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A MODEL OF SYMPTOMATIC INFANTILE SPASMS SYNDROME

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Abstract

Infantile spasms are characterized by age-specific expression of **epileptic** spasms, hypsarrhythmia and often result in significant **cognitive impairment**. Other epilepsies or autism often ensue especially in symptomatic IS (SIS). Cortical or subcortical **damage, including** white matter, **have** been implicated in the pathogenesis of SIS. To generate a model of SIS, we recreated this pathology by injecting rats with lipopolysaccharide and doxorubicin intracerebrally at postnatal day (P) 3 and with p-chlorophenylalanine intraperitoneally at P5. Spasms occurred between P4–13 and were associated with ictal EEG correlates, interictal EEG abnormalities and neurodevelopmental decline. After P9 other seizures, deficits in learning and memory, and autistic-like behaviors (indifference to other rats, increased grooming) were observed. **Adrenocorticotropic hormone (ACTH) did not affect** spasms. **Vigabatrin transiently suppressed spasms at P5**. This new model of SIS will be useful to study the neurobiology and treatment of SIS, including those that are refractory to ACTH.

Keywords

Infantile spasms; West syndrome; autism; animal model; epilepsy; lipopolysaccharide; doxorubicin; p-chlorophenylalanine; neonatal rat

Introduction

The infantile spasms (IS) syndrome is an age-related epileptic disorder characterized by spasms and hypsarrthythmia, described as high amplitude multifocal epileptiform discharges on a disorganized background(Hrachovy and Frost, 2003). Ictal correlates include the electrodecremental response but can be variable(Kellaway et al., 1979). The emergence of the spasms is often associated with developmental **decline** that may improve if spasms are treated

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effectively (Caplan et al., 2002; Goh et al., 2005). Many patients with IS eventually develop other intractable epilepsy syndromes (Rantala and Putkonen, 1999), mental retardation (Riikonen, 1982; Riikonen and Amnell, 1981)and autistic features(Rantala and Putkonen, 1999; Riikonen and Amnell, 1981) leading to lifelong debilitation. Under the current International League Against Epilepsy classification, IS are classified into symptomatic, cryptogenic and idiopathic (Watanabe, 1998). Symptomatic IS (SIS) result from pre-existing brain disorders, often multifocal pathology, and comprise the largest proportion of cases (Hrachovy and Frost, 2003; Watanabe, 1998). Multiple etiologies have been described, often as a combination of additive insults (Watanabe, 1998). In cryptogenic IS, a central nervous system dysfunction is suspected but remains unidentified[8]. In idiopathic cases the cause is suspected to be genetic.

IS are refractory to most conventional antiepileptic drugs. The recommended first line therapies are adrenocorticotropic hormone (ACTH) and, in patients with IS due to tuberous sclerosis, vigabatrin. ACTH **and vigabatrin are** not always effective and may have potentially **severe** side effects(Mackay et al., 2004). The prognosis is better when treatments are effective in controlling IS and even patients with SIS may not regress and continue to acquire developmental milestones at a rate determined by the underlying etiology(Caplan et al., 2002; Goh et al., 2005; Primec et al., 2006). For this purpose, it is crucial to develop successful animal models of SIS to study mechanisms and design **more** safe and effective treatments. Here we describe an animal model of SIS that satisfies many of the criteria for a successful model of the IS syndrome(Stafstrom et al., 2006). This model was developed based on evidence that structural or functional abnormalities in cortical or subcortical structures or their connections may be necessary to produce IS(Lado and Moshe, 2002). Doxorubicin (DOX) and lipopolysaccharide (LPS) were given intracerebrally at postnatal day (P) 3 and p-chlorophenylalanine (PCPA) was given intraperitoneally (i.p.) at P5. DOX is an antineoplastic agent that, when injected intraventricularly results in diffuse brain damage involving the forebrain and brainstem based on previous reports in adult rats(Siegal et al., 1988). Intracerebral injection of LPS in rat pups activates inflammatory cascades resulting in hypomyelination, white matter rarefaction and necrosis(Pang et al., 2003). PCPA depletes serotonin by inhibiting the enzyme tryptophan hydroxylase, which catalyzes the conversion of tryptophan to serotonin (Rattray et al., 1996). Low serotonin CSF metabolite, 5-hydroxyindoleacetic acid has been observed in patients with IS(Silverstein and Johnston, 1984).

We describe the seizure semiology, EEG features, neurodevelopmental deficits, neuropathology and the response of spasms to ACTH **and vigabatrin**.

Methods

Animals

These studies were done in the male offspring of timed pregnant Sprague Dawley rats. Female rats were excluded from this study to avoid gender-related developmental and phenotypic differences. The day of birth was considered as P0 and on P3 the litters were culled to 8 pups. Pups were divided into two experimental groups: 1) Pups that received DOX/LPS/PCPA (DLP pups) 2) handled control pups, which were monitored in parallel to DLP pups. In the subsequent experiments, handled control pups injected with vehicle and naïve pups were combined as no differences in seizure phenotype and neurodevelopment were found. Litters were culled to 8 pups at P3. The pups were kept with the dam throughout the experiments, unless they were monitored. Pups were maintained in a 12hr light/dark cycle with free access to food and water until P20 when they were sacrificed for histological studies. Animal care and use conformed to institutional policy and guidelines of the American Association for the Accreditation of Laboratory Animal Care. All procedures and experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Agents used

After extensive screening studies (supplementary materials) the doses of DOX (5μg/2.5 μl) and LPS (3μg/1.5 μl) were selected that produced the maximal frequency of spasms with minimal mortality. Intracerebral infusions of DOX and LPS were done stereotaxically at P3 under isoflurane anesthesia. Pups were positioned in a stereotaxic frame for neonatal rat surgery (Benchmark Angle One, MyNeurolab.com, St Louis MO). DOX was injected into the right lateral ventricle followed by LPS into the right parietal cortex. The following coordinates were used: DOX: 2.68mm anterior to lamda; 1.1mm lateral to sagittal suture; 3.3 mm deep; LPS: 2.55mm anterior to lambda; 1mm lateral to sagittal suture; 1.7 mm deep. After the injections the skin was closed with Vetbond © and the pups were allowed to recover on a heating pad before returning them to their mother. At P5, pups were injected with PCPA 200 mg/kg i.p.. Sterile saline was used as the vehicle for the three injections.

Behavioral and video-EEG monitoring

The behaviors of the pups were monitored using a video camera for 2hours twice daily from P4 (first post-operative day) until P20. The videos were analyzed for evidence of spasms and other seizures.

