Neonatal PCP Is More Potent than Ketamine at Modifying Preweaning Behaviors of Sprague-Dawley Rats

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Treatment with *N*-methyl-ᴅ-aspartate (NMDA) receptor antagonists, such as ketamine (KET) or phencyclidine (PCP), can trigger apoptotic neurodegeneration in neonatal rodents; however, little is known about the behavioral alterations resulting from such treatment. Here, rats were sc treated with saline; 10 mg/kg PCP on postnatal days (PNDs) 7, 9, and 11; 20 mg/kg KET (six injections every 2 h on PND 7); or a regimen of ketamine and 250 mg/kg ʟ-carnitine (KLC) both administered on PND 7 with additional 250 mg/kg doses of ʟ-carnitine given on PNDs 8–11. Postinjection, the home cage behavior of each pup was categorized on PNDs 7–11. Slant board and forelimb hang behaviors were examined on PNDs 8–11 and 12–16, respectively. The initial KET or KLC injections on PND 7 elevated abnormal home cage activity (i.e., paresis and paddling); however, KLC pup behavior returned to normal by the fourth injection, indicating the protective effects of ʟ-carnitine against NMDA antagonist toxicity. PCP treatment caused substantial abnormal home cage activity on each injection day (PNDs 7, 9, and 11). Latencies to turn on the slant board were significantly longer on PND 8 for KET- and PCP-treated pups and PND 10 for PCP-treated pups. On PND 12, the forelimb hang time of PCP-treated pups was significantly shorter. Body weight was decreased on PNDs 8–18 in PCP-treated pups and PNDs 8–10 in KET-treated pups. These data indicate that developmental NMDA antagonist treatment causes short-term behavioral alterations which appear related to motor coordination and may be cerebellar in nature. Furthermore, single PCP injections appear more potent at altering behavior than multiple injections of KET.

Key Words: ketamine; phencyclidine; neurodegeneration; forelimb hang; negative geotaxis; pup behavior.

The excitatory neurotransmitter glutamate activates ionotropic (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, kainate, and *N*-methyl-ᴅ-aspartate [NMDA]) and metabotropic (G protein linked) receptors and is essential for neuronal differentiation, migration, and survival (reviewed by Meldrum, 2000). Treatment of postnatal day (PND) 7 rats with noncompetitive NMDA receptor antagonists such as MK-801 (dizocilpine), phencyclidine (PCP), or ketamine (KET) results in increased neuronal degeneration (Hayashi *et al.*, 2002; Ikonomidou *et al.*, 1999; Scallet *et al.*, 2004; Wang and Johnson, 2005; Wang *et al.*, 2001). Specifically, multiple injections of 20 mg/kg KET caused increased numbers of Fluoro-Jade, terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling, and silver-stained cells in the hippocampus, thalamus, subiculum, caudate nucleus, and frontal, cingulate, parietal, and retrosplenial cortices (Ikonomidou *et al.*, 1999; Scallet *et al.*, 2004). Single injections of the same or higher doses (25–75 mg/kg) do not appear to cause similar neuronal cell death (Hayashi *et al.*, 2002; Scallet *et al.*, 2004).

Such NMDA antagonist–induced neurodegeneration has been shown to result in behavioral deficits as well. For example, neonatal PCP or MK-801 treatment causes later sensorimotor gating deficits as measured by prepulse inhibition (Harris *et al.*, 2003; Wang *et al.*, 2001) and impairs Morris water maze performance in juvenile and adult rats (Sircar, 2003; Sircar and Rudy, 1998). Neonatal PCP treatment has been described to cause increased sensitivity to later PCP treatment as well as transient deficits in spatial alternation performance (Wang *et al.*, 2001). Repeated neonatal MK-801 treatment results in long-term deficits in radial-arm maze performance (Kawabe *et al.*, 2007). Those behavioral deficits imply that the observed neurodegeneration following developmental NMDA antagonist treatment has long-term effects.

The description of long-term behavioral effects after developmental NMDA antagonist treatment led us to hypothesize that there may be acute effects observable during treatment. Here, the behavioral effects of PCP or KET were evaluated using those treatment regimens previously shown to produce significant neurodegeneration. Rat pups were treated on PND 7 with KET at 2-h intervals for six injections or on PNDs 7, 9, and 11 with PCP. As a preliminary exploration, the potential protective effects of ʟ-carnitine were measured in KET-treated rats since ʟ-carnitine appears to prevent glutamate neurotoxicity (Felipo *et al.*, 1994) and neurodegeneration in the frontal cortex of PND 7 rats (Zou *et al.*, 2008). Home cage behavior of the pups was rated using a comprehensive scoring system on PNDs 7–11 after each treatment. Slant board (negative

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geotaxis; PNDs 8–11) and forelimb hang (PNDs 12–16) behaviors were examined to assess potential early neurotoxicant-induced dysfunctions.

