
The acute effects of 3-nitropropionic acid on the behavior and spontaneous cortical electrical activity of rats

Anita Lukács, Andrea Szabó, Tünde Vezér, and András Papp

Department of Public Health, University of Szeged Faculty of Medicine,
Dóm tér 10., H-6720 Szeged, Hungary

Abstract. In this study, the acute effect of 3-nitropropionic acid was investigated on open field and startle behavior of rats, and on their cortical electrical activity. Spontaneous locomotor activity, acoustic startle response, and pre-pulse inhibition of acoustic startle were measured in male Wistar rats (10 weeks old, 180–200 g body weight) after a single dose of 10 or 20 mg/kg i.p. 3-nitropropionic acid. After the behavioral tests, the rats were anaesthetized, and spontaneous cortical electrical activity was recorded. The vertical, horizontal and local open field performance showed dose-dependent deterioration in the rats treated with 3-nitropropionic acid. The number of “noise-positive” startle responses showed non-significant changes, but the inhibition by pre-pulse was significantly reduced in the high dose animals. High dose also increased the proportion of low-frequencies in the cortical activity. 3-nitropropionic acid, known primarily to act in repeated doses (e.g., in animal models of Huntington’s disease) had also some clear-cut acute effects on behavioral and electrophysiological parameters of the treated rats.

The correspondence should be addressed to A. Lukács,
Email: lukacs@puhe.szote.u-szeged.hu

Key words: 3-nitropropionic acid, spontaneous open field activity, acoustic startle response, pre-pulse inhibition, cortical activity, rat

INTRODUCTION

3-nitropropionic acid (3-NP) is an irreversible inhibitor of succinate dehydrogenase (Alston et al. 1977) and interferes with mitochondrial ATP synthesis (Alexi et al. 1998, Lee et al. 2002, Ludolph et al. 1992). Human 3-NP intoxication, occasionally resulting from infestation of foodstuffs (sugar cane, cereals etc.) with moulds of the *Arthrimum* and *Aspergillus* genus (Patocka et al. 2000, Peraica and Domijan 2001, Scallet et al. 2001) producing 3-NP, resulted in damage to the basal ganglia and substantia nigra (Pei and Ebendal 1995). The similarity of such lesions to those observed in patients with Huntington's disease led to the development of animal models of this disease based on 3-NP. The striatal atrophy induced by 3-NP in experimental animals was similar to that seen in human Huntington's disease (Alexi et al. 1998, Sanberg et al. 1999).

The 3-NP induced shortage of ATP affects the turnover of amino acids (Klivényi et al. 2005), exerting direct effect on the levels of transmitters. Central dopaminergic and glutamatergic transmission seems to be important in the development of the lesions induced by 3-NP. By acting on N-methyl-D-aspartate (NMDA) receptors, 3-NP induces excitotoxicity (Pubill et al. 2001), contributing to the structural damage in the striatum, hippocampus etc., as determined by the density of NMDA receptors (Beal 1992). Behavioral effects possibly related to the damage, such as significant hypoactivity and reduced pre-pulse inhibition, were found in rats after subacute systemic injection of 3-NP (Kodsi and Swerdlow 1997). In our previous works, however, electrophysiological parameters were altered by a single dose of 3-NP (Szabó et al. 2005b, 2006). In the present study, our aim was to see the acute effects of intraperitoneally injected 3-NP on open field and startle behavior and on cortical electrophysiological parameters.

METHODS

Animals and drugs

Adult male Wistar rats (10 weeks old, 180–200 g body weight) were kept under controlled environmental conditions (22–24°C, 12 h light/dark cycle with light starting at 6:00 A.M.). Standard rodent chow and drinking water was given *ad libitum*. At start, 30 rats

were divided into three groups so that the average body weight in the groups was approximately the same. One group ($n=10$) received a single i.p. injection of 20 mg/kg 3-NP (high dose), another one ($n=10$) received 10 mg/kg 3-NP (low dose), and the third group was vehicle-injected control ($n=9$; one rat was lost for a reason unrelated to the experiment). 3-NP was dissolved in distilled water to 1 ml/kg body weight administration volume. There was no pH correction because of the instability of 3-NP at physiological pH and because no noteworthy discomfort, beyond that caused by the injection itself, was seen in the rats.

The time scheme of the experiment was as follows. Baseline behavioral data (open field, acoustic startle response and pre-pulse inhibition, see below) were recorded on the day preceding the treatment. The next day, the rats were given 3-NP or saline, one by one, with 10 min interval between two rats. Exactly 90 min later for each rat, the behavioral tests were repeated. On the following day, that is, 24 hours later, electrophysiological recording was done.

