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Ontogeny of Phencyclidine and Apomorphine-Induced Startle Gating Deficits in Rats

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MARTINEZ, Z. A., N. D. HALIM, J. L. OOSTWEGEL, M. A. GEYER AND N. R. SWERDLOW. *Ontogeny of phencyclidine and apomorphine-induced startle gating deficits in rats.* PHARMACOL BIOCHEM BEHAV **65**(3) 449–457, 2000.—NMDA antagonists and dopamine (DA) agonists produce neuropathological and/or behavioral changes in rats that may model specific abnormalities in schizophrenia patients. In adult rats, NMDA antagonists and DA agonists disrupt sensorimotor gating—measured by prepulse inhibition (PPI)—modeling PPI deficits in schizophrenia patients. In addition, high doses of NMDA antagonists produce limbic system pathology that may model neuropathology in schizophrenia patients. We examined these behavioral and neuropathological models across development in rats. Both the NMDA antagonist phencyclidine (PCP) and the DA agonist apomorphine disrupted PPI in 16 day pups, demonstrating early developmental functionality in substrates regulating these drug effects on PPI. In contrast, PCP neurotoxicity was evident only in adult rats. Brain mechanisms responsible for the PCP disruption of PPI, and PCP-induced neurotoxicity, are dissociable across development. © 2000 Elsevier Science Inc.

Apomorphine Development Neurotoxicity Phencyclidine Prepulse inhibition Schizophrenia Sensorimotor Startle

STUDIES have demonstrated deficits in sensory and sensorimotor gating in schizophrenia spectrum patients (1–4,14,16,32). One index of deficient sensorimotor gating in schizophrenia patients—prepulse inhibition (PPI) of the startle reflex—can also be measured across species, and is impaired in rats after acute treatment with specific drugs, including dopamine (DA) agonists (24,25,36,37,40) and NMDA antagonists (22,23). Conceptual models link the substrates responsible for these drug-induced PPI deficits in rats, with those that may underlie PPI deficits in schizophrenia patients (38,39). Thus, information regarding the neural basis for DA agonist or NMDA antagonist effects on PPI in rats may ultimately advance our understanding of the pathophysiological basis for reduced PPI in schizophrenia. The present studies were designed to assess two aspects of these drug-induced PPI deficits in rats: (a) their developmental time course, and (b) the developmental relationship between NMDA antagonist effects on PPI and NMDA antagonist-induced neurotoxicity, which has also been studied as a model for understanding the pathophysiology of schizophrenia [cf. (30)].

The PPI-disruptive effects of the direct D_1/D_2 agonist apomorphine (24) and the noncompetitive NMDA antagonist PCP (22) have been demonstrated by numerous laboratories using adult rats. One previous study demonstrated PPI-disruptive effects of apomorphine in 36-day-old prepubertal rats (21). No published studies have reported this drug effect in younger rats, and no studies of the PPI-disruptive effects of NMDA antagonists have been reported using juvenile rats. An ability of these drugs to disrupt PPI early in development would suggest functionality in brain substrates responsible for these drug effects; such information would be useful for narrowing the list of candidate substrates for these effects.

Some animal models for the pathophysiology of schizophrenia suggest that neonatal abnormalities in specific brain

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substrates lead to a delayed, postpubertal emergence of behavioral dysfunction (20,43,44). If prepubertal rats are sensitive to DA agonist- and NMDA antagonist-induced disruptions of PPI, the delayed postpubertal emergence of behavioral abnormalities in animal models of schizophrenia presumably would not reflect an immaturity within DA and NMDA systems that regulate PPI.

Another effect of NMDA antagonists—cytotoxicity characterized by vacuolization and other cellular changes—is developmentally sensitive, being evident in adult, but not juvenile rats (11). A second aim of this study was to examine the temporal relationship between the neurotoxic and PPI-disruptive effects of PCP. A developmental dissociation between these effects would suggest that the neural substrates responsible for these effects might also be dissociable.

We now report that the PPI-disruptive effects of apomorphine and PCP are evident by 16–18 days of age in rats; but, consistent with previous reports (11), only slight PCP-induced neurotoxicity was observed in pups, and not until 30 days of age. These findings suggest that the brain substrates regulating the apomorphine- and PCP-induced loss of sensorimotor gating are functional early in the developing rat brain, and that the brain substrates mediating PCP effects on PPI and PCP-induced neurotoxicity are developmentally dissociable.

METHOD

Experimental Animals

A total of 179 Sprague–Dawley rat pups weighing 30–130 g, 16 pregnant adult female Sprague–Dawley rats weighing 250–300 g, and 23 adult male Sprague–Dawley rats weighing 250–300 g were used in these experiments. Timed pregnant female rats were housed individually, and pups were housed with their mothers until 5–7 days after birth; at that time rat pups were sexed and "crossfostered," i.e., redistributed so that each litter was approximately the same size and contained an equal number of male and female pups. At 21 days, rat pups were weaned and housed in same-sex groups of three to five. Adult male rats were housed in same-sex groups of two or three. A reversed 12 L:12 D cycle was used (lights on at 1900, off at 0700 h) for at least 1 week prior to any testing or surgery. All testing and drug administration occurred between 1000 and 1700 h, during the dark circadian phase. Rats were handled prior to any procedures to minimize stress during behavioral testing, and were given ad lib access to food and water except during behavioral testing [for background for specific methods, e.g., circadian phase selection, animal handling procedures, see (13)].

Drugs

PCP (saline vehicle, 0.5, 1.0, or 1.5 mg/kg) or apomorphine (0.1% ascorbate/saline vehicle, 0.1, 0.25, or 0.5 mg/kg) was administered subcutaneously (SC) to rat pups. For studies assessing the effects of treatment with a neurotoxic dose of PCP, saline vehicle or PCP (5.0 mg/kg) was administering subcutaneously.

