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Effect of mitochondrial toxins on evoked somatosensory activity in rats

Communication

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Abstract: Mitochondrial toxins represent an interesting group of neurotoxicants related both to causation and modelling of central nervous damage. 3-nitropropionic acid (3NP), a neurotoxin of herbal and microfungal origin, inhibits succinate dehydrogenase leading thereby to various biochemical and morphological alterations in the brain. Experimental animals treated by 3NP are used to model Huntington's disease. Manganese, often present in occupational settings and as environmental pollutant, inhibits complex II and III of the mitochondria and is known to cause Parkinson-like CNS damage. In this work, rats were administered a single acute dose of Mn (50 mg Mn^{2+}/kg body weight) or 3-NP (20 mg/kg b.w.) and the alterations of the somatosensory cortical evoked potential elicited by stimulation of the whisker pad and the tail base were observed, together with the changes of the action potential in the tail nerve. Latency and amplitude of the two cortical responses changed in parallel, while those of the tail nerve response remained more or less unaltered. The two mitochondrial toxins studied seem to exert their action centrally, primarily on synaptic transmission, rather than peripherally. Recording of evoked activity could be used to follow-up the nervous system effects of mitochondrial toxins, but it requires further investigation.

Keywords: *Mitochondrial toxin • Neurotoxicity • Evoked potential • Nerve action potential • Rat*

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1. Introduction

Mitochondrial damage leads to energetic crisis, which may have severe consequences in an energy-demanding structure like the nervous system. Disturbance of cortical functions were observed in humans with inherited or idiopathic mitochondrial dysfunction, such as mitochondrial encephalomyopathy [1]. The EEG abnormalities (typically slowed activity [2]) observed in such patients were interpreted as a sensitive indicator of mitochondrial disorder in cells of the brain [3]. Nervous system phenomena caused by mitochondrial damage of toxic origin (by environmental agents or in experimental exposure) are not less well known. An important environmental mitochondrial toxicant, representing a major occupational health problem, is manganese. This metal is essential for living organisms in small amounts but toxic when overdosed. Exposure to abnormally high levels of manganese has traditionally been a risk factor of the metal and metal-processing industry. Population-level Mn exposure results from the use of methylcyclopentadienyl manganese tricarbonyl as an anti-knock petrol additive in certain countries, organic Mn compounds applied as fungicides, and spent dry cells in solid household waste [4].

The typical outcome of occupational Mn exposure, manganism, is a Parkinson-like syndrome characterized by functional and structural damages to the dopaminergic systems [5]. As evidenced by results of experiments in rats [6] this is caused by the blocking effect of Mn on mitochondrial complex II and III.

Other known mechanisms of action of Mn on the nervous system include block of voltage-dependent Ca-channels in neurons and presynaptic endings [7]. The release of excitatory transmitters and GABA were both reduced by moderate doses of Mn [8]. Inhibition of

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astrocytic glutamate uptake by Mn can, on the contrary, enhance synaptic transmission in the cortex [9].

Exposure by the substance 3-nitropropionic acid (3-NP) of environmental origin has been infrequent but resulted in development of an important disease model (see below). *Astragalus* species (*Leguminosae*) occasionally intoxicate grazing animals [10] while human intoxication may result from infestation of foodstuffs (sugar cane, cereals *etc.*) with moulds of the *Anthirium* and *Aspergillus* genus producing 3-NP. Hypodensity in the putamen and globus pallidus was consistently found on cranial CT images from humans after 3-NP intoxication [11]. In some cases, nucleus caudatus and claustrum were also affected. The morphological damage and decreased motor performance after 3-NP intoxication have been replicated in animals, which led to creation of an animal model of human Huntington's disease [12].

At the cellular level, 3-NP inhibits succinate dehydrogenase, a key enzyme of oxidative energy production [13] which effect develops fast and is not limited to the sites of morphological damage [14]. Beyond that, 3-NP was found to act on NMDA receptors thereby inducing excitotoxicity [15].