Epidural recordings were obtained from 8 DLP and 5 vehicle-injected control pups. P6–9 pups were anesthetized and positioned in the stereotaxic frame as described above and a circuit board (Pinnacle Technology) configured to obtain both EEG and EMG activity was affixed to the skull using Vetbond © and four stainless steel screws. EMG was recorded using two stainless steel subdermal electrodes overlying the trapezius muscles. Pups were individually placed in beakers warmed in a water bath and filled with bedding (31–33 ° C). Video-EEGs were recorded for 2h twice daily (9–11 AM and 3–5 PM) from P7 – 20 except for weekend days (1 daily session only at P7 and P8) using Pinnacle Technology and Stellate systems where appropriate.

Depth EEG recordings were obtained from the entorhinal cortex contralateral to the injection of DOX and LPS in three additional DLP pups that developed flexion spasms and in one non-injected control. In this study, the following coordinates were used: 4.0mm posterior to bregma, 3.3mm left of the sagittal sinus, 6.0mm deep to the skull surface. EEG recordings were obtained during a 45 min session starting from P9 once in two experimental pups and in three consecutive days in the other experimental pup and control pup.

Interictal EEG activities were analyzed in DLP pups and controls in 15 min epochs. To account for state-specific changes in spectral patterns, comparisons were done in the: “awake state” defined as the period the pup was exploring or moving around and the “resting state” when pups were either resting on their side (P7–9) or standing (after P9) without exploratory movements for at least 5 min. Segments with behavioral seizure activity were excluded. To adjust for age, EEGs were separated into three groups: P7–9, P10–13, P14–20. The following parameters were measured: a) discontinuity, defined as intermittent attenuation of the EEG background, at least 5 times lower than the baseline, for more than 10 sec; (b) occurrence of spikes, i.e. sharp waveforms greater than 1.5 times the baseline in amplitude which lasted less than 70msec; (c) Fast Fourier Transform (FFT) analysis was also performed with a 5.2 sec FFT length, 300 sec interval restart without overlapping.

Developmental tests

Pups in all groups underwent a battery of developmental behavioral tests that included the following: 1) Surface righting time (SRT) 2) Negative geotaxis (NG) and 3) Open field activity (OFA) (P3–P20) (Mikulecka and Mares, 2002) 4) Social chamber (P12) (modified from

(Bolivar et al., 2007) **(Crawley, 2004)**) 5) Barnes Maze (P16–19) (modified from(Barnes et al., 1980)).

Histology

The brains of 5 DLP pups were obtained at different time points to evaluate, qualitatively, the extent of lesions induced by DLP. The brains were fixed in formalin, sectioned and stained with thionin according to established protocols (see supplementary materials for methods).

Treatment with ACTH and Vigabatrin

Prior to treatment with ACTH the presence of spasms was documented after obtaining a 2h baseline monitoring in the morning and 1h pre-injection baseline in the afternoon. **A depot formulation of the tetracosactide**, Cosyntropin (Synacthène Retard, Novartis, Switzerland) was injected at a dose of 0.0125mg/kg/day i.p. (P4–P13), which is equivalent to 1.25U/kg/day of $ACTH_{1–39}$ (n=8 rats). Cosyntropin is the $ACTH_{1–24}$ fragment of ACTH, which has the same sequence in all known species [(http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=170660) (Uhler and Herbert, 1983)]. Equal volumes of vehicle were injected in 6 control rats. Higher doses (cosyntropin 0.025mg/kg/**dose given every 48 hours** i.p.) were lethal in 3/3 pups by P11. Pups were injected daily and monitored for 2h following the injection. Monitoring continued twice daily with two-hour sessions till P13 when they were sacrificed. To test the effects of vigabatrin, we used different doses ranging between 20mg/kg and 200mg/kg total daily dose. Because of significant sedation, resulting in poor feeding and early death, most experiments were done with the 20mg/kg i.p. twice daily regimen (n=6 pups), which was better tolerated. Results were compared with vehicle-treated pups (n=9 pups).

Statistics

Results are expressed as least square means +/− standard errors. The Student's *t*-test was used to compare the means of two groups of unpaired data; for multiple group comparisons, the one-way or multifactorial analysis of variance (ANOVA) was used with Tukey post hoc comparisons as appropriate. The critical significance level of all tests was $p < 0.05$.

Results

Spasms, other seizure types and responsiveness to ACTH and Vigabatrin

Flexion, extension or mixed flexion/extension spasms were observed in all DLP pups. Flexion spasms were characterized by the abrupt onset of flexion of the trunk with forward tonic extension of the limbs at the height of the spasm (Figure 1, video 1). The extension spasms were characterized by the sudden hyper-extension of the back with tonic posturing of the limbs (video 2). Spasms first appeared at P4, i.e. prior to the PCPA injection, indicating that PCPA is not necessary for the generation of spasms. However higher PCPA doses were associated with higher frequencies of spasms and therefore PCPA was maintained in our protocol. Spasms typically occurred during the resting state. Spasms were observed only between P4–P13, peaked between P4–P6 and subsequently declined (Figure 2). In controls, rare sporadic abrupt events consisting of brief flexion or extension were observed that resembled spasms were observed, but these were rare, did not appear as forced and were typically in the context of changing their position or stretching.

Other seizures were observed in 67 % in DLP pups starting from P9–20 that included behavioural arrest and wild-running (video 3) seen between P9–12. Tonic seizures, myoclonus with sudden drops, and stage 5 limbic seizures associated with forelimb clonus, rearing and falling seen after P13 (video 4). Myoclonic jerks were very frequent in DLP pups. Less frequent

myoclonic jerks were observed in control pups, as previously reported by other investigators (Blumberg et al., 2005). Behaviourally, they were distinguished from spasms in that they were very brief without tonic posturing.

All EEG-monitored DLP pups with spasms had spasms with electrographic correlates. In the epidural EEG recordings, electrodecremental responses (i.e. background attenuation) were noted in 27% of spasms with electrographic correlate (Figure 3). Spike and sharp wave discharges and/or fast rhythmic activities comprised the 73% of electrographic correlates of spasms. However, within the same DLP pups, 51% of captured behavioural spasms either did not have clear EEG correlate (42%) or were associated with artifact (9%). In the depth recordings, electrodecremental pattern time-locked with the spasms in all captured events (figure 4, video 5). In controls, none of the above electrographic events occurred even in the presence of the flexion or extension episodes described earlier.

Interictally, frequent spikes or runs of high amplitude spike and slow wave discharges at both the awake and resting state were observed only in P7–13 DLP pups, (Figure 5). Rare isolated spikes of unclear significance occurred in controls perhaps due to immaturity or the prior surgery. Discontinuity of the background activities was seen during the early period of spasms in DLP pups (36% of EEGs, 3/7 DLP P7–9 pups) and not in controls. This difference was significant during the awake state ($P<0.01$, chi-square). Spectral analysis showed increased alpha only during the resting state, i.e the state when spasms usually occurred in P7–9 DLP pups **(data not shown)**. At P7–13, a shift towards both alpha and theta activities was seen in DLP pups, at either awake or resting state.