Apparatus

All startle experiments utilized four startle chambers (SR-LAB; San Diego Instruments, San Diego, CA) housed in a sound-attenuated room with a 60-dB ambient noise level. For 28–42-day-old rat pups, and adult rats, each startle chamber consisted of a Plexiglas cylinder 8.7 cm in internal diameter resting on a 12.5 × 25.5-cm Plexiglas stand. For 14-, 16-, and 18-day-old rat pups, each startle chamber consisted of a smaller Plexiglas cylinder 3.75 cm in internal diameter resting on a 12.5 × 25.5-cm Plexiglas stand. Acoustic stimuli and background noise were presented via a Radioshack Supertweeter mounted 24 cm above the Plexiglas cylinder. Startle magnitude was detected and recorded as transduced cylinder movement via a piezoelectric device mounted below the Plexiglas stand. Acoustic stimulus intensities and response sensitivities were calibrated (using an SR-LAB Startle Calibration System) to be nearly identical in each of the four startle chambers (maximum variability <1% of stimulus range and <5% of response ranges). Response sensitivities were calibrated for adult and pup chambers separately, and recalibrated each time the chambers were changed, always within the <5% response range. Chambers were also balanced across all experimental groups. Sound levels were measured and calibrated with a sound level meter (Quest electronics: Oconomowoc, WI), A scale (relative to 20 N/M2), with microphone placed inside the Plexiglas cylinder. Methodological details can be found in published material (13).

Testing Procedures

In our testing apparatus, reliable measures of startle could first be obtained in pups at 14 days of age. At 14, 28, or 42 days of age, different groups of rat pups were exposed to a brief "matching" startle session. Adult rats (65–80 days of age) were exposed to this "matching" session 3–7 days prior to testing. Rat pups were placed in a startle chamber, and exposed to 5 min of 70-dB background noise followed by 17 PULSE trials of 40-ms 120-dB noise bursts ("PULSE ALONE") and 5 PREPULSE + PULSE trials consisting of a 20-ms 82-dB (12 dB above background) prepulse followed 100 ms by a 120-dB pulse (onset to onset). Data from this session were used to assign rat pups to balanced groups according to their average PULSE-ALONE startle magnitude.

For rat pups and juvenile rats, behavioral testing continued 2 days after the "matching" session. Sixteen- or 18-day-old rat pups were brought to the laboratory in their home cages, with their mothers to minimize stress before and after testing. Weaned rats (30- or 32-day-old pups and 44-day-old juvenile rats) were brought up to the lab with at least one littermate to minimize stress. After 30 min, rat pups and juvenile rats were treated with either PCP (saline vehicle, 0.5, 1.0, or 1.5 mg/kg) or apomorphine (0.1% ascorbate/saline vehicle, 0.1, 0.25, or 0.5 mg/kg) and placed immediately (apomorphine) or 10 min later (PCP) into the startle chambers, according to established procedures [e.g., (13,21,22,24,38)]. We previously reported that rat pups exhibit less robust PPI than adult rats (21). For this reason, the test session for the rat pups utilized a single relatively intense prepulse (15 dB above background) to elicit maximal levels of PPI.

Each test session was approximately 20 min long, and consisted of 5 min of 70-dB background followed by three trial types: PULSE ALONE noise bursts; PREPULSE trials (20-ms noise bursts 15-B above background, followed 100 ms by a PULSE); and NOSTIM trials (stabilimeter recordings obtained when no stimulus was presented). The session consisted of 21 PULSE trials, 21 PREPULSE trials, and 21 NOSTIM trials presented in a balanced order so that PREPULSE trials occurred immediately before and after both PULSE and NOSTIM trials.

Adult rats were brought to the laboratory for 30 min and then treated with saline or PCP (5 mg/kg) 10 min prior to a test session. Each session was approximately 19 min long, and

consisted of 5 min of 70-dB background of white noise followed by four trial types: PULSE ALONE noise bursts; and PREPULSE trials (20-ms noise bursts 3, 6, or 12 dB above the 70-dB background noise, followed 100 ms by a PULSE). The session consisted of four "blocks:" blocks 1 and 4 consisted of four PULSE-ALONE trials, and blocks 2 and 3 included both PULSE-ALONE (eight trials per block) and 3, 6, and 12 PREPULSE + PULSE trials (five trials each per block), presented in pseudorandom order with a variable intertrial interval (average of 15 s). In addition, interspersed between each stimulus trial, stabilimeter readings were recorded during periods where no stimulus was presented. These "NOSTIM" trials were used to assess gross motor activity during the test session, but were not included in the calculation of intertrial intervals.

Data Analysis

All startle data were analyzed using an analysis of variance (ANOVA) with drug treatment, sex, and age as between-subject factors, and block and trial type as within-subject repeated measures. All post hoc comparisons were conducted using the Tukey–Kramer test. Alpha was set at 0.05 for main effects and interactions using ANOVA and 0.01 for post hoc comparisons.

Treatment and Test Schedule

Experiment 1A: Effects of PCP and apomorphine on PPI in rat pups and juvenile rats. Sixteen- and 18-day-old rat pups: rat pups ($n = 75$) were tested on postnatal day 16 with acute administration of PCP (saline vehicle, 0.5, 1.0, or 1.5 mg/kg; ns = 18, 19, 19, and 19). Dose groups were then randomized, and rats were retested on postnatal day 18 after acute treatment with apomorphine (0.1% ascorbate/saline vehicle, 0.1, 0.25, or 0.5 mg/kg; ns = 18, 19, 19, and 19).

Thirty- and 32-day-old rat pups: rat pups ($n = 32$) were tested on postnatal day 30 following acute treatment with PCP (saline vehicle, 0.5, 1.0, or 1.5 mg/kg). Dose groups were then randomized, and rats were retested at postnatal day 32 after acute treatment with apomorphine (0.1% ascorbate/saline vehicle, 0.1, 0.25, or 0.5 mg/kg).

Forty-four- day-old juvenile rats: juvenile rats ($n = 32$) were tested on postnatal day 44 following acute treatment with PCP (saline vehicle, 0.5, 1.0, or 1.5 mg/kg). Apomorphine was not tested in this age group, because previous reports have already documented the results of such studies (21).

Experiment 2: Effects of a neurotoxic dose of PCP on rat pups and adult rats. Adult rats ($n = 17$) and rat pups ($n = 20$) were treated with saline vehicle or PCP (5 mg/kg) and tested in the startle paradigm. Other adult rats ($n = 2$) and rat pups ($n = 20$) were treated with saline or PCP but not tested in the startle paradigm. Brains were removed from animals approximately 4 h postinjection.

