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EEG changes as an indication of central nervous system involvement following cyclopentolate 1% eye drops; a randomized placebo-controlled pilot study in a pediatric population

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ABSTRACT

To compare EEG-patterns after instillation of cyclopentolate versus placebo eye drops. Prospective, randomized, placebo-controlled, and observational pilot study is presented. Ophthalmology outpatient clinic Dutch metropolitan hospital. Healthy 6- to 15-year-old volunteers with normal or low BMI requiring a cycloplegic refraction/retinoscopy. Randomized; 1 visit 2 drops cyclopentolate-1% and 1 visit 2 drops placebo (saline-0.9%). Single-blind: conducting researcher. Double blind: subjects, parents, clinical-neurophysiology staff, neurologist, and statistician. A 10-min baseline EEG-recording, drop-application, and follow-up to at least 45 min. Primary outcome: Detection of CNS changes, i.e. EEG-pattern changes, following two drops of cyclopentolate-1%. Secondary outcome: Determination of the extent of these pattern changes. Thirty-six cyclopentolate-1% saline-0.9% EEG registrations were made in 33 subjects; 18 males and 15 females. Three subjects were tested twice (interval 7 months). Nine out of fourteen (64%) of the 11- to 15-year-old children reported impaired memory, attention, alertness, as well as mind wandering following cyclopentolate. Drowsiness and sleep were seen in EEG-recordings of 11 subjects (33%) following cyclopentolate. We observed no drowsiness nor sleep during placebo recordings. The mean time to drowsiness was 23 min. Nine subjects arrived in stage-3 sleep but none arrived in REM-sleep. In subjects without sleep (N=24), significant changes compared to placebo-EEG were present for many leads and parameters. The main findings during awake eye-open recording were as follows: 1) a significant increase of temporal Beta-1,2 and 3-power, and 2) a significant decrease in: a) the parietal and occipital Alpha-2-power, b) the frontal Delta-1-power, c) the frontal total power, and d) the occipital and parietal activation synchrony index. The former finding reflects cyclopentolate uptake in the CNS, and the latter findings provide evidence for CNS suppression. Cyclopentolate-1% eye drops can affect the CNS and may cause altered consciousness, drowsiness, and sleep with concomitant EEG results in both young children and children in puberty. There is evidence that cyclopentolate has the potency to act as a short acting CNS depressant. Nevertheless, however, cyclopentolate-1% can safely be used in children and young adolescents.

KEYWORDS

Adverse reactions; anticholinergic syndrome; central nervous system; cyclopentolate; cycloplegics; objective refraction; retinoscopy; side effects

Introduction

Cyclopentolate 1% is the most frequently used cycloplegic eye drop for refractive measurements in pediatric ophthalmology. Cyclopentolate has been commercially available since 1951 and is considered safe for pediatric use.[1–4] Severe adverse events following administration are rare, they occur, seem dose-related, and involve the central nervous system (CNS).[2,4–6] Anticholinergic CNS adverse reactions include: behavioral disturbances, psychotic symptoms, ataxia, incoherent speech, restlessness, hallucinations, hyperactivity or drowsiness, seizures, disorientation in time and place, failure to recognize people, and amnesia.[1] Peripheral adverse reactions include: urinary retention, diminished gastrointestinal motility, tachycardia, hyperpyrexia, vasodilation, skin rash, decreased secretion in salivary and sweat glands, pharynx, bronchi, and nasal passages.[1] Milder adverse reactions are more common and involve mainly but not exclusively the CNS.[2,4–6] Both the more severe and the milder adverse reactions commonly occur 20–60 min after application, and subside within 2–6 h with no permanent sequelae.[6–9]

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In a previous observational study, we found a high incidence of drowsiness following cyclopentolate 1% in a cohort of 3- to 14-year-old children. It indicated a CNS involvement even in the milder cases.[6] We showed furthermore, that the risk for adverse reactions increases with younger age and in the presence of a low BMI. However, during puberty, a considerable amount of drowsiness was also reported.

Cyclopentolate is lipid soluble and crosses membrane barriers easily and thus is well distributed into the CNS and other organs.[9,10] About 30% to 80% of the instilled eye drops will be systemically absorbed by the conjunctiva and the mucosa of the richly vascularized nasopharynx.[11] Considerable cyclopentolate plasma concentrations can therefore be obtained. Pharmacological studies showed a wide range of peak plasma concentrations for individual cycloplegics in both adults and children. Lahdes et al.[8] describe a mean peak plasma concentration of 2.8 ± 1.3 ng/ml^{-1}, 15 ± 11 min after a 60-µl dose of cyclopentolate 1% in 8 adults. Kaila et al.[12] found peak concentrations ranging from 3.3 to 15.5 ng/ml^{-1} for the same regime in six adults. In six children, a median peak concentration of 2.9 ng/ml^{-1}, ranging from undetectable low to 5.8 ng/ml,$^{-1}$ was found after a 35-µl dose of cyclopentolate 1%.[13] Detectable concentrations were seen as early as 3 min after application; reflecting the rapid absorption of cyclopentolate.

Our cohort study[6] showed that the CNS is relatively often affected by cyclopentolate eye drops. Cyclopentolate acts on muscarinic receptors. There are five, M1 to M5, muscarinic subtypes identified. Cyclopentolate has no selectivity for any of these subreceptor types.[14] All sub-receptors are abundantly present in the dense layers of the CNS structures.[15] CNS receptor binding is the possible etiology for the cyclopentolate CNS adverse reactions. However, very little is known about the exact CNS changes that are occurring. CNS effects can be recorded by EEG monitoring in both changes of patterns as well as the time of onset of those changes. An EEG reflects cortical electrical activity. We started a pilot study on 6- to 15-year-old subjects. Subjects were followed with EEG recording after placebo and two doses of cyclopentolate 1%. This paper will describe and discuss the significant EEG changes we encountered in this cohort of children.

Methods

Ethical considerations

This study was conducted according to the principles of the Declaration of Helsinki (version 59th WMA General Assembly, Seoul, Republic of Korea, October 2008) the Dutch Agreement on Medical Treatment Act, and the Dutch Personal Data Protection Act and was ethically approved by the Medical Ethical Committee South-West-Holland and admitted to the European clinical trial database (EudraCT) as EUCTR201200114942-NL and Netherlands Trial Register (NTR) as NTR3446 (NL3278). Since the termination of the NTR in June 2022, 22 items of the initial registration were admitted to the International Clinical Trials Registry Platform.

Primary and secondary study outcomes

The primary outcome was defined as follows: Detection of EEG pattern changes, following two drops of cyclopentolate 1%. The secondary outcomes were defined as follows: detection of 1) which patterns change, 2) time of onset of EEG pattern change, 3) amount and depth of EEG pattern change, and 4) to detect factors that influence the onset of changes in EEG pattern.