MATERIALS AND METHODS

Animals

Sprague-Dawley dams ($n = 48$) had normal vaginal births and on the day of birth (PND 0), each litter was separated by sex, and four males and four females were randomly selected so that each litter was culled to eight. The dams with their natural litters (culled to four/sex/litter) were obtained on PND 0 from the breeding colony at the National Center for Toxicological Research (NCTR/FDA). Each dam was individually housed in a standard polycarbonate cage lined with wood chip bedding and provided with *ad libitum* food (NIH-31, Purina Mills, St Louis, MO) and water. The colony room was maintained at 22°C ± 1°C (mean ± SE) and 45–55% humidity on a 12-h light/dark cycle (7:00 A.M.–7:00 P.M.). Each pup was paw tattooed on PND 1 and also identified with a nontoxic marker on the dorsal side and tail tip on PND 4. All animal procedures followed the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and were approved in advance by the NCTR Institutional Animal Care and Use Committee.

Treatment

Ketamine hydrochloride (100 mg/ml solutions as Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) was diluted with saline to produce 2 mg/ml solutions. PCP (NIDA, Bethesda, MD) and L-carnitine (Sigma-Aldrich Corp., St Louis, MO) were dissolved in 0.9% saline. Ketamine hydrochloride (400 μl) and L-carnitine (500 mg) were diluted with 10 ml of saline to produce 40 mg/ml KET and 500 mg/ml L-carnitine solutions, respectively. These solutions were combined in a 50 ml conical tube to obtain the KLC dose (250 mg/kg L-carnitine and 20 mg/kg ketamine) injected on PND 7. Solutions were made weekly and kept refrigerated. The sc injections were done using a 25-gauge needle.

The within-litter treatment (one pup/sex/treatment/litter) was a particularly important aspect of the experimental design since it is well recognized that differences in maternal care can affect offspring behavior (Barron and Riley, 1985; Fleming *et al.*, 1999) and, at least in rats, pup behavior determines some aspects of maternal care (Marino *et al.*, 2002). Thus, similar to that described by Zissen *et al.* (2007), overall maternal care was controlled at the litter level in that each dam cared for a litter which contained pups of all treatment groups. However, as noted by Zissen *et al.* (2007), this cannot prevent or control for differential treatment of individual pups by the dam.

Treatment assignment was based on PND 4 body weight such that all groups had similar average body weights prior to treatment. The four groups were (1) 10 mg/kg PCP at 12:00 P.M. on PNDs 7, 9, and 11; (2) six injections of 20 mg/kg KET on PND 7 (8:00 A.M.–6:00 P.M.), separated by 2-h intervals; (3) six injections of 20 mg/kg KET and 250 mg/kg L-carnitine on PND 7 (8:00 A.M.–6:00 P.M.), separated by 2-h intervals followed by 250 mg/kg L-carnitine at 12:00 P.M. on PNDs 8–11; and (4) six injections of saline at on PND 7 (8:00 A.M.–6:00 P.M.), separated by 2-h intervals followed by saline at 12:00 P.M. on PNDs 8–11. The doses and treatment regimens were based on previous reports indicating that similar treatments caused neurodegeneration in rats (Ikonomidou *et al.*, 1999; Scallet *et al.*, 2004; Wang *et al.*, 2001). The L-carnitine dose was based on studies of its protective effects against 1-methyl-phenylpyridinium ion–induced apoptosis (Wang *et al.*, 2007). Thus, for each of the 48 litters, 1 male and 1 female were assigned to each treatment resulting in 48 pups/sex/treatment.

Body Weight

Body weights of the offspring were recorded on PNDs 4, 7, 8, 9, 10, 11, and 18. On PNDs 8–11, body weights were recorded after behavioral testing and prior to treatment.

Home Cage Pup Behavior

To determine the immediate effects of treatment, home cage behavior was assessed on PNDs 7–11. At each treatment time, the dam was placed in a holding cage. Each pup was then identified and when indicated, injected. Those pups not injected (e.g., PCP-treated pups at 8:00 A.M., 10:00 A.M., 2:00 P.M., and 4:00 P.M. on PND 7 and on PNDs 8 and 10 as well as the KET-treated pups on PNDs 8–11) were handled in a manner similar to the injected pups. Time of the last injection/handling for each litter was recorded, and the dam was returned to the home cage. Time from dam removal to replacement into the home cage was less than 120 s. At 5, 14, 23, and 32 min posttreatment, the behavior of each pup was assessed by one of two experimenters blind to treatment. Thus, there were four observations at five of the six treatment times on PND 7 (i.e., pups were observed after injections/handling at 8:00 A.M., 10:00 A.M., 12:00 P.M., 2:00 P.M., and 4:00 P.M., but not after the 6:00 P.M. injection/handling time). On PNDs 8–11, there were four observations following the 12:00 P.M. treatment time. Each pup was categorized as exhibiting one of 12 different behaviors (see Table 1) which were based on a previous scoring system (Goodwin and Barr, 2005). Only one behavior/pup/observation time was recorded.

