5 on bolus and total PCP testis concentrations are shown (see legend for Figure 2 for experimental details). The only significant reduction in testis bolus PCP concentrations occurred at the 20 min time point, as indicated by the # ( $p > 0.05$ ). The testis total PCP concentrations were significantly reduced in the antibody group at all time points except at the pre-PCP i.v. bolus time point, as indicated by the \* ( $p < 0.05$ ).

**Figure 5: Anti-PCP mAb affects PCP partitioning in tissues versus serum.** Brain (A) or testis (B) to serum PCP concentration ratios before and after a 1.0 mg/kg i.v. dose of PCP and a 100  $\mu\text{Ci}/\text{mg}$  PCP dose of [ $^3\text{H}$ ] PCP (as a tracer) given over 15 sec during a steady-state subcutaneous infusion of 18 mg/kg/d of PCP are shown as the ratios of averaged concentration values. The ratios from total PCP concentrations are shown as circles and the ratios from bolus PCP concentrations as squares. The ratios in the antibody group are indicated with black symbols and the ratios from the saline group with white symbols. The brain to serum ratios for total PCP concentrations were significantly reduced at all time points in the antibody group. The brain to serum ratios for bolus PCP measurements, and the testis to serum ratios for total and bolus PCP measurements were not different at the 30 sec time points, but were significantly reduced at all other time points in the antibody group.

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**Table 1. Comparisons of PCP Concentrations: Ratios of Average Antibody Group Concentrations to Saline Group Concentrations**

	<b>Baseline</b>	<b>Post-i.v. Bolus PCP</b>			
	<b>(Pre-i.v. Bolus)</b>	<b>0.5 min</b>	<b>2 min</b>	<b>5 min</b>	<b>20 min</b>
<b>Serum</b>					
Total PCP	11.2	2.9	3.2	4.6	5.2
Bolus PCP	N/A	1.8	2.1	3.7	3.7
<b>Brain</b>					
Total PCP	0.4	1.2	0.7	0.6	0.7
Bolus PCP	N/A	1.2	0.8	0.7	0.9
<b>Testis</b>					
Total PCP	0.6	0.7	0.6	0.7	0.6
Bolus PCP	N/A	1.0	0.8	1.2	0.7