Behavioral tests

The animals' motility was tested in an automated open field (OF) apparatus (ACTIFRAME, Gerb Electronic, Berlin, Germany). A 40 × 40 × 40 cm size OF box was used with two arrays (3 and 15 cm above floor level) of infrared sensors at 1.1 cm distance between the beams. The test was performed from 8.00 A.M. to 2.00 P.M., and the animals were first allowed to accommodate in the testing room for 30–40 min. Each animal had one session before, and another after, drug injection (see above), started by placing the rat into the center of the box and then recording spontaneous horizontal (running; total and 1st min), vertical (rearing) and local (mainly grooming) activity, for 10 min.

Acoustic startle response (ASR) is a fast involuntary contraction of facial and body muscles evoked by sudden and intense acoustic stimuli (Koch 1999), and pre-pulse inhibition (PPI) is the normal suppression of the ASR by a preceding stimulus (Braff and Geyer 1990). This test was performed in a commercially available reflex monitor (Responder X System, Columbus Instruments, Ohio, USA) with a plexiglass test cage (16 cm × 28 cm × 18 cm) having a piezo force sensor bottom. A muscle twitch, producing more than 50 g force on the bottom, was accepted as "noise-

positive response”, of which latency (from stimulus onset to passing the 50 g threshold), time to peak (from stimulus onset to peak of force on the cage bottom) and peak amplitude (value of this peak) were measured. The ASR test was performed immediately after the OF session. The rats were, one by one, put into the test cage and, after a 10 min acclimation period, a series of 10 consecutive tones (5 kHz, 110 dB, 200 ms, 15 s interval) were given as acoustic stimuli. Following 15 min rest in the test cage, pre-pulse inhibition (Koch 1999) was tested in a second, similar series, whereby a weaker acoustic (1 kHz, 73 dB, 500 ms), preceding the startling stimulus with 200 ms, was given. Stimulation and data acquisition was controlled by a PC.

Electrophysiological recording

Twenty-four hours after the injection of 3-NP, the rats were anesthetized by urethane (1000 mg/kg i.p.). The head of the animals was fixed in a stereotaxic instrument. The skull was opened to expose the left hemisphere; wounds were sprayed with 10% lidocaine, and the exposed cortex was covered with warm paraffin oil. After recovery from the surgery (30 min) silver electrodes were placed on the dura over the primary somatosensory, visual and auditory areas (Zilles 1982). Spontaneous electrical activity (electrocorticogram, ECoG) was recorded from these sites simultaneously by the Neurosys 1.11 software (Experimetria Ltd., UK) for six minutes (for details of ECoG recording and analysis, see Inštitóris et al. 2004). At the end of recording, the rats were sacrificed with an overdose of urethane.

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

Statistical analysis

The number of “noise-positive responses” in the ASR and PPI test was evaluated by χ^2 test ($P < 0.05$). Distribution of OF and other ASR/PPI data was checked for normality by Kolmogorov-Smirnov test. Results of measurements were tested by one-way ANOVA, *post-hoc* Scheffé test ($P < 0.05$). The ECoG results were tested for significance using the two-sample t-test with $P < 0.05$ as a limit. SPSS 9.0 software pack was used to the statistical analysis.

RESULTS

Open field activity

The rats’ spontaneous exploratory activity decreased dose-dependently 90 min after the administration of 3-NP. Following the injection of high dose 3-NP, significant decrease was seen in the length of run during the 10 min session (total distance run; ($F_{2,29}=9.747$; $P < 0.01$ vs. control, $P < 0.05$ vs. low dose) (Fig. 1A) and in the run length in the 1st minute ($F_{2,29}=8.53$; $P < 0.01$ vs. control, $P < 0.05$ vs. low dose) (Fig. 1B). The dose-dependent decrease of the speed of the running (Fig. 1C) was also significant in the high dose group ($F_{2,29}=6.044$; $P < 0.01$ vs. control).

