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# Effect of severe neonatal seizures on prepulse inhibition and hippocampal volume of rats tested in early adulthood

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## HIGHLIGHTS

- Deficits in PPI have been observed in patients with schizophrenia.
- Structural changes in the hippocampus have been implicated in schizophrenia.
- Rats pups subjected to seizures presented deficits in PPI when tested in adulthood.
- Hippocampal volume was reduced in rats that experienced severe neonatal seizures.
- These results suggest that early seizures may raise risk for later schizophrenia.

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## ABSTRACT

Several lines of evidence indicate that the risk of developing schizophrenia is significantly enhanced following postnatal exposure to environmental insults occurring during the critical periods of early central nervous system development. The hippocampus is a brain structure that has been associated with the neuropathology of schizophrenia. Neonatal epileptic seizures in rat pups can affect the construction of hippocampal networks. Patients with schizophrenia exhibit deficits in an operational measure of sensorimotor gating: prepulse inhibition (PPI) of startle. PPI is the normal reduction in the startle response caused by a low intensity non-startling stimulus (prepulse) which is presented shortly before the startle stimulus (pulse). The aim of the present study was to investigate if prolonged epileptic seizures, occurring during postnatal brain development, alter prepulse inhibition (PPI) response of acoustic startle reflex and hippocampal volume of rats tested later in life (post-pubertal phase). Pilocarpine-induced status epilepticus (SE) was induced in postnatal days (PNDs) 7–9 in rat pups. On PND56, the animals were tested in the acoustic startle/PPI paradigm. Hippocampal volume was measured in histological brain slices using the Cavalieri's principle. Dorsal and ventral hippocampi were measured bilaterally. Our results demonstrate that animals subjected to SE presented deficits in PPI when tested in adulthood. Dorsal hippocampal volume was reduced in rats that experienced severe neonatal seizures.



## 1. Introduction

Schizophrenia results from the cumulative interaction of a number of risk factors, some of which are neurodevelopmental. Human epidemiological studies have provided compelling evidence that the risk of developing schizophrenia is significantly enhanced following prenatal and/or perinatal exposure to environmental insults (*i.e.*, birth complications, infections, toxin exposure, maternal stress, hypoxia, *etc.*), occurring during the critical periods of early central nervous system development [14]. An interaction between early neurodevelopmental disturbances and periadolescent brain maturation seems to be necessary in order to trigger the onset of full-blown psychotic behavior, which typically emerges during adolescence or early adulthood [14,17].

The hippocampus is a brain structure that has traditionally been associated with the pathophysiology and neuropathology of schizophrenia [13,19]. Neonatal insult of the hippocampus in rat pups at postnatal day (PND) 7 disrupts development of the widespread cortical and subcortical circuitry in which the hippocampus participates. This triggers a number of behavioral,

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molecular and physiological changes reminiscent of a variety of aspects of schizophrenia that typically emerge late in adolescence (PND 35) and early adulthood (PND 56), resembling the delayed onset of psychotic symptoms in humans [13,15,17]. The rodent brain is less mature at the time of birth as compared to the human brain and undergoes rapid brain growth postnatally, with a peak around PNDs 7–10 [17]. The brain growth spurt occurring at birth in humans is centered around one week postnatal in rats, leading to the notion that the last trimester of human gestation corresponds to PNDs 1–10 in rats [14].

The relationship between schizophrenia and epilepsy has been of interest for many years. A common underlying etiology of epilepsy and psychosis is supported by the findings of Hesdorffer et al. [8]. They found that psychosis is both associated with increased risk for developing epilepsy, and following a diagnosis of epilepsy, the risk for developing psychosis is increased. Animal data indicate that epileptic seizures at an early developmental stage can dramatically affect the construction of hippocampal networks [10]. Experimental models have shown that neonatal seizures result in decreased neurogenesis, sprouting of mossy fibers, long-standing changes in signaling properties, and altered firing patterns of hippocampal neurons [10]. Accordingly, Santos et al. [20] demonstrated that multiple episodes of pilocarpine-induced *status epilepticus* (SE) in developing rats on PNDs 7–9 led to progressive epileptiform activity, persistent *in vitro* hyperexcitability, increased hippocampal apoptosis, and cognitive impairment in adulthood. Moreover, adult rats submitted to SE at PNDs 7–9 showed abnormal distribution of neocortical interneurons and altered expression of hippocampal glutamate and GABA receptors [22,23].