Inhibition of mitochondrial energy production and interference with glutamatergic transmission are two obvious common points in the action of the mentioned two agents, what justified their common investigation in an earlier work of the department [16]. Further, both effects are ubiquitous in the whole nervous system (in fact, the whole organism) suggesting that phenomena other than dopaminergic motor control could be affected by Mn or 3-NP, and be, hence, of practical importance as functional tests for following-up both the level of damage in Mn-exposed individuals and the development of disease in the Huntington model. Recording and analysis of peripheral and central evoked responses offers an approach for this purpose, as it can be performed in a non-invasive and repeatable way if the necessary technique is available. For exact interpretation, however, the damage indicated by alterations of the cortical response has to be localized within the complete sensory pathway. In this work, this question was addressed by administering the two agents in question to rats and comparing cortical and peripheral responses obtained in the same animal under identical conditions.

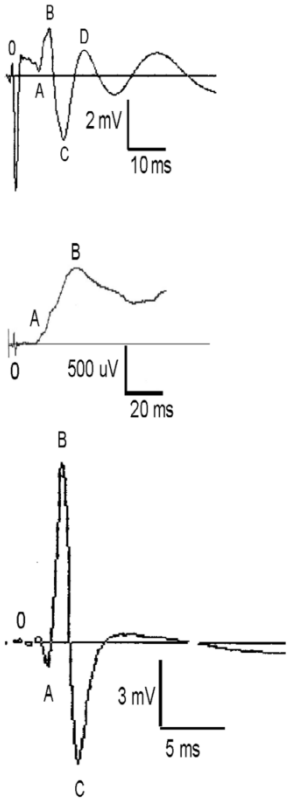

Figure 1. Examples of cortical and peripheral evoked activity and measurements on the curves. Top, cortical somatosensory evoked potential (SEP) elicited by whisker stimulation; onset latency: 0 (stimulus artefact) - A, amplitude: B-C. Middle, SEP elicited by tail stimulation; onset latency: 0 - A, amplitude: A-B. Bottom, tail nerve compound action potential; onset latency: 0 - A, amplitude: B-C.

2. Experimental Procedures

2.1 Animals, preparation and treatment

Adult (*ca.* 300 g) male Wistar rats were prepared for electrophysiological recording in urethane anaesthesia (1000 mg/kg b.w. ip. [17]). The head of the rat was clamped in a stereotaxic frame and the left hemisphere was exposed. Wounds were sprayed with 10% lidocaine and the exposed cortex was covered with warm paraffin oil. After *ca.* 30 min recovery, silver recording electrodes were placed on the somatosensory projection area of the whiskers (barrel field [18]) and of the tail. The contralateral whisker pad and the base of the tail was stimulated by electric pulses (just supramaximal strength: *ca.* 4 V, 0.05 ms, 1 Hz) delivered *via* a pair of needle electrodes. In the tail, another pair of needles was inserted 50 mm distally, to record the compound action potential of the tail nerve (for details, see [19,20]). In a recording session, a train of 20 stimuli was first delivered to the whiskers and somatosensory cortical evoked potentials (SEPs) were recorded from the barrel field. The tail was then stimulated with a train of 50 pulses, for recording cortical SEPs, and with another train of 20 stimuli, for recording compound action potential of the tail nerve. The recording sessions followed each other