**Figure 1**

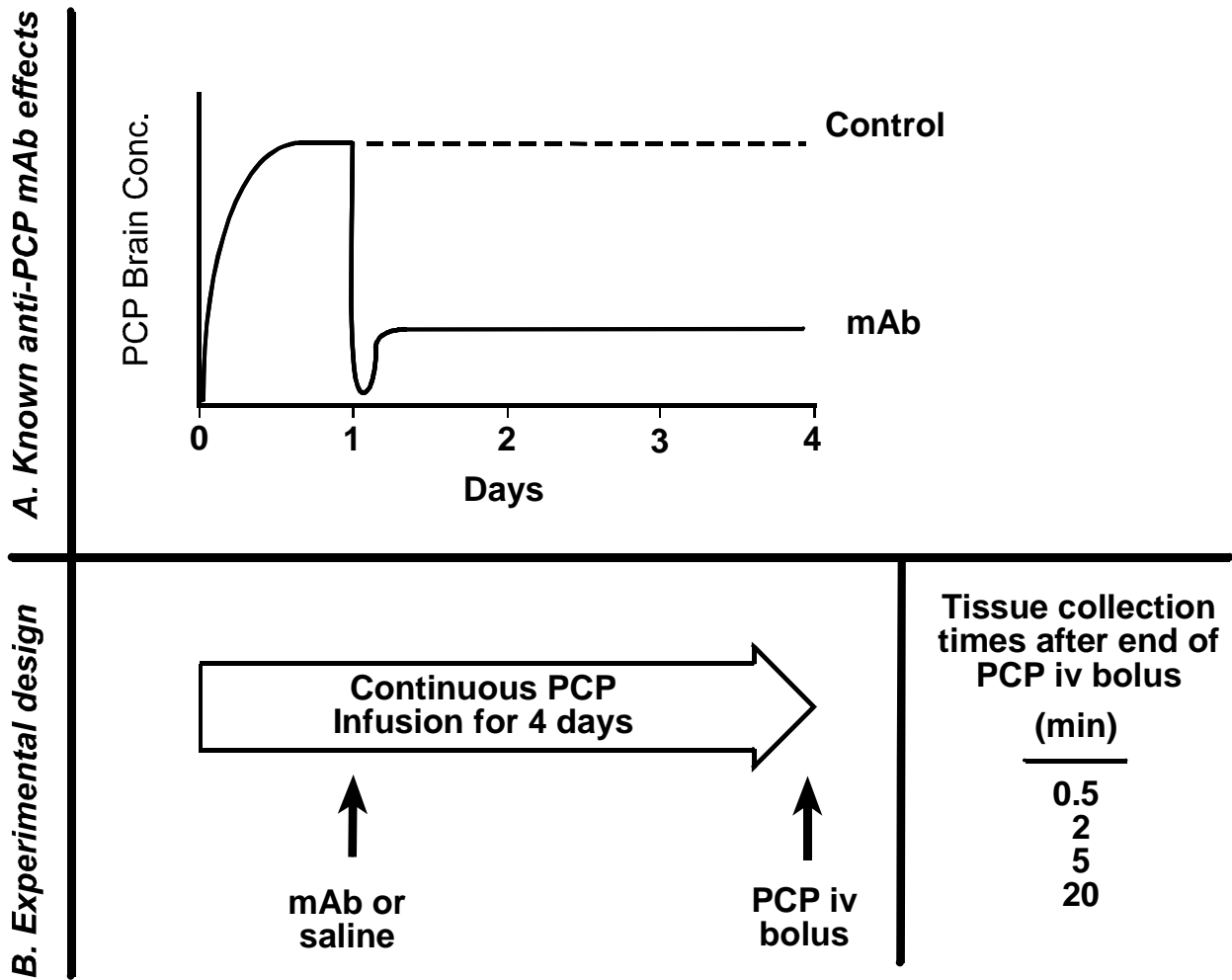