Electrographic seizure correlates to the other seizures were also identified in DLP pups. Selected examples are shown in Figure 6.

Cosyntropin did not suppress spasms (Supplementary materials Figure 1). Vigabatrin had a significant effect on spasms only at P5 ($P=0.0058$) (Figure 7). However, the associated sedation resulted in high mortality rate (50% mortality by P8, 100% mortality by P11).

Growth, mortality, developmental impairments and underlying neuropathology

There were no significant differences in the weights of DLP and control pups from P3 till P5, even though spasms had already emerged. After P5, DLP pups gained weight at a slower rate than controls (Figure 8). Mortality was highest during the period of spasms (53% till P14) and reached a plateau between P14 – P20. DLP pups had worse performance in OFA, which was sustained even after spasms stopped. Abnormal scores in NG and SRT were also seen in DLP pups in sporadic days especially during the period of spasms (Figure 8).

In the social chamber, DLP pups demonstrated overall fewer attempts to explore the rat chamber as compared to controls, in the presence of either a stranger or familiar rat (Figure 9A). This may suggest indifference to the presence of other rats, preferring to inhabit the middle compartment or may stem from the deficits in exploratory behavior described previously. DLP pups exhibited increased grooming behaviour, which are reminiscent of stereotypies (Figure 9B) (Turner et al, 2001). The increased grooming and **decreased numbers of approaches to** other rats may indicate autistic symptomatology. In the Barnes maze, DLP did not learn to find the target hole during the trial period whereas control pups showed a rapid learning curve (Figure 9C)

The brains of DLP pups showed multifocal pathology involving cortical and subcortical structures including the white matter tracts, most prominent at the right hemisphere and periventricular regions (Figure 10).

Discussion

We describe a multiple-hit model of SIS that recapitulates many of the features of human IS as far as phenotype, age specificity, ictal and interictal EEG abnormalities, multifocal pathology, developmental behavioral abnormalities and the occurrence of other seizure types once the spasms have stopped.

In humans, IS are age-specific with a peak onset between 3–7 months (Jeavons et al., 1973). Similarly DLP pups developed spasms between P4–13, classically considered to be equivalent to human neonatal period. However, the drastic improvement in OFA scores between P4–13 pups indicate that this period is equivalent to the developmental period when pups start to develop their ambulatory abilities, a motor milestone that is normally reached during the infantile stage in humans. No spasms were observed beyond P13 but instead we observed other seizure types. Evolution to other epilepsies, including the Lennox-Gastaut syndrome, is well described in human IS (Jeavons et al., 1973; Rantala and Putkonen, 1999; Riikonen, 1982).

Variable ictal discharges were observed with spasms, including electrodecremental-like responses. Similarly, in one study that characterized the ictal correlates of 5042 spasms, 11 ictal abnormalities were observed in association with IS(Kellaway et al., 1979). Classic electrodecremental response was seen in only 37.9 % of spasms. Similar to our model, they also described sharp/slow waves, voltage attenuation and fast activity as ictal correlates. In a proportion of spasms, ictal EEG correlates were not observed in our model. In part this is attributable to movement artifact making the EEG uninterpretable. However, IS in humans can occur without EEG correlates even in the same cluster of spasms where ictal correlates have been identified(Kellaway et al., 1979; Plouin et al., 1993). The reason for this is unknown but it has been suggested that some spasms may have a subcortical generator which can be associated with changes in electrical cerebral activity that do not always propagate to the EEG electrodes(Lado and Moshe, 2002). Although we cannot confirm that DLP pups have hypsarrhythmia as the small size and fragility of their skull limits the number of electrodes that can be placed; the interictal EEG was clearly abnormal suggestive of an epileptic encephalopathy.

In line with human IS, DLP pups showed deficits in the developmental tests in part due to the lesion, resultant neurological deficits and comorbid conditions but possibly also because of the underlying epileptic encephalopathy. In support, the abnormalities in SRT, OFA, NG started at P7, after the onset of spasms, and then partially improved after spasms disappeared. Autism is a neurodevelopment disorder characterized by fundamental core features which include inappropriate social interactions, poor communication skills and ritualistic repetitive behaviors (stereotypies) (Crawley, 2004). Autism is a commonly reported comorbidity in patients with SIS but not in patients with cryptogenic IS, suggesting that the underlying brain lesion or pathology may be more important in the expression of autistic symptomatology than infantile spasms (Riikonen and Amnell, 1981; Saemundsen et al., 2008). To test DLP pups for autistic features we used the social chamber which is an established paradigm to evaluate for abnormal social interactions in rodent models (Crawley, 2004). In this paradigm rodents with autistic features have abnormal approaches to a familiar and-or stranger rat. When tested between P16–19, a period when spasms had resolved but other seizure types were still present, DLP pups had decreased social interactions, as indicated by the reduced number of entries to the rat compartment. The underlying neurological deficits due to the induced brain lesion may contribute to the abnormal social behavior, by impairing the exploratory activity. However, this is also true for the human condition, as only infants with SIS have a greater risk to manifest autistic symptomatology. Pups with IS also demonstrated increased grooming which is thought to be consistent with stereotypies described in autism (Crawley, 2004; Turner et al, 2001). In addition DLP pups performed poorly in tasks requiring learning through visuospatial cues

consistent with profound cognitive deficits patterning human mental retardation. Such deficits in visuospatial learning can be at least partially expected by the presence of the right parietal and hippocampal lesion due to the DOX/LPS injections. The extent to which the prior spasms and the underlying epileptic encephalopathy contribute to these cognitive deficits will be best determined when effective antiepileptic therapies are identified in the DLP model.

A limitation of the model is the significant mortality but again 5–30% of the children with IS die and of these deaths 50% are disease-related (Appleton, 2001; Mackay et al., 2004; Rantala and Putkonen, 1999; Riikonen, 1982; Snead et al., 1983). Furthermore, mortality is greater in symptomatic cases(Dulac et al., 1997). At present, it is not possible to differentiate between the impact of spasms and other seizure types versus the structural injury of the brain or co-morbid conditions on these deficits. ACTH was ineffective in treating the spasms in the DLP model, in accordance with clinical reports indicating that human infants with SIS are less likely to respond to ACTH than infants with cryptogenic IS. Vigabatrin resulted in transient suppression of spasms in DLP pups but was also associated with significant mortality, as it caused significant sedation and consequently poor feeding and malnutrition. However, when **more** effective **and safer** therapies **are identified** that suppress the spasms and subsequent seizures in the DLP model, we will be able to determine whether the cognitive deficits and mortality observed in the DLP model can be partially improved by effective antiepileptic therapies, as reported in human patients with IS (Lux et al, 2005).

