Published in final edited form as:

J Primatol. 2015 December ; 4(2): . doi:10.4172/2167-6801.1000129.

Cerebrospinal Fluid Levels of Monoamine Metabolites in the Epileptic Baboon

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Abstract

The baboon represents a natural model for genetic generalized epilepsy and sudden unexpected death in epilepsy (SUDEP). In this retrospective study, cerebrospinal fluid (CSF) monoamine metabolites and scalp electroencephalography (EEG) were evaluated in 263 baboons of a pedigreed colony. CSF monoamine abnormalities have been linked to reduced seizure thresholds, behavioral abnormalities and SUDEP in various animal models of epilepsy. The levels of 3-hydroxy-4-methoxyphenylglycol, 5-hydroxyindolacetic acid and homovanillic acid in CSF samples drawn from the cisterna magna were analyzed using high-performance liquid chromatography. These levels were compared between baboons with seizures (SZ), craniofacial trauma (CFT) and asymptomatic, control (CTL) baboons, between baboons with abnormal and normal EEG studies. We hypothesized that the CSF levels of major monoaminergic metabolites (i.e., dopamine, serotonin and norepinephrine) associate with the baboons' electroclinical status and thus can be used as clinical biomarkers applicable to seizures/epilepsy. However, despite apparent differences in metabolite levels between the groups, usually lower in SZ and CFT baboons and in baboons with abnormal EEG studies, we did not find any statistically significant differences using a logistic regression analysis. Significant correlations between the metabolite levels, especially between 5-HIAA and HVA, were preserved in all electroclinical groups. While we were not able to demonstrate significant differences in monoamine metabolites in relation to seizures or EEG markers of epilepsy, we cannot exclude the monoaminergic system as a potential source of pathogenesis in epilepsy and SUDEP. A prospective study evaluating serial CSF monoamine levels in baboons with recently witnessed seizures, and evaluation of abnormal expression and function of monoaminergic receptors and transporters within epilepsy-related brain regions, may impact the electroclinical status.

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The authors declare no conflict of interest.

Keywords

Cerebrospinal fluid; Epilepsy; Monoamines norepinephrine; Respiratory dysfunction

Introduction

Monoamines norepinephrine (NE), serotonin (5-HT) and dopamine (DA) have been implicated in the modulation of seizure threshold in animal models of epilepsy [1–3]. Seizures induce the secretion of brainstem NE, 5-HT and DA, which then serve as an endogenous pathway to abort seizures, status epilepticus and prevent seizure recurrences [4]. While all three neurotransmitter systems have direct cortical influences, DA also modulates seizure activity also via the basal ganglia. Of the three, 5-HT and DA have the most consistent effects on seizure threshold. Their metabolites are increased both in epileptogenic tissue as well as in the cerebrospinal fluid in people with chronic epilepsy undergoing resective surgery [5]. In rodent hippocampi, 5-HT release was briefly increased immediately after onset of pilocarpine-induced status epilepticus, whereas its main metabolite, 5-hydroxyindolacetic acid (5-HIAA) increased only two days later, indicating that the 5-HT was being metabolized locally [6]. While the 5-HIAA increases in the CSF lasted weeks, both 5-HT and 5-HIAA levels eventually decreased to below baseline levels, reflecting possible injury to the raphe nuclei. Whether the raphe nuclei were damaged by status or pilocarpine is unclear, but the emergence of spontaneous seizures was thought to reflect decreased serotonergic inhibition of the hippocampus. The genetically epilepsy-prone rat provides further support for the monoaminergic effect on seizure threshold, demonstrating an increased frequency in seizures in association with a decrease of 5-HT and NE levels [2]. Nonetheless, the temporal relationship of brain and CSF concentration changes of monoamines and their metabolites is still poorly defined in chronic epilepsy and may vary due to genetic or etiological factors underlying the epilepsy, as well as the type of epilepsy syndrome, seizures and their severity or frequency.

