JASA Express Letters

OCTOBER 03 2023

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JASA Express Lett. 3, 104401 (2023)
https://doi.org/10.1121/10.0021187

JASA EXPRESS LETTERS ARTICLE pubs.aip.org/asa/jel

Enhanced suppression of otoacoustic emissions by contralateral stimulation in Parkinson's disease

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Abstract: Dopamine depletion affects several aspects of hearing function. Previous work [Wu, Yi, Manca, Javaid, Lauer, and Glowatzki, eLife **9**, e52419 (2020)] demonstrated the role of dopamine in reducing the firing rates of inner ear cells, which is thought to decrease synaptic excitotoxicity. Thus, a lack of dopamine could indirectly increase acoustic stimulation of medial olivocochlear efferents. To investigate that, here we studied contralateral suppression of distortion product otoacoustic emissions in a population of Parkinsonian patients, compared to an age-matched control group, both audiometrically tested. To rule out activation of the acoustic reflex, middle ear impedance was monitored during testing. The results show significantly stronger contralateral suppression in the patient group.

[Editor: Christopher Bergevin] https://doi.org/10.1121/10.0021187

Received: 21 June 2023 **Accepted:** 11 September 2023 **Published Online:** 3 October 2023

1. Introduction

The association between neurodegenerative pathologies and the auditory pathway has been recently investigated by several studies.[1–5] The presence of cochlear dopaminergic receptors in different elements of the peripheral auditory system is likely responsible for the observed statistical associations between neurological and audiological (both audiometric and otoacoustic) variables, although complete mechanistic models of these interactions are still missing. Otoacoustic variables showed high sensitivity to such effects, and a lateralizaton correlated to that of the motor symptoms of the patients.[2] Recently,[3] a significant association was found between the lack of contralateral dopamine availability, measured by a radioactive tracer diagnostic test called DaTSCAN, and the DPOAE level, only in the left-side predominant group of Parkinson's disease patients.

One of the elements of the auditory system that may be investigated to clarify this issue[4,5] is the medio-olivocochlear (MOC) efferent system, which modulates the gain of the cochlear amplifier, and therefore, the amplitude and phase of otoacoustic emissions (OAEs). Although other nonlinear mechanisms could play a minor role, the cochlear nonlinearity associated with the mechanoelectric transduction (MET) of the outer hair cells (OHC) is considered responsible[6] for the generation of intracochlear distortion product (IDP) traveling wave (TW) components in the cochlear regions where two or more frequency components simultaneously drive the nonlinear local amplifier. The IDP propagates in both directions along the cochlear longitudinal direction x, and may be linearly reflected by randomly distributed impedance irregularities (roughness), and by the impedance mismatch between the cochlea and the middle ear at the cochlear base.[7] Due to different mechanisms, a fraction of the TW propagates back towards the cochlear base,[8–11] partially gets through the middle ear, and may be eventually detected in the ear canal as distortion product OAEs (DPOAE). In the mammalian cochlea, the most studied DPOAEs are cubic intermodulation distortion products generated at frequency $2f_1$-f_2 by two tones of nearby frequencies f_1 and f_2. One may identify a "distortion" or "generator" DPOAE component, associated with the superposition of the backward IDP wavelets generated in the overlap region near $x(f_2)$, and a "reflection" component, associated with the fraction of the forward IDP waves that are amplified and reflected by roughness near the $x(f_{DP})$ place.[8] The distortion component phase is almost independent of frequency, as predictable for a wave-fixed generation mechanism in a scaling symmetric cochlea, while the group delay of the reflection component is predicted to be inversely proportional to frequency in the same scale-invariant limit.[12,13]

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The nonlinear compressive response of the cochlear amplifier is modulated by contralateral acoustic stimulation (CAS), which is generally an intense acoustic noise stimulus fed to the contralateral ear during a measure of the OAE response in the ipsilateral ear. This effect is mediated by the MOC efferent system, characterized by a short response time, which allows one to interleave several periods of acquisition with and without CAS within a single OAE measurement session, and to compare the two responses without any bias associated with slow changes of the response level due, e.g., to movement of the probe in the ear canal.