Slant Board Behavior (Negative Geotaxis)

Vestibular system integrity and Motor coordination were examined using a slant board test as previously described (Adams *et al.*, 1985). Briefly, between 7:30 and 9:00 A.M. on PNDs 8–11, the dam was removed and each pup was placed on its ventral side with its nose pointing toward the lower end of a sandpaper-covered 45° incline board. Each pup was allowed 60 s to complete a 180° turn. One trial/day was conducted, and the latency to turn or fall from the apparatus was recorded by a tester blind to treatment conditions.

Forelimb Hang Behavior

Muscle strength/coordination was examined using a forelimb hang test as previously described (Cada *et al.*, 2000). Briefly, between 7:30 and 10:00 A.M. on PNDs 12–16, the dam was removed and each pup was placed on a taut string stretched between two blocks of wood spaced 46 cm apart and 41 cm above a padded surface. One trial/day was conducted, and the latency to fall was recorded (maximum 60 s) by a tester blind to treatment conditions.

Statistical Analyses

Body weight. Offspring body weights were compared using ANOVAs with factors of treatment (control, KET, PCP, and KLC), sex, and the repeated measure of PND (JMP, Version 7.0; SAS Institute Inc., Cary, NC). Tukey *post hoc* tests were used to further analyze significant main effects or interactions.

Home cage pup behavior. Data from the five observation times on PND 7 (8:00 A.M., 10:00 A.M., 12:00 P.M., 2:00 P.M., and 4:00 P.M.) were analyzed separately from the single observation time on PNDs 8–11 (12:00 P.M.). Six behaviors were categorized as abnormal activity: fast activity, paddling, partial paddling, paresis, partial paresis, wall climbing. To analyze abnormal activity, each pup at each observation at each time was assigned a "1" if it exhibited any of the six abnormal behaviors or a "0" for any other behavior. Generalized linear models with a log link and Poisson distribution were used to analyze the counts for each of the two data sets (PND 7 only and PNDs 8–11) with factors of treatment, observation time (e.g., 8:00 A.M., 10:00 A.M.) (PND 7 analysis only), minutes posttreatment (e.g., 5, 14, 23, or 32 min), and sex.

Slant board behavior. Each pup could exhibit one of three outcomes: a successful turn within 60 s, a fall from the apparatus within 60 s, or an incomplete turn. A failure was categorized as a fall or an incomplete turn. The odds of failure were analyzed using a generalized linear model with repeated measures and a binomial distribution and logit link function. To analyze the latency to turn time, a Cox Proportional Hazards model was run in SAS (SAS Version 9.1; SAS Institute Inc.) using treatment, sex, and PND as factors. Pups that fell or did not complete the turn were accounted for in this analysis by adjusting the empirical distribution function.

TABLE 1
Behavior

Behavior[a]	Definition	No. of instances[b]
Quiet	No movement except for twitches and respiration	5929
Immobile	No movement except for respiration with the head up	234
Activity	Walking, sniffing, and rearing	3028
Fast activity	Running	3
Paddling	On dorsal side while moving forelimbs and hind limbs	418
Partial paddling	On dorsal side with three limbs moving	340
Maternal interference	Dam interacted with pup (e.g., licking, moving, or stepping on pup)	241
Paresis	On ventral side with hind limbs moving and stiff forelimbs	129
Partial paresis	On ventral side with hind limbs moving and one stiff forelimb	271
Grooming	Grooming any part of the body	1
Wall climbing	Standing on hind limbs against cage side while forelimbs are alternately moving back and forth	15
Unidentifiable	Pup could not be identified (typically, as a result of nursing and inability to identify via paw tattoo or markings)	3175

[a]These categories and the scoring system were modified from Goodwin and Barr (2005).

[b]The total number of observations for PNDs 7–11 home cage behavior was 13,244 (four posttreatment observations [5, 14, 23, and 32 min posttreatment] at each of five observation times on PND 7 (8:00 A.M., 10:00 A.M., 12:00 P.M., 2:00 P.M., and 4:00 P.M.), four posttreatment observations at each single treatment time on PNDs 9–11, maximum eight pups each of 48 litters).

