Journal of Biological Sciences

ISSN 1727-3048
Perinatal Exposure to Weak Magnetic Fields Delays the Asymmetry Ontogeny of Astroglia in the Parasolitary Nucleus: Implications for Sudden Infant Death

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Abstract: The aim of the study was to discern if Sudden Infant Death Syndrome (SIDS) reflects a transient vulnerability to stimuli during anomalous ontogeny of normal cellular interaction within brain stem structures such as the parasolitary (Psol) nucleus. Perinatal exposure equivalent to the strength and frequency of a magnetic field configuration correlated with SIDS was utilized to produce alterations in cellular asymmetry within the Psol nucleus. The number of neurons and nuclei of astroglia and oligodendroglia from n = 40, 5 and 15 day old rats that had been exposed to 0.5 Hz, 5 to 10 nT magnetic field between -3 to +7 days of birth and were counted at 1000 X. A robust and significant interaction (explaining 30% of the variance) between numbers of astroglia nuclei in the right vs. the left side of the nucleus, age and exposure to the field was demonstrated. These results indicate exposure to this specific intensity and frequency may delay the normal ontogeny of asymmetry-to-symmetry in cell numbers that affect the blood brain barrier and neuronal function. Greater attention to neuroaxial asymmetry in post-mortem measures of SIDS cases may be warranted.

Key words: SIDS, astroglia numbers, ELF magnetic fields, brain stem

INTRODUCTION

The sudden mortality of human infants - Sudden Infant Death Syndrome (SIDS), between the ages of 2 and 6 months (Waters et al., 1999; Moon et al., 2007) suggests a transient period of vulnerability coupled to ontogeny. The level of the solitary nucleus is one of the most frequent brain stem areas for which changes in measures of neuronal, glial, or receptor subtypes have been found (Kawai and Senba, 2000; Sawaguchi et al., 2003). Patterns of synaptic currents involved with respiratory neurons in this area are not stable but change during early postnatal life (Zec and Kinney, 2003). We have been pursuing the hypothesis that disruptions by subtle environmental stimuli of the normal ontogeny of structural symmetry (and consequently function) within the brain stem nuclei may contribute to neuroelectrical transients and sporadic autonomic crisis. Transgenic mice with reversible but excessive serotonin autoinhibition within the brain stem have been recently shown (Audero et al., 2008) to display sporadic bradycardia that frequently progressed to death.

Geomagnetic activity has been considered both a predisposing and precipitating factor for SIDS (Eckert, 1992). Daily and monthly levels of global geomagnetic activity and the numbers of cases of SIDS are moderately and positively correlated (O'Connor and Persinger, 1997, 1999; Persinger and O'Connor, 2001). The primary association involves numbers of days with specific bands of variation in geomagnetic intensities around 1 to about 10 nT. Experiments by Dupont et al. (2005) demonstrated fewer live births (M = 14.1, SD = 2.1) in rats exposed for two days before birth to 0.5 Hz, 5 to 10 nT magnetic fields compared to sham-field controls (M = 16.2, SD = 2.7). The proportions of death were actually greater for rats exposed to 500 nT fields of the same frequency. In other studies (Dupont et al., 2004) brains of 21 day old rats exposed perinatally for 3 days before to 7 days after birth to a variable range of 0.5 Hz intensities between 4 and 13 nT showed fewer neurons within the parasolitary (Psol) nucleus.

In the present study we selected this low intensity (5 to 10 nT) and frequency (0.5 Hz) as primary parameters because of their frequent presence on days in which SIDS occur (O'Connor and Persinger, 1997, 1999). In addition 0.5 Hz is within the range of stimulation rates that evoke reliable volleys in brain stem neurons involved with vestibular, respiratory and cardiovascular responses (Barmack and Yahkntisa, 2000) and with a specific prediction from a theoretical model involving proton...
resonance (Persinger, 2006). Weak, extremely low frequency magnetic fields can interact with unstable states (Whissell and Persinger, 2007a) and interact with Nitric Oxide (NO) functions (Whissell and Persinger, 2007b). Persinger et al. (2005) had shown that 50 nT but neither weaker (10 nT) nor stronger (500 nT) experimentally generated 7 Hz magnetic fields whose amplitudes were continuously increased and decreased in 46 steps every 6 min (0.3 mHz) increased sudden cardiac death in adult rats during the first 24 h following induction of epileptic seizures.