Organization of interventions

In this randomized single-blind placebo-controlled observational study a 22-channel EEG registration was conducted in subjects receiving in a randomized manner either two drops of cyclopentolate hydrochloride 1%, (unit-dose Chauvin-Bausch&Lomb-Pharma, Benelux) with an interval of 5 min in both eyes or two drops of saline 0.9% (placebo, unit dose PARI-GmbH, Germany), with an interval of 5 min in both eyes, in two consecutive visits. For cyclopentolate, the mean drop volume was 25 µl, therefore a total dose of 100 µl cyclopentolate was given. The time between both visits was 3 days at a minimum and 14 days at a maximum.

Randomization was organized by the hospital pharmacist using a computer-generated sequence. After inclusion, the subject received intervention according to the designated randomization: visit 1

cyclopentolate and visit 2 placebo or visit 1 placebo and visit 2 cyclopentolate. Single-blinded were conducting researcher (applicant of eye drops), subjects, and parents. Double-blinded were clinical-neurophysiology staff, neurologists, and statisticians.

Procedures

The subjects were healthy 6- to 15-year-old volunteers, requiring objective refraction because of the standard departmental protocol and with a normal or low BMI and without syndromes or diseases. After oral consent of subjects and oral and written consent of parents length and weight were determined. BMI was calculated according to the formula: BMI = weight/height. Subjects were divided into two categories: low BMI and normal BMI. According to the international cutoff values for under- and overweight by sex between 2 and 18 years.[16,17] For South-Asian subjects, cutoff values according to the guidelines of Wilde et al.[18] were used. Subjects were also subdivided into two age categories: 6–10 or 11–15 years. For this pilot study, we aimed to assess the feasibility of recording an EEG in children for 60 min. Furthermore, we included a sufficient enough number of subjects to be able to find EEG changes after cyclopentolate 1% eye drops. Therefore, recruitment was continued until in each age category of 12 subjects, and in each BMI subcategory of 6 subjects, an awake observational EEG was recorded.

Measurements

EEG recordings were performed in a lightened room at 3 pm to avoid circadian influences. EEG electrodes were attached according to the international 10–20 systems with additional electrodes for eye movement and respiratory recording (thermistor) and connected with a Nihon Kohden Neurofax system. EEG recordings were performed with Mason-Likar lead placements (Figure 1). We observed an alteration in the pattern of the routine 3 lead ECG during the EEG recordings. Therefore, we decided to extend the study protocol with a full

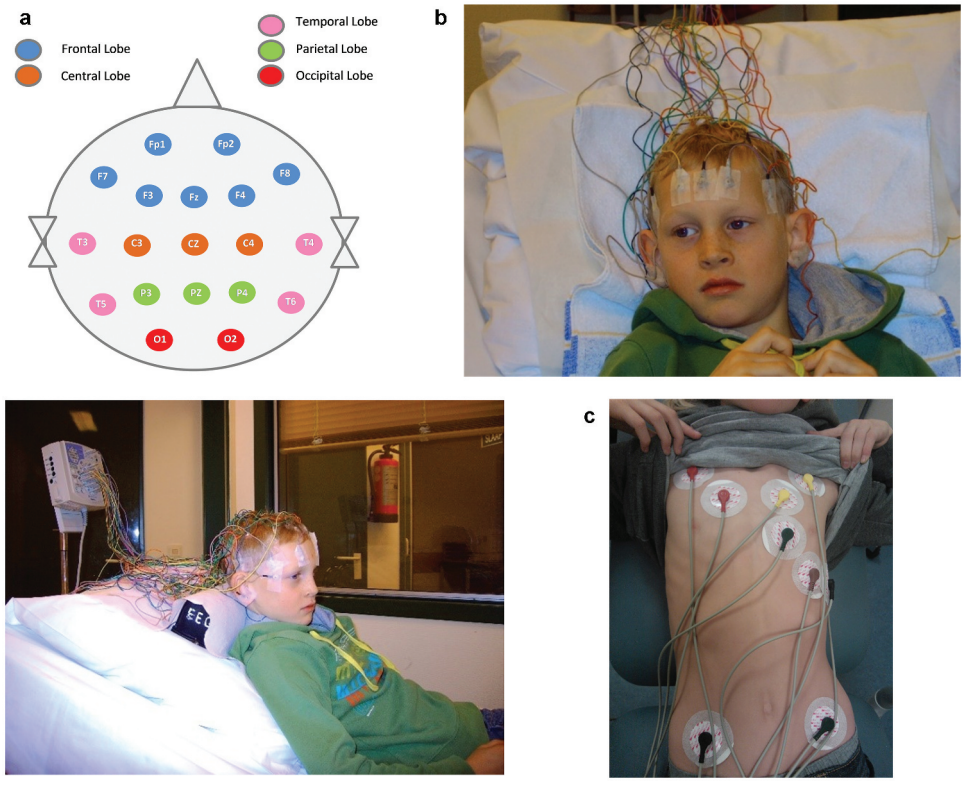

Figure 1. (a) Standard 10–20 EEG electrode names and positions. EEG electrodes: Z: Midline; FZ: Midline Frontal; CZ: Midline Central; PZ: Midline parietal. Even numbers, right hemisphere locations; odd numbers, left hemisphere locations: Fp: Frontopolar; F: Frontal; C: Central; T: Temporal; P: Parietal; O: Occipital. (b) Setup subjects. (c) 12 channel ECG electrodes placements.

continuous 12-channel ECG recording (Mason-Likar lead placements) using a Norav 1200 ECG device. Furthermore, before and after EEG/ECG recordings, blood pressure and pulse-oximetry measurements were performed using a Philips SureSigns-VS2+ device. The children were tested in a relaxed 45 degrees recumbent position on a bed.

Subjects were not restricted in their activities. In the first 10 min, the background pattern was judged with eyes open/eyes closed condition. The eyes closed measurements were made as a resting state EEG with no task or visual stimulation. After a 10 min baseline, EEG recordings of eye drops were applied; then, registrations were continued for at least 45 min. A double-blinded neurophysiologist adjudicated all EEG registrations. EEG recordings were visually assessed for changes in pattern (changes in arousal, increase, decrease, alpha, beta, delta, theta activity; waveform, amplitude, frequency, latency, etc.) and other abnormal transitional events compared to the normal pattern, with respect to the age of the person. Sleep latency for drowsiness and sleep stages 1, 2, and 3 and REM-sleep were calculated (according to rules of the American Academy of Sleep Medicine; AASM) from the time of the first eye drop instillation.