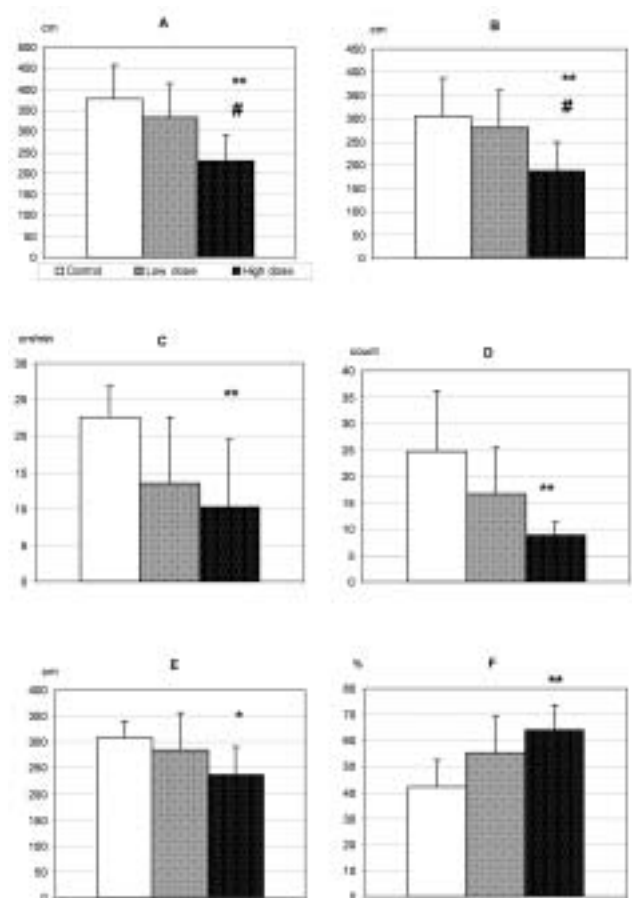


Fig. 1. The spontaneous horizontal [total distance run (A); run length in the first minute (B); speed of the running (C)], vertical [rearing (D)], and local [grooming (E)] exploratory activity of the rats, and the rate of their rest time (F), during the 10 min session in the open-field box, 90 min after 3-NP treatment. Abscissa: groups (see insert in (A) for bar patterns.) Mean + SD, *,** $P < 0.05$; 0.01 vs. control. # $P < 0.05$ high vs. low dose.

The number of rearings during the 10 min session was also significantly reduced in the high dose group ($F_{2,29}=8.996$, $P<0.01$ vs. control) (Fig. 1D); as was the summed local activity (i.e., grooming: $F_{2,29}=4.177$; $P<0.05$ vs. control) (Fig. 1E). In contrast to the indicators of motility, the rate of rest time during the 10 min session (Fig. 1F) increased significantly in the high dose group ($F_{2,29}=8.862$; $P<0.01$ vs. control).

Acoustic startle response and pre-pulse inhibition

Acute administration of 3-NP caused minor, non-significant changes in the number of “noise-positive responses” in the ASR test. In the PPI test (Fig. 2), inhibition was seen in the control but was absent in both treated groups. The differences in the number of responses were, however, below significance. The effect of 3-NP on the latency, time to peak and peak amplitude of the “noise-positive responses” was moderate (Table I). In the PPI test, however, there was significant difference in the latency ($F_{2,29}=3.95$; $P<0.05$) and time to peak ($F_{2,29}=5.011$; $P<0.05$) of the high dose rats vs. control, which was another indication of reduced ASR inhibition in the treated rats.

Cortical electrical activity

The effect of 3-NP on the electrocorticogram seemed to have an anomalous dose dependence with opposite changes in the low dose and high dose groups

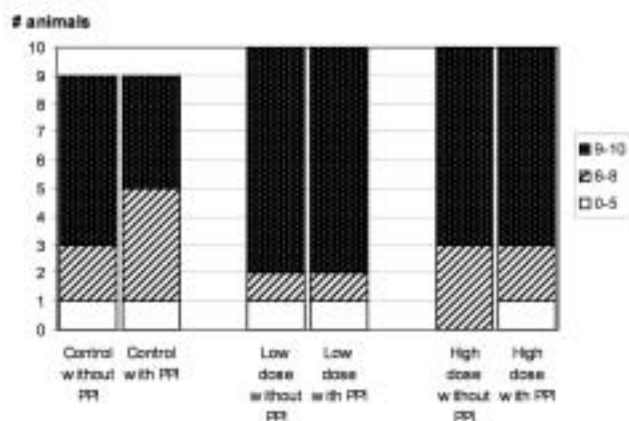


Fig. 2. Number of “noise-positive” startle responses without and with pre-pulse inhibition, 110 and 125 min after 3-NP injection, respectively (see Methods). Abscissa: groups. Ordinate: number of animals giving as many responses as indicated in the insert on the right.