Histology/Tissue Preparation

Approximately 4 h after treatment with saline vehicle or 5 mg/kg PCP, rat pups ($n = 10$ 16-day-old and $n = 10$ 30-day-old) and adult rats ($n = 19$) of both sexes were deeply anesthetized with 420 mg/kg chloral hydrate/distilled water solution and perfused through the heart with physiological saline, followed by a 1.5% gluteraldelhyde/1% paraformaldehyde solution, and decapitated. Brains were removed and fixed in aldehyde for 24 h, then cut into coronal sections using a razor-lade guillotine fashioned to a sliding stage (patent pending SD 99-024). Brains were then postfixed in 1.5% osmium tetraoxide for 1 h, dehydrated serially with ethanol,and cleared with propylene oxide. Sections (0.5 mm^3) of the posterior cingulate/retrosplenial (PC/RS) cortex were then removed from these sections and embedded in Embed (Epon 812, EMS Fort Washington, PA); NMDA antagonist-induced neurotoxicity has previously been observed within this region (8,9,11,12,28,29). Sections (0.5 μm) were cut via an ultramicrotome, mounted on slides, and stained with Azur II.

After reviewing this histologic material, an investigator (NDH) chose a representative field of view within the PC/RS from tissue within each drug, age, and sex condition (i.e., saline vs. PCP; 16-day-old pup vs. 30-day-old pup vs. adult; and male vs. female). Two other investigators "blind" to treatment condition (ZAM, NRS) counted the number of neuronal cell bodies in the representative view that were abnormal, based on the presence of cytoplasmic abnormalities previously described (11,12,28,29). Cell counts were compared for interrater reliability using a Spearman Correlation ($r = 0.90$, $p < 0.005$).

RESULTS

Experiment 1: Effects of PCP and Apomorphine on PPI in Rat Pups and Juvenile Rats

Effects on startle magnitude. PCP: across development, startle magnitude increased with age and PCP dose (Fig. 1, inset). ANOVA with dose of PCP, sex, and age as between-subject factors revealed a significant effect of PCP dose, $F(3, 115) = 4.11$, $p < 0.008$, no significant effect of sex, $F(1, 115) = 3.39$, NS, a significant effect of age, $F(2, 115) = 80.02$, $p < 0.0001$, and a significant dose × age interaction, $F(6, 115) = 2.22$, $p < 0.05$. No other interactions reached significance. In vehicle-treated rats, the increase in startle magnitude occured between 30 and 44 days of age, $F(1, 16) = 12.528$, $p < 0.003$.

Apomorphine: in 18- and 32-day-old animals treated with apomorphine, startle magnitude decreased with apomorphine dose (Fig. 2, inset), and was also slightly lower in female vs. male rats (data not shown). ANOVA with dose of apomorphine, sex, and age as between-subject factors revealed a significant effect of apomorphine dose, $F(3, 91) = 2.92$, $p < 0.04$, a significant effect of sex, $F(1, 91) = 5.01$, $p < 0.03$, a significant effect of age, $F(2, 91) = 32.27$, $p < 0.0001$, and no significant interactions.

Effects on PPI. PCP: PPI significantly increased with age, and PCP significantly reduced PPI across development (Fig. 1). ANOVA with PCP dose, sex, and age as between-subject factors revealed a significant effect of PCP dose, $F(3, 115) = 32.41$, $p < 0.0001$, no significant effect of sex, $F(1, 115) = 1.76$, NS, a significant effect of age, $F(2, 115) = 3.88$, $p < 0.025$, and no significant interactions.

Apomorphine: apomorphine significantly reduced PPI across development (Fig. 2). ANOVA with dose of apomorphine, sex, and age as between-subject factors revealed a significant effect of apomorphine dose, $F(3, 91) = 14.28$, $p < 0.0001$, no significant effect of sex, $F(1, 91) < 1$), a significant effect of age, $F(1, 91) = 3.99$, $p < 0.05$, and no significant interactions.

NOSTIM measures. Although NOSTIM data were used as a gross measure of motor activity, it is important to note that this measure is not comparable to more sophisticated measure of rat locomotion or stereotyped behavior. NOSTIM data are included as an additional behavioral measure of drug effects in these animals, across the developmental period of