EEG analyses

The cortical activity is represented by waves with different frequencies,:delta (<4 Hz), theta (4-<8 Hz), alpha (8–12 Hz), beta (12.5-30 Hz), and gamma (>35 Hz), waveforms, and amplitudes. These individual frequencies were further subdivided:, respectively, delta-1 (0.5–2.5 Hz) and delta-2 (2.5–4 Hz), alpha-1 (8.0–10.0 Hz), and alpha-2 (10.0–12.0 Hz), beta-1 (12.5–18.0 Hz), beta-2 (18.5–21.0 Hz), and beta-3 (21.5–30.0 Hz) and Gamma-1 (35–55 Hz) and Gamma-2 (60–120 Hz). Parameters were expressed in terms of power values for each lead. Furthermore, for each lead we determined: a) the Dominant Frequency wave, b) the Activation Synchrony Index wave, c) the total power and d) the ratio between theta and beta values.

To establish whether significant treatment effects can be detected on the repeated measured EEG parameters, each parameter has been analyzed with a mixed model analysis of covariance (ANCOVA) with treatment, time, period, and treatment by time as fixed factors and subject, subject by treatment and subject by time as random factors and the (average) baseline measurement as the covariate. The baseline is defined as the average value prior to dosing. All EEG parameters, except the dominant wave and the Activation Synchrony Index wave, are log-transformed before analysis and back-transformed after analysis. Their results can be interpreted as a percentage change. Heat maps were used for the visualization of the primary pharmacodynamics analysis of the EEG parameters. For each log-transformed EEG parameter and for each type of eye condition (closed and open), the colors in the heat maps represent the percentage change of cyclopentolate 1% with respect to placebo for the 19 leads. The color scale has the same range for all log-transformed EEG parameters. The dominant wave and the Activation Synchrony Index wave have an individual color scale. All calculations were performed using SAS for windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

We aimed to investigate the effect of cyclopentolate and placebo on EEGs of 24 awake subjects as sleep EEGs differ significantly from awake EEGs. Despite the bright illuminated room and the attached electrodes, unexpected spontaneous (deep) sleep occurred in our subjects, so we continued the inclusion until EEGs of 24 awake subjects were obtained.

In total 36 cyclopentolate 1% and 36 saline 0.9% EEG registrations were made in 33 subjects: 18 males and 15 females. Three subjects were tested twice (retinoscopy due to changes in refractive error, interval 7 months). Twelve subjects fell asleep during the cyclopentolate EEG recording. One twice-tested subject fell asleep during both cyclopentolate sessions. No subject fell asleep during placebo recordings.

A flowchart of the inclusion process and the EEG analyses is admitted in Figure 2. In Table 1 the group demographics are shown (sex, age, and BMI) as well as changes in behavior seen during clinical observation of the subjects, and results of visual assessment of the EEG recordings.

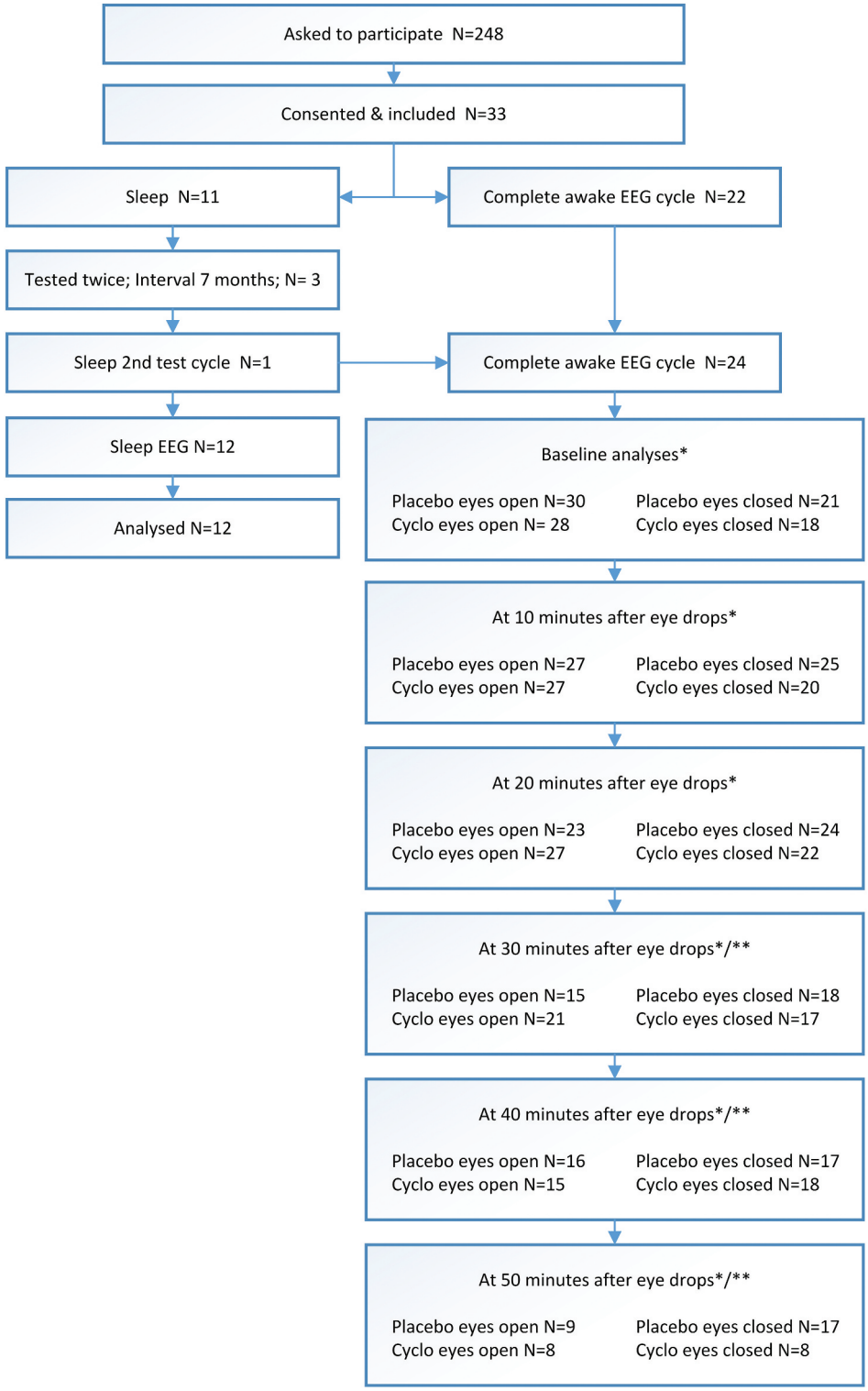

Figure 2. Flowchart included subject and EEG analyses process. *N*= represent the amount of EEGs analyzed at the consecutive time-points. * and ** reflect missing data for analyses; either through movement or other activities of the subject* or sleep**.

Table 1. Group characteristics: Admitted are : 1) sex, age, and BMI category, and 2) details of prior drowsiness or complaints following cyclopentolate 1% eye drops at an earlier visit, and 3) clinical observation details of changes in behavior during EEG recordings, and 4) results of visual assessments of the placebo and cyclopentolate EEG recordings.

