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Neurochemical changes in the rat prefrontal cortex following acute phencyclidine treatment: an in vivo localized ^1H MRS study[†]

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Abstract

Acute phencyclidine (PCP) administration mimics some aspects of schizophrenia in rats, such as behavioral alterations, increased dopaminergic activity and prefrontal cortex dysfunction. In this study, we used single-voxel ^1H -MRS to investigate neurochemical changes in rat prefrontal cortex in vivo before and after an acute injection of PCP. A short-echo time sequence (STEAM) was used to acquire spectra in a 32- μL voxel positioned in the prefrontal cortex area of 12 rats anesthetized with isoflurane. Data were acquired for 30 min before and for 140 min after a bolus of PCP (10 mg/kg, $n=6$) or saline ($n=6$). Metabolites were quantified with the LCModel. Time courses for 14 metabolites were obtained with a temporal resolution of 10 min. The glutamine/glutamate ratio was significantly increased after PCP injection ($p < 0.0001$, pre- vs. post-injection), while the total concentration of these two metabolites remained constant. Glucose was transiently increased ($\pm 70\%$) while lactate decreased after the injection (both $p < 0.0001$). Lactate, but not glucose and glutamine, returned to baseline levels after 140 min. These results show that an acute injection of PCP leads to changes in glutamate and glutamine concentrations, similar to what has been observed in schizophrenic patients, and after ketamine administration in humans. MRS studies of this pharmacological rat model may be useful for assessing the effects of potential anti-psychotic drugs *in vivo*.

Keywords

PCP; prefrontal cortex; glutamine; glutamate; ^1H MRS

[†]The authors declare that they have no conflict of interest related to the work presented in this paper.

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INTRODUCTION

Phencyclidine (PCP) is an NMDA (N-methyl-D-aspartic acid) receptor antagonist which induces behavioral symptoms in healthy subjects similar to those observed in schizophrenic patients. In rats, an acute injection of PCP induces locomotion hyperactivity (1,2) and alters prepulse inhibition (3,4). In addition, PCP increases brain dopamine release, along with serotonergic release, both of which have been reported previously in schizophrenic patients (5). These observations, along with numerous studies, have led to the concept that schizophrenia may result from the hypofunction of the NMDA receptors (6-10).

Because hypofrontality is a key feature of schizophrenia (associated primarily with cognitive and negative symptoms) (11), studies in animals have investigated the effects of PCP on the prefrontal cortex. For example, in rats, intracranial injections of PCP in the prefrontal cortex have been shown to induce hyperlocomotion, and to increase dopamine neurotransmission in nucleus accumbens (12). In addition, an acute intraperitoneal (i.p.) dose of PCP has also been shown by microdialysis to increase glutamate efflux in the prefrontal cortex (13). On the basis of these and other studies, it has been suggested that NMDA receptor hypofunction leads to decreased activity of inhibitory GABAergic interneurons (5,14-17), causing in turn the disinhibition of glutamatergic neurons projecting to the prefrontal cortex and an increase in glutamate release (13,18,19).

Energy metabolism and cerebral blood flow are also impaired in schizophrenia patients (20-25). Similarly, acute PCP-treatment in rats has been shown to alter energy metabolism and blood flow in prefrontal cortex. Glucose utilization and uptake have been shown to be altered in rats after a single injection of PCP (26-28). Recently, a perfusion-based MRI study showed dose-dependent effects on the blood flow in the prefrontal cortex after an acute systemic injection of PCP in isoflurane-anesthetized rats (29).

So far, neurotransmission in the PCP model has been studied using microdialysis (13,30,31), and brain glucose metabolism has been studied in histologic studies using ¹⁴C autoradiographic techniques (26-28). NMR spectroscopy has also been used to study the effect of chronic and acute MK801 (32-34) administration. In these latter studies, brain extracts have been studied using ¹³C labeled substances as tracers. Only one of these studies (34) reported *in vivo* data with MK-801 (albeit on the entire brain), and all the others were performed in brain extracts. High-field NMR spectroscopy allows the study of brain metabolism non-invasively *in vivo* by measuring a 'neurochemical profile' of metabolite concentration in a well-defined area of the brain (35). In the present study, we hypothesized that an acute injection of PCP would result in changes in the concentration of MR-observable metabolites involved in neurotransmission (glutamate, glutamine, GABA) and in energy metabolism (glucose, lactate) in the prefrontal cortex.