Forelimb hang behavior. To analyze the latency to fall, a Cox Proportional Hazards model was run using SAS (SAS Version 9.1, SAS Institute Inc., Cary, NC) with treatment, sex, and PND as factors.

RESULTS

Mortality and Body Weight

There were three deaths. One male KET pup died after the 2:00 P.M. PND 7 home cage behavior observation and before the 4:00 P.M. PND 7 treatment. One male KLC pup died on PND 8 after the 12:00 P.M. home cage behavior observation time and another male KLC pup died on PND 9 after the 12:00 P.M. home cage observation time.

Body weight analysis revealed a significant interaction of treatment with PND ($F_{18,2244} = 75.66$, $p < 0.0001$) (Fig. 1). *Post hoc* tests indicated that there were no significant body weight differences on PND 4 or 7 (before treatment). However, on PNDs 8, 9, and 10, the KET-treated group weighed less than the control group ($p < 0.05$). On PNDs 8–18, the PCP-treated group weighed less than the control group ($p < 0.05$). *Post hoc*
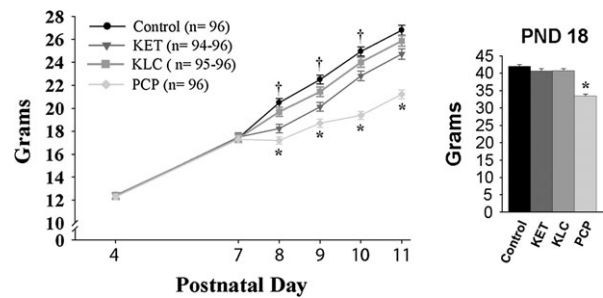

FIG. 1. Body weight on PNDs 4–18, averaged over sex. The larger graph shows body weight on PNDs 4–11 while the inset graph shows PND 18 body weight. *Indicates the PCP-treated group weighed significantly less than controls. †Indicates the KET-treated group weighed significantly less than controls.

tests of the significant sex by PND interaction ($F_{6,2244} = 3.62$, $p < 0.002$) demonstrated that males were significantly heavier than females on PND 18 only ($p < 0.05$) (mean ± SE for PND 18 males and females: 40.0 ± 0.5 and 38.4 ± 0.5 g, respectively). There was neither a main effect of sex nor a significant interaction of treatment with sex.

Home Cage Pup Behavior

PND 7 abnormal behavior. There was a significant interaction of treatment with observation time ($p < 0.0001$) (Fig. 2, large graph). KET-treated pups had elevated levels of abnormal activity at each of the five observation times ($p < 0.001$) compared with controls. KLC-treated pups exhibited higher abnormal activity levels at 8:00 A.M., 10:00 A.M., and 12:00 P.M. relative to controls ($p < 0.02$); by 2:00 P.M., however, their abnormal behavior was within the range of controls. Although the PCP-treated pups had somewhat higher counts of abnormal activity at 10:00 A.M. ($p < 0.004$), the PCP treatment at 12:00 P.M. caused a significant increase in abnormal activity levels ($p < 0.0001$) which remained elevated ($p < 0.0001$) throughout the last two observation times (i.e., 2:00 and 4:00 P.M.).

PNDs 8–11 abnormal behavior. A significant interaction of treatment with PND ($p < 0.0001$) indicated that PCP-treated pups exhibited elevated levels of abnormal activity on PNDs 9 and 11 (i.e., treatment days) relative to all other treatment groups ($p < 0.0001$) (Fig. 2, inset graph). Levels of abnormal behavior in KET-, KLC-treated, and control pups did not differ from one another on PNDs 8–11, nor did PCP-treated pups exhibit significant levels of abnormal behavior on PNDs 8 and 10 (i.e., days on which handling, but no PCP treatment, occurred).

Slant Board Behavior (Negative Geotaxis)