	Sex	Age	BMI	Prior drowsiness	Placebo EEG	Cyclo 1% EEG
1	Male[a]	8	Low	Unknown[b]	-	Visit 1: sleep
					-	Visit 2: sleep
2	Male	12	Low	Yes	-	Sleep
3	Male[a]	10	Normal	Yes	-	Visit 1: sleep
4	Female[a]	15	Low	Unknown[b]	-	Visit 1: sleep
5	Female	10	Low	No	-	-
6	Male	12	Normal	Yes	-	-
7	Female	10	Normal	Yes	-	-
8	Female	11	Low	Yes	-	Sleep
9	Male	8	Normal	No	-	Sleep
10	Male	11	Normal	No	-	Hyperactivity
11	Female	6	Normal	No	-	-
12	Female	10	Normal	No	-	-
13	Female	9	Normal	No	-	-
14	Male	8	Normal	No	-	-
15	Male	6	Normal	No	-	-
16	Male	10	Normal	No	-	Sleep
17	Female	10	Low	Unknown[b]	-	-
18	Female	8	Low	Unknown[b]	-	-
19	Female	6	Normal	Unknown[b]	Epileptic like activity	Sleep. Increased epileptic like activity.
20	Female	11	Normal	No	-	-
21	Male	11	Normal	No	-	-
22	Female	8	Normal	Yes	-	-
23	Male	15	Normal	Yes	-	Sleep
24	Female	8	Low	Yes	-	-
25	Male	13	Normal	No	-	-
26	Female	11	Normal	No	-	-
27	Male	14	Low	No	-	-
28	Male	11	Normal	No	-	-
29	Male	7	Low	Unknown[b]	-	Sleep
30	Male	14	Low	Yes	-	Sleep
31	Female	10	Low	Unknown[b]	-	-
32	Male	8	Low	Unknown[b]	-	-
33	Male	12	Low	Unknown[b]	-	-

[a]tested twice.
[b]first-time cyclopentolate 1% eye drops.

EEG pattern changes

Subjects were not restricted in their activities during recording and were not subjected to tasks. Children could freely play, talk, read, listen to music, and move on the bed, which had an impact on EEG outcomes. Not all data were usable for analyses. Figure 2 shows an overview of the number of subjects with data that were suitable for analyses at individual measurement time points.

In the awake subjects, many of the leads; both under eyes open and eyes closed circumstances, showed significant mean changes between placebo and cyclopentolate recordings. In Table 2 (eyes closed) and Table 3 (eyes open), we admitted an overview of the significant findings. Displayed are the mean lead signals of both interventions, the mean percentage of changes from baseline recordings, the p-value, and the accompanying 95% CI of the difference between placebo and cyclopentolate outcomes. Figure 3 visually reflects the mean percentage of change from placebo. Significant changes are marked with a *($p < .05$) or **($p < .01$).

Main findings during eyes-closed recording were as follows: a significant decrease of a) central, frontal, occipital, and parietal Beta-1-power, b) central, frontal, occipital, and Alpha-2-power, and c) the occipital activation synchrony index. As the eyes-closed EEG reflects a resting state EEG with no tasks or visual stimulation, the decrease in our significant parameters reflects the additive effect of cyclopentolate 1% on EEG values.

The main findings during awake eyes-open recording were as follows: 1) a significant increase of temporal Beta-1,2 and 3-power and frontal and central and frontal Theta/Beta-ratio, and 2) a significant decrease in: a) the parietal and occipital Alpha-2-power, b) the frontal Delta-1-power, c) the frontal total power, and d) the occipital and parietal activation synchrony index.

Table 2. Statistically significant ($p < .05$) changes of the individual leads and frequency in eyes closed from baseline recording between placebo (saline 0.9%) and cyclopentolate 1% eye drops in awake subjects.

	Lead Signal (LS) Means				LS Means change from baseline	
Parameter	Placebo	Cyclo 1%	Treatment *P*-value	Mean % change 95% CI limits	Placebo	Cyclo 1%
EEG Alpha-power 2 Cz Closed (uV/Hz)	0.937	0.856	.0452	−8.6% (−16.3%, −0.2%)	−2.5%	−10.9%
EEG Alpha-power 2 F4 Closed (uV/Hz)	1.155	1.054	.0121	−8.7% (−14.9%, −2.1%)	0.3%	−8.5%
EEG Alpha-power 2 O2 Closed (uV/Hz)	3.016	2.508	.0429	−16.8% (−30.4%, −0.6%)	1.6%	−15.5%
EEG Alpha-power 2 Pz Closed (uV/Hz)	1.561	1.341	.0336	−14.1% (−25.2%, −1.3%)	−1.8%	−15.6%
Activation synchrony index O1 Closed (%)	144.499	83.488	.0018	−61.0 (−97.6, −24.4)	21.7	−39.3
Activation synchrony index O2 Closed (%)	144.212	91.733	.0236	−52.5% (−97.5%, −7.5)	22.9	−29.6
EEG Beta-power 1 C3 Closed (uV/Hz)	0.277	0.253	.0144	−8.6% (−14.8%, −1.9%)	2.9%	−6.0%
EEG Beta-power 1 C4 Closed (uV/Hz)	0.277	0.253	.0138	−8.8% (−15.0%, −2.0%)	3.0%	−6.0%
EEG Beta-power 1 Cz Closed (uV/Hz)	0.307	0.268	<.0001	−12.7% (−17.6%, −7.6%)	6.9%	−6.7%
EEG Beta-power 1 F3 Closed (uV/Hz)	0.435	0.399	.0155	−8.3% (−14.4%, −1.7%)	7.4%	−1.5%
EEG Beta-power 1 F4 Closed (uV/Hz)	0.466	0.405	.0035	−13.0% (−20.4%, −4.9%)	12.6%	−2.0%
EEG Beta-power 1 Fz Closed (uV/Hz)	0.415	0.369	.0036	−11.1% (−17.6%, −4.1%)	8.0%	−4.0%
EEG Beta-power 1 O1 Closed (uV/Hz)	0.728	0.651	.0338	−10.5% (−19.2%, −0.9%)	0.2%	−10.3%
EEG Beta-power 1 O2 Closed (uV/Hz)	0.734	0.663	.0425	−9.7% (−18.2%, −0.4%)	−1.0%	−10.7%
EEG Beta-power 1 P3 Closed (uV/Hz)	0.477	0.435	.0321	−8.9% (−16.3%, −0.8%)	0.1%	−8.8%
EEG Beta-power 1 P4 Closed (uV/Hz)	0.463	0.417	.0054	−10.0% (−16.2%, −3.4%)	0.9%	−9.2%
EEG Beta-power 1 Pz Closed (uV/Hz)	0.442	0.395	.0046	−10.7% (−17.3%, −3.7%)	2.2%	−8.8%
EEG Beta-power 2 F4 Closed (uV/Hz)	0.732	0.634	.0119	−13.3% (−22.3%, −3.3%)	17.5%	1.8%
EEG Beta-power 2 Fz Closed (uV/Hz)	0.594	0.522	.0047	−12.2% (−19.6%, −4.2%)	9.6%	−3.8%
EEG Beta-power 3 Fz Closed (uV/Hz)	0.190	0.177	.0494	−7.1% (−13.6%, −0.0%)	5.3%	−2.2%
Dominant Frequency P3 Closed (Hz)	8.312	7.880	.0415	−0.43% (−0.85%, −0.02%)	0.08	−0.35
Dominant Frequency P4 Closed (Hz)	8.377	7.928	.0448	−0.46% (−0.89%, −0.01%)	0.15	−0.26
EEG Gamma-power 1 T5 Closed (uV/Hz)	0.154	0.187	.0427	+21.8% (0.7%, 47.2%)	−8.1%	+11.9%
EEG Theta-power 1 Cz Closed (uV/Hz)	0.966	0.870	.0295	−10.0% (−18.0%, −1.1%)	3.6%	−6.7%

