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Olfactory stimulation induces delayed responses in epilepsy

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ABSTRACT

Precipitation and inhibition of seizures and epileptic discharges by sensory stimuli are receiving increasing attention because they provide insight into natural seizure generation in human epilepsies and can identify potential nonpharmacological therapies. We aimed to investigate modulation (provocation or inhibition) of epileptiform discharges (EDs) in mesial temporal lobe epilepsy (MTLE) versus idiopathic generalized epilepsy (IGE) by olfactory stimulation (OS) compared with standard provocation methods. The underlying hypothesis was that any response would be more likely to occur in MTLE, considering the anatomical connections of the temporal lobe to the olfactory system. This multicenter, international study recruited patients with either MTLE or IGE who were systematically compared for responses to OS using an EEG/video-EEG protocol including a 30-min baseline, twice 3-min olfactory stimulation with ylang-ylang, hyperventilation, and intermittent photic stimulation. The 95% confidence interval (CI) for the baseline EDs in each patient was calculated, and modulation was assumed when the number of EDs during any 3-min test period was outside this CI. A total of 134 subjects (55 with MTLE, 53 with IGE, and 26 healthy controls) were included. Epileptiform discharges were inhibited during OS in about half the patients with both MTLE and IGE, whereas following OS, provocation was seen in 29.1% of patients with MTLE and inhibition in 28.3% of patients with IGE. Olfactory stimulation was less provocative than standard activation methods. The frequent subclinical modulation of epileptic activity in both MTLE and IGE is in striking contrast with the rarity of reports of olfactory seizure precipitation and arrest. Inhibition during OS can be explained by nonspecific arousal. The delayed responses seem to be related to processing of olfactory stimuli in the temporal lobe, thalamus, and frontal cortex.

1. Introduction

Modulation of epileptic activity by sensory stimuli has long been known and can be either provocative or inhibitory. It can be used for nonpharmacological treatments and provides insight into mechanisms of natural seizure generation. Habitual precipitation of seizures by such stimuli is called reflex epilepsy, which in the majority of cases is related to the visual system including photosensitivity [1]. Olfactory reflex epilepsy has never been described although olfactory auras are known since Gowers [2] and related to dreamy states by Jackson [3]. They occur in approximately 6% of patients with temporal lobe epilepsy (TLE) [4]. In addition, it is known that patients with TLE have impaired olfactory function and reduced olfactory bulb volume [5]. Stevens studied the influence of external factors on epileptiform discharges (EDs) in 100 patients, 61 of whom were exposed to olfactory stimulation (OS) [6]. Sixteen patients with TLE (26.2%) demonstrated "exaggerated

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spiking during or immediately after exposure to perfumed air", whereas no response was observed in the EEG of 22 patients with idiopathic generalized epilepsy (IGE) [6]. However, no seizures were provoked in any of these patients, and there are only a few case reports where seizures were triggered by aromatic oils [7] or by the inhalation of a paint thinner [8]. The counterpart of seizure precipitation, i.e., seizure inhibition by OS, has also been reported [2,9–12]. However, targeted investigations are scarce. We therefore undertook a systematic comparison of EEG responses to OS in patients with a well-established diagnosis of either mesial TLE (MTLE) or IGE, the underlying hypothesis being that any responses would be more likely to occur in MTLE, considering its functional anatomical relations to the olfactory system.

2. Materials and methods

2.1. Study description, approvals, and consents

This multicenter, international study was conducted between June 2009 and February 2015 at seven enrolling sites in Brazil, Lithuania, Denmark, Uruguay, and Turkey. It was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki, 2014) [13] and the Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Institutional review boards and ethics committees for each site approved the study protocol and informed consents. All subjects signed an informed consent form and voluntarily agreed to participate.

We consecutively included patients with a definite diagnosis of either MTLE with hippocampal sclerosis (HS) or IGE. Diagnosis was based on clinical history and seizure semiology supported by EEG or video-EEG and magnetic resonance imaging (MRI) or computed tomography (CT) scan consistent with these diagnoses and without evidence of progressive structural lesions in the central nervous system or progressive encephalopathy. All patients were on treatment with standard antiepileptic drugs. A control group comprised 26 healthy volunteers with no history of any neurological or psychiatric disease or complaints, with normal intellect, and with no family history of epilepsy. None of the patients and controls had any clinically relevant nasal condition or complaints of smell or taste dysfunction. Patients with olfactory auras were excluded because these were considered as potential confounders. A previous internationally validated questionnaire – the Multi-Clinic Smell and Taste Questionnaire (MCSTQ-SC) – was given to all participants in order to systematically evaluate the presence of any nasal, smell, or taste dysfunction [14].