Furthermore, all three monoamines underlie postictal and interictal behavioral changes in people with epilepsy and in genetic animal models of epilepsy [7]. Postictal affective psychoses are triggered by increased seizure severity or duration, when these neurotransmitter systems are maximally activated. These systems appear to be underactive interictally in humans with medically refractory epilepsies, and both depression and seizure control appears to improve with treatments applying serotonin reuptake inhibitors (SSRIs). Furthermore, 5-HT may be decreased in intractable human epilepsy and its supplementation may prevent sudden unexpected death in epilepsy (SUDEP) both in humans and animal models of epilepsy [7,8]. Respiratory dysfunction in murine and rodent models of epilepsy have been linked to serotonergic dysfunction [7,8], while administration of a selective SSRI in a DBA/1 mouse model of SUDEP completely suppressed seizure-induced respiratory arrest (S-IRA) after administration [9,10].

While the baboon provides an excellent model for genetic generalized epilepsy (GGE) as well as a model of SUDEP in humans [11,12], there is little data regarding the monoaminergic activity in epileptic baboons. More than 20% of the pedigreed baboon

colony housed at the Southwest National Primate Research Center (SNPRC, Texas Biomedical Research Institute, San Antonio, Texas), have been witnessed to have generalized myoclonic, absence and/or tonic-clonic seizures [13]. In addition to witnessed seizures, craniofacial trauma (CFT), such as orbital bruising or muzzle lacerations, may be due to seizure activity [14,15]. The baboons demonstrate generalized ictal and interictal epileptic discharges on scalp EEG and about 40% of the epileptic baboons are also photosensitive. This is in line with rates of photosensitivity in wild *Papio hamadryas papio* in West Africa ranging from 10–60% [16]. While this study demonstrated that photoconvulsive thresholds could be pharmacologically altered via changes in serotonergic activity, but it is not known whether epileptic baboons have lower monoamine levels than their asymptomatic counterparts [17]. SUDEP has been reported in epileptic baboons of this pedigree, based upon evidence of sudden death in otherwise healthy baboons, and pulmonary edema being the only pathological finding in almost all of the baboons [12]. Based upon murine models of SUDEP, it is possible that the epileptic baboons may also demonstrate serotonergic dysfunction, and, if this were the case, the baboon could provide a model that is more effectively translated to humans. Therefore, we were interested in the evaluating the relationship of seizures and EEG markers for epilepsy, such as generalized interictal epileptic discharges (IEDs) or photosensitivity (PS), with cerebrospinal fluid (CSF) concentrations of monoamine metabolites, including 3-hydroxy-4-methoxyphenylglycol (MHPG), 5-HIAA, and homovanillic acid (HVA). We aimed to do this retrospectively, combining two separately acquired datasets of electroclinical phenotypes [14] and CSF studies [18]. In order to test the hypothesis that epileptic baboons will have altered levels of the monoamine metabolites, we compared their CSF concentrations based upon clinical, EEG and electroclinical classification. We compared metabolite levels between baboons with a history of witnessed seizures (SZ), craniofacial trauma (CFT), and asymptomatic controls (CTL), between baboons with IEDs or PS on scalp EEG, and between well-characterized electroclinical categories, such as baboons with seizures and/or CFT (Sz +CFT) compared to CTL baboons, each with either abnormal EEG studies, including both IEDs and PS (IED+PS), or normal EEG studies.

Methods

Animal selection

We identified 263 (196 F, 67 M) baboons (*Papio hamadryas anubis*, *P. h. cynocephalus*, and their hybrids) living in a multigenerational colony maintained at the Southwest National Primate Research Center (SNPRC) in San Antonio, Texas that had undergone both a CSF examination and a scalp EEG as part of separate studies evaluating heritability of monoamine levels and interictal epileptic abnormalities, respectively [14,18]. The pedigreed baboons at the SNPRC are housed in group cages, either designated as breeding or all male cages. The seizure animals were identified by the review of veterinary records [13]. Only those animals were classified as epileptic that had a history of at least one witnessed seizure or evidence of peri-ictal behavioral changes and interictal epileptic discharges (IEDs) on scalp EEG. This methodology underestimates seizure occurrences in the pedigree and recurrences in the individual animals, as the baboons are not being continuously observed, and clinically more subtle seizures types, such as absence and myoclonic seizures, that are

not typically recognized by the veterinary technicians. Craniofacial trauma (CFT) can also be a symptom of seizure-related falls, but as it can be a consequence of combat, it is a sensitive but not specific marker for epilepsy [14,15]. Because CFT's clinical implications and their association with an increased prevalence of EEG abnormalities than in CTL [14,15], we decided to include this group in our analyses.