A considerable increase in Beta activity was present. Beta activity occurs rarely in children during wakefulness, and especially an increase in activity is always seen as a medication effect.[19] We found signs of an altered state of consciousness, i.e., CNS involvement. First, significantly decreased parietal and occipital alpha power was present during eyes open conditions. A significant decrease in eye open measurements can be considered a sign of visual neglect or inattention.[20–23] Parietal Alpha activity is seen during auditory attention and a decrease indicates auditory neglect and decreased visual spatial attention.[24,25] Second, a significantly decreased eyes open (pre)frontal delta power was found. Delta activity increases in the (pre)frontal lobe during tasks, the more demanding and complex the more activity.[26] Hence, low level or reduced attention is reflected in the frontal values of our subjects. Third, we found a significantly increased Theta/Beta ratio (TBR) in the frontal and central regions. The TBR has been related to attentional control and is significantly higher during mind wandering episodes than during task performance.[27] An increased frontal TBR reflects a reduced top-down control over thoughts.[27] Finally, a significant decrease in the occipital and parietal activation synchrony index (ACI) was presented. The ACI quantitatively represents interhemispheric synchrony,[28] which is important for normal brain function and is essential for motor, perceptual, and cognitive functions. Good visual attention, recognition, and working memory demand an occipital and parietal interhemispheric synchronization of the Alpha 2 band.[29–31] The significant ACI decrease is most likely associated with the significant decrease in the Alpha 2 power we found.

Adverse reactions, drowsiness, and sleep

Nine out of 14 (64%) of the 11- to 15-year-old children reported adverse reactions such as impaired memory, impaired alertness, i.e., focus/attention difficulties, as well as mind wandering following cyclopentolate 1% application. In addition, one of these subjects showed hyperactivity. One young child had epileptiform EEG activity during cyclopentolate recording (first visit) as well as during placebo recording (secondvisit). This child was referred to a neurologist.

Striking is that 11 subjects, despite the extensive wire ring of EEG – and ECG electrodes, the brightly illuminated room, and the ambient sounds spontaneously fell asleep, and only in

Table 3. Statistically significant ($p < .05$) changes of the individual leads and frequency of eyes open from baseline recording between placebo (saline 0.9%) and cyclopentolate 1% eye drops in awake subjects.