Analysis of the odds of failure (falling or incomplete turn) indicated a significant interaction of treatment by PND ($\chi^2 = 32.05$, df = 9, $p < 0.0002$) (data not shown). *Post hoc* tests
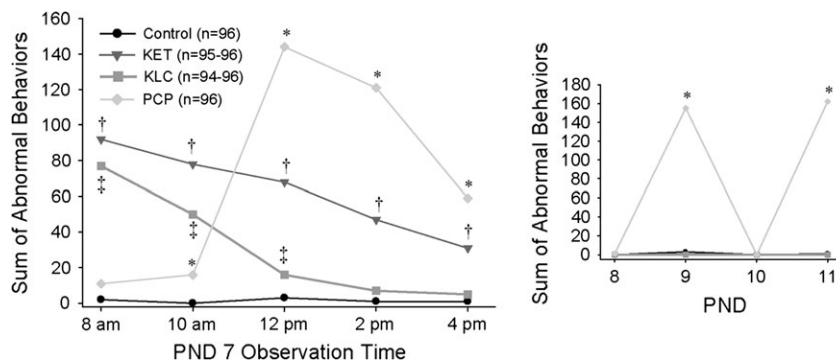
FIG. 2. Level of abnormal home cage pup behavior on PNDs 7–11. The larger graph shows abnormal behavior on PND 7 while the inset graph shows abnormal behavior on PNDs 8–11. *Indicates higher levels of abnormal activity in the PCP-treated group relative to the control group. †Indicates higher levels of abnormal activity in the KET-treated group relative to the control group. ‡Indicates higher levels of abnormal activity in the KLC-treated group relative to the control group.

on each PND indicated that on PND 8, the KET- and PCP-treated groups had increased odds of failure ($p < 0.05$) (mean ± SE for control, KET-, KLC-, and PCP-treated groups: 0.19 ± 0.04, 0.39 ± 0.05, 0.21 ± 0.04, and 0.63 ± 0.05 proportion failing to turn, respectively). On PND 10, the PCP-treated group again had increased odds of failure ($p < 0.05$) (mean ± SE for control, KET-, KLC-, and PCP-treated groups: 0.17 ± 0.04, 0.21 ± 0.04, 0.22 ± 0.04, and 0.39 ± 0.05 proportion failing to turn, respectively). On PND 11, the proportion failing to turn was less than 0.25; however, relative to the control group, the KLC-treated group was more likely to fail ($p < 0.05$) (mean ± SE for control, KET-, KLC-, and PCP-treated groups: 0.13 ± 0.03, 0.16 ± 0.04, 0.24 ± 0.04 and 0.14 ± 0.04, respectively).

For illustrative purposes, Figures 3A–3D shows the proportion of each treatment group making a successful turn during the time allotted on PNDs 8–11. Analysis of latency to turn indicated a significant interaction of treatment by PND ($\chi^2 = 68.69$, df = 9, $p < 0.0001$). *Post hoc* tests indicated that on PND 8, KET- and PCP-treated pups had longer latencies to turn than controls ($p < 0.05$) (mean ± SE for control, KET, KLC, and PCP groups: 16.8 ± 1.5, 25.4 ± 2.1, 17.2 ± 1.4, and 40.4 ± 2.0 s, respectively) (see also Fig. 3A). On PND 10, the PCP-treated group had a longer latency to turn ($p < 0.05$) (mean ± SE for control, KET, KLC, and PCP groups: 12.0 ± 1.0, 13.0 ± 1.5, 13.8 ± 1.7, and 25.3 ± 2.3 s, respectively) (see also Fig. 3C). Neither the main effect of sex nor any interactions with sex were significant.

Forelimb Hang Behavior

For illustrative purposes, Figures 4A–4E show the proportion of each treatment group that fell from the forelimb hang apparatus during the time allotted on PNDs 12–16. Analysis of latency to fall indicated a significant interaction of treatment with PND ($\chi^2 = 27.78$, df = 12, $p < 0.006$). *Post hoc* tests revealed that on PND 12, relative to controls, the PCP-treated group had a shorter latency to fall ($p < .05$) (mean ± SE for

control, KET-, KLC-, and PCP-treated groups: 15.5 ± 1.2, 14.5 ± 1.1, 15.1 ± 1.2, and 13.0 ± 1.1, respectively) (see also Fig. 4A). On PND 16, the KLC- and PCP-treated groups had longer latencies to fall ($p < 0.05$) (mean ± SE for control, KET-, KLC-, and PCP-treated groups: 12.0 ± 0.9, 14.6 ± 1.3, 15.0 ± 1.2, 15.6 ± 1.3, respectively) (see also Fig. 4D). There was neither a significant main effect of sex nor any significant interactions with sex.