Patients were excluded if they were below 13 years of age; were smokers; were pregnant women; had any nasal, smell, or taste dysfunction as assessed with the MCSTQ-SC; had cardiovascular and pulmonary disease or other clinically relevant conditions that might interfere with hyperventilation; had a history of ylang-ylang essential oil allergy; or had nonepileptic events, including psychogenic seizures.

2.2. EEG/VEEG protocol

Noninvasive EEG or video-EEG was recorded using 19–32 EEG electrodes placed according to the International 10–20 or 10–10 electrode system, with an inferior temporal electrode chain and/or sphenoidal electrodes when applicable (MTLE-HS). All patients and controls were submitted to the protocol summarized in Table 1. The recording started with the subject lying awake and relaxed on a bed, with eyes closed. The EEG during the entire protocol was continuously monitored to ensure that drowsiness or sleep did not occur. After baseline and olfactory stimulation, intermittent photic stimulation (IPS) and hyperventilation (HV) were performed according to standard protocols [15,16], adjusted to a total duration of 5 min. According to local standard procedures, IPS was not performed in all patients with MTLE-HS. Each test condition was separated by 15-minute intervals. The total duration of the recording was 1.5–2 h per patient.

Table 1
Video-EEG protocol.

1. Baseline — 30 min of EEG recording (awake, relaxed state, eyes closed). Epileptiform discharges (EDs) were counted in each 3-minute time window, and a mean number of EDs of the total of 10 time windows was obtained.
2. First olfactory stimulus (OS) — OS1 — 3 min normally breathing with the odorant stimulus (EDs counted) — adaptation of olfactory cells was avoided by approaching and distancing the cotton ball containing the essential oil slowly toward and from the nostrils.
3. Post-OS1 — 15 min of recording — EDs were counted for each 3-minute time window, and a mean number of EDs of the total 5 time windows was obtained.
4. Second OS — OS2 — 3 min normally breathing with the odorant stimulus (EDs counted).
5. Post-OS2 — 15 min of recording — EDs were counted for each 3-minute time window, and a mean number of EDs of the total 5 time windows was obtained.
6. Hyperventilation (HV) — 5 min of hyperventilation (inspiring and expiring through the mouth) — ED occurrences in the last 3 min were counted.
7. Post-HV — 15 min of recording — EDs were counted for each 3-minute time window, and a mean number of EDs of the total 5 time windows was obtained.
8. Intermittent photic stimulation (IPS) — photic stimulation with frequencies of 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 60, 50, 40, 30, 25, and 20 Hz (eyes opened for 5 s and closed for 5 s in each frequency and interval between flashes of 7 s).
9. End of protocol.

The olfactory stimulus consisted of ylang-ylang essential oil (*Cananga odorata*) as suggested by Betts [11]. Ylang-ylang has a pleasant fragrance and produces little or no trigeminal activation [17]. Its essential oil is obtained by steam distillation from fresh matured ylang-ylang flowers and is used in the cosmetic and pharmaceutical industries as an active component of antibacterials and in aromatherapy. Its chemical composition is predominantly of volatile terpenes and benzenoid and phenylpropanoid components [18–20]. An odorant solution at 10% was obtained by diluting 0.5 ml of the essential oil (stored in 20-ml yellow glass bottles) in 4.5 ml of an odorless solvent (glycerine, paraffin, or mineral oil) immediately before use. Afterwards, 1 ml of this odorant solution was dropped on a cotton ball and applied near patients' both nostrils simultaneously. All subjects received this stimulation for 3 min twice (first olfactory stimulus — OS1, second olfactory stimulus — OS2), with each stimulation followed by 15 min of rest (post-OS1 and post-OS2 periods, respectively) to account for any late response. Because of its volatility, the solution was used just once. Great attention was paid to ensure that all subjects during stimulation kept a constant quiet breathing rate. All EDs fulfilling established criteria [21] were visually identified and counted in the recordings under each test condition (Table 1): spike, spike-and-slow wave, sharp wave, sharp-and-slow wave, polyspike, and polyspike-and-slow wave. All EEGs of each group were recorded and read by one experienced and board-certified clinical neurophysiologist according to the International Federation of Clinical Neurophysiology [22] and American Clinical Neurophysiology Society [23] guidelines and consensus statements. Foreseeable difficulties were discussed between the raters beforehand in a virtual session. If any doubts arose during individual evaluations, the traces were referred to one of the authors (S.B.) for final rating.