MATERIALS AND METHODS

Animal preparation

All experiments were performed according to the guidelines for the care and use of laboratory animals at the University of Minnesota and were approved by the Institutional Animal Care and Use Committee (IACUC). Twelve healthy male Sprague-Dawley rats (250-300 g, Harlan, Madison, WI, USA) were used in this study. Rats were anesthetized in an induction chamber using isoflurane (5%) with a mixture of O₂ and N₂O as carrier gases (in the ratio 0.3:0.7 L/min, respectively). Rats were then placed on a heating pad and kept under gaseous anesthesia (isoflurane 2%) using a nose cone. An intraperitoneal line was placed for PCP (n=6) or saline (n=6) solution injection. External electrodes for ECG monitoring were placed on the front paws and a pressure sensor for respiration monitoring was positioned under the abdomen of the

animal, below the diaphragm (SA Instruments, Inc., Stony Brook, NY, USA). The animals were then positioned prone in a custom-designed holder for MRS experiment. Isoflurane concentration was adjusted between 1.2 and 2% to maintain a stable respiration rate. Temperature was controlled by a rectal thermal probe and maintained at physiological values (37 °C) using warm water circulation. Baseline spectra were acquired 30 min before and 140 min after the injection of PCP or saline (10 mg/kg i.p.). PCP (Sigma, St. Louis, MO, USA) was dissolved in sterile saline NaCl 0.9% solution to yield a 2 mg/mL concentration.

Spectroscopy

All experiments were conducted on a 9.4 T/31 cm horizontal bore magnet equipped with a Varian console (Varian, Palo Alto, CA, USA). A surface transmit-and-receive radiofrequency probe consisting of two quadrature ^1H coils was used. A short-echo-time STEAM sequence with outer volume suppression, described elsewhere (36), was used for 3D-localization, with the following timing modification compared to the cited article: TE=2 ms, TR=4.5 s. A volume of interest of 32 μL ($4 \times 2 \times 4 \text{ mm}^3$) containing mainly prefrontal cortex was positioned in the rat brain using transverse and sagittal RARE images for anatomical guidance. Localized shimming in the voxel of interest was performed using FAST(EST)MAP (37), leading to a water linewidth ranging from 11 to 16 Hz. For every three sets of 128 single-shot scans (each set being acquired in 9 min and 44 s), a water reference scan was acquired for compensation of eddy currents and to serve as a concentration reference for absolute quantification.

Post-processing

Each individual single-shot scan was saved separately. One spectrum consisted of 128 scans added after correction for phase, frequency drift and eddy currents of individual scans. This yielded a signal-to-noise ratio between 18 and 20 for NAA as determined by the LCModel.

Quantification of metabolites

The LCModel fitting software (Stephen Provencher, Inc., Oakville, ON, Canada) was used to quantify the metabolites in the frequency domain, using the basis set (including an experimentally measured 'metabolite-nulled' macromolecule spectrum) as discussed previously by Pfeuffer *et al.* (35).

Statistics

For each metabolite separately, a repeated measures ANOVA was fitted using PROC MIXED in SAS (SAS Institute, Inc., Cary, NC) with effects for group, time, group $\times$ time interaction, and a random effect for rats to account for inter- and intra-individual variability. Contrasts of least squares means were used to test (A) PCP *versus* saline at each time point and (B) PCP (or saline) pre-injection compared to PCP (or saline) post-injection, i.e. averaging over a longer acquisition time to achieve sufficient signal-to-noise ratio before and after injection, respectively. Pre-injection averages were obtained by averaging the data acquired during the first 30 min; post-injection averages were taken over time periods during which the concentration of the considered metabolite was stable. For each of (A) and (B), *p*-values reported have been adjusted for multiple comparisons using the Tukey-Kramer method. Percentage changes in the concentration of metabolites before and after injection were also calculated for each rat to account for inter-individual variability, and two-sample *t*-tests were carried out to assess the difference between groups.