Theoretically, the IDP generation changes induced by CAS may affect the DPOAE components differently. The IDP level reduction in the $x(f_2)$ overlap region tends to decrease the level of both components, while the decrease in the cochlear gain in the $x(f_{DP})$ region further decreases the level of the reflection component only, whose group delay is also sensitive to changes in the cochlear tuning induced by CAS.[10] The distortion component level could be affected in a complex way by cochlear tuning changes. Indeed, the level of the distortion DPOAE component depends non-monotonically on the width of the overlap region due to interference between the wavelets of different phase coming from different places within this region. This is the reason for the "resonant" shape of the DPOAE level-ratio functions (for the distortion component only, see Sisto *et al.*[14]) and may become a confounding effect in DPOAE CAS experiments.

Wu *et al.*[15] demonstrated in rodents that dopamine released by lateral olivocochlear (LOC) efferent neurons down-regulates auditory nerve fiber (ANF) firing rates, with a protecting effect against synaptic excitotoxicity. The LOC effect, whose characteristic time is longer than that of the MOC effect, is the result of a fine-tuned balance between the antagonist roles of acetylcholine and dopamine. We hypothesize that the lack of dopamine characteristic of the Parkinsonian patients could imply higher ANF firing rate and, consequently, stronger activation of the MOC reflex, and consequent increased DPOAE contralateral suppression.

The DPOAE response is also obviously affected by the transmission of the middle ear, as regards both the intensity of the stimuli actually fed to the cochlea and the fraction of the OAE response getting back through the middle ear. For this reason, any activation of the acoustic reflex (MEM) could affect the results of a CAS experiment. On the other hand, the amplitude of the OAE response changes due to CAS tends to increase with increasing CAS level,[16] improving the statistical significance of the comparisons. Therefore, we used a relatively high white noise level (in our case, 80 dB SPL), while monitoring changes in the middle ear transmission in real time during the DPOAE measurements, and excluded from the analysis the (few) cases in which a significant difference in the middle ear energy reflectance was observed.

2. Methods

Pure tone audiograms and high frequency-resolution complex DPOAE spectra were recorded with and without CAS in both ears of 90 patients affected by Parkinson's disease and in a matched control group of 41 subjects. We recruited outpatients with a diagnosis of idiopathic Parkinson's disease, from the Parkinson's Disease Center of the University of Rome "Tor Vergata." Patients and controls reporting professional exposure to high noise levels and/or middle ear disorders were excluded from the analysis. Additional information exclusion criteria, patient selection, neurological evaluation, Parkinson's disease severity and progression, and motor disability level is available in a previous study on a largely overlapping population.[2]

For the Parkinsonian patients, each ear was classified as ipsilateral (I) and contralateral (C) with respect to the side affected by the worse motor symptoms. This definition, already adopted in previous studies on OAEs and Parkinson's disease using subsets of the same population,[2,3] must not create confusion with the standard CAS definition of ipsilateral and contralateral ear as, respectively, that of the OAE measurement and that of the noise stimulus.

The customized acquisition setup, programmed in LABVIEW (National Instruments), and consisting of two NI4461 data acquisition boards, uses two swept tone stimuli (ascending linear chirps) $f_1(t)$ and $f_2(t)$, of levels $(L_1, L_2) = (65, 55$ dB SPL), to elicit the complex DPOAE response, which is recorded at frequency $f_{DP}(t) = 2f_1 - f_2$, in the 1–5 kHz range, with 20 Hz frequency resolution. Each acquisition is divided in 50%-overlapping, Hanning-windowed frames of duration 50 ms, and the optimal speed of the DP chirp is set to 800 Hz/s in order to get a frequency resolution of the Fourier analysis of each frame that matches the f_{DP} change between subsequent frames. A total measurement time of 3 min was necessary for testing each ear, corresponding to the acquisition of an average of 15 coherently averaged "clean" frames for each frequency bin.

In the same measurement session, the audiometric threshold was also evaluated for all ears in the [0.125–8 kHz] range with 5 dB accuracy using a Madsen Astera2 clinical audiometer. All measurements were performed in an audiometric booth.

A data rejection threshold was set at SNR = 3 dB. To reduce the bias of aging, the age match between the SNR-selected data subsets of patients and controls was restored by excluding from the analysis the subjects with age < 45 years. Time-frequency filtering[17] of the complex spectra was used to unmix distortion (or zero-latency, ZL) and first reflection components, with a further SNR advantage varying between 6 and 12 dB in the 1–5 kHz range. This way, the SNR of the ZL DPOAE component included in the analysis is actually quite high, between 9 and 15 dB, and the selected ears have almost normal hearing, e.g., the 83% of audiometric data were in the normal hearing range with 13% in the mild range of loss. This is important to separate the effect of presumed lack of dopamine from that of hearing loss, which is correlated

to Parkinson's disease and to the DPOAE SNR.[2] The effect of CAS was evaluated for each ear by computing the half-octave band DPOAE level with and without CAS for the ZL DPOAE component. Note that, although we label the DPOAE bands with their center f_{DP} frequency, as the generation place of the ZL DPOAE component is near the $x(f_2)$ place, the clinically relevant frequency is the corresponding f_2, approximately equal to $1.5f_{DP}$.