Recently, St-Pierre et al. (2007) reported a doubling of mortality following inductions of epileptic seizures in 21 to 25 day old (weanling) rats if they had been exposed perinatally to a 5 nT, 7 Hz magnetic field. The mortality of rats that had been exposed to different windows of strengths below or above (up to 500 nT) this narrow window did not differ from controls. We report here that perinatal exposure to 0.5 Hz, 5 to 10 nT magnetic fields produced a specific delay in the asymmetry of astroglia but not oligodendroglia or neurons within the Pso1 nucleus. It was selected, of the many possible regions that have been shown to display pathophysiology, because of its proximity to and sometimes interposition with the solitary nucleus with which it shares and receives projections (Barmack et al., 1998).

MATERIALS AND METHODS

Animals: A total of 40 male and female albino Wistar rats that had been born in the laboratory within the various experimental conditions served as subjects. All procedures were completed in 2006 under approved research protocols at the Paul Field Animal Care Facility at Laurentian University, Sudbury, ON, Canada.

Procedure: In four separate blocks of experiments over a one year period a total of 20 Wistar pregnant females were exposed singly in plastic cages approximately two to three days before expected birth (as inferred by sperm plugs) to seven days after birth to a coil in which a 5 to 10 nT, 0.5 Hz sine wave magnetic field was generated or to an orthogonally-oriented, non-activated coil in the same room. We selected the sine-wave rather than square-wave option because of the similar morphology to Po1 (continuous pulsations) generated during specific conditions within the geomagnetic field. Characteristics of the coil have been published elsewhere (McKay et al., 2003; Dupont et al., 2005; St-Pierre et al., 2007). The light-dark cycle was 12:12 with the onset of light at 08:00 h. Temperature was maintained within 1 deg of 21°C.

The field was generated in the E-W direction for two blocks of experiments and in the N-S direction for two blocks. The activated coil (and hence orientation) was alternated with each block. A FEDA FM-300 Vector magnetometer placed in the coil and coupled to a laptop computer maintained a continuous record of the normal geomagnetic (background) and experimental fields. During the period between 3 days before birth to 7 days after birth the experimental field was discontinued for 30 min once every 4 h (hence 21 h of field exposure per day) because we have found that 30 min of no field following protracted presentations can be as effective as 30 min of field presentation in adults for producing analgesic effects (Martin et al., 2004). Similar observations have been found for analgesia (Prato et al., 2005) in adult mice. The induced field within the perpendicular coil employed as a reference in any given block was not detectable by instrumentation (less than 1 nT).

On postnatal days 5 and 15 (8 days after the termination of the field exposures) one pup from each litter was randomly selected. The brain was removed within four min and fixed in ethanol-formalin-acetic acid (Persinger, 1983). After processing and paraffin embedding 10 μm coronal sections were obtained from the medulla at the level of the easily identifiable Pso1 nucleus. The sections were stained with toluidine blue. The numbers of neurons and nuclei of astroglia and oligodendroglia were counted at 1000 μm within a 0.1 mm by 0.1 mm grid (containing 6×6 smaller squares) that was focused at the center of the Pso1 nucleus for two non-consecutive slides for the left and right side of the brainstem. The cytometrist was blind to the experimental conditions of the rats.

Statistical analysis: Four way analyses of variance with three between subject levels: treatment (sham field vs. 5 to 10 nT), gender (male vs. female), age (5 vs. 15 days) and one within subject measure (left vs. right parasolitary nucleus) were completed for each of the cell types. Post hoc analyses involved combinations of paired t-tests and Tukey's set at p<0.05. All analyses involved SPSS software on a VAX 4000 computer.