	Lead Signal (LS) Means				LS Means change from baseline	
Parameter	Placebo	Cyclo 1%	Treatment *P*-value	Mean % change 95% CI limits	Placebo	Cyclo 1%
EEG Alpha-power 2 O2 Open (uV/Hz)	1.950	1.647	.0092	−15.5% (−25.4%, −4.4%)	6.5%	−10.0%
EEG Alpha-power 2 P3 Open (uV/Hz)	1.172	1.053	.0113	−10.1% (−17.2%, −2.5%)	1.8%	−8.5%
EEG Alpha-power 2 P4 Open (uV/Hz)	1.156	1.028	.0035	−11.1% (−17.8%, −4.0%)	3.0%	−8.5%
EEG Alpha-power 2 Pz Open (uV/Hz)	1.153	1.032	.0092	−10.5% (−17.6%, −2.8%)	1.9%	−8.8%
Activation synchrony index O1 Open (%)	39.442	27.992	.0274	−11.5% (−21.5% −1.4%)	13.5232	2.0731
Activation synchrony index O2 Open (%)	40.321	28.051	.0204	−12.3% (−22.6%, −2.0%)	14.4970	2.2273
Activation synchrony index P3 Open (%)	33.020	25.634	.0131	−7.4% (−13.1%, −1.7%)	8.6610	1.2750
Activation synchrony index Pz Open (%)	32.150	26.798	.0027	−5.4% (−8.8%, −1.9%)	5.5364	0.1848
EEG Beta-power 1 Fz Open (uV/Hz)	0.434	0.396	.0208	−8.7% (−15.5%, −1.5%)	4.5%	−4.7%
EEG Beta-power 2 T4 Open (uV/Hz)	0.975	1.187	.0171	21.7% (3.7%, 42.8%)	−20.7%	−3.5%
EEG Beta-power 2 T5 Open (uV/Hz)	0.772	0.893	.0255	15.7% (1.9%, 31.4%)	−15.3%	−2.0%
EEG Beta-power 2 T6 Open (uV/Hz)	0.745	0.858	.0230	15.2% (2.0%, 30.1%)	−15.9%	−3.1%
EEG Beta-power 3 T4 Open (uV/Hz)	0.405	0.510	.0177	26.0% (4.3%, 52.3%)	−24.0%	−4.2%
EEG Beta-power 3 T5 Open (uV/Hz)	0.275	0.356	.0031	29.5% (9.5%, 53.2%)	−22.9%	−0.1%
EEG Beta-power 3 T6 Open (uV/Hz)	0.270	0.335	.0064	24.4% (6.6%, 45.1%)	−21.1%	−1.9%
EEG Delta-power 1 F3 Open (uV/Hz)	3.085	2.474	.0088	−19.8% (−31.7%, −5.9%)	−14.5%	−31.4%
EEG Delta-power 1 F4 Open (uV/Hz)	3.073	2.726	.0254	−11.3% (−20.1%, −1.6%)	−14.6%	−24.2%
EEG Delta-power 1 F7 Open (uV/Hz)	5.135	4.498	.0208	−12.4% (−21.6%, −2.2%)	−12.5%	−23.3%
EEG Delta-power 1 F8 Open (uV/Hz)	5.553	4.471	.0035	−19.5% (−30.0%, −7.4%)	−9.3%	−27.0%
EEG Delta-power 1 Fp1 Open (uV/Hz)	6.116	4.904	.0019	−19.8% (−29.7%, −8.5%)	−3.6%	−22.7%
EEG Delta-power 1 Fp2 Open (uV/Hz)	6.152	5.112	.0078	−16.9% (−27.2%, −5.2%)	−5.0%	−21.1%
EEG Delta-power 1 Fz Open (uV/Hz)	2.843	2.450	.0103	−13.8% (−22.8%, −3.8%)	−10.7%	−23.1%
Dominant Frequency C3 Open (Hz)	7.590	7.229	.0014	−0.4% (−0.6%, −0.2%)	0.1441	−0.2169
Dominant Frequency C4 Open (Hz)	7.570	7.233	.0035	−0.3% (−0.6%, −0.1%)	0.1208	−0.2160
Dominant Frequency Fz Open (Hz)	6.805	6.604	.0415	−0.2% (−0.4%, −0.01%)	0.1069	−0.0944
Dominant Frequency O1 Open (Hz)	7.792	7.280	.0001	−0.5% (−0.8%, −0.3%)	0.3731	−0.1390
Dominant Frequency O2 Open (Hz)	7.794	7.284	.0001	−0.5% (−0.8%, −0.3%)	0.3763	−0.1335
Dominant Frequency P3 Open (Hz)	7.701	7.197	<.0001	−0.5% (−0.7%, −0.3%)	0.2868	−0.2177
Dominant Frequency P4 Open (Hz)	7.720	7.221	.0001	−0.5% (−0.7%, −0.3%)	0.3069	−0.1929
Dominant Frequency Pz Open (Hz)	7.519	7.103	<.0001	−0.4% (−0.6%, −0.3%)	0.2273	−0.1890
Dominant Frequency T5 Open (Hz)	7.482	7.170	.0071	−0.3% (−0.5%, −0.09%)	0.2125	−0.0990
Dominant Frequency T6 Open (Hz)	7.610	7.213	.0009	−0.4% (−0.6%, −0.2%	0.2037	−0.1937
EEG Gamma-power 1 O1 Open (uV/Hz)	0.195	0.234	.0314	19.7% (1.7%, 40.9%)	−22.2%	−6.9%
EEG Gamma-power 1 T4 Open (uV/Hz)	0.341	0.449	.0193	31.7% (5.0%, 65.3%)	−26.4%	−3.0%
EEG Gamma-power 1 T5 Open (uV/Hz)	0.204	0.294	.0009	43.7% (16.7%, 76.9%)	−31.0%	−0.8%
EEG Gamma-power 1 T6 Open (uV/Hz)	0.205	0.278	.0025	35.5% (11.8%, 64.2%)	−27.1%	−1.2%
Total power F3 Open (uV/Hz)	0.555	0.460	.0138	−17.1% (−28.4%, −4.1%)	−14.1%	−28.8%
Total power F4 Open (uV/Hz)	0.554	0.498	.0293	−10.1% (−18.2%, −1.1%)	−12.5%	−21.4%
Total power F7 Open (uV/Hz)	0.870	0.780	.0378	−10.4% (−19.1%, −0.7%)	−12.1%	−21.2%
Total power F8 Open (uV/Hz)	0.933	0.773	.0070	−17.2% (−27.5%, −5.4%)	−9.5%	−25.0%
Total power Fp1 Open (uV/Hz)	1.039	0.852	.0020	−18.0% (−27.2%, −7.7%)	−3.7%	−21.0%
Total power Fp2 Open (uV/Hz)	1.045	0.885	.0087	−15.3% (−24.9%, −4.5%)	−5.0%	−19.5%
Total power Fz Open (uV/Hz)	0.509	0.449	.0089	−11.8% (−19.6%, −3.4%)	−9.4%	−20.1%
Theta/Beta Ratio C3 Open (%)	241.909	279.392	.0075	15.5% (4.2%, 28.0%)	−3.6%	11.4%
Theta/Beta Ratio C4 Open (%)	241.978	279.468	.0078	15.5% (4.2%, 28.0%)	−3.4%	11.5%
Theta/Beta Ratio F3 Open (%)	271.715	309.468	.0473	13.9% (0.2%, 29.5%)	−0.9%	12.8%
Theta/Beta Ratio Fz Open (%)	422.291	506.310	.0106	19.9% (4.7%, 37.3%)6	−7.4%	11.0%
Theta/Beta Ratio T4 Open (%)	196.557	155.649	.0225	−20.8% (−35.1%, −3.4%)	26.9%	0.5%

cyclopentolate 1%. The children awoke spontaneously 30 to 50 min after falling asleep. Drowsiness preceded the sleep. The mean onset of drowsiness was 22.8 min after the first eye drop. The majority (75%) reached sleep stage 3. All these children went rapidly from sleep stage 2 to sleep stage 3. In our subjects, the mean time to sleep stage 3 was only 7 min. In 5 of the 9 subjects, this was even less than 5 min. None completed the sleep cycle and arrived in REM sleep. Low BMI was present in six subjects (mean age 11.2 years) and normal BMI in 5 (mean age 9.8 years). The ratio of male to female was 2.33:1. Furthermore, 43% of the subjects with low BMI had drowsiness and fell asleep compared to 26% of the subjects with a normal BMI. Logistic regression, however, revealed that neither sex, age, BMI, or prior drowsiness in cyclopentolate 1% was a risk factor for falling asleep (all $p > .05$). Table 4 reflects the demographics as well as the sleep stages and latencies of drowsiness and the consecutive sleep stages of the eleven subjects who fell asleep.