RESULTS

Figure 1(a) shows a sagittal MR image of the rat brain, on which the prefrontal cortex area was identified for acquisition of the spectroscopic data. Typical *in vivo* localized spectra acquired

in the animals 40 min after the bolus of saline (b) and PCP (c) are also shown. Visual inspection shows no obvious changes except for a decrease in lactate signal in animals treated with PCP (Fig. 1c). For quantitative assessment, absolute concentrations of metabolites were obtained for each spectrum with the LCModel, and time courses of these concentrations were obtained. Changes were observed in the following metabolites: glutamate, glutamine, glucose, lactate, and possibly GABA. These changes are described in more detail below. Cramér-Rao Lower Bounds (CRLB) are provided for those metabolites (Table 1). No change was observed in the concentration of any other metabolites following PCP treatment (namely macromolecules, creatine and phosphocreatine, glutathione, *myo*-inositol, lactate, *N*-acetylaspartate, *N*-acetylaspartylglutamate, phosphorylethanolamine, taurine, choline, and glycerophosphocholine). In addition, there were no significant differences between preand post-injection for control rats (saline injection) in any of the metabolites observed.

Glutamine and glutamate

Figure 2 shows results for glutamate and glutamine. The total pool (glutamate $\pm$ glutamine) was stable in both groups, before and after injection of PCP (Fig. 2a). The change in glutamine β glutamate concentration after injection was not significantly different between the two groups (saline, $1\pm2\%$; PCP, $(1\pm5)\%$ change post- vs. pre-injection, mean $\pm$ SD).

In contrast, the glutamine/glutamate ratio was significantly increased by 17% after PCP injection compared to baseline ($F=18.93$, $df=166$, least square mean 0.05 , $t=7.83$, $p<0.0001$) and compared to saline-treated rats ($F=0.553$, $df=10$, $p=0.00016$, Fig. 2c and d). The increased glutamine/glutamate ratio reflected a simultaneous 12% increase in glutamine (Fig. 2e and f) and a 5% decrease in glutamate (Fig. 2g and h). The increase in glutamine concentration was significant compared to both baseline ($F=30.45$, $df=166$, least square mean $=0.37$, $t=5.94$, $p<0.0001$, post- vs. pre-PCP) and control (saline) animals after injection ($F=4.038$, $df=10$, $p=0.0007$, Fig. 2e and f). The decrease in glutamate concentration was also significant compared to baseline ($F=26.64$, $df=106$, least square mean $=-0.49$, $t=-5.06$, $p<0.0001$, post- vs. pre-PCP) and control ($F=3.69$, $df=10$, $p=0.034$). As the total concentration of glutamine β glutamate was stable, the observed increase in glutamine concentration compensated for the decrease in glutamate concentration.

Glucose and lactate

Figure 3 shows the time courses of concentrations of glucose and lactate (Fig. 3a and c, respectively). Percentage changes in concentrations after injection compared to baseline are also reported for both saline and PCP-treated rats (Fig. 3b and d). Glucose increased significantly immediately after the injection until 30 min (from 2.2 ± 0.6 to 3.3 ± 0.9 $\mu\text{mol/g}$ wet weight of tissue, mean $\pm$ SD; $F=26.23$, $df=106$, least square mean $=1.00$, $t=7.06$, $p<0.0001$, pre- vs. post-PCP) and this change in concentration was significantly higher compared to saline-treated animals ($F=0.143$, $df=10$, saline, $-4\pm24\%$, PCP, $43\pm23\%$, $p=0.0055$).

Lactate decreased significantly (from 2.4 ± 0.4 to 1.1 ± 0.4 $\mu\text{mol/g}$ wet weight of tissue, $F=46.49$, $df=57$, mean $\pm$ SD; least square mean $=-1.24$, $t=-9.09$, $p<0.0001$, pre- vs. post-PCP), and was significantly lower in PCP compared to saline ($-54\pm7\%$ and $6\pm20\%$ respectively, $F=0.784$, $df=10$, $p=0.00022$, PCP vs. saline). Lactate concentration remained low from 30 to 90 min after the bolus was given.

GABA

The LCModel software also fitted GABA in almost all individual rats treated with saline at each time point (CRLB $28\pm9\%$, mean $\pm$ SD), and in PCP animals before injection ($32\pm7\%$, mean $\pm$ SD). After injection of PCP, however, CRLB for GABA were above 50%, suggesting that the total GABA concentration was below the detection threshold. In the animals receiving

saline, CRLB were $24 \pm 5\%$ (mean $\pm$ SD), comparable to those observed before injection. The fact that GABA was fitted with less precision after PCP injection suggests a decrease in GABA concentration, but the sensitivity on GABA, clearly, was not sufficient in individual animals to draw firm conclusions. Therefore, in order to estimate the change in GABA with better signal-to-noise ratio, we added the spectra of all six rats at each time point and quantified the summed spectra with LCModel. Concentration for GABA for these sums after injection were $0.8 \pm 0.1 \mu\text{mol/g}$ wet weight (CRLB = $13 \pm 2\%$) in control and $0.5 \pm 0.1 \mu\text{mol/g}$ wet weight (CRLB = $24 \pm 3\%$) in PCP rats, suggesting that indeed the total pool of GABA decreases after the injection of PCP.