2.3. Statistical analysis

A sample size of 43 patients in each group (MTLE-HS and IGE) was considered necessary to detect a significant difference in olfactory modulation of epileptic discharges between groups with a power of 80% and a two-sided test at a significance level of 5%. Statistical analysis was performed using IBM® SPSS® software package for Mac (standard version 21.0) and Microsoft Excel® software package for Windows (2014). Descriptive analysis was made to characterize the sample. Quantitative variables were expressed as mean ± standard deviation (SD), and qualitative variables were expressed as percentage values. The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. Wilcoxon test was used to compare the occurrences of EDs between the baseline period and testing periods within each group (MTLE-HS and IGE). To compare individual responses (provocative × inhibitory) between MTLE-HS

Table 2
Subjects' demographic data.

		Control (N = 26)	MTLE-HS (N = 55)	IGE (N = 53)	p[a]
Gender	Female	18 (69.2%)	28 (50.9%)	37 (69.8%)	0.09
	Male	8 (30.8%)	27 (49.1%)	16 (30.2%)	
Age groups	<20 years	3 (11.5%)	0 (0.0%)	24 (45.3%)	<0.001*
	21–30 years	14 (53.8%)	9 (16.4%)	20 (37.7%)	
	≥31 years	9 (34.6%)	46 (83.6%)	9 (17.0%)	

IGE = idiopathic generalized epilepsy, MTLE-HS = mesial temporal lobe epilepsy with hippocampal sclerosis.
* Statistically significant as $p < 0.05$.
[a] Pearson's chi-square test.

and IGE, Pearson's chi-square test was used. Binary logistic regression was used to analyze prediction of belonging in each group (MTLE-HS × IGE) that had increased EDs (as a dependent variable regarding intervention) controlled for diagnosis, age, and gender (predictor variables).

To determine ED activation and inhibition in individual patients, we calculated the 95% confidence interval (CI) of EDs per 3-minute epoch of baseline. A test condition was considered provocative when the number of EDs during that test exceeded the 95% CI; inhibition was assumed when it fell below this interval. This procedure accounts for the spontaneous fluctuations of EDs in the baseline period of each patient [24].

The primary endpoint of this study was therefore to investigate, with these statistical methods, the modulation (provocation and inhibition) of EDs in focal and generalized epileptic syndromes by olfactory stimulus and compare it with standard provocation methods.

A p-value <0.05 was considered statistically significant.

3. Results

A total of 134 subjects (55 with MTLE-HS, 53 with IGE, and 26 controls) were included. Their demographics are detailed in Table 2. The mean age (SD) was 38 years (9.7) for the group with MTLE-HS, 23 years (8.2) for the group with IGE, and 28 years (8.9) for controls, with an age range of 13–64 years for patients and 16–53 years for controls.

3.1. Group responses

The responses of EDs to different test conditions are shown in Table 3. No EDs were seen in any control subject under any test condition. In the patients' baseline, considerable spontaneous fluctuations of EDs were observed. Patients responded differently to OS and to conventional provocative methods. Compared to baseline, the group with MTLE-HS presented a statistically significant reduction of EDs during OS1 and OS2, while increased EDs were observed during HV. In the group with IGE, there was a statistically significant increase of EDs during HV and IPS, while a significant reduction of EDs was observed post-OS2.

3.2. Individual responses

The response to each test condition was analyzed individually using, as a criterion of provocation and inhibition, a spike density above or below the 95% CI range calculated from the individual baseline spike density. Provocative or inhibitory responses to each test condition are shown in Tables 3 and 4. Most subjects in both the group with MTLE-HS and the group with IGE presented inhibitory responses during OS1 and OS2, an example of which is shown in Fig. 1A. However, these were followed by antithetical responses between groups in the post-OS periods (Fig. 1B–C). While more subjects presented provocation of EDs in the group with MTLE-HS, more subjects had an inhibitory response in IGE. This effect was more pronounced after OS 1. There was no difference in the response of 25 subjects with right MTLE and 27 subjects with left MTLE (3 were bilateral).