The MOC reflex should yield a reduction of the DP level. Thus, negative level differences are expected, but we did not exclude from the analysis the few positive values, which, in principle, are not necessarily due to artifacts. We excluded instead from the analysis the few ears in which a significant change of the middle ear energy reflectance was systematically induced by CAS, because this phenomenon could be attributed to the activation of the acoustic reflex and could mask the MOC effect. Although we did not directly estimate the energy reflectance, we monitored the difference between the pressure level measured in the ear canal at the stimulus frequencies with and without CAS and attributed to the acoustic reflex any difference exceeding 1 dB.

2.1 Statistical analysis

All statistical analyses were performed using the software R (version 4.0.3, R Foundation for Statistical Computing). A significance criterion $p \leq 0.05$ was conventionally adopted. The difference between the ZL DPOAE response amplitude measured with contralateral suppression and without it was evaluated in four half-octave frequency bands. A t-test (one tail, for heteroskedastic samples) was performed in each of the four frequency bands to compare and test the significance of the difference between patients and controls. The ipsilateral and contralateral ear with respect to the worse motor symptoms were separately compared to the control subjects.

Multivariate mixed-effect linear regression models were also fitted to the data. In this approach, particularly useful in case of non-independent measures performed on the same subject, the subject is considered as a random variable. All ZL DPOAE levels were treated as a unique variable, being the half-octave frequency band a four-level factor (as in Sisto *et al.*[2]) a factor named frequency was added in order to define the frequency band. Age was treated as a continuous variable whilst the factor "ear" was considered at the aim of studying the possible differences between the right and left side. A two-level factor "diagnosis" was defined to distinguish the patients from the controls. In the case of patients, the factor "laterality," distinguishing ipsilateral and contralateral ears with respect to the body side that was more affected by motor symptoms, was introduced as in the previous papers. The mixed-effect models do not produce a determination coefficient, the statistical significance of the model itself was tested by fitting two models, one including the fixed effect factor of interest and the other one without it. The two models are compared by means of an analysis of variance (ANOVA) test.

3 Results

The selection rules listed above selected a much smaller data subset (61 ears of patients and 27 ears of controls), differently populated in the four bands, due to the different SNR. The resulting groups of patients and controls had the same mean age of 62 years, with standard deviations of 7 and 8 years, respectively. Across the explored frequency range, the suppression effect of CAS is larger in the patients (see Fig.1), and, particularly, in their ipsilateral ears (i.e., those on the same side of the body affected by the worse motor symptoms). The difference between patients and controls reaches statistical significance ($p < 0.015$) only in the 2.8 kHz frequency band. The audiometric hearing levels of the selected ears in the 1.5–6 kHz range (clinically corresponding roughly to the frequency range of the four half-octave DPOAE bands) are reported in Fig.1(b), confirming that the strict SNR-based selection rule of the present study yielded audiometrically comparable subsets of ears, with mild hearing loss, or none at all.

The multivariate fixed effect model comparing the patients and the controls simultaneously in all frequency bands did not give a significant effect for the factor diagnosis, i.e., between patients and controls. If only the ipsilateral ear of the patients were considered, a significant coefficient was found for the factor diagnosis. The average increase in the DPOAE suppression in the ipsilateral ears, with respect to the control ears, was 0.32 dB ($p < 0.05$). The model containing the factor diagnosis and the model without it were compared by means of an ANOVA test. The comparison gave a significant result ($p < 0.05$).

4. Discussion

The MOC reflex is expected to suppress the DPOAE amplitude during contralateral acoustic stimulation (CAS). The MOC system is in fact able of adjusting the working point of the cochlear amplifier, reducing the gain and linearizing the gain function as the background sound level increases. The neurotransmitter involved in the efferent MOC is acetylcholine. Although the role of dopamine in the cochlea has not been completely clarified, several animal studies have been performed. Among them, Darrow *et al.*[18] studied the dopaminergic innervation of the mouse inner ear, and Maison *et al.*[19] demonstrated the effect on the DPOAE reduction of the targeted depletion of dopamine D2 receptors in knockout mice.