There were 263 (196 female, 67 male) baboons with complete CSF metabolite measures and electroclinical data. These included 140 CTL (119 female, 21 male), 103 CFT (63 female, 40 male) and 20 SZ (14 female, 6 male) baboons (Table 1). The SZ animals had averages of at least 2 (range 1–6) witnessed seizures and 2 (range 2–12) craniofacial trauma. The CFT baboons had an average of at least 2 (range 1–10) injuries. Scalp EEG demonstrated generalized IEDs in 128 (49%) and photosensitivity in 66 (25%) of the baboons (Table 2). While these numbers indicate a high prevalence of genetic predisposition for epilepsy, it is in line with previous reports from this pedigree [16]. The CSF and EEG studies were not performed in close temporal association, the EEG studies lagging behind the CSF studies by a range of 2–6 years. All procedures adhere to the Animal Welfare Act [19] and were approved by the Institutional Animal Care and Use Committees of the Texas Biomedical Research Institute and by the Institutional Animal Care Committee of University of Texas Health Science Center at San Antonio.

CSF collection and monamine assays

Cerebrospinal fluid (CSF) was drawn once from the cisterna magna of each study baboon within 30 minutes after sedation with RAAK (rompun, acepromazine, atropine, and ketamine) [18]. The CSF samples were immediately placed on wet ice, and within 60 minutes they were centrifuged to pellet any contaminants, including blood cells. Aliquots of the supernatant were split into multiple cryogenic tubes and frozen at -80°C less than 90 min after collection. After storage at -80°C , samples were shipped to the Columbia University (New York, New York) and assayed for MHPG, 5-HIAA and HVA [20]. A measured aliquot of each sample was mixed with an equal volume of cold mobile phase. The mixture was then filtered at centrifugation (6000 g for 40 min at 4°C) and part of the filtrate transferred to a 300-FL microinjection insert. This material was then analyzed by high performance liquid chromatography with electrical detection, which allows simultaneous measurement of MHPG, 5-HIAA and HVA. The precision of this methodology is high and was validated with specific gas chromatographic-mass spectrometric (GS-MS) assays for each metabolite [20]. Overall, monoamine metabolite levels are relatively stable and more reliable than monoamine levels in the CSF [5] because monoamines are rapidly metabolized in brain tissue [6].

Scalp EEG

The methods for the scalp EEG studies were described by our group previously [16]. The surface electrodes are placed according to the standard international 10–20 electrode placement system at FP1, FP2, T8, C4, Cz, C3, T7, O1, O2, A1 and A2 positions. Electrodes were also placed bilaterally beside the eyes to monitor lateral eye movements (EOG1, EOG2), over the deltoids or paraspinal muscles to monitor skeletal muscle activity, and a single electrode monitored the heart rhythm (ECG). EEG studies were acquired on a laptop-

based acquisition machine (Grass-Telefactor, U.S.A., XLTEK, Canada, and Nihon-Kohden, Japan). Electrode impedances were below 10 kOhms. Ketamine was used to sedate animals in order to transfer them to a primate chair for electrode placement and EEG recording at a dose of 5–6 mg/kg. At these doses, ketamine can activate interictal and ictal epileptic discharges, with minimal effect on photosensitivity. As a subset of epileptic baboons may also be photosensitive, intermittent light stimulation (ILS) was performed at frequencies ranging between 1–30 Hz in two sessions at least 15 minutes apart. Photoepileptic responses were determined if the baboons demonstrated myoclonic or generalized tonic-clonic seizures with ILS, or if the IEDs were time-locked to stimulus onset or doubled in their rate to a pre-ILS baseline sample [14]. The same dose of ketamine was administered prior to the baboons' removal from the primate chair. Results of the EEG studies have been published previously [14].