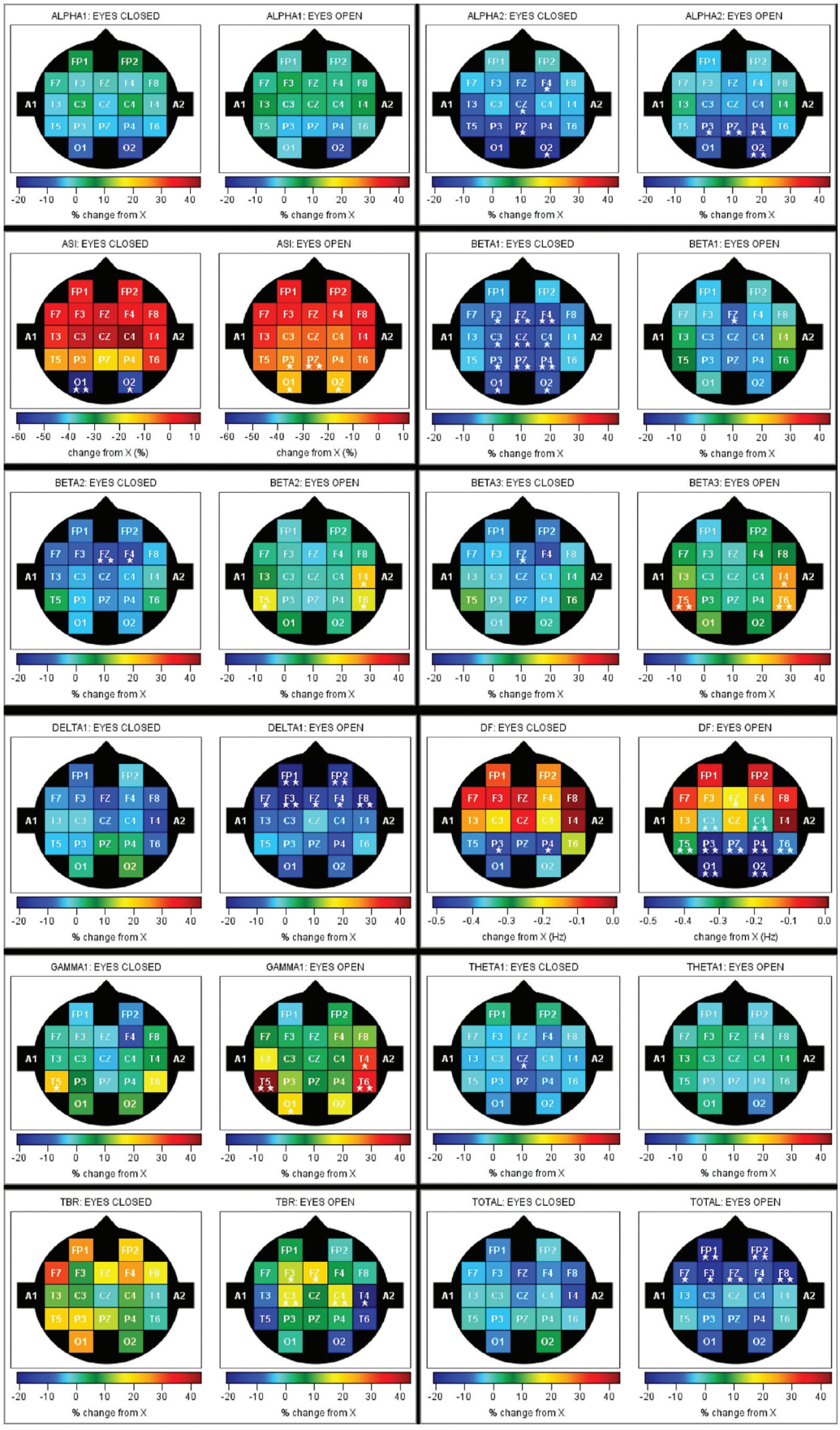

Figure 3. The significance level of the percentage change of contrast in the awake subjects is represented by stars: One star for percentage change from placebo with a p-value <.05 and two stars for percentage change from placebo with a p-value <.01. DF: dominant Frequency ASI: Activation Synchrony Index

Table 4. Overview of characteristics of subjects that had drowsiness and/or fell asleep. Displayed are sex, age in years, BMI category (low or normal), time and mean time (min) to drowsiness, and time and mean time (min) to sleep stages 1, 2, and 3.

	Subject	Sex	Age	BMI	Drowsiness	Sleep stage 1	Sleep stage 2	Sleep stage 3	REM-sleep
1	1	Male-1*	8	Low	36	43	46	48	-
		Male-2*	9	Low	31	39	44	49	-
2	2	Male	12	Low	10	15	-	-	-
3	3	Male-1*	10	Normal	16	28	28	37	-
4	4	Female-1*	15	Low	10	20	22	41	-
5	8	Female	11	Low	15	26	28	32	-
6	9	Male	8	Normal	27	39	46	52	-
7	16	Male	10	Normal	26	35	37	41	-
8	19	Female	6	Normal	14	24	27	39	-
9	23	Male	15	Normal	22	33	44	-	-
10	29	Male	7	Low	48	57	61	62	-
11	30	Male	14	Low	14	23	31	-	-
	Mean				**22.8**	**31.8**	**37.6**	**44.6**	-

*Visit 1 or 2.

Discussion

The main aim of this pilot study was to objectify EEG changes after cyclopentolate 1% eye drops. We succeeded in this aspect. Our pilot study showed without any doubt that cyclopentolate 1% can affect the CNS in both younger children and children in puberty. We were, however, surprised by the amount of significantly altered EEG parameters. Significant changes in cortical activity were present for almost all brain wave frequencies.

As discussed in the report of our previous study,[6] children have an increased risk for drug-related adverse events. They receive a greater dose relative to blood volume and body weight, a higher cutaneous blood flow, and less dense tissues thereby facilitating a more profound and rapid absorption of drugs.[11,13,32,33] Furthermore, a limited serum protein-binding capacity is presented. Metabolic systems and organs in children are not fully developed, and thereby clearing is slower.[32,33] The former and the latter result in higher availability of cyclopentolate in the blood plasma and a prolonged half-life of this specific drug.[32,33] The CNS adverse reactions are facilitated due to their large brain mass in relation to body volume and the higher blood–brain barrier permeability in children.[19,20] During puberty hormonal changes, rapid restructuration of the brain, and increased physical growth might explain the susceptibility to adverse reactions.[13,32,34]

The ophthalmic cycloplegics atropine, cyclopentolate, scopolamine, and homatropine belong to the group of tertiary amines. Tertiary amines are lipid soluble, cross-membrane barriers, and are well distributed into the CNS and other organs. To be able to pass the blood-brain-barrier (BBB) through lipid-mediated free diffusion certain drug characteristics are necessary. We admitted an overview provided by Pajouhesh and Lenz[35] in Table 5 and added the known corresponding characteristics of the ophthalmic cycloplegics in this table.

As Table 5 reflects, scopolamine and cyclopentolate share strong BBB penetrating properties. Wherein atropine and homatropine eye drops mainly peripheral adverse reactions occur and higher doses are required for central effects, scopolamine is known for its central effects.[36–38] Scopolamine produces CNS depressive effects, such as drowsiness and amnesia, and tends to promote sleep and, in induced sleep, it is often dreamless.[37,38] In contrast to cyclopentolate, scopolamine is well investigated in many aspects.

In the study of Liem-Molenaar et al.,[39] using 0.5 mg intravenous scopolamine, 88 of the 90 subjects experienced anticholinergic symptoms. Besides dry mouth, nausea, and palpitations, central adverse reactions, such as dizziness, drowsiness, and concentration problems were reported. Cognitive testing showed significant impairment of memory, reduced attention, and awareness. Also, in our study population, exactly these specific adverse reactions were reported. Alvarez-Jimenez et al.,[40] using the same regime, showed an increase in the effects in elderly persons, which they explained as due to a lower clearance of the scopolamine in the elderly, which is in this aspect comparable to children. Liem-Molenaar et al.[39] and Alvarez-Jimenez et al.[40] found a mean maximum plasma concentration of 3.9 to 5.2 ng/ml^{-1} at 15 min in normal BMI subjects.

