FUNCTIONAL NEUROTOXIC EFFECTS IN RATS ELICITED BY 3-NITROPROPIONIC ACID IN ACUTE AND SUBACUTE ADMINISTRATION

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Abstract: *Biochemical and morphological alterations caused by 3-nitropropionic acid in the brain of experimental animals are well described. Changes possibly induced by 3-NP in electrophysiological functional characteristics of the central nervous system are less well known. In this study ten weeks old male Wistar were subacutely and acutely treated with 3-NP. For recording, the animals' left hemisphere was exposed in urethane anesthesia. Silver electrodes were placed on the cortical (sensory foci) and tungsten needles in the subcortical (caudatum, globus pallidus) recording sites. Spontaneous electrical activity and sensory (somatosensory, visual and auditory) evoked potentials were recorded. Following subacute treatment, changes were first of all in the slowest and fastest frequencies of the spontaneous activity. The change was different in the cortical vs. subcortical sites. In the sensory evoked potentials after subacute treatment, the most characteristic change was an increase of the latency, seen in all sensory areas. In the acutely treated animals, the amplitude of the somatosensory evoked potential decreased after giving 3-NP. With double stimuli, the relation of the two responses was treatment - and interval - dependent. It needs further studies to find the possible connections between the biochemical effects of 3-NP and the functional neurotoxical changes described above.*

KEY WORDS: *3-nitropropionic acid, cortical activity, subcortical activity, rat*

1. INTRODUCTION

The substance 3-nitropropionic acid (3-NP) is naturally found in *Astragalus* species (*Leguminosae)* [7]. Human intoxication may result from infestation of foodstuffs (sugar cane, cereals etc.) with moulds of the *Anthirium* and *Aspergillus* genus producing 3-NP. Human exposure to 3-NP, even in low doses, causes acute encephalopathy followed by dystonia [9].

The morphological and functional effects of 3-NP intoxication have been replicated in animal experiments [2]. Decrease of motor performance was seen [17] with degeneration of primarily the striatum but also the hippocampus and thalamus [1,10]. At the cellular level, 3-NP inhibits succinate dehydrogenase, a key enzyme of oxidative energy production [5] which effect develops fast and is not limited to the

sites of morphological damage [3]. Beyond that, 3-NP was found to act on NMDA receptors thereby inducing excitotoxicity [12].
It seems reasonable that the latter two effects of 3-NP are reflected in the electrical activity of the brain. The aim of this work was therefore to see to what extent the neurophysiological investigation system established in our laboratory is suitable to detect functional changes caused by 3-NP administration in rats.

2. METHODS

The effects of 3-NP were investigated in three different time schemes. For subacute experiments, ten weeks old male Wistar rats (10 in a group) received 10 (low dose) and 15 (high dose) mg/kg b.w. 3-NP ip. on 5 consecutive days and were kept for further 4 weeks before recording. Control animals were untreated. For acute exposure, the animals received 20 mg/kg 3-NP ip. and were kept for 24 hrs. To see immediate effects, the rats were first prepared for recording (see below) and 20 mg/kg 3-NP ip. was given to the prepared animal after a few control records. For recording, the animals were anaesthetized with urethane and were placed in a stereotaxic instrument. The left hemisphere was exposed and silver electrodes were placed on the primary somatosensory, visual and auditory areas. One steel needle electrode each was inserted in the caudato-putamen and the globus pallidus. Spontaneous electrical activity (electrocorticogram, ECoG) was recorded from these sites simultaneously for 6 min, and the relative spectral power of the frequency bands was determined.

Stimulus-evoked activity was then recorded via the surface electrodes. Somatosensory stimulation was done by a pair of needles inserted into the whiskery skin. Visual stimulation was performed by flashes (1 Hz, 60 lux) delivered by a flash generator via an optical fiber directly into the contralateral eye of the rat. For acoustic stimulation, clicks (1 Hz, 40 dB), were applied into the ear of the rat.

3. RESULTS

The most conspicuous effect in the ECoG was the increase in the delta activity seen in all cortical foci after subacute, acute and immediate 3-NP treatment (Fig. 1). In the theta and alpha bands, there was a decrease in all three treatment schemes. In the fast bands (beta2 and gamma), there was a mild increase in the subacutely treated animals and a massive decrease in the acutely treated ones. In the immediately treated rats, this alteration needed ca. an hour to appear. The difference between power spectra in the three cortical sites was insignificant. In the basal ganglia, however (recorded only in the animals with subacute treatment) the changes were opposite, i.e. the activity in the fastest bands was increasing and in the slow bands, decreasing.

Subacute treatment with 3-NP caused a general slow-down of the cortical activity, also reflected in the increased latency of the sensory evoked potentials (Fig. 2). The alteration of the duration of the responses was mild. In acute 3-NP exposure, there was no consequent effect on the latency but the duration of the potentials was increased. Double-pulse somatosensory stimulation with different inter-stimulus intervals was used to reveal any fatigue or dynamic interaction in the sensory system. The decrease in the amplitude of the second vs. first evoked potentials (Fig. 3, top) was hardly different in acutely 3-NP treated vs. control rats, but the latency increase was less in the treated animals. In immediate exposure, decrease of the

amplitude and increase of the latency appeared with a long delay. The second evoked potential seemed to be facilitated depending on the inter-stimulus interval (Fig. 3, middle and bottom).