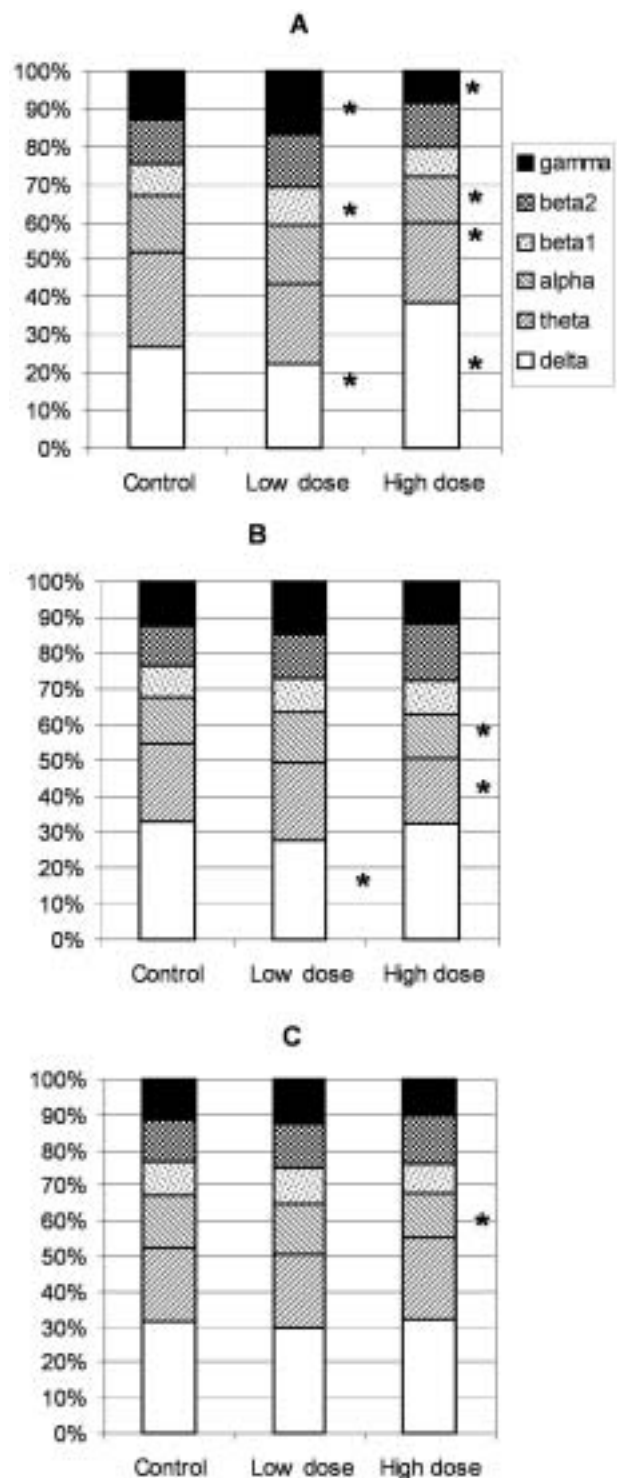


Fig. 3. Spontaneous cortical somatosensory (A), visual (B), and auditory (C) activity in the control and treated groups, 24 hours after 3-NP injection. Abscissa: groups. Ordinate: relative power of the bands (see insert in A for the bar patterns). * $P<0.05$ vs. control.

Table I

Measured parameters of the "noise-positive" acoustic startle response without and with PPI after 3-NP treatment			
	Control	Low dose (10 mg/kg)	High dose (20 mg/kg)
ASR			
Latency (ms)	16.43 ± 1.06	18.10 ± 2.34	16.31 ± 1.28
Peak amplitude (g)	478.48 ± 252.6	312.28 ± 157.64	383.57 ± 121.08
Time to peak (ms)	24.95 ± 1.25	25.30 ± 1.31	24.44 ± 0.67
PPI			
Latency (ms)	18.22 ± 1.94	17.84 ± 1.91	16.17 ± 1.21*
Peak amplitude (g)	360.41 ± 210.02	272.61 ± 134.51	410.17 ± 152.27
Time to peak (ms)	25.64 ± 1.33	24.47 ± 1.35	24.04 ± 0.56*

* $P < 0.05$ vs. control

compared to control. In the somatosensory area (Fig. 3A), delta activity decreased in the low dose ($t_{19}=2.13$, $P < 0.05$ vs. control), but increased in the high dose group ($t_{19}=2.15$, $P < 0.05$ vs. control). Beta1 and gamma went up in the low (beta1: $t_{19}=2.31$, $P < 0.05$ vs. control; gamma: $t_{19}=2.24$, $P < 0.05$ vs. control) and down in the high dose group (beta1: $t_{19}=2.42$, $P < 0.05$ vs. control; gamma: $t_{19}=2.34$, $P < 0.05$ vs. control). In the visual area (Fig. 3B), decrease of the slow to middle bands was seen in both treated groups but not uniformly (significant decrease of delta in the low dose ($t_{19}=2.29$; $P < 0.05$ vs. control), and alpha and theta in the high dose group (alpha: $t_{19}=2.33$, $P < 0.05$ vs. control; theta: $t_{19}=2.41$; $P < 0.05$ vs. control). In the auditory area (Fig. 3C), only the decrease of the alpha band in the high dose group was significant ($t_{19}=2.28$; $P < 0.05$ vs. control).