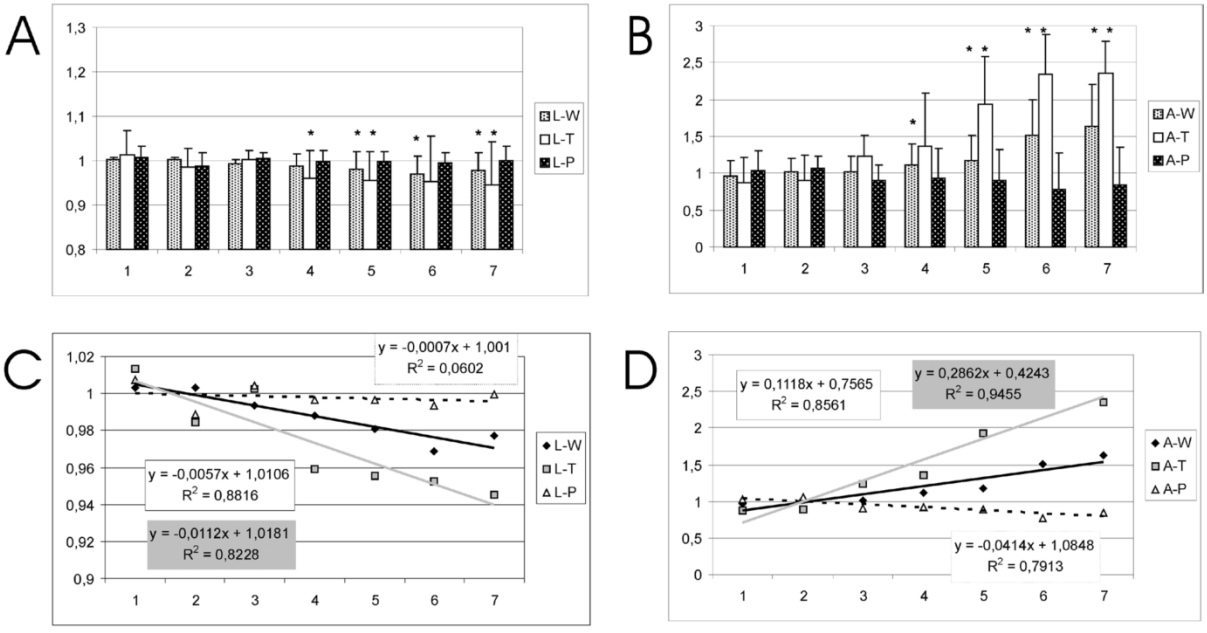

Figure 2. Effects of acute Mn administration on the evoked responses. (A): Latency of the cortical EP obtained by stimulation of the whiskers (L-W) and the tail base (L-T), and of the tail nerve action potential (L-P, "P" for peripheral). Abscissa: measurements; 1-3: before Mn injection (control), 4-7: after Mn injection (50 mg Mn/kg b.w.). Mean+SD, n=10, data normalized to the average of the three controls. * $P<0.05$ *vs.* baseline (averaged controls). (B): Amplitude of the cortical EP obtained by whisker (A-W) and tail base stimulation (A-T), and of the tail nerve action potential (A-P). (C) and (D): Time trend of latency (C) and amplitude (D) visualized by fitted straight lines. Corresponding lines and inserts – equation and R^2 value – have the same graphical marking.