Statistical analysis—Metabolite concentrations were compared among CTL, CFT and SZ groups, and between the sexes (one metabolite at a time) using a two-way ANOVA followed by a Tukey's post-hoc multiple comparison tests (Table 1; left three data columns). The correlations between pairs of monoamine metabolites for each of these groups were determined using the Pearson's correlation analysis (Table 1; three right data columns). The alpha level was set at 0.05 for all analyses. The metabolite concentrations were then compared for EEG markers of epilepsy among IED+, IED–, PS+ and PS– groups (one metabolite at a time) using one-way ANOVA with a Tukey's post-test multiple comparison tests (Table 2; left three data columns). The correlations between pairs of monoamine metabolites for each of these groups were determined using the Pearson's correlation analysis (Table 2; three right data columns). Finally, we compared the levels of metabolite among electroclinical categories, SZ+CFT with or without IED+PS and the CTL group with or without IED+PS using a one-way ANOVA with a Tukey's post-test multiple comparison tests (Table 3; left three data columns). The correlations between pairs of monoamine metabolites for each of these groups were determined using the Pearson's correlation analysis (Table 2; three right data columns). These last categories aimed to include the electroclinically most abnormal (SZ+CFT/IED+PS) and least affected (CTL/NL EEG) categories for comparison. The GraphPad Software, Inc. (La Jolla, CA, USA) statistical analysis package (Prism 6.02) was used for these comparisons and correlations. The logistic regression analysis was done using MedCalc software (Ostend, Belgium).

Results

A two-way ANOVA was used to determine how the CSF level of individual monoamine metabolites is affected by the baboons' clinical status and sex (Table 1). While the CSF levels of each metabolite were generally the lowest in the SZ group followed by the CFT group, the analyses did not yield any significant differences in the main effect of clinical status: $F(2,256)=0.3666$; $p=0.6935$ (MHPG); $F(2,256)=0.7077$; $p=0.4937$ (5-HIAA); $F(2,256)=1.504$; $p=0.2243$ (HVA). While male baboons consistently exhibited lower levels of monoamine metabolites compared to their female counterparts in each clinical group, there was no significant interactions between clinical status and sex: $F(2,256)=1.980$; $p=0.1401$ (MHPG); $F(2,256)=0.4925$; $p=0.6117$ (5-HIAA); $F(2,256)=0.2483$; $p=0.7803$

(HVA). The corresponding post-test Tukey's multiple comparison tests also did not yield significant differences between pairs of factors for each metabolite ($p>0.05$; Table 1; left three data columns).

A one-way ANOVA was used to determine how the CSF level of individual metabolites is affected by the baboons' photosensitivity (PS) and the presence of IEDs (Table 2; left three data columns). The CSF levels appeared to be the lowest for the PS+ groups followed by the IED+ group, but again these differences were not statistically significant: $F(3,522)=0.5667$; $p=0.6372$ (MHPG); $F(3,522)=0.1408$; $p=0.9355$ (5-HIAA); $F(3,522)=0.04342$; $p=0.9880$ (HVA). The post-test Tukey's multiple comparison tests did not detect statistically significant differences among any pairs of groups ($p>0.05$; Table 2; left three data columns).

Finally, a one-way ANOVA was also used to determine the dependence of the CSF level of individual metabolites on electroclinical factors among SZ+CFT vs CTL with or without IED+PS obtained from scalp EEG measurements (Table 3; left three data columns). The SZ+CFT group had lower metabolite levels than the CTL group, regardless of their EEG status, while the CTL baboons with abnormal EEG had increased levels of metabolites as compared to the CTL animals with normal EEG studies. However, these differences for MHPG and HVA were not statistically significant: $F(3,170)=1.1520$; $p=0.3297$ (MHPG); $F(3,170)=2.331$; $p=0.0761$ (HVA) (Table 3; left three data columns). While the differences for 5-HIAA were statistically significant ($F(3,170)=3.3233$; $p=0.0238$) suggesting that this set of clinical factors impacts the levels of this metabolite, the post-test Tukey's multiple comparison test did not detect statistically significant differences among any pairs of groups ($p>0.05$; Table 3; left three data columns).