3.3. Effects of standard activation methods and comparison to olfactory stimulation

In MTLE-HS, the significantly highest spike density was with HV while in IGE, it occurred with HV and IPS (Tables 3 and 4). On the contrary, ED inhibition was also observed during HV in both groups and during IPS predominantly in MTLE-HS (Table 4).

Significant prediction of increased EDs (controlled for diagnosis, age, and gender) was obtained only at IPS by binary logistic regression analysis. If one had an IGE diagnosis, there was a 6-times-higher chance

Table 3
Number of epileptic discharges in each 3-minute time window under olfactory stimulus and other test conditions (data divided for MTLE-HS and IGE).[a]

Group		N	Mean (±SD)	Minimum	Maximum	Percentiles[b]			p[c]
						25th	50th (median)	75th	
MTLE-HS[d]	Baseline	55	13.6 (29.88)	0	145.9	0.3	1.9	9.8	–
	OS1	55	9.9 (20.71)	0	120.0	0	1.0	6.0	0.02*
	OS2	54	9.7 (17.18)	0	70.0	0	1.0	11.0	0.04*
	HV	55	17.9 (36.09)	0	194.0	0	4.0	13.0	0.008*
	IPS	25	13.8 (30.38)	0	115.0	0	0	9.0	0.08
	Post-OS1	55	14.5 (29.03)	0	155.2	0.4	2.2	11.6	0.12
	Post-OS2	54	13.1 (29.60)	0	166.4	0.2	1.6	10.0	0.62
	Post-HV	55	13.6 (29.04)	0	143.4	0.2	1.6	8.0	0.74
IGE	Baseline	53	30.4 (77.95)	0	443.8	0.05	1.5	11.7	–
	OS1	53	24.0 (67.18)	0	407.0	0	2.0	11.0	0.10
	OS2	53	21.1 (63.37)	0	390.0	0	0	8.5	0.02
	HV	53	46.7 (103.63)	0	532.0	0	5.0	38.5	<0.001*
	IPS	53	49.3 (99.93)	0	420.0	0	5.0	43.5	0.001*
	Post-OS1	53	25.1 (82.46)	0	531.2	0	0.4	6.0	0.22
	Post-OS2	53	21.7 (71.95)	0	452.2	0	0.4	3.2	0.005*
	Post-HV	53	30.4 (74.36)	0	402.6	0	1.2	15.0	0.62

HV = hyperventilation, IGE = idiopathic generalized epilepsy, IPS = intermittent photic stimulation, MTLE-HS = mesial temporal lobe epilepsy with hippocampal sclerosis, OS1 = first olfactory stimulation, OS2 = second olfactory stimulation, SD = standard deviation.
* Statistically significant as $p < 0.05$.
[a] In the control group, no epileptic discharge occurred in any condition in any individual.
[b] Nonparametric distribution — median and interquartile ranges.
[c] Wilcoxon test (dependent test for two repeated measures) comparing EDs in each test condition with baseline.
[d] Only 25 individuals with MTLE-HS underwent to IPS, and 1 patient had his OS2 and post-OS2 test conditions excluded from the analysis because he had a complex partial seizure during these time windows.

Table 4
Number of patients who experienced excitatory or inhibitory response (increased or decreased number of epileptic discharges compared with the 95% CI baseline range) in each test condition.

		Diagnosis				p[a]
		MTLE-HS		IGE		
		N	%	N	%	
OS1 test	Below 95% CI baseline range	28	50.9%	22	41.5%	0.59
	In 95% CI baseline range	20	36.4%	22	41.5%	
	Above 95% CI baseline range	7	12.7%	9	17.0%	
OS2 test	Below 95% CI baseline range	26	47.3%	28	52.8%	0.69
	In 95% CI baseline range	15	27.3%	16	30.2%	
	Above 95% CI baseline range	14	25.4%	9	17.0%	
HV test	Below 95% CI baseline range	8	14.5%	6	11.3%	0.88
	In 95% CI baseline range	20	36.4%	20	37.7%	
	Above 95% CI baseline range	27	49.1%	27	50.9%	
IPS test	Below 95% CI baseline range	10	40.0%	4	7.5%	0.001*
	In 95% CI baseline range	11	44.0%	23	43.4%	
	Above 95% CI baseline range	4	16.0%	26	49.1%	
Post-OS1	Below 95% CI baseline range	9	16.4%	19	35.8%	0.02*
	In 95% CI baseline range	28	50.9%	26	49.1%	
	Above 95% CI baseline range	18	32.7%	8	15.1%	
Post-OS2	Below 95% CI baseline range	7	12.3%	11	20.8%	0.24
	In 95% CI baseline range	33	61.1%	35	66.0%	
	Above 95% CI baseline range	14	25.9%	7	13.2%	
Pooled post-OS1 and 2[b]	Below 95% CI baseline range	16	14.5%	30	28.3%	0.001*
	In 95% CI baseline range	61	55.5%	61	57.5%	
	Above 95% CI baseline range	32	29.1%	15	14.2%	
Post-HV	Below 95% CI baseline range	11	20.0%	10	18.9%	0.97
	In 95% CI baseline range	30	54.5%	30	56.6%	
	Above 95% CI baseline range	14	25.5%	13	24.5%	