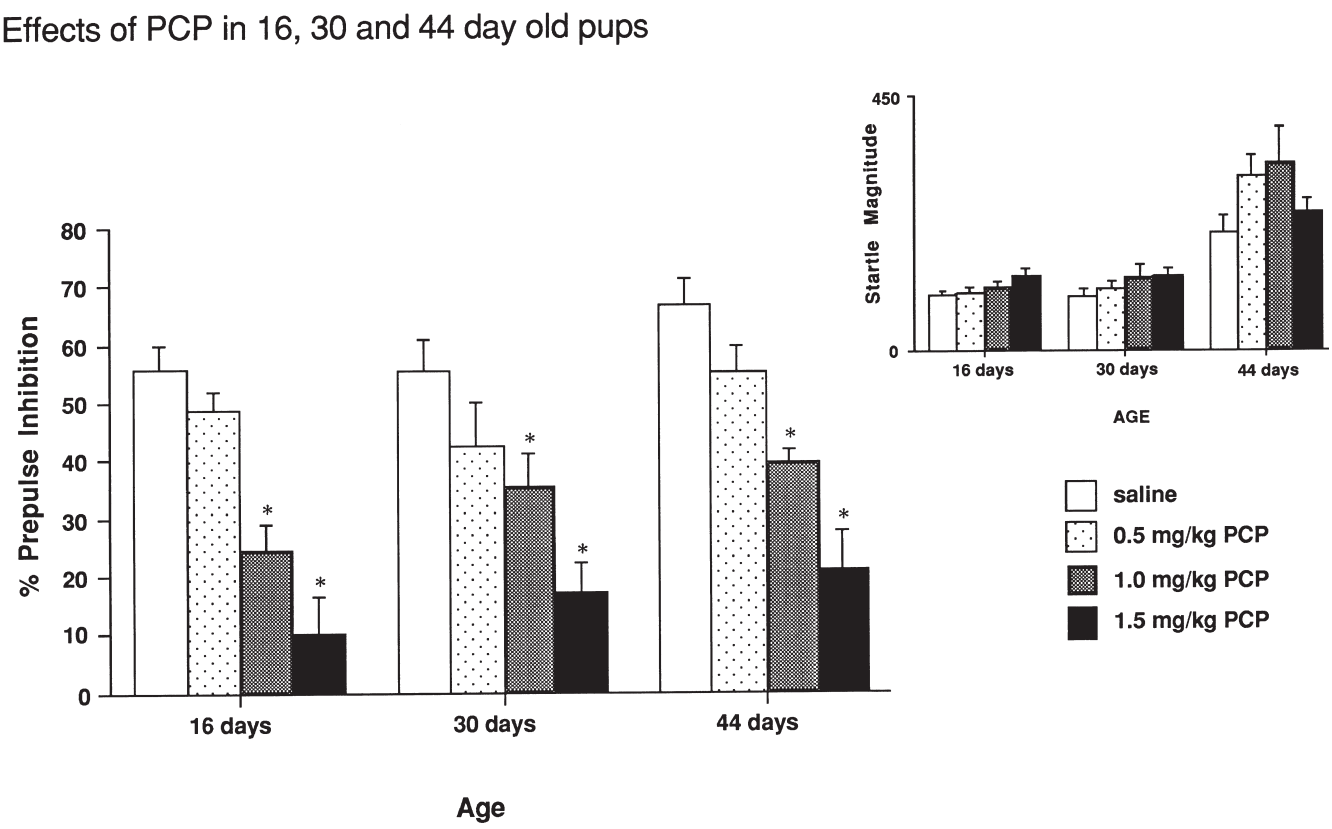

FIG. 1. Effects of PCP on PPI in 16- and 32-day-old rat pups and 44-day-old juvenile rats. Error bars represent SEM. PCP produced a reduction of PPI in male and female rats. This reduction was statistically significant at the 1.0 and 1.5 mg/kg doses. *$p < 0.01$ vs. vehicle, by Tukey–Kramer post hoc comparison, following significant main effect of dose by ANOVA. Inset: mean startle magnitude.

startle testing. Across development, PCP significantly increased NOSTIM activity, and NOSTIM activity was greater in males than in females. ANOVA with dose of PCP, sex, and age as between-subject factors revealed a significant effect of PCP dose, $F(3, 115) = 9.55$, $p < 0.0001$, a significant effect of sex, $F(1, 115) = 4.02$, $p <$ [0.05, no significant effect of age, $F(2, 115) < 1$, and no significant interactions (Table 1). Apomorphine significantly increased NOSTIM activity in both 18- and 32-day-old rats, and NOSTIM activity was lower in 32-day-old rats compared to 18-day-old rats. ANOVA with dose, sex, and age as between-subject factors revealed a significant effect of apomorphine dose, $F(3, 91) = 4.89$, $p < 0.004$, no significant effect of sex, $F(1, 91) < 1$, a significant effect of age, $F(1, 91) = 8.72$, $p < 0.004$, and no significant interactions (Table 1).

Experiment 2: Effects of a Neurotoxic Dose of PCP on Rat Pups and Adult Rats

Histology. Sensitivity to PCP-induced neurotoxicity within PC/RS cortex increased with age (Fig. 3). No abnormal cells were observed in any saline-treated rats, and few or no abnormal cells were observed in PC/RS cortex after PCP treatment in 16-day-old pups. A small number of abnormal cells (approximately 10%) were observed in the PC/RS cortex of PCP-treated 30-day-old pups; in PCP-treated adults, over 95% of all cells were observed to be abnormal. In adults, abnormalities observed in PC/RS neurons were pronounced, and consistent with previous reports, included "foamy" cytoplasmic changes with vacuolization (10,11,26,27) (Fig. 3).

Behavior. Sixteen-day-old pups: treatment with a neurotoxic dose (5 mg/kg SC) of PCP significantly decreased startle magnitude in 16-day-old pups (Fig. 4A, inset). ANOVA with PCP dose and sex as between-subject factors revealed a significant effect of PCP dose, $F(1, 16) = 5.64$, $p < 0.04$, no significant effect of sex, $F(1, 16) = 3.39$, $p < 0.09$, and no significant dose × sex interaction, $F(1, 16) < 1$. PCP also significantly reduced PPI in 16-day-old pups (Fig. 4A). ANOVA with PCP dose and sex as between-subject factors revealed a significant effect of PCP dose, $F(1, 16) = 22.78$, $p < 0.0002$, no significant effect of sex, $F(1, 16) < 1$, and no significant dose × sex interaction, $F(1, 16) < 1$.

Adult rats: treatment with neurotoxic doses (5 mg/kg SC) of PCP had no significant effects on startle magnitude (Fig. 4B, inset). ANOVA revealed no significant effect of PCP dose, $F(1, 14) = 1.37$, NS). In contrast, PCP significantly reduced PPI (Fig. 4B). ANOVA revealed a significant effect of PCP dose, $F(1, 14) = 91.79$, $p < 0.0001$, a significant effect of prepulse intensity, $F(2, 14) = 8.78$, $p < 0.001$, and no significant dose × intensity interaction, $F(2, 14) = 2.76$, $p < 0.08$. Because different startle test session were used in pups and adult rats for this neurotoxicity study (see above, Method section), it is not meaningful to compare these particular PPI findings across age groups.

DISCUSSION

There is a paucity of information related to the developmental time course of drug effects on PPI in rats. The ability of drugs to modify PPI at a particular developmental stage in-