Several other etiology-based models of infantile spasms have been proposed to model human IS which are reviewed in (Stafstrom, 2009). The corticotropin-releasing hormone (CRH) model was based on the hypothesis that ACTH mediates its effects by suppressing CRH(Brunson et al., 2001). However, intraventricular administration of CRH to neonatal rats results in primarily “limbic” seizures with no reported spasms (Brunson et al., 2001). Another model involves i.p. injections of N-methyl-D-aspartic acid (NMDA) which result in acute, age-specific, expression of spasms that do not last beyond the period of NMDA exposure(Velisek et al., 2007). Prenatal exposure to betamethasone renders the NMDA-induced spasms sensitive to ACTH(Velisek et al., 2007). The tetrodotoxin model, generated by infusions of tetrodotoxin to cortical or hippocampal regions, recapitulates the ictal and interictal EEG patterns in IS, **and models cryptogenic or symptomatic IS** (Stafstrom, 2009), but the seizures are observed in rats starting at P21(Lee et al., 2008). Deletion of the *aristaless-related homeobox* (ARX) gene in GABAergic interneurons in mice results in limbic seizures and subsequently spasms in adulthood(Marsh et al., 2009). Knockin mice with expansion of the polyalanine tract of the ARX gene manifest spasm-like myoclonus starting at P7, ictal and interictal EEG patterns, evolution to spontaneous seizures, low anxiety, impaired learning and social interactions (Price et al., 2009). The pharmacosensitivity of the tetrodotoxin and ARX models is not known yet. Administration of baclofen or γ-butyrolactone to the Ts65Dn mouse model of Down syndrome results in brief myoclonic jerks **resembling extensor spasms** acutely in both infant and adult mice(Cortez et al., 2009). These were significantly reduced by vigabatrin, the rodent $ACTH_{1–24}$ fragment, ethosuximide, valproic acid and the $GABA_B$ antagonist CGP 35348. The existing models include therefore both acute models of spasms (NMDA-induced spasms, baclofen or γ-butyrolactone-induced spasms in Ts65Dn mice) as well as models in which spasms are expressed over a longer period of time (DLP, tetrodotoxin, ARX models). The availability of different **acute or chronic** etiology-based models of IS with differential sensitivity to available candidate therapies will be important to improve our understanding of this heterogeneous disease, identify common pathogenetic pathways and candidate therapies with either restricted or broader range of indications for the different types of IS, prior to proceeding with the clinical testing.

In this model, spasms are associated with cognitive impairment and autistic features and evolve to other seizure types. One of the advantages is that the spasms occur specifically in the early

postnatal period. The mechanisms underlying seizure generation as well as the effects of drugs may be completely different in the developing brain compared to adults(Kaindl et al., 2006; Veliskova et al., 2004). The reliable appearance of spasms for several days will be invaluable in testing drugs with immediate or delayed efficacy in ACTH-refractory SIS as well as identifying other age-specific antiepileptogenic therapies to treat this catastrophic condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACTH	adrenocorticotropic hormone
ARX	**aristaless-related homeobox**
DOX	Doxorubicin
EEG	electroencephalogram
EMG	electromyogram
FFT	Fast Fourier Transform
IS	infantile spasms
DLP pups	DOX/LPS/PCPA injected pups
LPS	lipopolysaccharide
NG	Negative geotaxis
NMDA	N-methyl-D-aspartic acid
OFA	Open field activity
PCPA	p-chlorophenylalanine
P	postnatal day
SIS	symptomatic infantile spasms
SRT	Surface righting time

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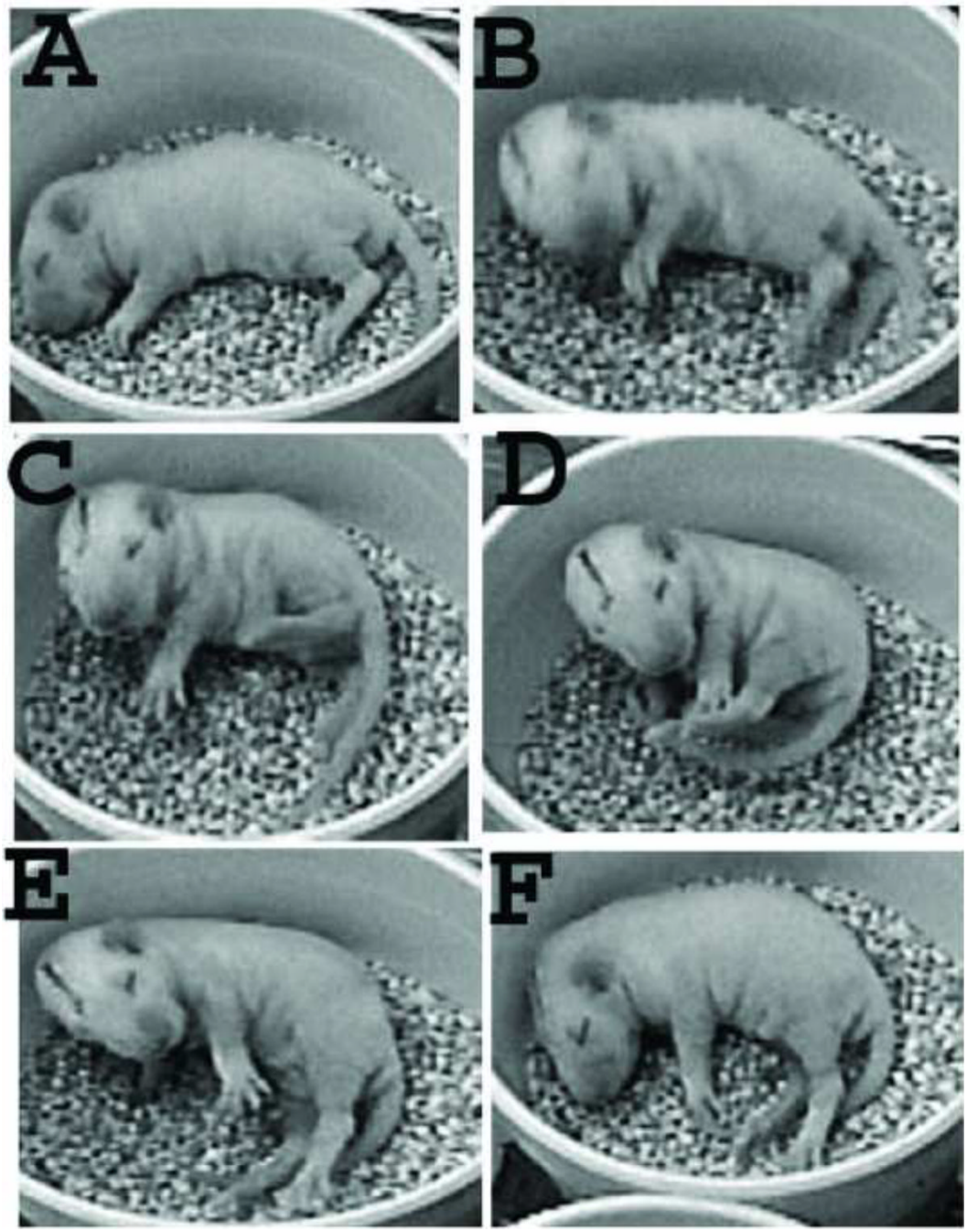

Figure 1.
Flexion spasm in a P9 rat following injections of DOX and LPS at P3 and PCPA at P5. (A) The pup is lying on its side prior to the spasm. (B) The pup raises its head abruptly at the start of the spasm. (C) This is followed by flexion of the trunk, extension of the left forelimb and flexion of the left hindlimb. The head is still raised. (D) The trunk is maximally flexed and there is extension of the left forelimb and both hindlimbs (E) The end of the spasm starts with relaxation of the trunk, however there is continued extension and abduction of the left forelimb. The left hindlimb is still mildly extended. (F) Postictal state. The pup is resting in a mildly flexed position prior to the emergence of a second spasm.