HV = hyperventilation, IGE = idiopathic generalized epilepsy, IPS = intermittent photic stimulation, MTLE-HS = mesial temporal lobe epilepsy with hippocampal sclerosis, OS1 = first olfactory stimulation, OS2 = second olfactory stimulation.

* Statistically significant as $p < 0.05$.

[a] Pearson's chi-square test.

[b] Pooled post-OS1 and 2 = post-OS1 and post-OS2 summed up together.

to have increased EDs during IPS (controlled for all other variables in the regression model, Table 1) compared to MTLE-HS.

3.4. Precipitation of seizures

Epileptiform discharges and seizures are different classes of epileptic events that do not necessarily occur in parallel. Therefore, registered seizures were analyzed separately.

In five patients (4 with IGE and 1 with MTLE), seizures were observed during the investigation. The four patients with IGE had a total of three myoclonic and six absence seizures which appeared randomly distributed over the various phases of investigation. The only focal seizure with loss of consciousness and automatisms observed occurred during OS2 in a patient whose highest spike count per 3 min was during the immediately preceding post-OS1 where the average spike density in OS/post-OS1 was significantly increased to 23.8/min from 18.8/min at baseline. This suggests a possible temporal lobe olfactory reflex seizure although the patient was not aware of previous seizure induction by smells.

4. Discussion

Whereas our findings do not indicate the existence of olfactory reflex epilepsy as a clinical entity, they are in accordance with literature indicating that olfactory seizure precipitation may occasionally occur. The only suspected olfactory reflex seizure observed in our investigation was a MTLE seizure, whereas in the most convincing case report in literature [8], generalized paroxysmal discharges and bilateral myocloni were precipitated. Another literature case concerned a patient with TLE [7], but here, the seizure, a first and only breakthrough seizure 8 years after successful TLE surgery, occurred several hours after the exposure to aromatic oils. This creates some concern about the role of the stimulus although no other provoking factors could be found. Likewise, the role of OS in a patient with absences and generalized spike–wave precipitated by smelling acetone [25] appears not entirely clear. In this case, the epileptic response was immediate.

Our results confirm the old observation of Stevens [6] that olfactory stimuli induce activation of focal epileptiform discharges in TLE "during or immediately after" exposure. This happened in 26% of Stevens' cases and in 26–33% of ours after OS (post-OS1 and post-OS2 test conditions). Also, in the few available reports of olfactory reflex seizures [7,8], these occurred with delay, even considerable. Unlike Stevens [6], we also observed an increase of EDs after OS in patients with IGE although significantly less frequently (13–15%).

