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Brief postnatal exposure to phenobarbital impairs passive avoidance learning and sensorimotor gating in rats

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ABSTRACT

Phenobarbital is the most commonly utilized drug for the treatment of neonatal seizures. However, mounting preclinical evidence suggests that even brief exposure to phenobarbital in the neonatal period can induce neuronal apoptosis, alterations in synaptic development, and long-lasting changes in behavioral functions. In the present report, we treated neonatal rat pups with phenobarbital and evaluated behavior in adulthood. Pups were treated initially with a loading dose (80 mg/kg) on postnatal day (P)7 and with a lower dose (40 mg/kg) on P8 and P9. We examined sensorimotor gating (prepulse inhibition), passive avoidance, and conditioned place preference for cocaine when the animals reached adulthood. Consistent with our previous reports, we found that three days of neonatal exposure to phenobarbital significantly impaired prepulse inhibition compared with vehicle-exposed control animals. Using a step-though passive avoidance paradigm, we found that animals exposed to phenobarbital as neonates and tested as adults showed significant deficits in passive avoidance retention compared with matched controls, indicating impairment in associative memory and/or recall. Finally, we examined place preference conditioning in response to cocaine. Phenobarbital exposure did not alter the normal conditioned place preference associated with cocaine exposure. Our findings expand the profile of behavioral toxicity induced by phenobarbital.

1. Introduction

Phenobarbital (PB) is the most commonly utilized drug for the treatment of neonatal seizures [1–3] despite growing concerns about its efficacy [4,5] and safety in neonatal or infant populations. For example, prolonged early-life exposure to phenobarbital as a treatment for febrile seizures has been associated with reduced IQ [6,7]. Comparable studies examining shorter exposures have not been performed.

Preclinically, there is mounting evidence that even brief exposure to phenobarbital during early postnatal life can have long-lasting effects on brain development in rodents. For example, when given even once to postnatal day (P)7 rats, phenobarbital induced a profound increase in neuronal apoptosis throughout a variety of cortical (e.g., frontal and parietal cortices) and subcortical (e.g., hippocampus, nucleus accumbens, amygdala, and thalamus) structures [8–10]. This effect has been well documented by several groups, with the period of vulnerability lasting until ~P10–P14 [8]. Early-life phenobarbital exposure is also associated with changes in the cortical proteome, including genes associated with synaptic function and regulation of oxidative stress [11].

Importantly, P7 exposure to PB also induces changes in nervous system function. For example, between P10 and P18, there is normally a robust increase in the number of functional excitatory and inhibitory synapses in the striatum [12]. By contrast, when rats were exposed to PB on P7, striatal synaptic development was stunted [12,13]. Interestingly, when the timing of PB exposure was shifted to P10, normal maturation patterns were found [12].

We [12–16] and others [17–21] have also reported functional effects of early-life PB exposure on adult behavior. One of the most consistent findings is impaired spatial memory in PB-exposed animals tested as adults; exposure from P6 to P10, from P7 to P14, or from P2 to P21 all disrupted adult spatial memory in the Morris water maze or radial arm maze [15,17,20,21]. Deficits in other memory tasks (i.e., delayed alternation [21], fear conditioning [13,15], reversal learning [12]) have also been reported after PB exposure in early life. Additional behavioral changes following acute or subacute neonatal exposure include impaired prepulse inhibition (PPI) [13–15], hypersensitivity to the

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locomotor-enhancing effects of amphetamine [14,16], decreased anxiety-like behavior, and reduced social exploration [15].

The present study had two objectives. The first objective was to better approximate a clinical schedule of PB exposure. Previous studies have used either acute [12,14] or prolonged [13,15,18,20,21] exposure to PB. While a single exposure is useful for examining the "worst-case" scenario of drug toxicity (i.e., even a single dose is sufficient to alter behavior), it does not mirror clinical recommendations [3]. Conversely, longer exposures can exceed both the therapeutically relevant dose (due to drug accumulation) and the developmentally equivalent [22] time period during which treatment would occur. To reduce these confounds, here, we examined the effects of subacute administration of PB (P7, P8, and P9) on subsequent adult behavior. Pups received a loading dose on P7 and half doses on P8 and P9 to avoid drug accumulation.

The second objective of this study was to examine a previously unexplored behavioral domain: psychostimulant reinforcement. We chose this measure because of the enhanced locomotor response PB-exposed rats display to psychostimulants [14,16] and because of the profound apoptosis that occurs in limbic structures that mediate reward [10]. As a basis for comparison, PPI was also examined, which is impaired by both acute and chronic exposures and, thus, serves as a positive control for the present study [13–15]. We also chose a step-through passive avoidance task as a measure of associative learning. Associative learning is impaired by chronic exposure [13,15] but has yet to be examined after acute or subacute exposure.