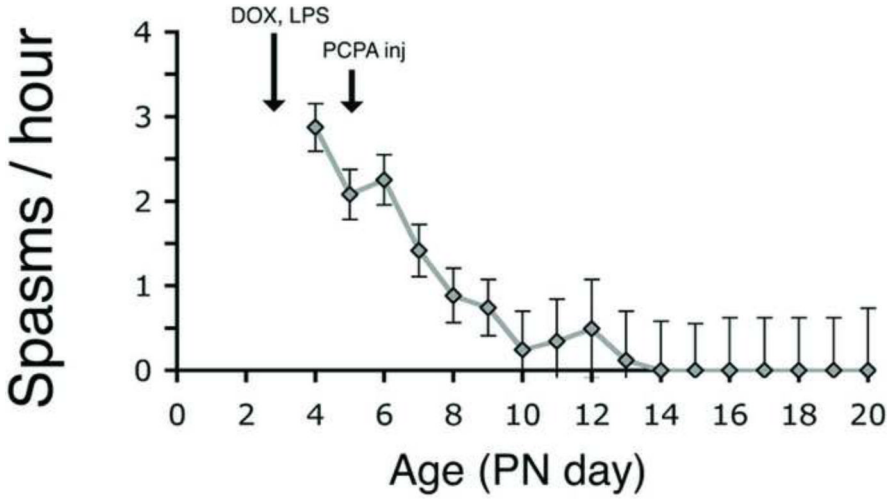

Figure 2.
Age-specific appearance of spasms in DLP pups. Daily monitoring with two daily 2h-sessions was done starting at P4 till P20 in DLP pups. Spasms appear at P4, peak during P4–7 and disappear after P13.

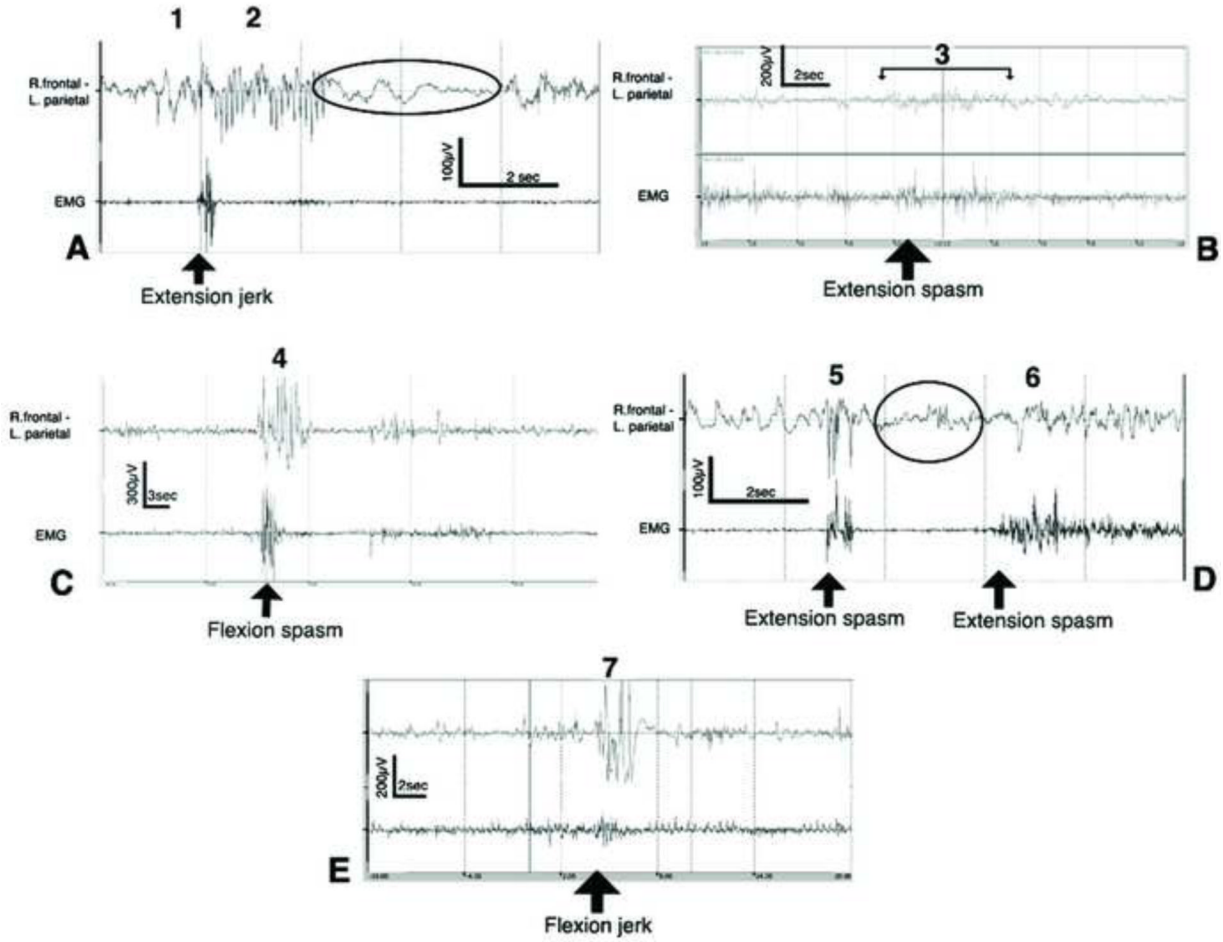

Figure 3.

Examples of ictal EEG patterns in P7–9 DLP pups using epidural recordings. The montage utilized here is bipolar: the upper channel is the right frontal-left parietal derivation and the lower channel is the EMG channel to demonstrate the occurrence of spasms (burst of EMG). Spasms in DLP pups showed different EEG ictal correlates, including preictal rhythmic delta (#1) followed by fast (8–9Hz) rhythmic activity (#2) and attenuation (circled) (panel A); fast low amplitude rhythmic activity (#3, panel B); burst of polyspikes (#4) (panel C); polyspikes (#5) followed by attenuation (circled) followed by a second spasm without an EEG correlate (#6) (panel D); high amplitude polyspike and slow wave discharge with attenuation with fast activity electrodecremental response) (#7) (panel E).