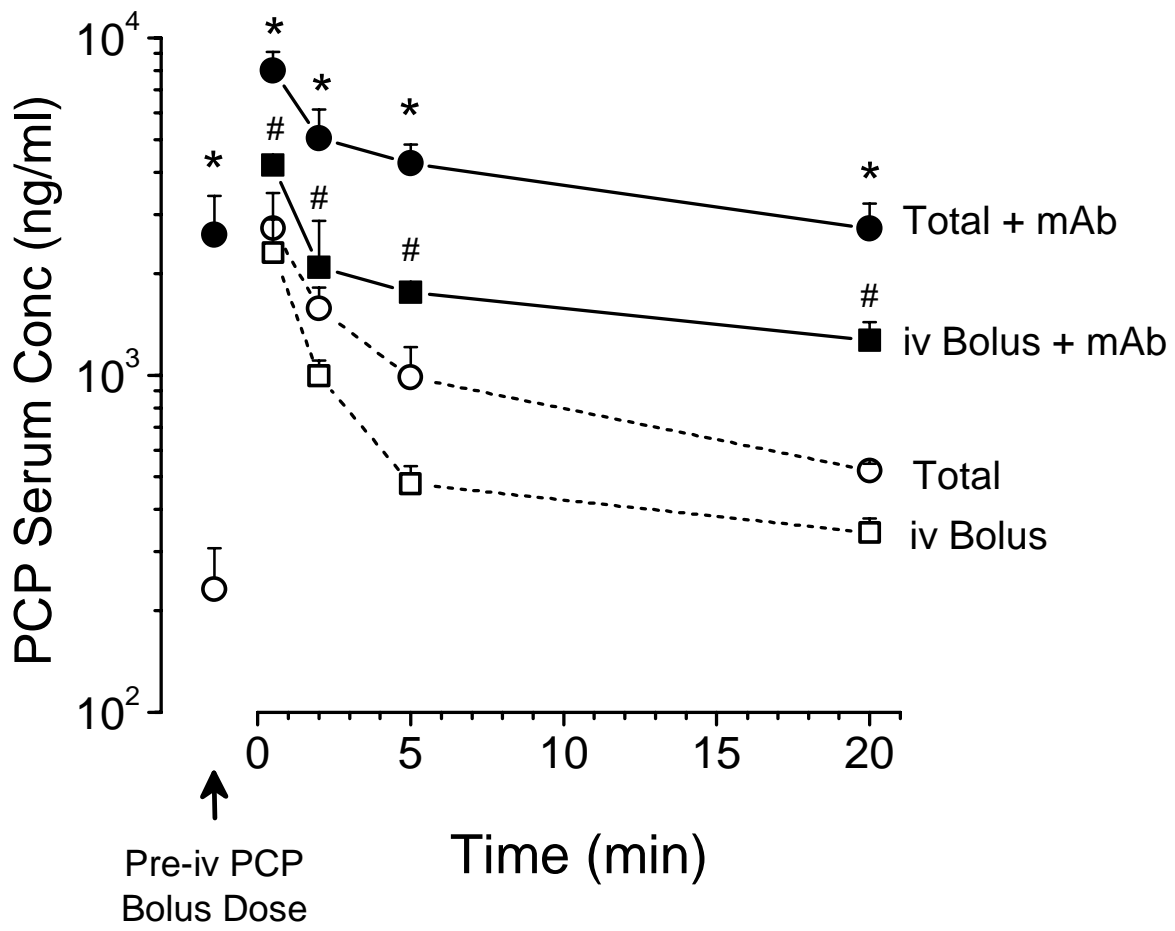
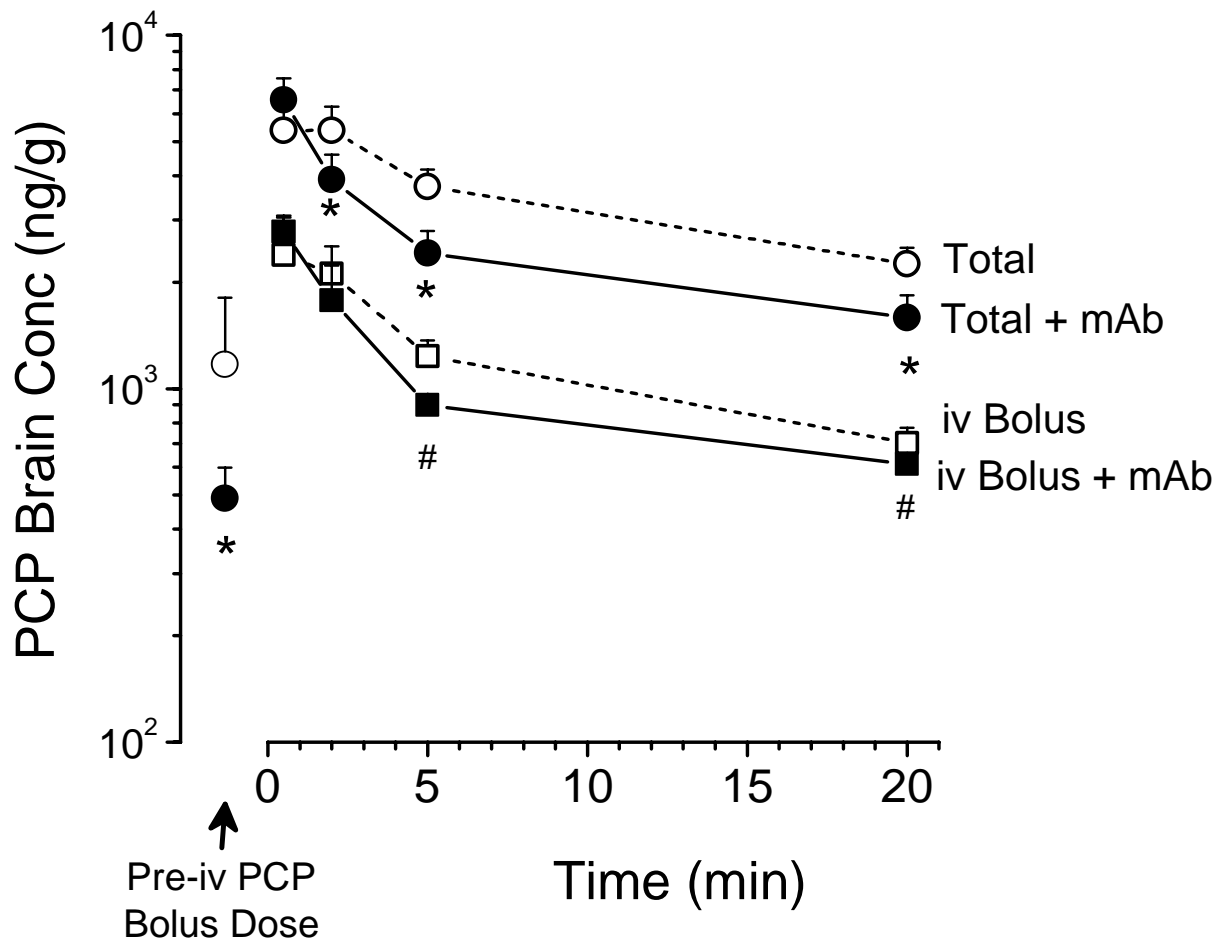