2. Materials and methods

2.1. Animals

Timed-pregnant Sprague–Dawley rats (Harlan Laboratories, Frederick, Maryland) were housed in the Georgetown University Division of Comparative Medicine. Animals were maintained in a temperature-controlled room (21 °C) with a 12-h light cycle (0600–1800 lights on). Food and water were available ad libitum. A total of 30 pups (a mix of male and females) were used, and date of parturition was designated P0 for all pups. Treatment was counterbalanced across litters and sex, and all manipulations occurred during the light phase. One animal from the PB-treated group was excluded from data analysis because it was a statistical outlier using Dixon's test ($P < 0.009$). All experiments were approved by the Georgetown University Animal Care and Use Committee.

2.2. Drug treatment

Phenobarbital sodium (5-ethyl-5-phenyl-1,3-diazinane-2,4,6-trione; Sigma) was dissolved in saline at a concentration of 8 mg/ml and administered intraperitoneally. Pups were treated on postnatal day (P)7, P8, and P9. We employed a loading dose of 80 mg/kg on P7, followed by a lower dose of 40 mg/kg on P8 and P9. Loading doses are commonly used clinically for neonates [23–25]. Doses were selected based on pharmacodynamic equivalence. A loading dose of 80 mg/kg was selected because this dose, but not lower doses, provides complete protection against seizures evoked by pentylenetetrazole in P7 rats [26]. Moreover, this dose, but not lower doses, prevented mortality associated with kainic acid treatment in P7 rats [27]. A lower dose of 40 mg/kg was selected because it is the lowest dose that provides complete protection against tonic seizures and partial protection against clonic seizures evoked by pentylenetetrazol [26]. Control pups received equivalent volumes of vehicle.

2.3. Behavioral testing

Animals were weaned into same-sex cages of 2–3 rats at P21 and maintained until adulthood when testing began (P60+). Prior to each behavioral test (described below), animals were allowed to acclimate to the testing room for at least 30 min. Tests were performed in the order described below.

2.3.1. Prepulse inhibition (PPI)

Prepulse inhibition testing was conducted as we have previously described [13–15,28]. Briefly, testing was conducted using the SR-LAB system (San Diego Instruments). Startle chambers were ventilated and illuminated, with continuous background noise (65 dB). Broadband noise pulses were generated by a high-frequency loudspeaker within the chamber. Each chamber contained a clear nonrestrictive Plexiglas cylinder resting on a platform. A piezoelectric accelerometer attached to the platform detected motion produced by startle responses.

Animals were allowed to acclimatize within the Plexiglas chamber for 5 min. Following the acclimation period, a startle-inducing broadband noise pulse ("Pulse Alone"; 120 dB, 30 ms) was presented five times to habituate the animals to the testing procedure. These trials were excluded from data analysis. Rats were then presented with either the startle pulse alone ("Pulse Alone") or 100 ms after a 30-ms prepulse ("Prepulse + Pulse") that was 3, 6, or 12 dB above the background noise. Each session consisted of a total of 40 trials (10 Pulse-Alone trials, 10 of each Prepulse trial) presented in pseudorandom order. Trials were separated by an average of 15 s (range: 5–25 s). Startle magnitude was calculated as the average of the startle responses to the Pulse-Alone trials. Prepulse inhibition was calculated according to the following formula: %PPI = (1 − (startle response for Prepulse + Pulse trials / startle response for Pulse-Alone trials)) × 100.

2.3.2. One-trial step-through passive avoidance task

As a measure of learning and memory, a passive avoidance task was employed. Conditioning and testing occurred in a standard rat shuttle cage (Coulbourn Instruments, H10-11R-PA). The cage (20″ × 10″ × 12″, width × depth × height) was divided evenly into a dark chamber and a light chamber. The light chamber was illuminated by a ceiling-mounted light bulb. A computer-controlled drop door separated the chambers.

On the first day of passive avoidance training, rats were placed into the light side of the apparatus (the door was closed to prevent entry into the dark side) for 180 s. On the second day, rats were placed into the light side of the apparatus. After 30 s, the door was lifted, allowing access to the dark chamber. When the animals crossed the dark chamber, the door was lowered (to prevent reentry into the light chamber), and a mild footshock (0.4 mA, 2-s duration) was administered. Animals were removed from the dark side within 30 s of the conclusion of the conditioning trial.

On days 3 and 4, animals were tested for retention. They were placed in the light side of the chamber, and after 30 s, the door to the dark side was lifted, allowing access. Latency to enter the dark chamber was recorded. If the rat did not enter the dark side within 300 s of the door opening, the trial was terminated, and a latency of 300 s was assigned.

2.3.3. Conditioned place preference (CPP)

Rats were tested using a place preference conditioning paradigm as previously described [29]. The apparatus consisted of two triangular compartments that shared one wall, with a rectangular door connecting the two chambers. Distinctive tactile and visual cues were used to differentiate the two chambers: (1) pellet bedding with dots on the walls and (2) corncob bedding with vertical stripes on the walls. The location of the rat was monitored by a camera suspended over the chambers and tracked and recorded by ANY-maze (Stoelting).

In a pretreatment baseline preference test, rats were placed in the doorway at the center of the chamber facing the dotted chamber. The rats were allowed free access to both compartments for 10 min. Animals with an unconditioned bias (>75% preference) to either chamber were withdrawn from further experiments. Five of thirty rats were removed on the basis of this criterion.