By contrast, significant correlations were detected among pairs of metabolites in multiple groups (Tables 1–3; right three data columns). Specifically, correlations of MHPG and HVA and 5-HIAA and HVA concentrations were statistically significant in all clinical and electroclinical groups. The Pearson's correlation coefficient (r) calculated for the 5-HIAA/HVA pairs indicated the strongest correlations across all comparisons. The highest coefficient was detected in the SZ and PS groups, as well as the control group with abnormal EEG (CTL/IED+PS).

To determine possible association of monoamine metabolites with electroclinical status and sex, logistic regression analyses were conducted. This analysis failed to detect significant association ($p>0.05$), while the odds ratios for each group (O.D.~1.0) suggested that similar concentrations of monoamine metabolites should be expected in female and male baboons as well as baboons with and without seizures and/or IEDs.

Discussion

This retrospective study compared cerebrospinal fluid (CSF) monoamine metabolite levels of norepinephrine (NA; 3-hydroxy-4-methoxyphenylglycol or MHPG), serotonin (5-HT; 5-hydroxyindolacetic acid or 5-HIAA), and dopamine (DA; homovanillic acid or HVA) between baboons with seizures (SZ), craniofacial trauma, suspicious for seizure activity (CFT) and asymptomatic control (CTL) baboons, also evaluating potential interactions with

sex in each of these groups. Furthermore, we evaluated the relationship of monoamine metabolites with EEG markers for seizures, such as the presence and absence of interictal epileptic discharges (IEDs) or photosensitivity (PS). Finally, the metabolite levels were also compared between the two groups with the strongest electroclinical phenotype for epilepsy (SZ+CFT) and the weakest, namely the CTL with normal EEGs. While there were decreases in monoamine metabolite levels in males compared to females, in SZ+CFT baboons compared to CTL (Table 1), and in animals with abnormal EEG compared to those with normal EEG studies (Table 2), this study did not detect statistically significant differences between these groups.

By contrast, monoamine metabolites, particularly 5-HIAA and HVA, were increased in CTL animals with abnormal EEG compared to those with normal studies (Table 3), although again these differences were not statistically significant. Statistically significant correlations among all three major monoamine metabolites were detected in the majority of clinical groups tested. A logistic regression analysis failed to detect significant association of the CSF levels of the monoamine metabolites and electroclinical status of baboons. Therefore, the electroclinical status of baboons cannot be used as predictors of the CSF concentrations of monoamine metabolites and vice versa.

A logistic regression analysis failed to detect significant association of the CSF levels of monoamine metabolites with sex and electroclinical status of baboons. The odds ratios (O.D.~1.0) suggested that similar concentrations of metabolites should be expected in female and male baboons as well as baboons with and without seizures or IEDs. Therefore, sex and electroclinical status of baboons cannot be used as predictors of the CSF concentrations of monoamine metabolites and vice versa. An important clinical implication of these results is that if experimental baboons and mice are any guidance, the brain monoaminergic activity may have a greater association with SUDEP than seizures and the related EEG activity [10,21–23].

The levels of neurotransmitters and metabolites in the CSF can be informative in certain brain disorders [21]. The CSF concentrations of MHPG, 5-HIAA and HVA reflect the overall rate of turnover of the neurotransmitters within the central nervous system. However, the CSF metabolite levels may be affected by neuronal activity extrinsic to each monoamine system. The underlying mechanisms underlying this difference is not obvious, but female baboons tended to have higher levels of all CSF monoamine metabolites overall. In humans, females tend to have higher CSF 5-HIAA and HVA levels as well, but not elevated MHPG levels [22]. Whether environmental factors may have affected the MHPG levels in the baboons is unclear, but the females almost all came from breeding cages, while the male baboons tended to come from all-male cages. The absence of significant differences of monoamine MHPG, 5-HIAA and HVA levels between the three groups was unexpected as these neurotransmitter systems are most commonly implicated in seizure modulation [4,7]. Hippocampal 5-HT is increased acutely in a rodent pilocarpine model of status epilepticus, with subsequent rise of 5-HIAA levels both in hippocampus and CSF [6]. Subsequently, both levels decreased below baseline levels despite the evolution of spontaneous seizure activity, which was ascribed to decreased production of 5-HT in the brainstem. As the asymptomatic baboons with abnormal EEG studies (CTL/IED+PS) are genetically