DISCUSSION

NMDA receptor antagonists can affect psychological and physiological functions in humans, and it is becoming clear that the musculoskeletal system can be altered as well (Brunson *et al.*, 2001; Kakizawa *et al.*, 2000; Wolff and Winstock, 2006). In the current study, neonatal rats were treated with KET, PCP, or ketamine + L-carnitine (KLC) and assessed for home cage pup behavior, slant board behavior, and forelimb hang time on PNDs 7–11, 8–11, and 12–16, respectively. The single 10 mg/kg PCP injection on PNDs 7, 9, and 11 decreased body weight gain and produced high levels of abnormal activity as well as altered slant board and forelimb hang behavior. The PCP effects appeared more severe than those caused by the prolonged 20 mg/kg KET treatment on PND 7. These data suggest that three injections of 10 mg/kg PCP over PNDs 7–11 were more potent at modifying preweaning behaviors than were repeated injections of 20 mg/kg KET on PND 7. The addition of L-carnitine to the KET treatment regimen appeared to ameliorate KET-induced adverse effects on body weight, home cage pup behavior, and slant board behavior. These results more completely describe the acute effects of NMDA antagonist treatment in rat pups and may be associated with the neuronal degeneration known to occur with similar treatment.

Increased levels of abnormal home cage behavior occurred within 5–32 min after the initial PCP and KET injections on
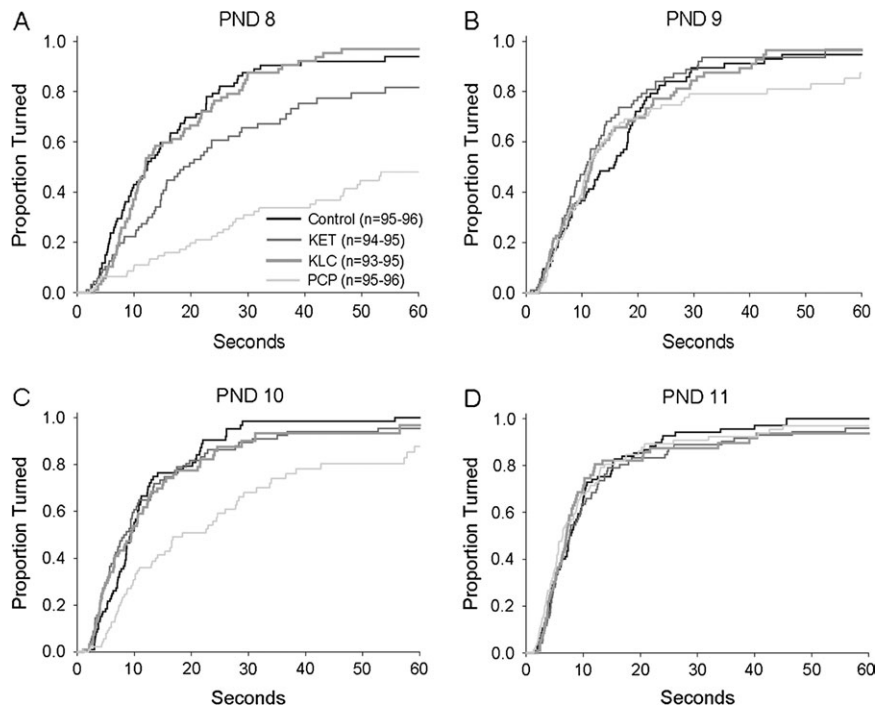
FIG. 3. Proportion of each treatment group making a successful slant board turn during the allotted 60 s. Each of the four panels (A–D) represents a single test day and is similar to a typical ''survival curve.'' Here, the ''survival'' estimates were inverted such that higher curves correspond to a higher proportion of animals making successful turns (e.g., on PND 8, approximately 43% of the control group had turned successfully at the end of 10 s). Note that a smaller proportion of the PCP-treated group successfully turned within the allotted 60-s trial on PNDs 8 and 10. See text for description of statistically significant effects.

PND 7. These abnormal behaviors were primarily paddling and paresis; other behaviors such as wall climbing rarely occurred. This is the first study to quantitatively describe the acute behavioral effects of developmental NMDA antagonist treatment; however, paddling has been described in PND 7 mice treated with 40 mg/kg KET (Young *et al.*, 2005) and in adult rats treated with 2–15 mg/kg PCP (Chen *et al.*, 1959; Sturgeon *et al.*, 1979). Further, KET can cause paresis, ataxia, and temporary paralysis in humans (Wolff and Winstock, 2006). Thus, the specific types of abnormal activity induced in neonatal rats by the NMDA antagonists here resemble those described for similarly treated adults.

The highest level of KET-induced abnormal behavior occurred after the initial injection (i.e., at 8:00 A.M.) on PND 7. Subsequently, levels declined at each 2-h observation time even though each was accompanied by an additional 20 mg/kg injection. Still, the rate of decline was shallow enough such that abnormal behavior levels at the fifth treatment (i.e., 4:00 P.M.) remained elevated. By 12:00 P.M. on the following day, there were no measurable levels of abnormal home cage behavior in the KET-treated group. This pattern of initially high levels of PND 7 abnormal behavior with a decline throughout the day was very similar to that exhibited by the KLC group, albeit to a lesser extent. While the initial injection of ketamine with L-carnitine elevated abnormal behavior levels, the rate of decline throughout the remainder of the day was fairly steep

and by the fourth injection (i.e., 2:00 P.M.), abnormal behavior levels were comparable to and remained at control levels. Thus, while the initial KLC treatment was associated with an increase in abnormal home cage behavior, the addition of L-carnitine somewhat ameliorated the effects of KET. Neither KET nor KLC had lasting effects on home cage pup behavior.