Figure 2

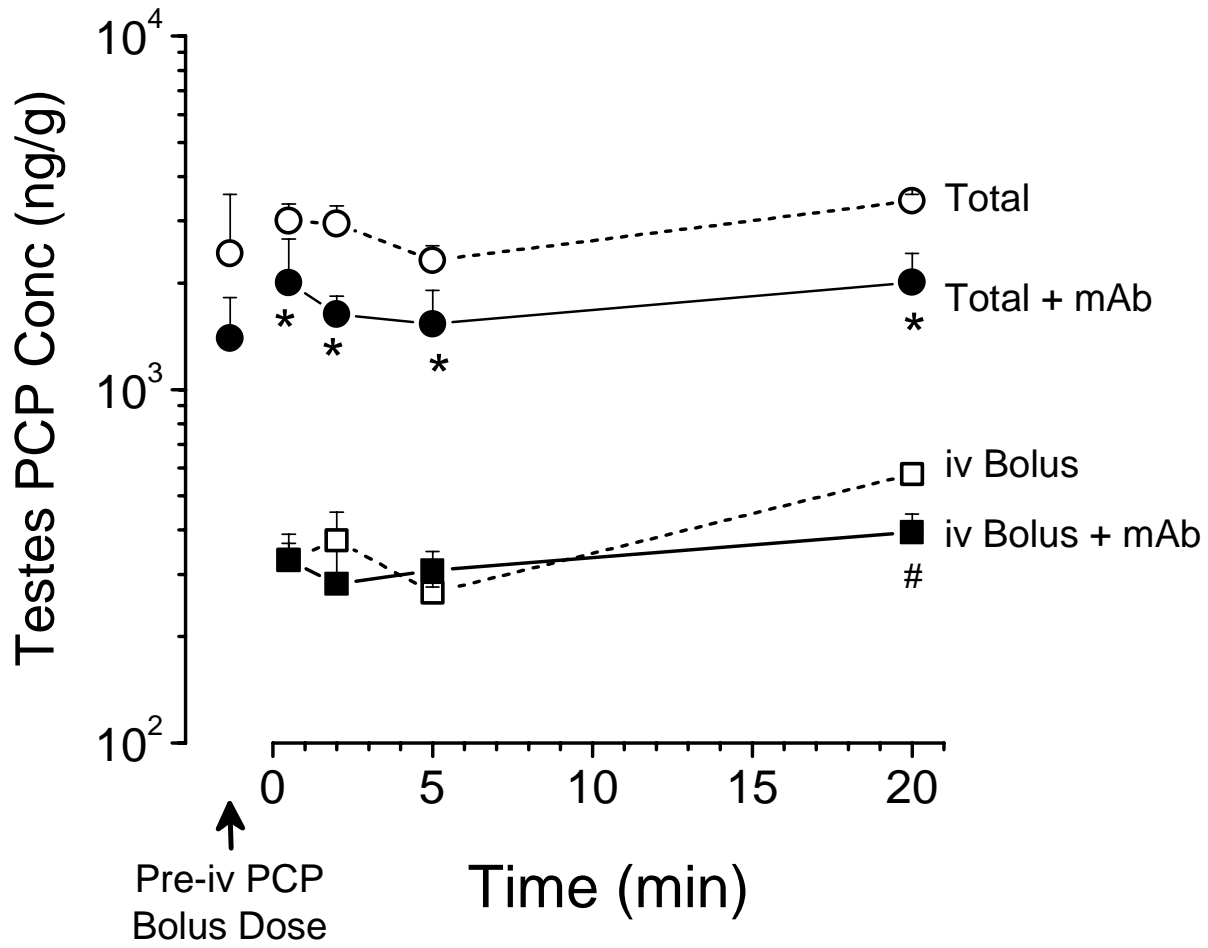


**Figure 3**





**Figure 4**



**Figure 5**