RESULTS

There were no statistically significant differences between treatments (all Fs = 1.30) for the numbers of neurons except for the significantly greater density within the 5 day old pups compared to the 15 day old pups (F(1,30) = 7.09, p = 0.01; 19% of variance explained). The means and standard deviations for the numbers of neurons 0.01 mm⁻² of the two ages were 28.5 and 5.1 and
Fig. 1: Right/Left Side Ratios of Numbers of neurons, nuclei of oligodendroglia and nuclei of astroglia in rats that had been exposed to either the 5 to 10 nT, 0.5 Hz magnetic field or reference setting at 5 days (8 days of field exposure) or 15 days (8 days after termination of field exposure) of age. Vertical bars indicate standard errors of the mean.

25.6 and 5.4, respectively. None of the within subject (left vs. right side) and between subject interactions were statistically significant. There were also no statistically significant effects for the differences between groups or the two-way interactions for the numbers of nuclei of oligodendroglia or astroglia.

However, there was a strong statistically significant interaction (F(1,30) = 9.91, p<0.01; 25% of variance explained) between exposure to the magnetic field, age of the brain (5 vs. 15 days) and the numbers of astroglia within the left and right Psol nuclei. Post hoc analyses, as shown in Fig. 1, indicated that the primary source of this interaction was due to the elimination of the asymmetry in the numbers of astroglia between the right and left sides of the Psol nuclei in the rats exposed to the magnetic field compared to controls at 5 days of age (after 8 days of continuous exposure to this field configuration). However by the time the rats were 15 days of age (8 days after the termination of the field), the rats that had been exposed demonstrated the right-sided increase of astroglia cells whereas the controls showed equal numbers.

The effect was still evident when the measures were expressed as right side/left side ratios for numbers of astroglia nuclei within the nucleus. This statistically significant interaction between age and treatment (F(1,30) = 10.98, p<0.01) explained 30% of the variance in this ratio. After initial covariance for the ratio of neurons and nuclei of oligodendroglia in the left vs. right side of the Psol nucleus, the statistically significant interaction between treatment and age (F(1,28) = 13.49, p<0.001).

Fig. 2: Mean numbers of nuclei of astroglia per mm² (×100) within the left and right parasolitary nucleus of rats exposed to either a 5 to 10 nT, 0.5 Hz field or reference condition at 5 days (8 days of field exposure) or 15 days (8 days after termination of field exposure) of age. Vertical bars indicate standard errors of the mean.

remained. For comparison, the right-to-left sided ratios for neurons and nuclei for oligodendroglia and astroglia in the Psol nucleus are shown in Fig. 2. There were no statistically significant differences in asymmetry for neurons or nuclei of oligodendroglia as a function of treatment or age. The major effect involved only the astroglia.

**DISCUSSION**

The results of this study indicated that 8 days of exposure to 0.5 Hz, 5 nT magnetic fields during development from three days before birth to 7 days after birth in the rat did not affect the numbers of neurons within the Psol nucleus. Instead the exposure affected the ratio of astroglia cells on the right vs. left side of the structure. After about 8 days of continuous exposure to the 5 nT, 0.5 Hz magnetic field configuration the asymmetry (right side dominance) of astroglial cells displayed by the reference group was not observed. By 8 days after the termination of the exposure this asymmetry (right side dominance) was present.

We interpret this age-dependent pattern as the consequence of the delaying effect of the 5 nT, 0.5 Hz magnetic field upon normal development in this area. Our interpretation would be compatible with the conclusions of Rodriguez et al. (1994) who found a decrease in asymmetry of dopaminergic neurons in the midbrain.
during postnatal development. On the basis of altered catecholaminergic dendritic development in SIDS cases, Takushima and Becker (1991) also suggested a delay in neuronal maturation manifested as subtle decreases in neurotransmitter interactions. One example was the alpha-2 adrenergic receptors (Ozawa et al., 2003). Such transient asymmetry in microstructure and function between the left and right side of the brain stem could contribute to positive feedback loops (Barmack and Yakhnitsa, 2000) from a variety of precipitating stimuli that could lead to sudden cardiac death or respiratory cessation following over stimulation of vestibular input from a number of direct and indirect afferents.