predisposed to epilepsy, the trend of decreasing 5-HIAA and HVA levels in these baboons compared to the SZ+CFT group suggests that the differences are more likely to be driven by environmental factors, including seizures and their social or behavioral consequences (Table 3). Though the differences are statistically insignificant, it is possible that the higher metabolite levels in the CTL/IED+PS baboons may reflect a modulatory effect on seizure threshold of monoamines. The reduced 5-HIAA levels in SZ+CFT baboons as compared to the CTL group with normal EEG studies is consistent with the previously reported inhibitory effects of serotonin on seizure threshold in the epileptic baboon [17]. The relationship between these metabolite levels and seizures, i.e. whether the decreases of 5-HIAA and HVA reflect recurrent seizure activity or actually predispose the animals to seizures, is still unclear. Most of the clinical groups support significant correlations between 5-HIAA and HVA. These results are consistent with previous reports that demonstrated a close association of 5-HIAA and HVA levels in the CSF of non-human primates, healthy humans and across psychiatric conditions, possibly, due to intimate interactions between these two monoamine systems [18,23–27]. Thus, this and previous studies suggest that the two monoaminergic systems appear to be functionally linked, regardless of the underlying disease and conditions.

There are several serious limitations of this retrospective study, but their recognition will be essential for designing future prospective studies. The first and foremost methodological issue was the utilization of two separate datasets, with CSF and EEG collected sometimes years apart. There are age-related changes in CSF monoamines and their metabolites, which may contribute to variability [28]. But more importantly, environmental effects that range from exposure to acute or chronic stress as well as seizures, all have acute and chronic effects on neurotransmitter systems. Furthermore, as the baboons are not observed continuously, the effect of chronic stressors is difficult to evaluate. Seizures also affect neurotransmitter turnover, and the degree and direction of the change may depend upon the timing of the seizures, their severity and frequency and type [4–6]. Due to the high prevalence of craniofacial trauma among epileptic baboons as well as animals that were never witnessed to have seizures by veterinary technicians, it is clear that most GTCS are not witnessed [14,15]. Furthermore, as most of the seizure types are subtle, such as myoclonic or absence seizures, and hence not recognized, which may also affect neurotransmitter turnover. Hence, it is difficult to determine the effect of seizure activity on monoamine metabolite levels in baboons unless they are measured soon after the witnessed event. Other limitations are that scalp EEG studies do not identify IEDs in all seizure animals and IEDs can be identified in numerous baboons with merely a genetic risk for epilepsy, which is widely distributed in this pedigree. Photosensitivity is a more specific marker for the diagnosis of epilepsy and needs to be utilized in addition to IEDs [14]. Finally, only averaged whole-brain CSF concentrations of metabolites were analyzed in this study and thus, and subtle brain region-specific abnormalities remained undetected. Thus, in order to overcome some of these challenges, a prospective study is needed in baboons with acute seizures, coordinating scalp EEG and CSF collections of monoamine metabolites (which are temporally more stable than monoamines), ideally performing serial measurements to demonstrate potential shifts in their levels in peri-ictal, postictal and interictal periods.

In summary, this study was guided by our hypothesis that the CSF levels of major monoamine metabolites (i.e., dopamine, serotonin and norepinephrine) are associated with electroclinical status and can be used as clinical biomarkers applicable to seizures or epilepsy in baboons. Although the data supported a general trend of decreased levels of MHPG, 5-HIAA and HVA in baboons with seizures or craniofacial trauma, and in baboons with abnormal EEG studies, the differences among most groups were not statistically significant. Moreover, similar concentrations of metabolites should be expected in female and male baboons as well as baboons with and without seizures or IEDs. Thus, sex and electroclinical status of baboons cannot be used as predictors for CSF concentrations of monoamine metabolites and vice versa.