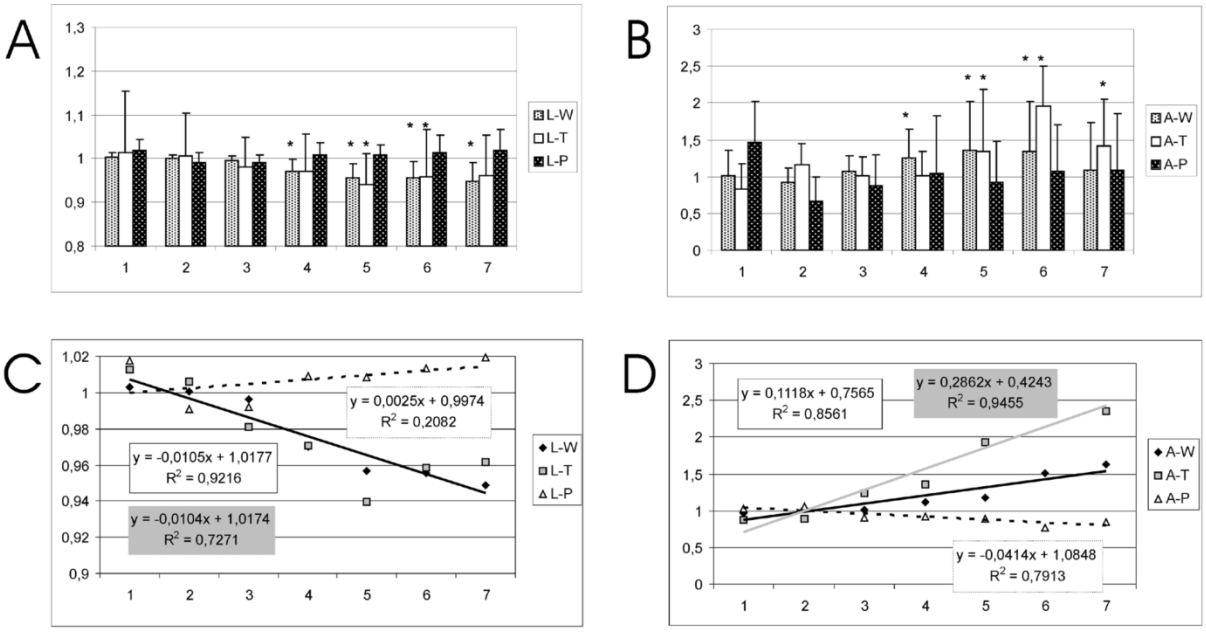

Figure 3. Effects of acute 3-NP administration on the evoked responses. (A): Latency of the cortical SEP elicited by whisker (L-W) and tail base stimulation (L-T), and of the tail nerve action potential (L-P). Abscissa: measurements; 1-3: before, and 4-7: after 3-NP injection (20 mg /kg b.w.). Mean+SD, n=10, data normalized to the average of the three controls. * $P<0.05$ *vs.* baseline (averaged controls). (B): Amplitude of the cortical SEP elicited by whisker (A-W) and tail base stimulation (A-T), and of the tail nerve action potential (A-P). Time trend of latency (C) and amplitude (D) visualized by fitted straight lines. Corresponding lines and inserts – equation and R^2 value – have the same graphical marking. Note that in C, the fitted lines for A-W and A-P are exactly overlapping.