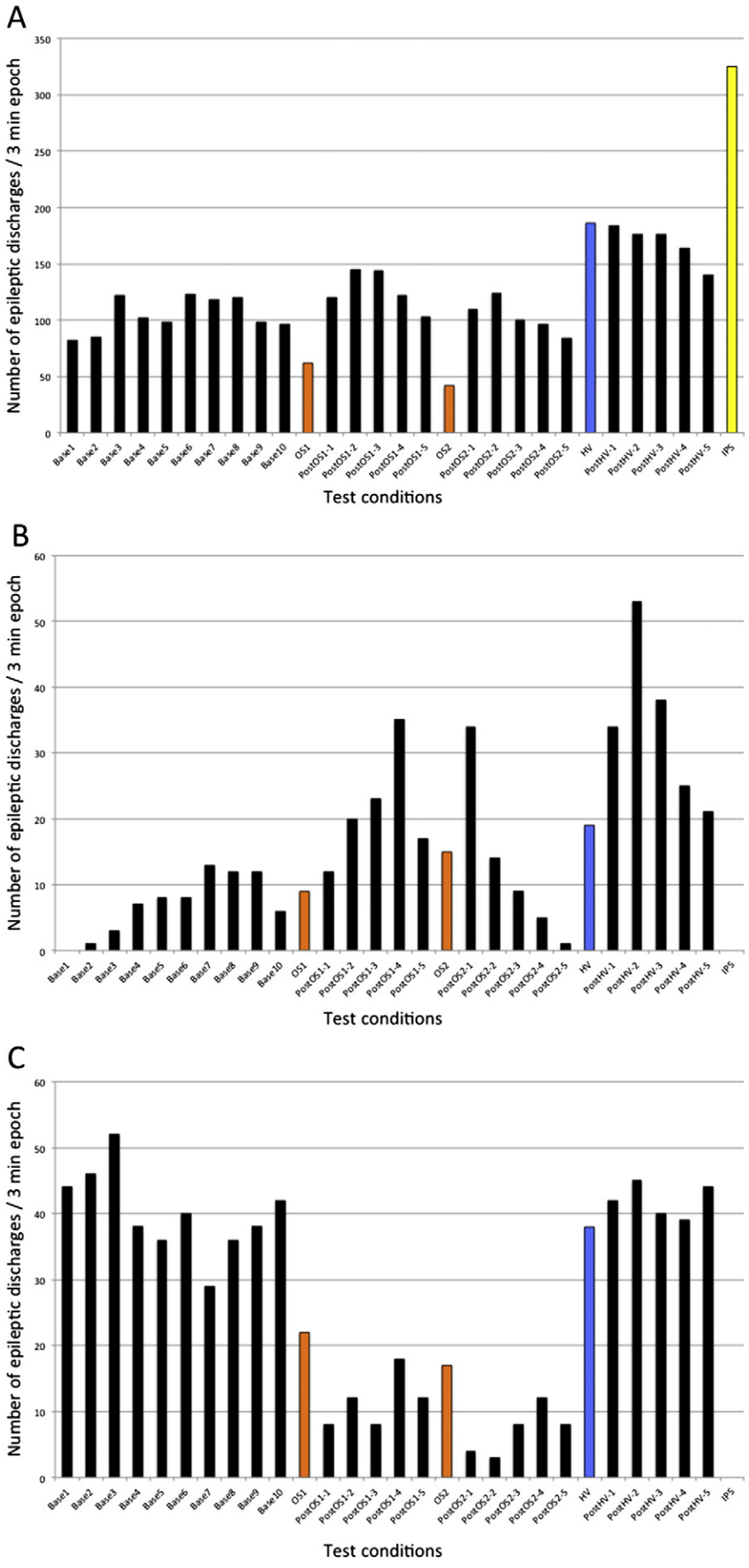
In accordance with observations of alternate responses where identical stimuli could both precipitate or arrest seizure activity [24,26], we found both provocation and inhibition of EDs by the same stimulus. However, among our patients, we observed predominantly inhibition in IGE while provocation of discharges was observed in MTLE-HS. There is not much in the literature to compare this with. Stevens [6] did not consider the possibility of inhibition. Gowers [2] was the first to describe seizure arrest by "a strong olfactory impression" and found it most frequently successful if the patient had an olfactory aura. The best described and spectacular report of successful treatment by consequent application of OS [10] concerns a patient with TLE. These reports are strongly supported by our experimental findings. But there are, to our knowledge, no reports of counteraction of generalized seizures by smells. The reason can simply be that minor generalized seizures (absences and myoclonic) are too brief to invite arresting procedures, whereas primarily generalized tonic–clonic seizures have no aura phase where an abortive stimulus could be applied.

The most consistent finding across groups in our study was a reduction of EDs during OS. In many instances, spike density would then return to baseline immediately after termination of the stimulation (Fig. 1A). This inhibition cannot unequivocally be ascribed to the OS as it can be fully explained by nonspecific arousal by the test procedure as such [20]. However, a nonspecific, arousal-related effect cannot explain the changes observed in the 15-min periods after the end of stimulation. During the poststimulation periods, the provocation or inhibition was often not homogeneous but seemed to indicate an individually variable dynamic build-up and regression (Fig. 1B–C).There is no analogy to this time course in any other human sensory reflex seizures. The responses to eye closure, striped patterns, and somesthetic and kinesthetic stimulation are immediate, whereas photosensitive and musicogenic seizures require some extended exposure but still occur during the stimulation rather than afterwards. The known neurophysiology of the processing of smells in the brain at present seems not to provide an easy explanation for the temporal development of these ictogenic and antiictogenic responses.