DISCUSSION

In this study, we report the effect of an acute systemic bolus of PCP on the concentration of multiple metabolites in the rat prefrontal cortex in vivo. Using a short-echo time localized ^1H MRS technique, time courses of several key metabolites were obtained. An acute, 10 mg/kg intraperitoneal injection of PCP led to an increase in glutamine over glutamate ratio, a transient increase in glucose along with a decrease in lactate. Data also suggest that GABA is decreased. Measurements were conducted in a relatively small volume in the prefrontal cortex. Except for glucose and lactate, which exhibited up to 70% variation in their concentration, detected changes were subtle but highly significant.

A major hypothesis for the pathophysiology of schizophrenia is that NMDA receptor hypofunction and therefore glutamatergic dysfunction are primary events leading to hyperdopaminergia (5,16,38). During neurotransmission, glutamate is released by neurons in the extracellular space and taken up by astrocytes, where the conversion of glutamate to glutamine occurs via the glutamine synthase. Then, glutamine is transported back to neurons, where it is converted to glutamate. After a systemic i.p. injection of the NMDA-receptor antagonist PCP (4), we observed no change in the total pool glutamate+glutamine, but we observed a concomitant increase in glutamine and decrease in glutamate concentration. The decrease in glutamate concentration therefore appears to be compensated by the increase in the glutamine pool. This suggests that the NMDA receptor blockade leads to a shift in the glutamate-glutamine cycle. Brenner *et al.* (32) hypothesized that the function of glutamine synthetase, the enzyme responsible for the conversion of glutamate into glutamine, might be affected by the oxidative stress that has been shown to be associated with NMDA receptor blockade (39,40). Our observation is in agreement with this hypothesis.

The glutamine increase observed here is consistent with previous observations in humans in the anterior cingulate cortex after ketamine infusion (41) and in different cortical regions of rats receiving another NMDA receptor antagonist, namely MK-801 (32,42). The same authors (32,42), also report an increase or no effect in the concentration of glutamate in the rat, depending on the region of the brain observed after an injection of MK-801. The response to MK-801 is strongly region-dependent (42), which may explain the different results on glutamate obtained in this study. PCP and MK-801 might also have different effects on brain metabolites, as it has been shown to be differently regulated by nitric oxide synthase inhibition (39). Recently, Moghaddam *et al.* (13) observed an increase in extracellular glutamate efflux using microdialysis in the prefrontal cortex after a systemic PCP injection. However, in contrast to microdialysis, which measures extracellular concentrations, ^1H MRS measures the total concentration of metabolites. Therefore direct comparison of results between the two techniques is difficult. One possible interpretation is that the increase in glutamate efflux may deplete the neuronal glutamate pool. Increased glutamate release would also result in increased glutamate uptake and glutamine synthesis in astrocytes, which would be consistent with the increase in glutamine concentration observed in our study. Such a change in the glutamine/

glutamate ratio may therefore reflect changes in neurotransmission and has in fact been observed in multiple pathological conditions in animals and humans (43-47).

Interestingly, similar alterations in the glutamine/glutamate ratio have been observed in schizophrenic patients. An increase in glutamine over glutamate ratio has been reported in the medial prefrontal cortex (48), the anterior cingulate and thalamus (45) in never-treated patients with schizophrenia. An increased glutamine/glutamate ratio has also recently been observed in the anterior cingulate in patients with schizophrenia at an early onset of the disease and not affected by treatment with antipsychotics (49). Indeed, it has been suggested that the aggravation of schizophrenia in patients results from the repeated exposure to hyperglutamatergia (11). According to our results, the use of a single dose of NMDA receptor antagonist such as PCP mimics the metabolic effects of an early, untreated (hyperglutamatergic) schizophrenic state in patients. This model could therefore be useful for the investigation of the glutamine-glutamate metabolism and neurotransmission mimicking the metabolic profile observed in drug-naïve patients with schizophrenia.