The effects of PCP treatment on home cage pup behavior differed quantitatively, but not qualitatively, from those of KET and KLC. Specifically, the single PND 7 injection elevated abnormal behavior levels well above those of the initial KET injection, and 4 h postinjection, abnormal behavior levels were still high. Further, while there appeared to be a type of tolerance to repeated KET treatment on PND 7, PCP treatment on PNDs 9 and 11 elevated abnormal behaviors to the same extent as the initial injection on PND 7. Still, similar to KET and KLC treatments, the effects of PCP on home cage pup behavior had no lasting effects since level of abnormal home cage behavior was indistinguishable from controls on PNDs 8 and 10. These differences in levels of home cage abnormal activity may be related to differences in the half-lives of KET and PCP. In adult rats, iv treatment with PCP results in a half-life of 3–5 h (Shelnutt *et al.*, 1999) whereas a half-life of less than 1 h is obtained with iv or im treatment with KET (Edwards *et al.*, 2002; Williams *et al.*, 2004). Thus, it is not surprising that PCP-induced abnormal home cage behavior was still evident 4 h posttreatment on PND 7.
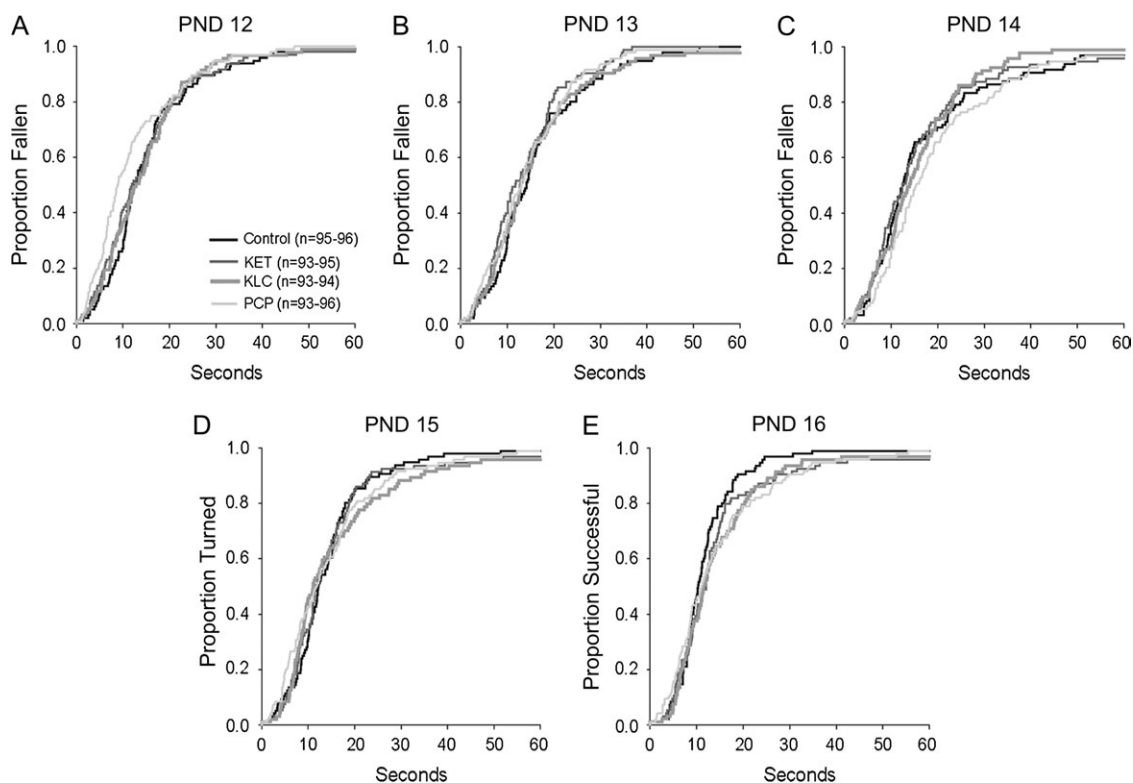
FIG. 4. Proportion of each treatment group falling from the forelimb hang apparatus during the allotted 60 s. Each of the five panels (A–E) represents a single test day and is similar to a typical ''survival curve.'' Here, the ''survival'' estimates were inverted such that higher curves correspond to a higher proportion of animals falling (e.g., on PND 8, approximately 27% of the control group had fallen at the end of 10 s). Note the shorter fall latencies in the PCP-treated group on PND 12 and the longer latencies to fall in the PCP- and KLC-treated groups on PND 16. See text for statistically significant effects.