However, processing of olfactory information is distributed across a wide range of cerebral structures involving, for example, the piriform and entorhinal cortices, amygdala, thalamus, cerebellum, and orbitofrontal cortex [27]. This fact can probably explain why responses were not only seen in MTLE but also IGE whose ictogenic mechanisms involve the thalamus and frontal cortex [28]. The effect was less pronounced after OS2, which may indicate that the response can habituate.

In the case shown in Fig. 1B, a similar delayed response is seen after HV, which is rather unusual. The typical carryover effects after provocation by HV (Fig. 1A, C) can be explained by the hypercapnia produced by voluntary HV outlasting the HV test condition. However, during OS, our patients were closely monitored to avoid HV.

Whereas our study confirmed the well-known relation of photosensitivity to IGE [29] and showed the habitual provocative effect of HV for both generalized and focal EDs, our methods also allowed us to quantify the inhibitory effect that these standard stimulation methods may have in some patients. The explanation for this may simply be the arousal effects the procedures have in patients where no activation occurs [24]. It is intriguing, however, that an inhibitory


A
Number of epileptic discharges / 3 min epoch
Test conditions
B
Number of epileptic discharges / 3 min epoch
Test conditions
C
Number of epileptic discharges / 3 min epoch
Test conditions

effect of IPS was significantly more frequent in MTLE than in IGE, which seems to indicate that there could be other factors deserving to be investigated.

Among the limitations of this study, it would have been useful to add control or sham stimulations to the protocol as well as unilateral OS and a period of sleep. However, it seemed not pragmatically possible to add more investigations to an already extensive protocol. Ideally, this study should have been conducted with untreated patients with active epilepsy, which for obvious reasons was not possible. All patients were treated with drugs that are likely to modify the level of spike activity and, in IGE that was adequately treated, the response to IPS. It is less likely, but cannot be excluded, that drugs in some instances may have also modified the response to OS. Also, a possible limitation of the study is that data on the respiratory rate during OS were not collected. Nevertheless, since the patients were monitored real-time to avoid hyperventilation, it is very unlikely that this influenced our results.

To conclude, EDs respond to olfactory stimulation in about half the patients with both MTLE and IGE. However, in MTLE, the response is mostly provocative and, in IGE, mostly inhibitory. In spite of the frequency of these responses, the precipitation of seizures by OS is rare, and we found no indication of olfactory reflex epilepsy as a clinical entity. These findings are in accordance with the scant literature about this subject to which they provide novel insight.

Our most surprising finding is that, after a frequent, presumably nonspecific, intermediate fall in spike activity during OS, significant specific responses (either provocative or inhibitory) developed in a dynamic process after termination of the stimulation, during the following 15 min and perhaps even beyond. This may explain puzzling literature reports of delayed seizure precipitation and confirms earlier findings of activation of EDs during and immediately after OS. In spite of widespread networking in olfactory perception, the understanding of the neurophysiology of olfaction at present offers no easy explanation of this time course which differs also from what is known from other human sensory reflex epileptic responses.

The ultimate goal of the study of natural inhibitory mechanisms is the development of new therapeutic approaches. For this, our finding of delayed effects after OS is of paramount importance. As a next step, we plan to explore the therapeutic potential of OS in acute situations like seizure series and clusters and to prevent reflex epileptic seizures.

As a spin-off finding, our approach of systematically comparing excitatory with inhibitory effects of stimulation methods also indicated increased inhibition by IPS in MTLE, which might be worth further study.

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Conflicts of interest

The authors declare that they have no conflict of interest with respect to the work submitted in this article. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yebeh.2016.05.022.

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Fig. 1. A) Example of spike reduction restricted to the two 3-min phases of olfactory stimulation in a patient with idiopathic generalized epilepsy. This response is presumably nonspecific and due to arousal by the procedure. B) Activation of epileptic discharges in a patient with mesial temporal lobe epilepsy and C) inhibition in a patient with idiopathic generalized epilepsy as examples of the dynamic development of epileptic discharge responses in the 15-min poststimulation periods. Responses to the following hyperventilation and posthyperventilation periods and photic stimulation where applicable are shown for comparison. Base = baseline, HV = hyperventilation, IPS = intermittent photic stimulation, OS1 = first olfactory stimulation, OS2 = second olfactory stimulation.

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