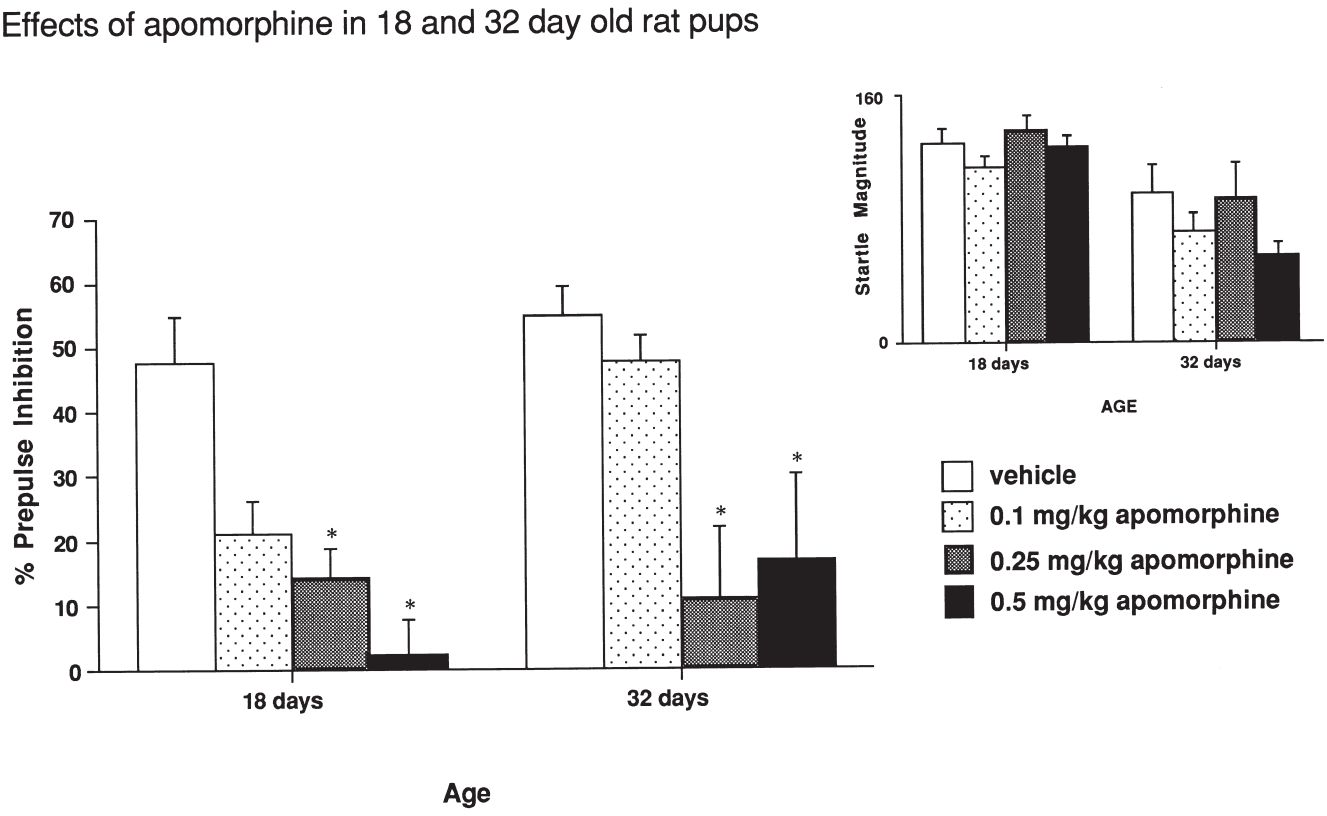

FIG. 2. Effects of apomorphine on PPI in 18- and 32-day-old rat pups. Error bars represent SEM. Apomorphine produced a reduction of PPI in male and female rats. This reduction was statistically significant at all the 0.5 and 0.25 doses. *$p < 0.01$ vs. vehicle, by Tukey–Kramer post hoc comparison, following significant main effect of dose by ANOVA. Inset: mean startle magnitude.

dicates functionality within specific brain systems that regulate PPI. Because these drug effects have been used in models for understanding the pathophysiology of schizophrenia, their developmental course is quite relevant to the evolving neurodevelopmental hypotheses for this disorder.

In the present study, both PCP and apomorphine significantly disrupted PPI in rats by 16–18 days of age. It is very conceivable that brain substrates responsible for these effects predate this age, but earlier measures of PP and drug sensitivity could not be assessed in our present startle apparatus. At this point in development, reports based on both behavioral (17,18) and volumetric studies (42) indicate that prefrontal cortex afferents and efferents are still developing. Although the neural basis for the PCPdisruption of PPI is not known, the present findings indicate that this behavioral effect does not require full maturity within the prefrontal cortex. The ability of apomorphine to disrupt PPI is thought to depend greatly on the function of DA receptors within subcortical DA terminal fields in the nucleus accumbens and striatum (36). Although D_2/D_3 DA receptors are not present at adult levels until after 21 days of age (34), the present findings indicate that by 18 days of age, DA receptors are present earlier in numbers capable of regulating PPI, presumably via their impact on descending circuitry that innervates the primary startle pathway [cf.(16,39)].

The present findings demonstrate that the effects of PCP and apomorphine on PPI are not dependent on postpubertal

TABLE 1

MEAN (SEM) NOSIM VALUES FOR EXPERIMENT 1

Group	*n*	Acute Drug Dose			
PCP dose		vehicle	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg
16 day olds	75	0.20 (0.10)	0.07 (0.05)	1.35 (0.31)	1.50 (0.46)
30 day olds	32	0.10 (0.10)	0.00 (0.00)	1.31 (1.30)	0.66 (0.22)
44 day olds	32	0.02 (0.02)	0.01 (0.01)	0.21 (0.10)	2.16 (0.76)
Apomorphine dose		vehicle	0.1 mg/kg	0.25 mg/kg	0.5 mg/kg
18 day olds	75	0.20 (0.13)	1.21 (0.29)	2.87 (0.58)	1.18 (0.31)
32 day olds	32	0.03 (0.02)	0.05 (0.03)	0.60 (0.51)	0.76 (0.47)

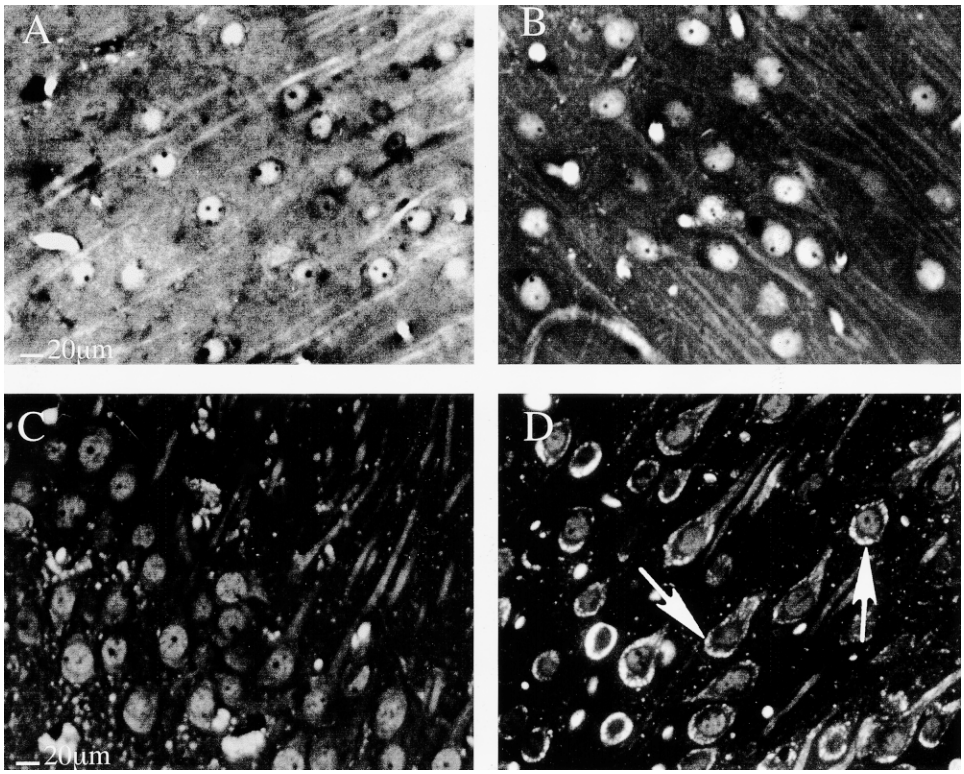

FIG. 3. Neurons from PC/RS in 16-day-old male rat pups 4 h after subcutaneous administration of saline vehicle (A) or 5 mg/kg PCP (B). Neurons from PC/RS in adult male rats 4 h after subcutaneous administration of saline vehicle (C) or 5 mg/kg PCP (D).