The behaviour of the efferent MOC system in Parkinsonian patients has been recently evaluated,[4,5] finding no significant differences between patients and healthy controls. In the present paper, general increase in the DPOAE suppression due to CAS was found in the patients with respect to the control subjects, more clearly visible in an intermediate

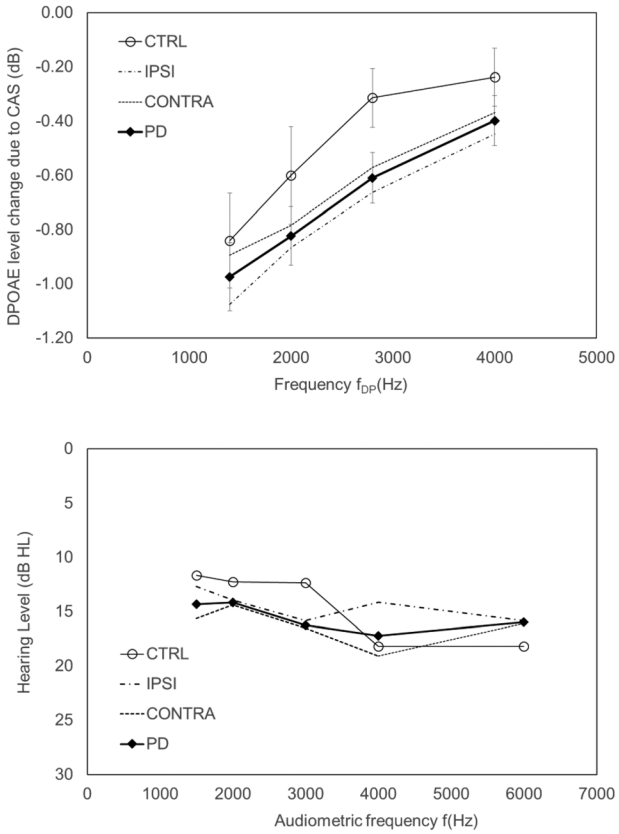

Fig. 1. (Top) Average ZL DPOAE level change due to CAS in controls (open circles) and in Parkinsonian patients (black diamonds). The dashed and the dashed-dotted lines separately refer, respectively, to the contralateral and ipsilateral ears of the patients. Error bars (representing standard errors) are reported, for clarity, only for the controls and for the patients. (Bottom) Average audiometric hearing levels of the patients and of the controls in five half-octave-spaced audiometric bands, roughly corresponding to the f_2 range explored by the DPOAE measurements.

frequency band. When all the frequency bands are simultaneously compared, the statistical significance of the enhanced suppression is not reached when all the ears of the patients are compared to the controls. If only the ears of the patients ipsilateral to the side more affected by the motor symptoms are compared to the controls the statistical significance of the enhanced suppression is reached in the multivariate approach. Interestingly, in our previous studies the ear ipsilateral to side of the body more affected by motor symptoms was found more injured than the contralateral one.[3] Therefore, we suggest that introducing such a distinction between the ears based on the side more affected by the motor symptoms may be generally useful in audiological studies to detect such small systematic differences between controls and patients.

The role of sound in stimulating the synthesis of dopamine has been recently clarified.[1] The dopaminergic LOC input to the auditory nerve fibers (ANFs) is dynamically regulated in order to increase the dopamine when the sound level increases. This effect has been studied in an animal model using rodents, mice, and rats. Sound was demonstrated to upregulate an enzyme that is responsible for the synthesis of dopamine in the LOC efferent system. It was demonstrated that the dopamine has the role of protecting the synapses from the excitotoxicity induced by acetylcholine. In particular, dopamine down-regulates ANF firing rates (whereas acetylcholine up-regulates it) protecting the synapses from excess of stimulation. Dopamine has therefore a crucial role acting as a filter capable of reducing the excitotoxicity at the level of the synapses. One can speculate that in Parkinsonian patients, the reduced or totally absent capability of dynamic dopamine synthesis causes a potential damage to the synapses, no more protected against excitotoxicity, which would explain the hearing deficit of Parkinsonian patients observed in several studies.[1–3] Such a mechanism would also explain the results of the present study. Indeed, for the same noise stimulation level, a higher firing rate would imply in the patients a stronger MOC effect, and a larger suppression by CAS. In this scenario, the efferent feedback system would react to the dysfunctionality consisting in the absence of dynamical capability of synthesis of dopamine with a (slightly) stronger reduction of the gain of the cochlear amplifier.