The slant board and forelimb hang behaviors measured here are typical of those utilized in developmental neurotoxicity studies and were included since developmental PCP treatment has been shown to cause later motor coordination deficits (Sircar, 2003; Sircar and Rudy, 1998; Wang *et al.*, 2001; Wiley *et al.*, 2003). Although home cage pup behavior on PND 8 (conducted approximately 4 h after slant board behavior tests) indicated no abnormal behavior in the PCP- and KET-treated pups, both groups were slower to turn on the slant board on that day. Further, PCP-treated pups were slower to turn on PND 10, but not on PNDs 9 and 11, days on which the test was conducted prior to PCP treatment. This pattern indicates that the behavioral effects of the PCP treatment were still detectable via slant board behavior approximately 20, but not 44, h later. PCP, but not KET, treatment appeared to affect muscle strength as latency to fall in the forelimb hang test was decreased on PND 12. Finally, the repeated saline injections appeared not to affect behavior since slant board and forelimb hang results of the control group here were similar to those of previous studies (Ferguson *et al.*, 2003).

There is some evidence of sexual dimorphism in the effects of PCP and anesthetics such as isoflurane and phenobarbital. The half-life of PCP is longer in adult female Sprague-Dawley rats than in males after iv treatment, and this appears to be due to sex differences in metabolism (Shelnutt *et al.*, 1999). On the other hand, neonatal treatment with isoflurane or phenobarbital resulted in more severe neurotoxicity in male Sprague-Dawley rats when examined at adulthood (Rothstein *et al.*, 2008). There was no evidence of sexual dimorphism in response to the KET, KLC, or PCP treatments in the preweaning assessments conducted in the present study; however, assessments continued through adulthood, and it is possible that sex differences in sensitivity to these compounds may be apparent after puberty.

These NMDA antagonist-induced behavioral alterations may be related to increased cerebellar apoptosis. While apoptotic elimination of cerebellar granule cells is a natural process in rodent pups (Wood *et al.*, 1993), inadequate stimulation of these cells or insufficient mossy fiber connections may increase the rate of apoptotic granule cell elimination. For example, treatment with the competitive NMDA receptor antagonist CGP 39551 on PNDs 7–11 increased DNA fragmentation levels in the inner granule layer and caspase-3 (a proapoptotic protease) levels in the rodent cerebellum (Monti and Contestabile, 2000). Further, developmental treatment with noncompetitive NMDA receptor antagonists such as MK-801 or KET resulted in increased numbers of multiple climbing fiber innervations in the cerebellum, increased apoptotic cerebellar cell death, and mild motor coordination impairments in mice

(Kakizawa *et al.*, 2000; Rudin *et al.*, 2005). Thus, the KET and PCP treatments here are likely to have altered cerebellar development which may have resulted in impaired motor coordination expressed as paddling and paresis, longer slant board turn latencies, and shorter forelimb fall latencies.

The mechanism by which L-carnitine ameliorated the adverse effects of KET treatment on body weight and behavior is not clear nor is it clear what effects L-carnitine may have had irrespective of the effects of KET. A group treated solely with L-carnitine (i.e., without KET) was not included as the focus of the study was on the effects of KET and PCP. The protective effects of L-carnitine may be mitochondrially mediated since L-carnitine protects against age-dependent mitochondrial decay (Hagen *et al.*, 2002). NMDA antagonist treatment causes neuronal cell death through the Bax-cytochrome c-caspase pathway (Yoon *et al.*, 2003) in which Bax is translocated into the outer mitochondrial membrane. Thus, L-carnitine may have prevented the KET-induced behavioral modifications by prohibiting the release of cytochrome c which would prevent caspase activation and apoptosis. As evidence, L-carnitine inhibits apoptosis in murine MC3T3-E1 osteoblastic cells by decreasing the release of cytochrome c and the activation of caspase-9 and caspase-3 (Xie *et al.*, 2007).

These results provide a more complete description of the acute behavioral effects of developmental NMDA antagonist treatment. The exact mechanism by which PCP and KET induce such effects is unknown but is likely related to the increased neuronal cell death caused by these compounds and may be regionally specific to the cerebellum. Future studies will examine adolescent and adult behaviors in these subjects as well as cerebellar morphology.

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