changes in the rat central nervous system. These drug effects must, thus, be mediated by brain processes that differ fundamentally from those responsible for the delayed postpubertal emergence of behavioral abnormalities observed in other animal models of the pathophysiology of schizophrenia, including those resulting from neonatal lesions of the ventral hippocampus (20,21). Evidence for apomorphine and PCP effects on gross motor activity (NOSTIM measures) confirms the sensitivity of other brain substrates to apomorprhine and PCP in 16–18-day-old pups. Numerous reports have confirmed the independence of drug effects on gross motor activity (e.g., NOSTIMs or startle magnitude) and PPI [cf. (35)]; in the present studies, there was a clear dissociation between drug effects (both apomorphine and PCP) on startle magnitude and PPI, and certain patterns of drug effects on NOSTIM activity (e.g., "inverted-U" dose effects on NOSTIM activity of PCP in 30-day-old pups and apomorphine in 18-day-old pups, sex differences, etc.) were clearly absent in measures of PPI.

The ability to characterize sensitivity to the PPI-disruptive effects of apomorhine and PCP in rat pups may provide important logistical benefits in studies utilizing PPI as a phenotypic marker for identifying candidate genes in neuropsychiatric disorders. Reports have identified differences in apomorphine sensitivity across rat strains (33,41), and within strains, across rat suppliers (33), suggesting that subtle genetic drift might lead to marked changes in this phenotype. For example, a recent report (15) noted that Sprague–Dawley-derived rats from one supplier (Bantin-Kingman, Hull, UK) were insensitive to the PPI-disruptive effects of apomorphine, in stark contrast to the present findings, and to numerous other reports with Sprague–Dawley-derived rats from another supplier (Harlan Laboratories, Indianapolis, IN) [e.g., (21,23, 26,38)]. Initial pharmacogenetic strategies have been successfully applied to PPI, using apomorphine sensitivity as a defining phenotype (7), and the present studies suggest that generational characteristics of this phenotype might be detectable as early as 16 days of age, which should facilitate rapid "throughput" and economical experimental designs.

No published data document the age of onset of PPI deficits in individuals who develop schizophrenia. In one scenario, PPI deficits might be expected to precede the onset of schizophrenia symptoms, because these deficits are also detected in unaffected family members and individuals with schizotypal personality disorder (4,5), suggesting that these PPI deficits may be a marker for a schizophrenia predisposition or trait. Alternatively, it is possible that the drug-induced loss of PPI in rat pups reflects experimentally induced neurochemical conditions (e.g., hypoglutamatergic or hyperdopaminergic states) similar to ones that evolve in schizophrenia (and perhaps in unaffected family members), only postpuberty. If this is the case, we might expect normal levels of PPI in children at risk for schizophrenia. Clearly, longitudinal studies of at-risk populations will add to our understanding of the developmental relationship between brain systems regulating PPI, and those responsible for the emergence of schizophrenia symptoms.

In the present study, there was a dissociation between the developmental time course for PCP effects on PPI and that for PCP-induced neurotoxicity. Specifically, while rat pups exhibited an "adult level" sensitivity to the PPI-disruptive ef-