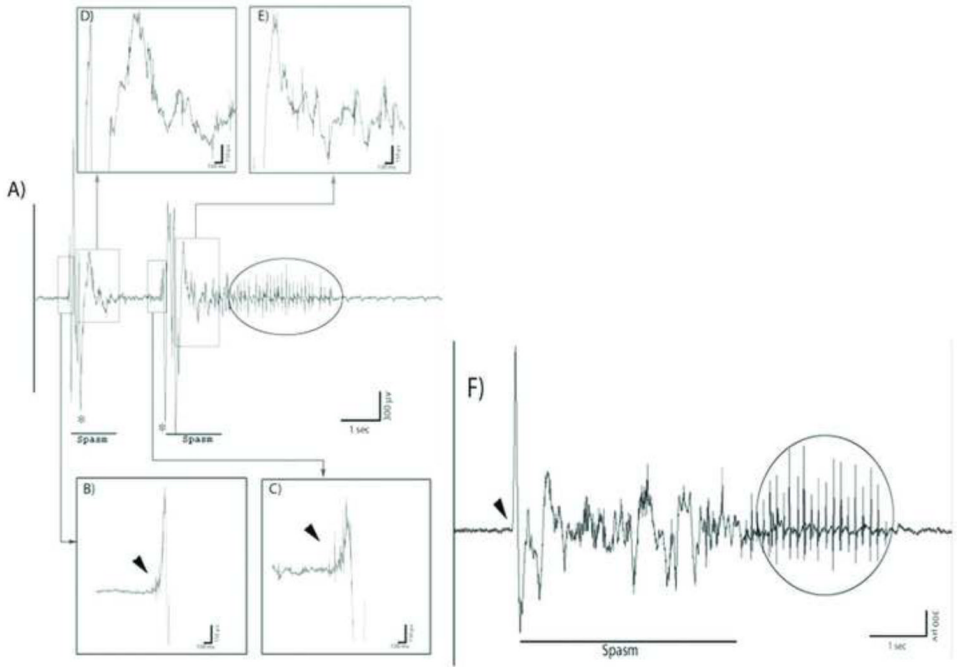

Figure 4.

Depth EEG recording from the entorhinal cortex during a cluster of 2 spasms in a P9 pup (video 5).

Panel A: The ictal discharge is characterized by the initial appearance of rapid polyspike activity, which precedes the behavioral seizure (inserts B and C, arrowheads, expanded time frame). A movement artifact obscures the onset of the spasm (asterisk). The EEG correlate during the latter part of the spasm consists of a slow wave with overriding polyspikes (inserts D and E, expanded time frame). *Panel F:* EEG recorded during another spasm. Please note the lack of polyspike activity preceding the spasm. The arrow points to the time the rapid polyspikes seen in the spasms depicted in Fig. 4A, inserts B and C. The different patterns observed at the ictal onset may indicate that there are various generators of the spasms. The second spasm in Figure 4A and the spasm in 4F are followed by a focal discharge (circled). This has also been described to occur in some humans with SIS.

Figure 5.
Interictal EEG abnormalities in DLP pups.
Epidural EEG recorded in a pup with spasms show high amplitude spike/polyspike and slow wave activity not associated with any behavioral manifestations. Horizontal bar= 2 sec, Vertical bar= 200 μV.

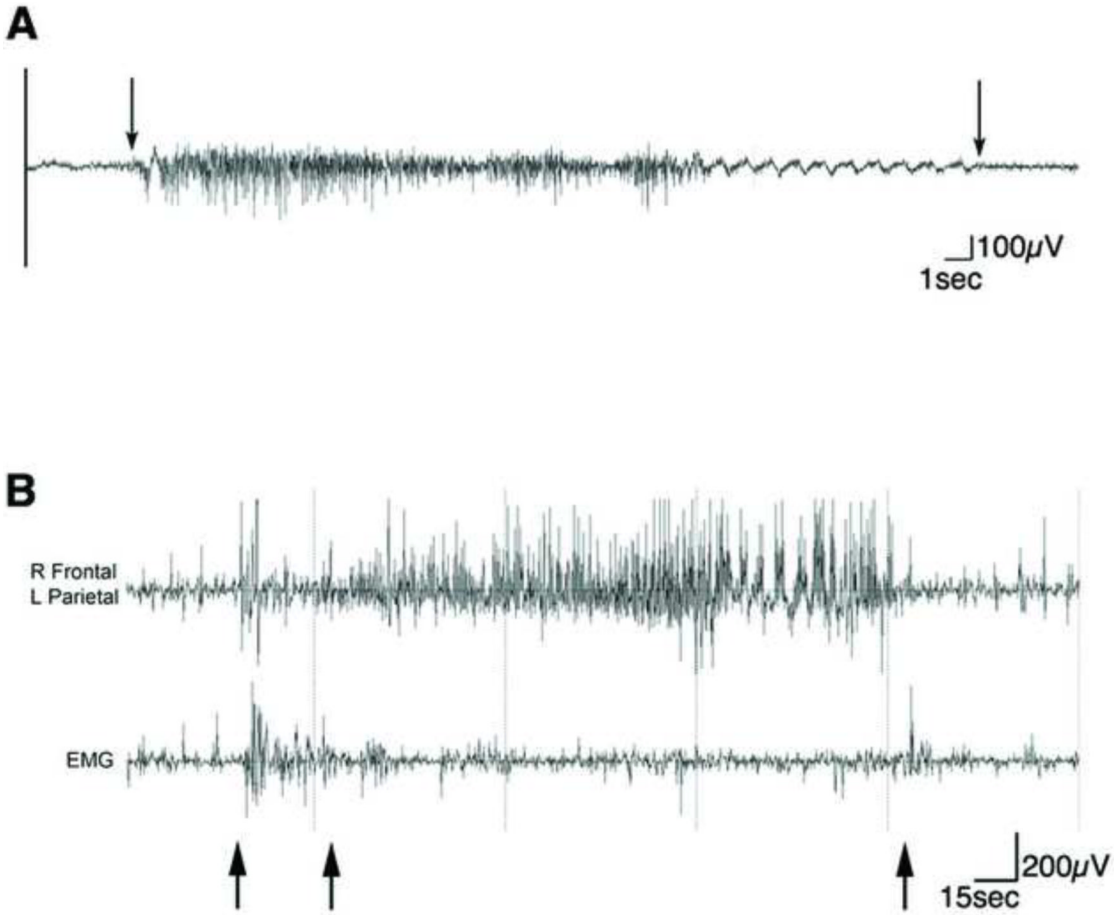

Figure 6.
Ictal patterns of other seizure types in DLP pups.
Panel A: EEG seizure discharge obtained from depth electrode recording from the entorhinal cortex of a P11 pup with prior spasms. The arrows indicate the beginning and the end of the seizure. During the seizure the rat exhibited behavioral arrest without any clonic movements.
Panel B: Epidural electrode recording in a P9 pup with spasms. During the seizure the pup was displaying “wild running” behavior between the 2 first arrows, followed by behavior arrest (video 3). The electrographic seizure pattern ends at the third arrow.