Along with the increase in glutamine over glutamate ratio, we observed that GABA was no longer detected in the neurochemical profiles after PCP injection. Similarly, Homayoun *et al.* (17) recently observed a decrease in activity of GABAergic interneurons following an injection of MK-801 in the prefrontal cortex of conscious rats. However, due to the high dose used in this study, PCP could also act on GABAergic interneurons, and it is therefore difficult to rule out a direct effect of PCP compared to an indirect effect of NMDA receptors blockade.

Glucose concentration increased drastically (up to 70%) within 20 min after the injection of PCP, and decreased rapidly thereafter. This increased glucose concentration may be due to either increased uptake through the blood-brain barrier or to decreased utilization of glucose by the brain, or both. In the PCP rodent model, autoradiographic studies have shown that local cerebral glucose utilization (LCGU) increases, decreases or is unchanged depending on the brain region, time after injection, and the dose of PCP injected (26-28). In particular, Weissman *et al.* (27) report that LCGU in the prefrontal cortex is decreased 2 min after PCP injection, and is later increased at 60 min. Therefore, an early decrease in glucose utilization may have led to the increased pool of glucose observed in this study, with glucose uptake occurring normally or being even increased in the prefrontal cortex, as observed with other NMDA receptor antagonists 30 min after injection (50). Then, a subsequent increase in glucose utilization may occur, allowing the glucose pool to decrease. This latter increase in LCGU has been shown to persist for nearly 3 h in the prefrontal cortex (26), and may account for the trend of a decreased glucose pool observed here.

Our results also show a drop in lactate concentration after the injection of PCP. Using the more specific NMDA receptor antagonist MK-801, Loubinoux *et al.* (34) observed an increase in the brain lactate pool in experiments conducted in conscious rats when utilizing a non-localized ¹H MRS technique. Brenner *et al.* (32) also observed an increase in lactate pool in extracts of both temporal lobe and frontal cortical regions in rats, 20 min after injection of MK-801. The apparent discrepancy between those results and our results may be due to the difference in the effects of PCP vs. the effects of MK-801 (39). In addition, in contrast to the above-mentioned studies, our measurements were done under isoflurane anesthesia. Isoflurane is known to increase the baseline lactate concentration in the brain (51), which explains the high levels of lactate seen in control conditions in our study. The fact that lactate decreases after treatment with PCP, together with an increase in glucose, may also be consistent with the transitory decrease in glucose utilization (27).

A few limitations of our study are worth mentioning. First, we used an acute PCP injection. Although acute PCP injection has been used as an animal model of schizophrenia in several

studies (12,13,52), chronic administration of PCP might be a more relevant animal model of schizophrenia than acute treatment. Ultrastructural changes are observed in neurons as early as 4 h after an acute injection of PCP (52), but chronic alterations of the brain connectivity observed in the disease are not mimicked by a single injection of PCP. Therefore, more work is needed to determine whether the changes we observed here after an acute injection also occur during chronic treatment and how they are relevant to the pathophysiology of schizophrenia. Second, we used a relatively high dose of PCP, which may lead not only to blockade of NMDA receptors, but also to non-specific effects of PCP, such as direct neurotoxicity, in particular on GABAergic interneurons. Third, we studied only the prefrontal cortex. Further studies in other areas of the brain would be of interest. In spite of these limitations, our study demonstrates that PCP treatment leads to changes in the total concentration of key metabolites involved in energy metabolism and neurotransmission. This underscores the potential of magnetic resonance spectroscopy to study animal models of schizophrenia longitudinally, an approach that may prove useful to assess the efficacy of new anti-psychotic drugs.

In conclusion, localized ^1H MRS allowed a non-invasive exploration of the effect of PCP on the concentration of several metabolites in a relatively small region of the brain, along with good temporal resolution. While microdialysis provides an insight into monoamines efflux in the extracellular space, ^1H MRS provides complementary information on the brain metabolism by giving access to the total tissue concentration of metabolites. To our knowledge, this is the first study using magnetic resonance to investigate changes in metabolism in intact rats following an acute injection of PCP. This technique may prove useful to assess the effect of metabotropic glutamate receptor agonists, which has been shown to abolish the glutamate efflux observed with PCP (13).

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Figure 1.