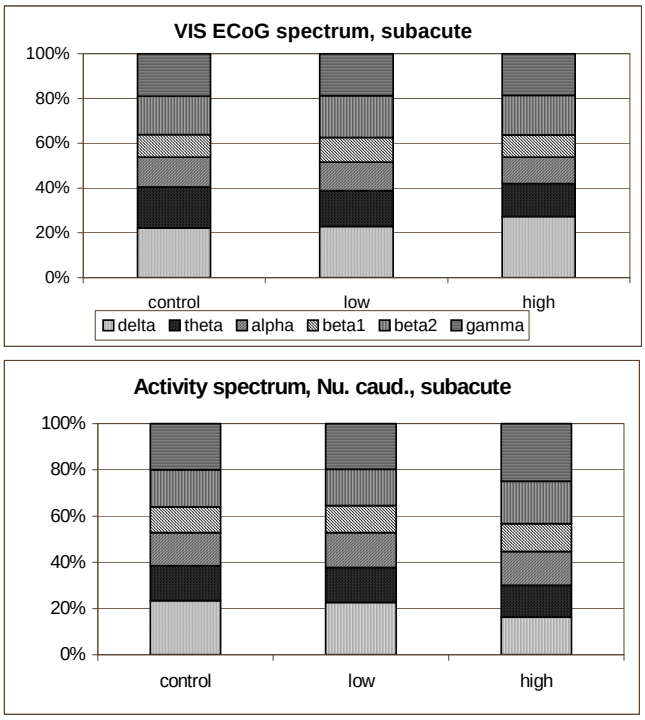


Fig. 1.
Changes of the spontaneous activity of the visual cortex (top) and the nu. caudatus (bottom) after 4 weeks exposure to 3-NP.

4. DISCUSSION

3-NP induced several alterations in the spontaneous and evoked cortical electrical activity of treated rats.

It is known that 3-NP causes energy insufficiency in the neurons by impeding mitochondrial oxidation [5]. A similar state can be induced by hypoxia. In human volunteers, breathing low-oxygen gas mixture caused an EEG shift to lower frequencies [18]. Also, in human cases of inherited or idiopathic mitchondrial dysfunction, like mitochondrial encephalomyopathy (ME), cortical functions were affected [11]. EEG abnormalities [15] and alteration of certain visual evoked potential components [6,13] were seen in ME patients. The increase of evoked potential duration was similar to what we have seen in the subacutely treated rats. In the EEG, the main abnormality of ME patients was slowed activity [14]. In the 3-NP treated rats, however, the low-frequency activity was decreased. The results obtained with double-pulse somatosensory stimulation reflect probably a kind of disinhibition, similary to what was found in the somatosensory and motor cortex of humans [8].
Another known effect of 3-NP, inhibition of glutamate uptake [16] may lead to imbalance between excitation and inhibition, producing the disinhibition mentioned above, and finally to excitotoxicity, in which long-term potentiation of NMDA-mediated excitation has probably a key role [4].

The overall effect of 3-NP on the cortical activity is complex, involving elements of depression and excitation. Further studies are needed to reveal which of the known effects of 3-NP is specifically responsible the observed effects, and how they are related to biochemical and/or histological alterations.