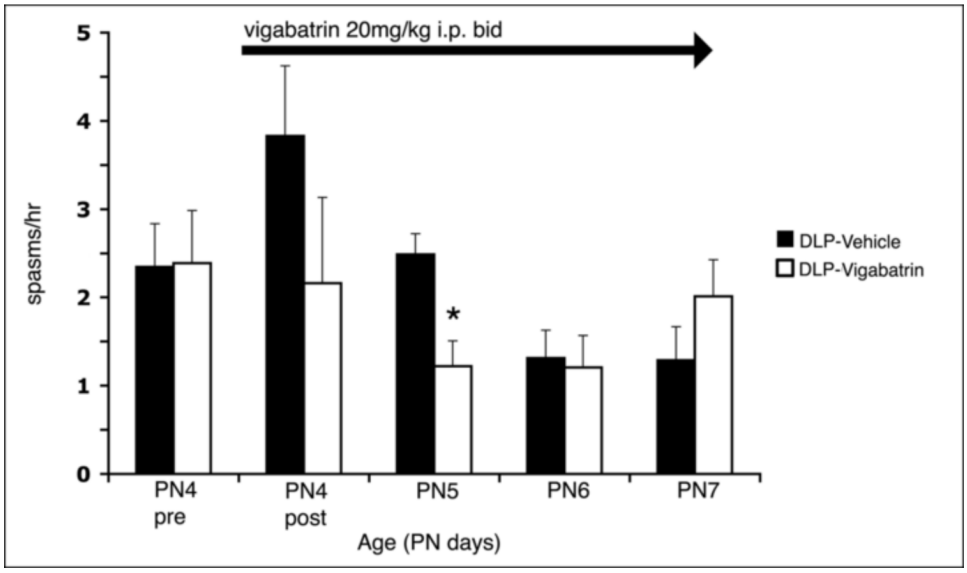

Figure 7.

Vigabatrin transiently suppresses DLP spasms.

Vigabatrin treatment (20 mg/kg/dose i.p. twice daily (bid), n=6 male pups), starting at P4) significantly reduced the frequency of spasms at P5 only. The results were compared with vehicle-treated male pups (n=9 pups). * P=0.0058 compared to controls.

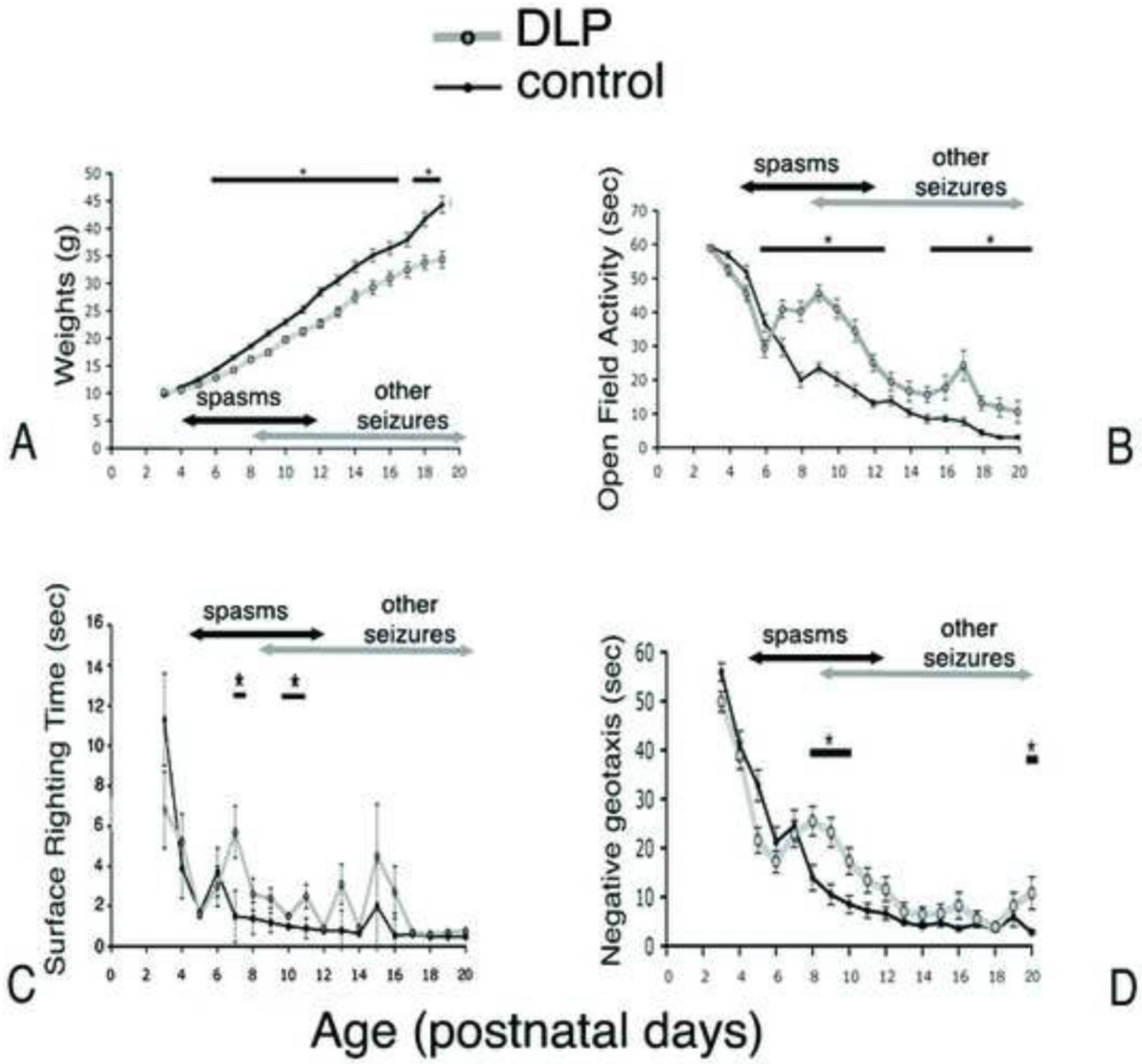

Figure 8.

Abnormalities in developmental milestones in DLP pups.

DLP pups showed deceleration of weight growth after P5 (panel A), worse scores in OFA after P7 (panel B), and scattered days with worse scores in SRT (panel C) and NG (panel D), after P7, compared to controls (CON).