(a) Sagittal view of the rat brain. The box delineates the area in which spectra were acquired (32 μL). (b, c) Typical *in vivo* ^1H NMR spectra acquired in the prefrontal cortex area 40 min after an injection of saline (b) or PCP (c). Each spectrum corresponds to an acquisition of 128 single scans. A different line broadening was applied to each spectrum to obtain comparable linewidth for the NAA peak between spectra. Lactate (at 1.32 ppm) was strongly reduced in the animal receiving PCP (c). tCr, creatine + phosphocreatine; Glx, glutamate + glutamine; Glu, glutamate; Gln, glutamine; NAA, *N*-acetylaspartate; GABA, γ -aminobutyric acid; PE, phosphorylethanolamine; *myo*Ins, *myo*-inositol; Lac, lactate. Please note that glucose resonances overlap with *myo*Ins and PE peaks and are therefore not directly observable.

Figure 2.

(a) Time course of the total concentration of glutamine + glutamate. The arrow indicates the time of PCP injection. Glutamine + glutamate concentration (saline, $n = 6$, PCP, $n = 6$; mean $\pm$ SEM) is shown in $\mu\text{mol/g wet weight}$ of tissue over time (min). (b) Percentage difference in the concentration of glutamine and glutamate between before (PRE) and after (POST) the injection of PCP (saline, $1 \pm 2\%$; PCP, $1 \pm 5\%$; mean $\pm$ SD). (c) Time course for the ratio glutamine/glutamate (mean $\pm$ SEM). The ratio was significantly higher after the injection in the PCP group (least square mean = 0.05, $t = 7.83$, $p < 0.0001$, PRE- vs. POST-PCP). (d) Percentage change in the ratio glutamine/glutamate in saline-treated ($-3 \pm 5\%$) and PCP ($\pm 17 \pm 7\%$) rats, $*p = 0.00016$. (e) Time course for the concentration of glutamine. Glutamine concentration is

shown in $\mu\text{mol/g}$ wet (mean $\pm$ SEM) over time (min). Glutamine concentration increased significantly after PCP injection (least square mean 0.05, t 7.83, $p < 0.0001$, PRE- vs. POST-PCP). (f) Percentage difference in the concentration of glutamine between before (PRE) and after (POST) the injection (saline, $(-3\pm3)\%$; PCP, $(12\pm7)\%$; mean $\pm$ SD, $*p = 0.034$). (g) Time course for the concentration of glutamate. Glutamate concentration (in $\mu\text{mol/g}$ wet weight, mean $\pm$ SEM) decreased significantly after PCP injection (least square mean = -0.49, $t = 5.06$, $p < 0.0001$, PRE- vs. POST-PCP). (h) Percentage difference in the concentration of glutamine between before (PRE) and after (POST) the injection (saline, $(1\pm2)\%$; PCP, $(-5\pm5)\%$; mean $\pm$ SD, $*p=0.034$). For (a), (c), (e), and (g), open squares are saline-treated (control) animals ($n = 6$); closed squares, PCP animals ($n = 6$). For (b), (d), (f), and (h), open bars are control animals and closed bars are PCP animals

Figure 3.

(a) Time course of glucose concentration. Glucose was significantly increased immediately after the injection until 30 min (least square mean = 1.00, $t = 7.06$, $p < 0.0001$, PRE- vs. POST-PCP). (b) Percentage change in the concentration of glucose between before (PRE) and after (POST) the injection of saline (control) and PCP in rats (* $p = 0.0055$). (c) Time course for lactate concentration. Lactate concentration was stable after the injection of saline, but it dropped significantly after the injection of PCP (mean = 0.05, $t = 7.83$, $p < 0.0001$, PRE- vs. POST-PCP). (d) Change in lactate concentration between PRE and POST injection in control and PCP rats (* $p = 0.00022$, PCP vs. control). For (a) and (c), open squares are control animals ($n=6$); closed squares, PCP animals ($n=6$). For (b) and (d), open bars are saline-treated (control) animals, closed bars are PCP-treated animals. In (a) and (c), the arrow indicates the moment of injection. Glucose and lactate concentrations are reported in $\mu\text{mol/g}$ wet weight of tissue over time (min). All data are mean $\pm$ SEM with $n=6$ in both the control and PCP groups.

Table 1

Average of the Cramér-Rao lower bounds for glu-tamine, glutamate, glucose, and lactate in both groups, before and after injection

	Control (n=6)		PCP (n=6)	
	Pre (%)	Post (%)	Pre (%)	Post (%)
Gln	6±0	7±0	7±0	6±0
Glu	2±0	2±0	3±0	3±0
Gln+Glu	2±0	2±0	2±0	2±0
Glc	22±6	22±9	20±7	14±5
Lac	7±2	7±1	7±1	19±4