These results suggest that the rate of monoaminergic metabolism (i.e., synthesis, uptake and clearance) may not significantly correlate with the epileptic condition in baboons. This conclusion, however, does not necessarily exclude the monoaminergic system as a potential source of pathogenesis in epilepsy and SUDEP. While the global monoaminergic and epileptic conditions may not significantly correlate, the expression and/or function of monoamine receptors and transporters within epilepsy-related brain regions may significantly impact seizure activity and warrants a thorough investigation. Furthermore, the level of monoaminergic activity (especially, serotonergic) may be more relevant to SUDEP than epilepsy, as evidenced by the results of recent studies that used seizure-prone sudden-death-susceptible DBA/1 mice [29–31].

Acknowledgments

This study was supported by the National Institute of Neurological Disorders and Stroke (NIH/NINDS 1 R21 NS065431 to CAS and 1 R01 NS047755 to Jeff T. Williams), the National Institute of Health Grant No. R01-MH65462 (Jeff Rogers), the base grant from the Southwest National Primate Research Center (P51-RR013986), and was conducted in facilities constructed with support from Research Facilities Improvement Grants C06 RR013556, C06 RR014578, and C06 RR015456.

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Table 1
Cerebrospinal fluid monoamine metabolites and their correlations compared by clinical classification.

Clinical Groups	EEG Findings	MHPG (nM)	5-HIAA (nM)	HVA (nM)	Pearson correlation, r		
					MHPG vs. 5-HIAA	MHPG vs. HVA	5-HIAA vs. HVA
All Females (N=196)	IEDs 91 (46%) PS 41 (21%)	89.7 ± 21.0	239.5 ± 99.7	579.4 ± 189.5			
All Males (N=67)	IEDs 37 (55%) PS 25 (37%)	79.6 ± 18.1	208.4 ± 72.1	548.3 ± 151.8			
All baboons (N=263)	IEDs (49%) PS (25%)	87.3 ± 20.7	231.9 ± 94.5	571.7 ± 181.2	0.277***	0.352***	0.635***
Female Controls (N=119)	IEDs 51 (43%) PS 19 (16%)	91.0 ± 19.1	249.4 ± 105.1	600.9 ± 190.2			
Male Controls (N=21)	IEDs 10 (50%) PS 8 (40%)	76.22 ± 19.0	204.8 ± 68.9	558.1 ± 151.2			
All Controls (N=140)	IEDs 61 (44%) PS 27 (19%)	88.7 ± 19.7	242.7 ± 101.5	594.5 ± 185.0	0.352***	0.330***	0.658***
Female CFT (N=63)	IEDs 31 (49%) PS 17 (27%)	86.3 ± 22.7	220.7 ± 85.6	529.7 ± 168.0			
Male CFT (N=40)	IEDs 22 (55%) PS 13 (33%)	81.3 ± 17.6	210.2 ± 74.5	543.1 ± 153.8			
All CFT (N=103)	IEDs 53 (51%) PS 30 (29%)	84.4 ± 20.9	216.6 ± 81.2	534.9 ± 162.0	0.122	0.317***	0.531***
Female Seizure (N=14)	IEDs 8 (57%) PS (29%)	92.2 ± 27.4	203.1 ± 87.4	522.5 ± 245.3			
Male Seizure (N=6)	IEDs 5 (83%) PS 4 (67%)	68.1 ± 15.3	199.0 ± 73.7	489.7 ± 128.1			
All Seizure (N=20)	IEDs 13 (65%) PS 8 (40%)	85.0 ± 26.6	201.9 ± 73.7	512.7 ± 213.8	0.370	0.523*	0.893***