The acoustic startle response (ASR) is expressed by a rapid contraction of the facial and skeletal muscles evoked by a sudden and loud acoustic stimulus. The ASR in rats is a useful model for the study of the plasticity of sensorimotor information processing in mammals, and is subject to a variety of influences which modify its response magnitude. Pre-pulse inhibition (PPI) refers to the normal reduction in the magnitude of the ASR caused by a low intensity non-startling stimulus (the prepulse) which is presented shortly before the startle stimulus [6]. It is thought that the prepulse activates an inhibitory process that attenuates or "gates" the startle response. The amount of PPI thus provides an operational measure of sensorimotor gating [21]. Deficits in PPI have been observed in several neuropsychiatric disorders including schizophrenia where evidence for sensory gating deficits and stimulus overload are observed [6]. In rats, deficits in PPI can be induced by pharmacological or developmental manipulations (for an extensive review, see Geyer et al. [6]).

Thus, based on the assumption that the clinical features of schizophrenia typically appear in adolescence and early adulthood [11], the aim of the present study was to investigate whether prolonged epileptic seizures, during a period of critical brain development PNDs 7–9, alter PPI response of acoustic startle reflex and the volume of the hipocampal formation in rats tested in early adulthood (PND 56).

## 2. Materials and methods

All experimental procedures were previously approved by the Animal Care and Use Committee of our institution (CEP n° 1603/09). Newly born male Wistar rats (UNIFESP-EPM, São Paulo, Brazil) were maintained in controlled environmental conditions (light/dark cycle of 12 h, 07:00–19:00 h; room temperature of 22–24 °C and relative humidity of 50–60%) with their respective dams. The age of the animals was determined starting from the day of birth (postnatal day zero, PND 0). A total of 36 male Wistar rats were used. They were randomly selected in each colony and assigned in different groups (see below).

### 2.1. Induction of status epilepticus (SE)

For status epilepticus (SE) induction, rat pups were isolated from their dams and received intraperitoneal injections of pilocarpine hydrochloride 2% (Merck, 380 mg/kg), in the seventh (PND 7), eighth (PND 8) and ninth (PND 9) days of postnatal life as previously reported [22,23]. This period is critical for brain development in which the nervous system becomes more susceptible to seizures [25]. After each injection, pups were observed for the characterization of seizures, duration of SE, occurrence of tonic episodes, and mortality. Tonic seizures was characterized by increased tone in the extensor muscles, jaw clenching or mouth opening, contraction of the respiratory and abdominal muscles, high-pitched cry and brief periods of apnea. Animals with prolonged tonic seizures (TS) were assigned in the group *SE with TS* ($N = 6$). Pups without severe tonic seizures constituted the *SE without TS* group ($N = 10$). After recovery, animals were returned to their respective dams. The *saline group* ($N = 8$) consisted of pups that received saline solution 0.9% instead of pilocarpine in the same volume (1 ml/kg). The *control group* ($N = 12$) consisted of pups without manipulation. These pups stayed undisturbed in the home cage with their dams until PND 21. The use of the control pups served not only to assess the specific effects of the seizures, but also those associated with maternal separation.

At the conclusion of the protocol on PND 9, all pups were returned to their dams where they remained, undisturbed, until weaning on PND 21. The male rats were subsequently housed socially with two to three animals from the same litter belonging to the same experimental group. They were on PND 56 at the beginning of the PPI testing. PND56 was selected in order to correspond with the typical age of onset of schizophrenia in early adulthood [13,15,17].

### 2.2. Startle chambers

Two commercial startle chambers devices (Insight Equipment, Brazil) were used simultaneously to record the amplitude of the acoustic startle response in the PPI test. The equipment consisted of a wire-mesh cage (16.5 cm × 5.1 cm × 7.6 cm) which was connected to a stabilimeter (response platform, 36.5 cm × 11.5 cm × 4.5 cm) with four thumb nail-screws, inside a ventilated, sound-attenuated chamber (48 cm × 48 cm × 45 cm). Noise bursts were presented *via* a high-frequency loudspeaker located 24 cm far from the wire-mesh cage. The startle reaction of the rat within the wire-mesh cage generated a pressure on the stabilimeter, and signals were amplified, digitized and analyzed by the software of the startle measurement system (Insight Equipment, Brazil), and interface assembly, which also digitized, and recorded stabilimeter readings. Calibrations were performed weekly to maintain accurate acoustic stimuli presentations and ensure equivalent sensitivities of the response platforms over the test sessions. Animal behavior was recorded by an infrared camera (Safety View) located behind the stabilimeter, allowing the discrimination of all possible behaviors, with the signal being relayed to a video and a monitor in another room *via* a closed circuit.

### 2.3. Testing startle and PPI

On PND 56, animals were tested in the acoustic startle/PPI paradigm. The acoustic startle session consisted of a 5 min acclimation period in the startle chamber with a constant background noise (65 dB) that continued throughout the remainder of the session, followed by 52 presentations of acoustic stimuli to measure acoustic startle. The 52 acoustic trials consisted of: twenty-two 40 ms