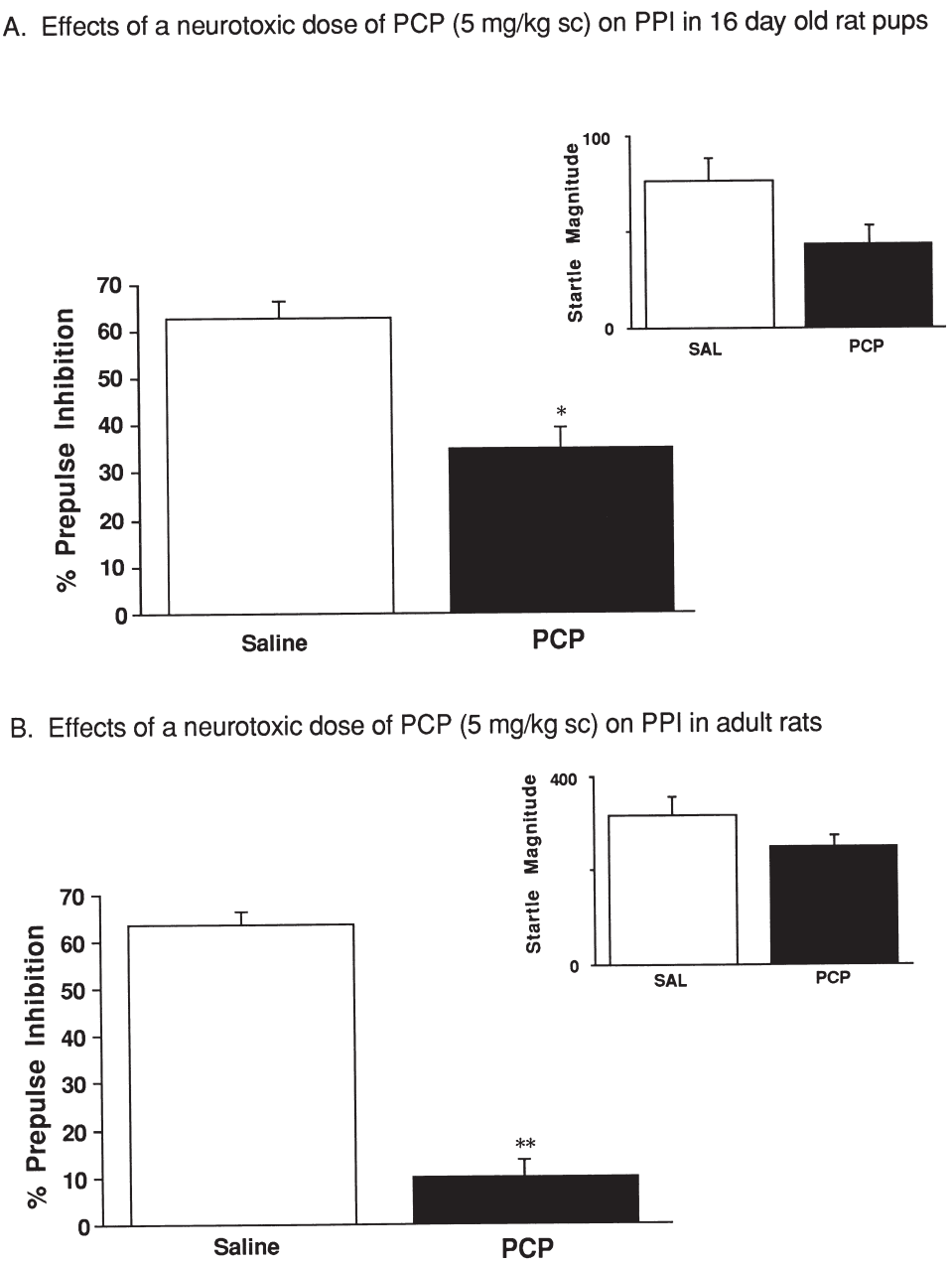

FIG. 4. (A) Effects of a neurotoxic dose (5 mg/kg SC) of PCP on PPI in 16-day-old rat pups, elicited by 15-dB prepulses. Error bars represent SEM. PCP significantly reduced PPI in male and female rat pups. Inset: mean startle magnitude. (B) Effects of a neurotoxic dose (5 mg/kg SC) of PCP on PPI in adult male rats, elicited by 3-, 6-, and 12-dB prepulses. Error bars represent SEM. For ease of presentation, PPI values are presented as the average across all three prepulse intensities (3, 6, and 12 dB above background). PCP significantly reduced PPI in adult male rats. Inset: mean startle magnitude. $*p < 0.05$, $**p < 0.01$ vs. vehicle by ANOVA.

fects of PCP that was stable across development, sensitivity to the neurotoxic effects of PCP was not readily observable in 16-day-old pups, and only emerged later in development. This latter observation is consistent with previous reports of negligible cytotoxicity produced by the noncompetitive NMDA antagonist dizocilpine (MK-801) in rat pups (11), and might conceivably be related to developmental changes in the sensitivity to postketamine emergence phenomena in humans (6,10,19,27,31,35). The present findings indicate that the PPI-disruptive and neurotoxic effects of PCP are mediated by different brain substrates, or at least different neuronal mechanisms. These findings of a dissociation between neurotoxic and behavioral effects of PCP are also consistent with recent work from our laboratory, which demonstrates intact PPI fol-

lowing sustained treatment with PCP at a time when neuroanatomic, neurochemical, and metabolic evidence of neurotoxicity is found (26). However, this apparent dissociation between neurotoxic and behavioral effects does not preclude the possibility that diminished glutamate function in schizophrenia may contribute both to the observed loss of PPI and to the reported cytoarchitectural abnormalities in this disorder. If this is the case, the present results indicate that the PPI deficits might be detectable at an earlier stage of development (as in the present 16-day-old pups), prior to the postpubertal maximal sensitivity to the neurotoxic effects of reduced glutamate activity. Again, such an hypothesis could be best tested via longitudinal measures of PPI in children and adolescents at risk for developing schizophrenia.