The concentrations of metabolites (MHPG, 5-HIAA and HVA in nM, i.e., pmol/mL) were compared between groups of asymptomatic controls (CTL) baboons, baboons with a history of craniofacial trauma (CFT), and seizure baboons, and between genders in each group using a two-way ANOVA with post-hoc Tukey's multiple comparison tests for each metabolite (left three data columns). IEDs interictal epileptic discharges; PS photoepileptic responses on scalp EEG. The results are presented as mean $\pm$ S.D. Pearson (r) correlation analyses of CSF metabolite concentrations in the right three data columns ($\alpha < 0.05$). Bold values indicate significant differences (* $p < 0.05$). The alpha level was set at 0.05:

* $p < 0.05$;

** $p < 0.01$;

p<0.001. The Pearson (r) correlation analyses (right three data columns) were employed to evaluate differences among groups.

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Table 2
Cerebrospinal fluid monoamine metabolites and their correlations compared by EEG classification.

EEG Data	MHPG (nM)	5-HIAA (nM)	HVA (nM)	Pearson correlation, r		
				MHPG vs. 5-HIAA	MHPG vs. HVA	5-HIAA vs. HVA
IED ± (N=128, F 71%)	85.6 ± 21.1	228.7 ± 94.5	564.8 ± 166.3	0.281 ***	0.346 ***	0.649 ***
IED– (N=135, F 78%)	87.9 ± 20.5	230.1 ± 92.8	565.0 ± 194.4	0.277 ***	0.368 ***	0.644 ***
PS ± (N=66, F 62%)	84.7 ± 19.2	223 ± 107.4	557.9 ± 173.2	0.410 ***	0.471 ***	0.766 ***
PS– (N=197, F 79%)	87.5 ± 21.3	231.5 ± 88.5	567.2 ± 183.8	0.233 ***	0.323 ***	0.602 ***

The concentrations of metabolites (MHPG, 5-HIAA and HVA in nM, i.e., pmol/mL) were compared between baboons with (IED ±) and without (IED–) interictal epileptic discharges or with (PS ±) or without (PS–) photoepileptic responses (PS) on scalp EEG using a **one-way ANOVA with post-hoc Tukey’s multiple comparison tests** for each metabolite (left three data columns). F, Females. The results are presented as mean ±/– S.D. The Pearson (r) correlation analyses of CSF metabolite concentrations are shown in the right three data columns. Bold values indicate significant differences (*p<0.05). The alpha level was set at 0.05:

* p<0.05;
** p<0.01;
*** p<0.001.

Table 3

Cerebrospinal fluid monoamine metabolites and their correlations compared by electroclinical classification.

EEG Findings	MHPG (nM)	5-HIAA (nM)	HVA (nM)	Pearson correlation, r		
				MHPG vs. 5-HIAA	MHPG vs. HVA	5-HIAA vs. HVA
Sz ± CFT/IED ± PS (N=27, F 56%)	81.3 ± 22.0	204.0 ± 84.5	504.1 ± 114.3	0.182	0.388 *	0.687 ***
Sz ± CFT/NI EEG (N=47, F 64%)	88.3 ± 20.5	207.5 ± 68.6	533.9 ± 180.6	0.077	0.319 *	0.656 ***
CTL/IED ± PS (N=24, F 75%)	91.0 ± 16.3	261.1 ± 136.1	617.7 ± 202.4	0.579 **	0.457 *	0.828 ***
CTL/NI EEG (N=76, F 88%)	88.6 ± 21.0	249.3 ± 102.7	582.5 ± 196.9	0.351 **	0.393 ***	0.626 ***

The concentrations of metabolites (MHPG, 5-HIAA and HVA in nM, i.e., pmol/mL) were compared between baboons with seizures and craniofacial trauma (SZ ± CFT) and control (CTL) baboons, in either case with abnormal EEGs with IEDs and PS (IED ± PS) and normal EEGs, using a one-way ANOVA with post-hoc Tukey's multiple comparison tests for each metabolite (left three data columns). F Females; NI Normal. The results are presented as mean $\pm$ S.D. The Pearson (r) correlation analyses of CSF metabolite concentrations in the right three data columns ($\alpha < 0.05$). Bold values indicate significant differences (* $p < 0.05$). The alpha level was set at 0.05;

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.001$.