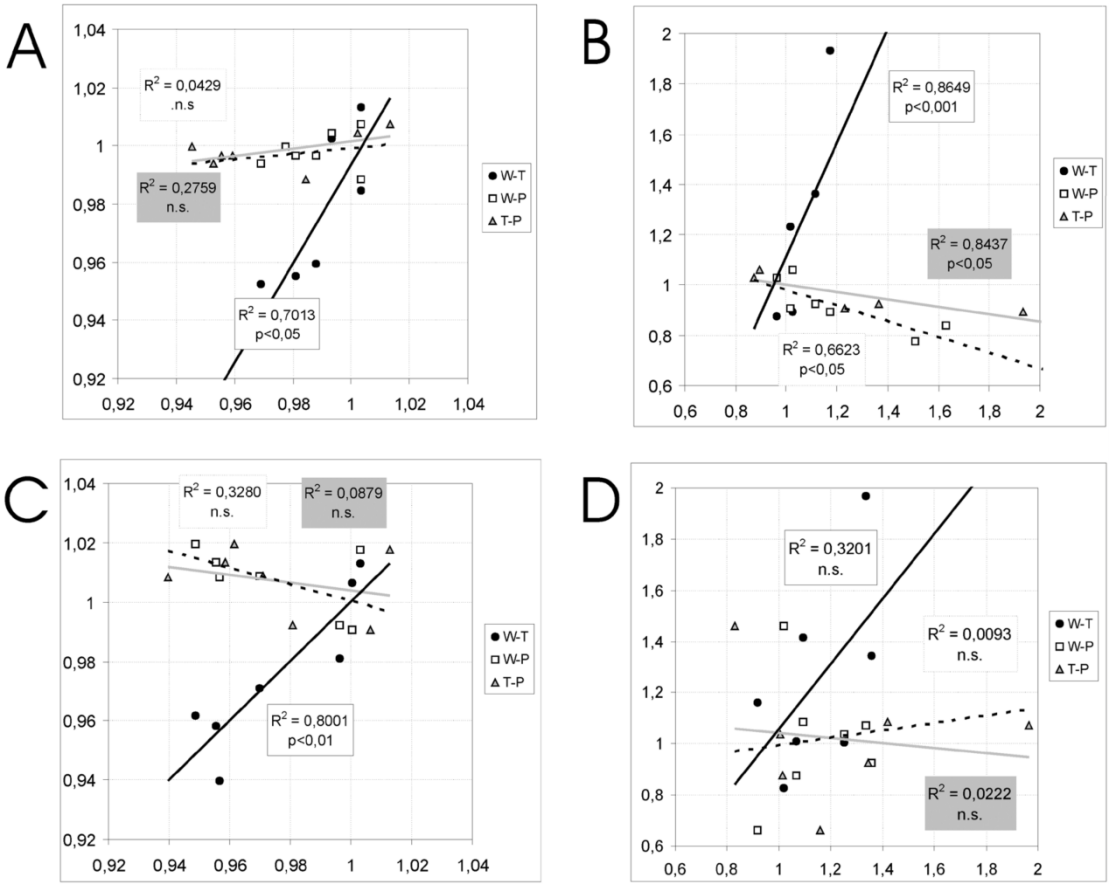

Figure 4. Correlation diagrams of the evoked response parameters. (A), latencies; (B), amplitudes from Mn-treated rats. (C), latencies; (D), amplitudes from 3-NP-treated rats. Corresponding lines and inserts have the same graphical marking. In the insert explaining the symbols: W, whisker response; T, tail response; P, peripheral response; data on the abscissa belong to the response indicated by the letter standing first.

at 30 min intervals. Right after the 3rd session, Mn or 3-NP was injected ip. and four additional sessions were recorded. After the 7th session was finished, the animal was sacrificed by an overdose of urethane. As observed in previous, related studies [19] the approximate time limit after which the animal's general state began slowly to deteriorate, in spite of keeping it warm by the heated support plate (37°C) of the stereotaxic apparatus was four hours after preparation. It was, on the other hand, also observed [19,20] that the doses applied had no noteworthy acute general toxicity.

The dose of Mn was 50 mg/kg b.w. (in form of $MnCl_2$), and of 3-NP, 20 mg/kg b.w., based on earlier works [16,19,20]. Both agents were dissolved in distilled water to 1 ml/kg dosing volume (solution of 3-NP was freshly made every time due to its instability), and were tested in a group of 10 animals each. During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

2.2 Evaluation and statistical analysis

Recording and evaluation of the electrical activity was PC-based, using the NEUROSYS 1.11 software (Experimetria Ltd., UK). SEPs and nerve action potentials were automatically averaged, and onset latency and peak-to-peak amplitude was then determined manually by means of the measuring cursors of NEUROSYS 1.11 (Figure 1). To make data from different animals comparable, all measurements from each rat were normalized to the mean of its own controls (the first three records). From the normalized values, group average was calculated and plotted against time, to visualize pre- *vs.* post-administration differences and divergent time trends. It was supposed that similar or dissimilar changes in the cortical responses by whisker and tail stimulation on one hand, and in the tail nerve action potential on the other, would give an indication of the origin and/or localization of the observed changes. Additionally, identical parameters of two records (e.g., latency of SEP from the whisker and tail projection) were plotted against each other and a straight line was fitted

to indicate correlation. Statistical analysis was done by SPSS 15.0, using the two-sample t-test (after *vs.* before differences) and the general linear model / univariate ANOVA (GLM; for differences in time trends).