Table 5. Overview of drugs characteristics needed to be able to penetrate the blood–brain barrier according to Pajouhesh and Lenz (2005). Displayed are the values of the individual characteristics of cycloplegics used in (pediatric) ophthalmology. In green marked the values meeting the criteria of Pajouhesh and Lenz.

Drugs Characteristics	Pajouhesh and Lenz, 2005	Atropine Sulphate[a]	Scopolamine Hydrobromide[b]	Cyclopentolate Hydrochloride[c]	Tropicamide[d]	Homatropine Hydrobromide[e]
Molecular weight (g/mol)	<450	677	384	291	284	356
Minimal hydrophobicity (LogP)	<5	1.8	1.4	2.4	1.3	1.9
Hydrogen bond donors	<3	4	2	2	1	2
Hydrogen bond acceptors	<7	12	5	4	3	4
Number of rotatable bonds	<8	10	5	7	6	4
pKa	neutral or basic with favorable pKa 7.5	9.4	7.8	7.9	5.2	9.9
Polar Surface Area (PSA)	< 60-70 A^2	182	62	50	53	50
Metabolic stability (half-life)	>80% remaining after 1 hour	3 hours	4.5 hours	111 min.	14 min.	8 hours
P-gp interaction	Not an efficient P-glycoprotein substrate (in vivo)	Unknown. Insufficient information/scientific literature/studies available				
Affinity serum albumin ligand	K_d <10 µm (= low)					
Permeability	>1 x 10^{-6} cm/sec					

[a]https://pubchem.ncbi.nlm.nih.gov/compound/5927 (Atropine Sulfate).
[b]https://pubchem.ncbi.nlm.nih.gov/compound/Scopolamine-HBr.
[c]https://pubchem.ncbi.nlm.nih.gov/compound/22162 (Cyclopentolate Hydrochloride).
[d]https://pubchem.ncbi.nlm.nih.gov/compound/5593 (Tropicamide).
[e]https://pubchem.ncbi.nlm.nih.gov/compound/6419941 (Homatropine Hbr): Accessed 28-08-2022.

The plasma-binding properties of cyclopentolate 1% eye drops also seem comparable to scopolamine. In adults, 60 µl cyclopentolate 1% resulted in a peak plasma concentration of 2.8 to 3.3 ng/ml^{-1} at 15 min,[8] recalculating an 4.7 to 5.5 ng/ml^{-1} in 100 µl. In children 35 µl cyclopentolate 1% resulted in a mean plasma concentration of 2.9 ng/ml^{-1}.[13] In our well-informed and cooperative subjects, there was no resistance, i.e., squeezing of the eyes and/or crying. Thus, it is likely that the upper limit of 80% absorption as postulated by Lee and Robinson[11] was obtained, rendering a mean plasma concentration of about 6.6 ng/ml^{-1} in 100 µl.[13] In this respect, our findings; such as the sleep, the significant changes in cortical activity at all brain wave frequencies in our awake subjects, the complaints of our subjects, and the findings of our previous study,[6] are not surprising.

At this moment, there are no scientific reports available for EEG pattern changes in short-acting ophthalmic cycloplegic eyedrops. However, EEG pattern changes after intravenous administration of scopolamine are well described.[39–41] In all studies alpha, beta, theta, and delta waves changed. A correlation between a decrease in attention and lethargy and changes in spontaneous EEG patterns was established. Several studies showed that in scopolamine EEG- and cognitive changes occurred in even small doses, representing their strong BBB penetrating properties.[39,40] Correspondingly, a PET study by Frey et al.[42] showed scopolamine retention in the CNS. As early as 5 to 8 min after intravenous injection, scopolamine was visible in the frontal and occipital cortices. At 30 min significant retention was present in the occipital-, frontal- and parietal cortex, as well as the pons, thalamus, and caudate nucleus.

Cyclopentolate 1%, sharing similar BBB penetrating properties, even in eye drops, produces similar EEG pattern changes, and similar CNS depressive effects such as impaired memory, decreased attention, alertness, as well as mind wandering, drowsiness, and tends to promote sleep. Deep sleep following cyclopentolate 1% occurred unexpectedly in our study population. During normal sleep, a person usually progresses through three stages non-REM-sleep and REM-sleep. The mean time to drowsiness and sleep in minutes was in concordance with the time of onset of adverse reactions reported in the literature and in our previous study.[6–9,43] At the age of 5, the sleep cycles have matured to adult length.[44,45] Normally, sleep stage 2 in our age population lasts about 20 to 40 min.[46,47] In our population, this was a factor 3–6 shorter. In normal sleep, sleep stage 3 is followed by REM-sleep. REM sleep was absent in our subjects. Sleep cycle patterns in cyclopentolate have not been investigated up to now. Scopolamine sleep cycle patterns, however, are well investigated. Significantly prolonged latency to REM sleep and shortened duration of REM sleep after intravenous and oral use was established.[48,49] In transdermal use, the latency was similar to placebo, but REM activity, density, and intensity were significantly decreased.[50]

In our study, as is customary in most ophthalmic practices, the subjects received a double dose of cyclopentolate 1% in each eye. An increased risk for adverse reactions; especially moderate-to-severe drowsiness, in repeated cyclopentolate 1% eye drop is established.[6] We postulate that the upper limit of absorption in our cooperative subjects and thereby high plasma concentrations are the cause of the deep sleep in many of our subjects. Since the use of a double dose of cyclopentolate 1% is customary for the measurement of refractive errors, especially in children and teens, it is advisable to reconsider this regime to minimize side effects. For example, it is shown that one drop of cyclopentolate, if necessary supplemented with one drop of tropicamide 1% in pigmented subjects, is most of the time equally effective and decreases the risk for adverse reactions.[6,43,51]

In conclusion, we showed that two drops of cyclopentolate 1% eye drops can affect CNS function, and if affecting the CNS, in general seems to act as a CNS depressant.

In this pilot study, we deliberately chose to first determine the feasibility of extended EEG recording in (young) children before adding more complex tasks such as cognitive, and motor function testing (i.e., pharmacodynamics), or the burden and stress involved in blood sampling needed for plasma concentration analyses (i.e., pharmacokinetics). To gain more insight, further research, in a larger population, combining EEG with pharmacokinetic and pharmacodynamic data, is necessary.

Limitations of the study

- Our study had some form of bias. A percentage of children and parents who agreed to participate were known patients. Some of them already had experienced the side effect drowsiness during an earlier visit. This group of subjects may perhaps be more prone to side effects.

Conclusions and implications for ophthalmic health care professionals

This pilot study shows that cyclopentolate 1% is capable of affecting the CNS function in both younger children and children in puberty. It appears to act as a CNS depressant. Nevertheless, however, cyclopentolate 1% eye drops can safely be used in children and young adolescents. A moderate altered state of consciousness and drowsiness is the most common adverse reaction that can be expected. The symptoms are mild and there are no indications whatsoever of long-lasting health effects, but awareness of the short, passing adverse reactions is warranted.

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