Unlike the experiment by Dupont et al. (2004) we did not find a significant difference in the numbers of neurons within the parasolitary nucleus. However, the brains of the rats in that study were 21 days old compared to 5 and 15 days in the present study. Although, their (Dupont et al., 2004) effect may be related to the additional slow amplitude-variations of their 0.5 Hz field, the reduced neuronal density may also reflect the long term consequences of disruption of the normal alterations of asymmetry in astroglial numbers. Numbers of astroglia affect neuronal density as well as synaptogenesis (Ullian et al., 2001). It may be relevant that more spontaneous neonatal deaths were noted as well within the nanoTesla fields of that study (Dupont et al., 2004) compared to much higher (microTesla) intensities.

The Pson nucleus has been described as a new vestibular nucleus located dorsal to the nucleus solitarius and tractus solitarius. Many of the structures implicated in SIDS postmortems send inputs to the Pson nucleus. It receives ipsilateral secondary vestibular afferent projections from vestibular nuclei, bilateral descending projections from the fastigial nuclei and cerebellar Purkinje cells located in the ipsilateral uvula-nudulus. It also receives input from the posterior hypothalamus (Cavdar et al., 2001) and serotonin-mediated vasopressor inputs from the midline medulla (Potas et al., 2003). Serotonergic neurons are sensitive sensors of CO₂ that maintain pH. An increase or decrease in blood concentration of a little as 100 nM of free protons can be fatal (Richerson, 2004).

Cytometric patterns characterized as active astroglialosis in the dorsal vagal and solitary nuclei have often been reported in the medulla of SIDS infants compared to controls (Sawaguchi et al., 2003). Recent research has shown that astrocytes are more than a passive synctium and play a significant if not major role in information processing. For example blocking astroglial responses increased the neuronal response to stimulation in the visual cortices (St-Pierre et al., 2008).

Prenatal exposures to very weak (10 to 200 nT) physiologically-patterned magnetic fields produced long-term changes in blood chemistry and subtle anomalies in cellular organization within the hippocampus (Schummers et al., 2008). Prolonged exposures to weak, physiologically-patterned magnetic fields affect NO (nitric oxide) levels (Akdag et al., 2007). NO reduces blood pressure in the solitary nucleus (Lin et al., 1999, Wu et al., 2002) within which there is reciprocal regulation of NO and glutamate (Lin et al., 2000); NO is directly (as is adenosine) involved in hypoxia induced vasodilation (Sharp and Bermanin, 2004). Weak magnetic, time-varying, pulsed magnetic fields also affect mu opiate receptors (Thomas et al., 1997) which may be relevant considering the association between opiate usage in mothers and SIDS (Kinney et al., 1998) and the recent discovery of endomorphin neurons and mu-receptors within the Pson and solitary nuclei (Wang et al., 2002).

Although, the biophysical mechanism by which such weak magnetic fields could affect cellular function must still be elucidated, Persinger (2006) has suggested that proton resonance may be sensitive to specifically tuned intensities and frequencies. The reason 5 nT was selected in the present study was because of the solution from this resonance equation. The resonance frequency for a 5×10⁻⁷ T field influencing a charge of 1.6×10⁻²⁷ kg is 0.5 Hz. It may be relevant that the average intensity of the interplanetary magnetic field embedded within the solar wind, whose fluctuations in density and velocity are the major correlates of geomagnetic activity, is between 5 and 10 nT. The voltage gradient if reconnecting flux lines in the geomagnetic field is about a microV/cm, well within the range of detection by some biological systems (Adey, 1988).

Proton channels are present within neurons, microglia, granulocytes, connective tissue and a variety of epithelium (Decoursey, 2003). We suggest that protracted modulation of proton channels by chronic exposure to frequency and intensity-tuned magnetic fields during ontogenesis could affect the expression of neuronal asymmetries and transiently alter the microstructure and function within vital regions of the brain stem. Because sudden unexpected death in infants involves less than 1% of the age-range population during the critical period, there are obviously several variables that must converge before a fatality occurs.

ACKNOWLEDGMENTS

Thanks to Mr. Mathew D. Hunter for his technical support and contributions. Portions of this research was supported by grants from the Canadian Foundation
for the Study of Infant Deaths for the years 2004 and 2000 (Dr. S. Segal Research Grant).

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