3. Results

3.1 Rats treated with Mn

The onset latency of the SEPs decreased after ip. injection of Mn. As seen in Figure 2A, the SEP elicited by stimulating the whisker pad and of the tail base both had shortened latency in response to Mn application, the peripheral nerve response showed, however, no similar change. Taking all normalized pre- *vs.* post-administration latency data of the SEPs or of the tail nerve response as separate sets, and comparing them by t-test, the differences were significant ($P<0.05$) for both cortical responses; and the time trends of these (Figure 2C) were also significantly different from that of the tail nerve response if tested by GLM ($F[2,18]=10.472$, $P<0.01$). Similar significant differences were obtained with the normalized amplitude data (Figure 2B,D): increase in the SEPs but minimal change in the tail nerve response (trends: $F[2,18]=55.397$, $P<0.001$).

Corresponding parameters of the signals from the three recording sites were plotted in correlation diagrams. Latency and amplitude of the SEPs obtained by whisker or tail stimulation were both in good correlation, as evidenced by the fitted straight line of substantial steepness and high coefficient of determination (R^2 in Figure 4A,B). When either of the EPs was paired with the data of the tail response, a nearly flat trend line was obtained.

3.2 Rats treated with 3-NP

In the 10 rats treated with 3-NP, the changes in the parameters of the evoked responses were much like those obtained with Mn injection. The cortical responses had significantly decreased latency and increased amplitude in the post- *vs.* pre-administration period (Figure 3A,B) but not the tail nerve response. The time trends of the latency data were also significantly different (GLM: $F[2,18]=11.405$, $P<0.001$). For the amplitude data, the difference in the time trends did not reach the level of significance (probably due to the higher scatter of individual measurements; Figure 3C,D). The diagrams in Figure 4C and D indicated clear correlation between the latency and amplitude data of the SEPs by whisker and tail stimulation (without significance in case of the amplitude, see above). The changes of the tail nerve response were, however, without any connection with the cortical responses also in the 3-NP treated animals

4. Discussion

The changes of the cortical EPs to acute application of Mn and 3-NP were similar to those described in earlier papers of the department [19,20]. The kind of changes in the cortical and peripheral responses suggested that these were due to some specific action of Mn or 3-NP, and not to systemic toxicity. In the latter case, one would expect rather a general decline of activity in form of longer latency and lower amplitude. A change caused solely by the elapsing time could also be ruled out, based on untreated parallel controls in a related work [21]. Also, the treatment used had only a moderate effect on the spontaneous cortical activity [19,20].

The similar changes caused by the two, chemically dissimilar, agents tested in the present study suggest some common action. Mn inhibits mitochondrial complex II and III [22,23]; and 3-NP, complex II [13]. Such an effect should be present in the whole organism after systemic (here: intraperitoneal) application, and should therefore affect peripheral nerves also. As, however, no significant change in the parameters of the tail nerve response was found, the energetic shortage due to mitochondrial inhibition must have been not the only responsible effect. Cortical EPs result from excitation of pyramidal cells by glutamatergic specific afferent pathways. 3-NP inhibits glial uptake of glutamate [24], while Mn inhibits both its uptake [9] and breakdown [25]. This can acutely lead to more intense cortical response (over a longer period, an opposite change develops, possibly because of desensitization [20,26]). On the other hand, in human subjects with mitochondrial disorders, increased cortical excitability was found [27] which is another link between the agents tested and the changes of cortical responses, and underlines the importance of the mitochondrial effect.

Based on the results it can be concluded that the two mitochondrial toxins caused the alterations observed in the cortical SEPs rather by a central action focussed on synaptic transmission, and less by acting on impulse conduction along the nerve fibres. It seems possible to follow-up the damage by recording evoked activity but the relationship of neuro-functional and biochemical alterations needs further elucidation.

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