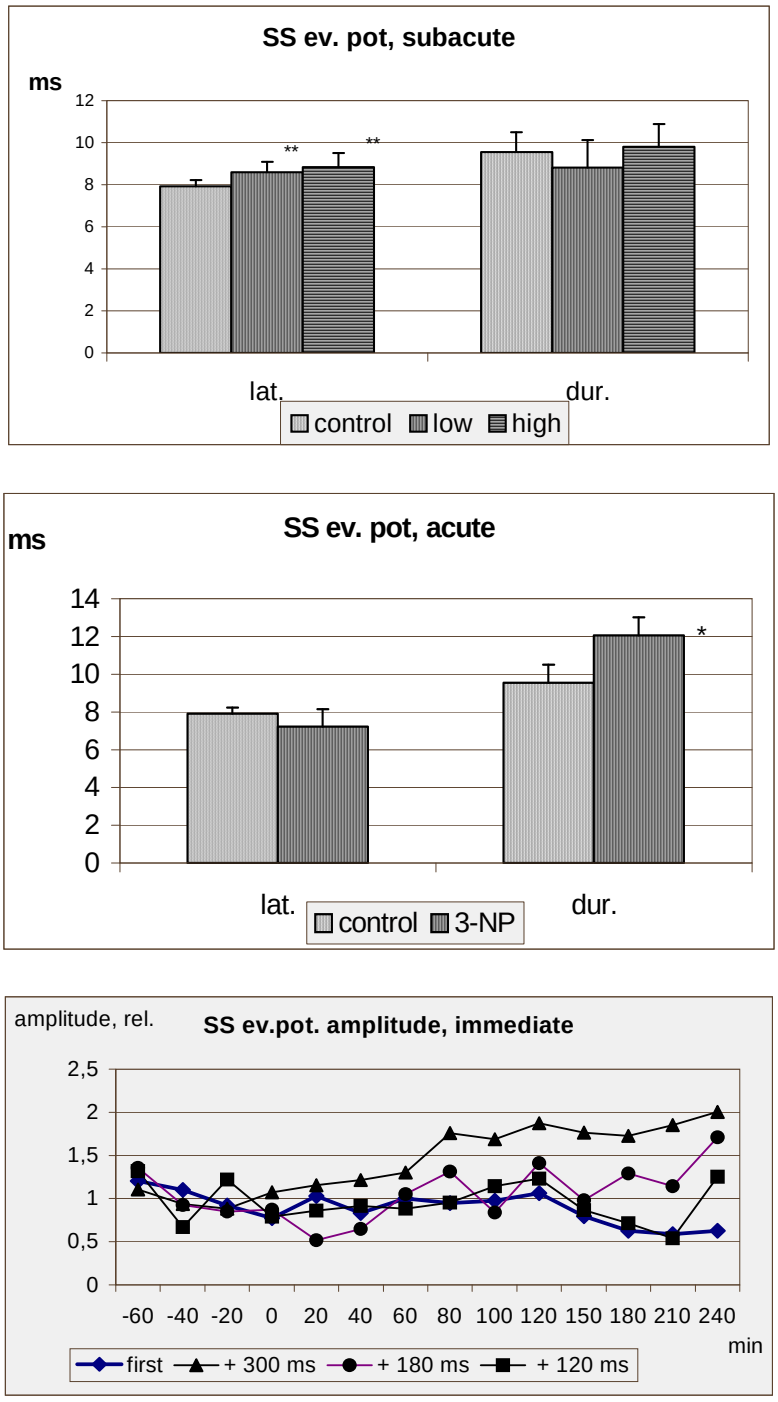
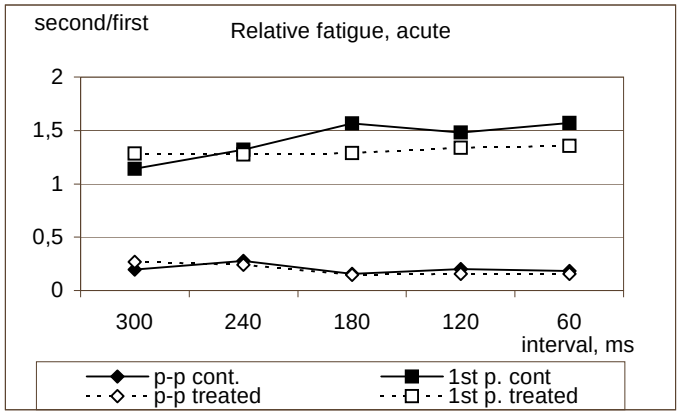
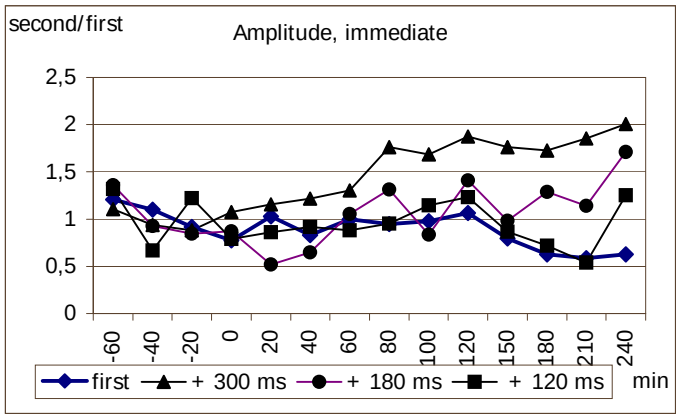
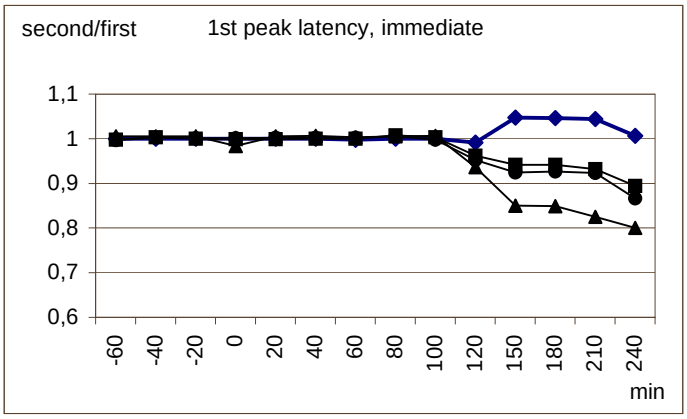


Fig. 2.
Alteration of the somatosensory evoked potential in subacute and acute treatment (top, latency and duration) and in immediate effect (bottom, amplitude)







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