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REFERENCES

1. Bolino, F.; Di Michele, V.; Di Cicco, L.; Manna, V.; Daneluzzo, E.; Casacchia, M.: Sensorimotor gating and habituation evoked by electro-cutaneous stimulation in schizophrenia. Biol. Psychiatry 36:670–679; 1994.
2. Braff, D.; Grillon, C.; Geyer, M.: Gating and habituation of the startle reflex in schizophrenic patients. Arch. Gen. Psychiatry 49:206–215; 1992.
3. Braff, D.; Stone, C.; Callaway, E.; Geyer, M. A.; Glick, I. D.; Bali, L.: Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 15:339–343; 1978.
4. Cadenhead, K. S.; Geyer, M. A.; Braff, D. L.: Impaired startle prepulse inhibition and habituation in schizotypal personality disordered patients. Am. J. Psychiatry 150:1862–1867; 1993.
5. Cadenhead, K. S.; Geyer, M. A.; Swerdlow, N. R.; Shafer, K.; Diaz, M.; Clementz, B. A.; Braff, D. L.: Sensorimotor gating deficits in schizophrenic patients and their relatives. Biol. Psychiatr. Abstr. 43:10S; 1998.
6. Cronin, M. M.; Bonsfield, J. D.; Hewett, E. B.; Boulton, T. B.: Ketamine anaesthesia for radiotherapy in small children. Anaesthesia 27:135–142; 1972.
7. Ellenbroek, B. A.; Geyer, M. A.; Cools, A. R.: The behavior of APO-SUS rats in animal models with construct validity for schizophrenia. J. Neurosci. 15:7604–7611; 1995.
8. Ellison, G.: Neuronal degeneration following both competitive and non-competitive NMDA antagonists. Neuroreport 5:2688–2692; 1994.
9. Ellison, G.; Switzer, R. C.: Dissimilar patterns of degeneration in brain following four different addictive stimulants. Neuroreport 5:17–20; 1997.
10. Elliot, E.; Hanid, T. K.; Arthur, L. J. H.; Kaj, B.: Ketamine anaesthesia for medical procedures in children. Arch. Dis. Child. 51:56–59; 1976.
11. Farber, N. B.; Wozniak, D. F.; Price, M. T.; Labruyere, J.; Huss, J.; St. Peter, H.; Olney, J. W.: Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: Potential relevance to schizophrenia? Soc. Biol. Psychiatry 38:788–796; 1995.
12. Fix, A. S.; Horn, J. W.; Wightman, K. A.; Johnson, C. A.; Long, G. C.; Storts, R. W.; Farber, N.; Wozniak, D. F.; Olney, J. W.: Neuronal vacuolization and necrosis induced by the noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist MK(+)801 (Dizocilpine Maleate): A light and electron microscopic evaluation of the rat retrosplenial cortex. Exp. Neurol. 123:204–215; 1993.
13. Geyer, M. A.; Swerdlow, N. R.: Measurement of startle response, prepulse inhibition, and habituation. In: Crawley, J. N.; Skolnick, P., eds. Current protocols in neuroscience. New York: John Wiley & Sons; 1998:8.7.1–8.7.15.
14. Grillon, C.; Ameli, R.; Charney, D. S.; Krystal, J.; Braff, D.: Startle gating deficits occur across prepulse intensities in schizophrenic patients. Biol. Psychiatry 32:939–943; 1992.
15. Kinney, G. G.; Wilkonson, L. O.; Saywell, K. L.; Tircklebank, M. D.: Rat strain differences in ability to disrupt sensorimotor gating are limited to the dopaminergic system, specific to prepulse inhibition, and unrelated to changes in startle amplitude or nucleus accumbens dopamine receptor sensitivity. J. Neurosci. 19:5644–5653; 1999.
16. Koch, M.; Schnitzler, H. U.: The acoustic startle response in rats—Circuits mediating evocation, inhibition and potentiation. Behav. Brain Res. 89:35–49; 1997.
17. Kolb, B.; Nonneman, A. J.: Functional development of prefrontal cortex in rats continues into adolescence. Science 193:335–336; 1976.
18. Kolb, B.; Nonneman, A. J.: Sparing of function in rats with early prefrontal cortex lesions. Brain Res. 151:135–148; 1978.
19. Krestow, M.: The effect of post-anaesthetic dreaming on patient acceptance of ketamine anaesthesia: A comparison with thiopentone-nitrous oxide anaesthesia. Can. Anaesth. Soc. J. 21: 385–389; 1974.
20. Lipska, B. K.; Weinberger, D. R.: Delayed effects of neonatal hippocampal damage on haloperidol-induced catalepsy and apomorphine-induced stereotypic behaviors in the rat. Dev. Brain Res. 75:213–222; 1993.
21. Lipska, B. K.; Swerdlow, N. R.; Geyer, M. A.; Jaskiw, G. E.; Braff, D. L.; Weinberger, D. R.: Neonatal excitotoxic hippocampal damage in rats causes post-pubertal changes in prepulse inhibition of startle and its disruption by apomorphine. Psychopharmacology (Berlin)122:35–43; 1995.
22. Mansbach, R. S.; Geyer, M. A.: Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology 2:299–308; 1989.
23. Mansbach, R. S.; Geyer, M. A.: Parametric determinants in prestimulus modification of acoustic startle: Interaction with ketamine. Psychopharmacology (Berlin) 105:162–168; 1991.
24. Mansbach, R. S.; Geyer, M. A.; Braff, D. L.: Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology (Berlin) 94:507–514; 1988.
25. Martinez, Z. A.; Ellison, G. D.; Geyer, M. A.; Swerdlow, N. R.: Effects of sustained cocaine exposure on sensorimotor gating of startle in rats. Psychopharmacology (Berlin) 142:253–260; 1999.
26. Martinez, Z. A.; Ellison, G. D.; Geyer, M. A.; Swerdlow, N. R.: Effects of sustained phencyclidine exposure on sensorimotor gating of startle in rats. Neuropsychopharmacology 21:28–39; 1999.
27. Modvig, K. M.; Nielsen, S. F.: Psychological changes in children after anesthesia: A comparison between halothane and ketamine. Acta Anaesth. Scand. 21:541–544; 1977.
28. Olney, J. W.; Labruyere, J.; Price, M. T.: Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244:1360–1362; 1989.
29. Olney, J. W.; Labruyere, J.; Wang, G.; Wozniak, D. F.; Price, M. T.; Sesma, M. A.: NMDA antagonist neurotoxicity: Mechanism and prevention. Science 254:1515–1518; 1991.
30. Olney, J. W.; Farber, N. B.: Glutamate receptor dysfunction and schizophrenia. Arch. Gen. Psychiatry 52:998–1007; 1995.
31. Page, P.; Morgan, M.; Lok, L.: Ketamine anaesthesia in paediatric procedures. Acta Anaesth. Scand.16:155–160; 1972.
32. Perry, W.; Braff, D.: Information-processing deficits and thought disorder in schizophrenia. Am. J. Psychiatry 151:363–367; 1994.

33. Rigdon, G.: Differential effects of apomorphine on prepulse inhibition of acoustic startle reflex in two rat strains. Psychopharmacology (Berlin)102:419–421; 1990.
34. Stanwood, G. D.; McElligot, S.; Lu, L.; McGonigle, P.: Ontogeny of dopamine D3 receptors in the nucleus accumbens of the rat. Neurosci. Lett. 223:13–16; 1997.
35. Sussman, D. R.: A comparative evaluation of ketamine anesthesia in children and adults. Anesthesiology 40:459–464; 1978.
36. Swerdlow, N. R.; Braff, D. L.; Geyer, M. A.; Koob, G. F.: Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. Biol. Psychiatry 21:23–33; 1986.
37. Swerdlow, N. R.; Braff, D. L.; Masten, V. L.; Geyer, M. A.: Schizophrenia-like sensorimotor gating abnormalities in rats following dopamine infusion into the nucleus accumbens. Psychopharmacology (Berlin) 101:414–420; 1990.
38. Swerdlow, N. R.; Braff, D. L.; Taaid, N.; Geyer, M. A.: Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. Arch. Gen. Psychiatry 51:139–154; 1994.
39. Swerdlow, N. R.; Geyer, M. A.: Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr. Bull. 24:285–302; 1998.
40. Swerdlow, N. R.; Mansbach, R. S.; Geyer, M. A.; Pulvirenti, L.; Koob, G. F.; Braff, D. L.: Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. Psychopharmacology (Berlin) 100:413–416; 1990.
41. Swerdlow, N. R.; Varty, G. B.; Geyer, M. A.: Discrepant findings of clozapine effects on prepulse inhibition of startle: Is it the route or the rat? Neuropsychopharmacology 18:50–56; 1998.
42. Van Eden, C. G.; Uylings, H. B. M.: Postnatal volumetric development of the prefrontal cortex in the rat. J. Comp. Neurol. 241:253–267; 1985.
43. Weinberger, D. R.: Implications of normal brain development for the pathogenesis of schizophrenia. Arch. Gen. Psychiatry 44:660–669; 1987.
44. Weinberger, D. R.; Lipska, B. K.: Cortical maldevelopment, antipsychotic drugs, and schizophrenia: Search for common grounds. Schizophr. Res. 16:87–110; 1995.