DISCUSSION

In the present study, treated rats exhibited dose-dependent hypoactivity after a single intraperitoneal injection of 3-NP. In the high dose group, the decrease of the local, vertical and horizontal exploratory activity was significant. In the literature, hypoactivity was reported to develop several days after application of 3-NP systemically (Borlongan et al. 1995, Koutouzis et al. 1994a) or into the striatum (Koutouzis et al. 1994b). In Borlongan and coauthors (1995), the effect of the first i.p. 3-NP injection was opposite to ours but the

increased activity score in that study was the average of a whole-night recording which is not comparable to the 10 min session in the morning as performed in our study.

Several works revealed that acute exposure to 3-NP produced neurotoxic damage to the striatum of rats. The dorsal aspect of the striatum may play a significant role in the control of nocturnal spontaneous locomotor activity in rats (Borlongan et al. 1995). Following 3-NP administration, increased DA level was observed in the rat striatum (Nishino et al. 1997). Inhibition of succinate dehydrogenase by 3-NP develops within 2 hours after injection (Massieu et al. 2001), leading to decreased energy production, resulting in elevated DA (Johnson et al. 2000). This, in turn, can acutely desensitize the receptors in motor control centers such as the globus pallidus and substantia nigra (Oberlander et al. 1987). With the decreased efficacy of dopaminergic transmission, locomotor activity decreases also (Fink and Smith 1980).

According to the cognitive map theory (O'Keefe and Nadel 1978), exploratory activity in the OF is initiated, by the novelty of the environment, in the hippocampus (Crusio 2001). Hippocampus is another structure damaged in 3-NP treated animals (Beal 1992) although several reports (e.g., Kodsí and Swerdlow 1997) claim the opposite. Decreased ATP synthesis causes a loss of function of Na^+/K^+ -ATPase, leading to membrane depolarization, resulting in Ca^{2+} influx due,

among others, to release of the Mg^{+} block of NMDA receptors. This cascade, finally resulting in excitotoxic cell death, may cause initial dysfunction manifested in decreased exploration.

On the number of ASR responses, no significant acute effect of 3-NP was seen, but PPI was markedly reduced in the treated rats. In the circuits responsible for ASR and PPI – first of all in the caudal pontine reticular nucleus, the main sensomotor interface of the ASR circuit (Koch 1999) – glutamatergic transmission has a major role, as shown by reduced inhibition after application of glutamate antagonists (Krase et al. 1993, Miserendino and Davis 1993). Our observations, taking the effect of 3-NP on NMDA receptors (Beal 1992) in consideration, are in line with that. In human cases of 3-NP intoxication, the nigrostriatal dopaminergic system (involved in motor behavior: Fink and Smith 1980) was damaged (Pei and Ebendal 1995) underlining the role of 3-NP induced dopaminergic dysfunction in the acute effects of the drug on OF behavior. The effect of 3-NP on glutamatergic transmission, together with the direct effect on energy supply, may also explain the alterations of the ECoG. Why this changed dissimilarly in the low vs. high dose group was not clear but the effect to increase cortical activity (decreased glutamate breakdown) and to decrease it (shortage of ATP) might well have different dose dependence. Such an effect of the same doses of 3-NP on the ECoG in subacute application was described by Szabó and others (2005a,b).

CONCLUSION

Our results showed that 3-NP had an effect on locomotor activity and ASR pre-pulse inhibition within two hours, and on the cortical electrical activity, within 24 hours, following a single i.p. injection of the substance. These effects can, at least partly, be deduced from the mechanisms which give rise to the histological alteration (striatal etc. damage) and behavioral effects (motor slowing, movement dysfunction, etc.) seen after repeated application of the agent, which fact may be relevant to the Huntington's disease model based on 3-NP.

ACKNOWLEDGEMENT

This work was supported by the Hungarian ETT grant No. 08356.

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Received 13 April 2006, accepted 14 September 2006