*: P<0.05 compared to controls.

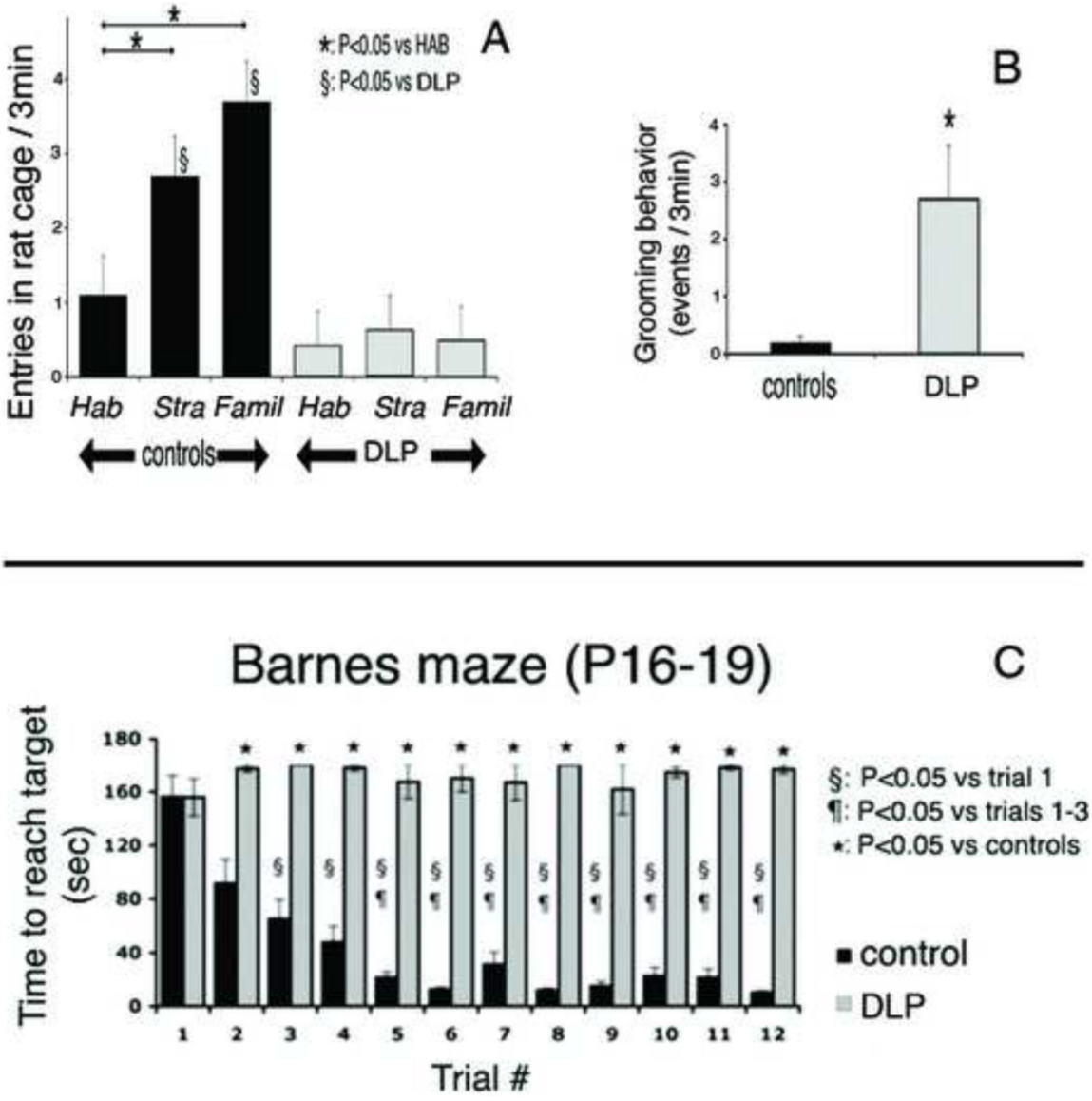

Figure 9.

Abnormalities in sociability, grooming behavior, and visuospatial learning in DLP-treated pups with spasms.

Panel A: The description of the social chamber test is described in the supplementary materials. Controls increased in the number of entries to the rat chamber in the presence of a stranger or familiar rat ($P<0.05$). In contrast, no change in the number of entries was seen in DLP pups with spasms. *Hab*: habituation; *Stra*: in the presence of a stranger rat; *Famil*: in the presence of a familiar rat.

Panel B: DLP pups showed increased grooming behavior reminiscent to stereotypies.

*: $P<0.05$ compared to controls.

Panel C: During the Barnes maze testing, pups underwent a training period during P16–P19 with 3 trials per day where they were trained to find a target open hole leading to a dark box. Control pups learned the task already by the fifth trial (2^{nd} day). In contrast, DLP pups were unable to find the target.

*: indicate $P<0.05$ compared with the controls tested on the same day.

§: indicate $P<0.05$ compared with same group performance in trial 1.

¶: indicate P<0.05 compared with same group performance in trials 1–3.

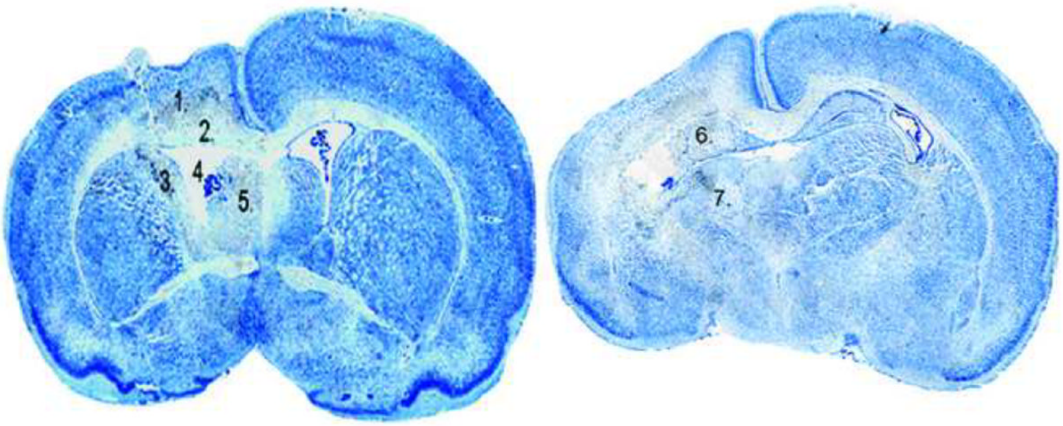

Figure 10.
Thionin stained coronal sections of a P11 pup with spasms showing showing mostly right hemispheric and periventricular injury that includes 1) thinning of the right hemi-cortex and diffuse damage to the 2) corpus callosum fibers, 3) striatum, 4) periventricular areas, 5) septum, 6) hippocampus and 7) thalamus on the right.