We further note that the effect is observed after having selected the ears with high DPOAE SNR, and, consequently, normal or close to normal hearing, as shown in Fig.1. If we repeat the analysis using a lower SNR threshold, we include hearing impaired subjects, and the effect is masked by the systematically decreased mechanical response of the

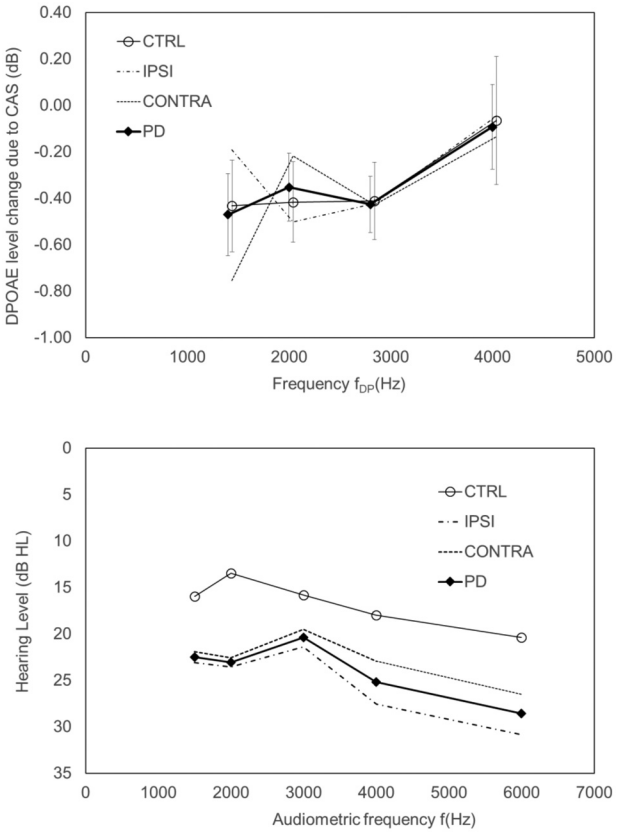

Fig. 2. Same as Fig.1, with a SNR data selection threshold increased by 10 dB. A significant difference between hearing levels in patients and controls masks the effect shown in Fig.1. A small dummy frequency shift was added to the control data in the top panel to visually differentiate the error bars.

cochlea in the Parkinson's disease patients. Indeed, to test the robustness of the result, the threshold for the SNR-based data selection rule was changed in order to include in the analysis also ears with significant levels of hearing loss. The observed effect gradually disappeared as the threshold was increased by up to 10 dB [see Fig. 2(a)]. When this threshold level condition is reached, the number of accepted ears approximately doubles in both populations with respect to Fig. 1. By doing so, one not only accepts noisy data that increase the statistical uncertainties, but also introduces a bias, because in this case the average hearing level is significantly worse in the patients [see Fig. 2(b)], as previously demonstrated in Sisto *et al.*[2] Our explanation of this behaviour is that worse hearing level means lower input to the efferent system by the same noise level and, consequently, lower DPOAE suppression by CAS, and that the two opposite effects tend to balance themselves unless very high SNR data, or comparable average audiometric levels, are selected. We also remark that the typical SNR of elderly subjects, particularly if affected by Parkinson's disease, is typically low. For this reason, the additional SNR improvement provided by time-frequency filtering could be necessary to bring to evidence such small differential effects.

5. Conclusion

Advanced otoacoustic emission acquisition and filtering techniques permitted the detection of small differences between the average suppression of DPOAE induced by CAS in patients and in controls, by selecting high SNR data. The stronger CAS suppression observed in the patients is consistent with the reduced effectiveness of the ANF firing rate regulation mediated by dopamine.

Acknowledgments

This work was supported by INAIL Grant No. BRiC 2022 ID08.

Author Declarations

Conflict of Interest

The authors declare no conflict of interest.

Ethics Approval

Written informed consent was given by all participants after having received an extensive disclosure of study purposes, according to the Declaration of Helsinki. All procedures were approved by the ethics committee of the University of Roma "Tor Vergata" (protocol No. 7/18, Feb. 7th, 2018).

Data Availability

The data will be made available in anonymous form upon request to the corresponding author, as well as all the MATLAB and R analysis codes.

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