CCK-8S Systemic Administration Blocks the 7-Nitroindazole-induced Effects on the EEG of Striatum and Globus Pallidus: a FFT Analysis in the Rat

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Abstract. *Background: Nitric oxide (NO) and cholecystokinin (CCK) are involved in the modulation of several neurotransmitter systems in the basal ganglia, and a functional interaction between their modulatory effects could be hypothesised. Materials and Methods: We studied the effects exerted by the administration of 7-nitroindazole (7-NI) (50 mg kg^{-1} i.p.), a selective inhibitor of neuronal NO synthase, on the depth EEG activity of the striatum and of the globus pallidus in both not pre-treated and sulphated CCK octapeptide (CCK-8S)-treated (100 nM kg^{-1} i.p.) rats. Striatal and pallidal depth EEG power spectra were examined by means of a Fast Fourier Transform analysis. Results: Striatal depth recordings showed a marked increase of % power of slow standard rhythms after 7-NI systemic treatment. In contrast, pallidal recordings revealed an increase of % power of rapid standard rhythms after i.p. injection of 7-NI. The same modifications were not evidenced in CCK-8S pre-treated rats after 7-NI administration. Conclusion: The results show an influence exerted by peripheral CCK on the nitrergic modulation of the bioelectric activities of the striatum and of the globus pallidus. This effect could be of particular interest in the light of NO and CCK involvement in the neuroprotective mechanisms.*

The presence of diffusible nitric oxide (NO) (1) has been largely shown in the context of the central nervous system (CNS). In fact, it plays a key role in the modulation of several neurotransmitters, particularly on the excitatory

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glutamate and on the inhibitory GABA (2, 3). Moreover, several lines of evidence have demonstrated that NO is involved in NMDA-receptor-mediated neurotoxicity in different areas of the CNS (4-6). In particular, NO synthase (NOS), activated by Ca^{2+} influx, produces the neurotoxic peroxynitrite (ONOO-), through a reaction with superoxide anion (O_2^-) (7). On the other hand, NO is also considered to exert an efficacious neuroprotective role through a down-regulatory effect on the redox site of the NMDA receptor complex during all the conditions associated with an *overstimulation* of NMDA receptors (8). In previous studies, we explored the role of NO in the modulation of the normal bioelectric activity of cortical (9) and subcortical (10, 11) structures, as well as the induction of hyperactivity phenomena due to a significant reduction of NO cerebral levels (12, 13). In the CNS, the gastrointestinal peptide cholecystokinin (CCK) exerts a well known neuro-modulatory action which shares several analogies with the NO system (14, 15). In particular, CCK binds two receptor subtypes: CCK-A, particularly abundant in the gastrointestinal tract and CCK-B, highlighted in the brain (16). Interestingly, the peripheral administration of sulphated cholecystokinin octapeptide (CCK-8S) is able to influence the activity of several areas in the brain, particularly in the basal ganglia (17-21). In fact, systemic CCK-8S crosses the blood-brain barrier (22, 23) and it can directly interact with several CNS areas (24, 25). At the same time, it has been demonstrated that peripheral CCK injection exerts an indirect regulatory influence on CNS areas, modulating other neurotransmitter systems such as glutamate, dopamine, GABA, serotonin *etc*. through the functional involvement of vagal afferents (26-29).

A wide range of actions have been proposed for CCK in various areas of the CNS. In particular, a significant role of the systemic administration of CCK was evidenced in modulating both hyperactivity phenomena and the dopaminergic output from the midbrain, which

Table I. *Basal values of striatal and pallidal EEG power.*

EEG bands	Not pre-treated (Group C)				CCK-8S pre-treated (Group D)			
	1-4 Hz	*4-8 Hz*	*8-13 Hz*	*13-30 Hz*	*1-4 Hz*	*4-8 Hz*	*8-13 Hz*	*13-30 Hz*
Striatum	37.2±9.6	44.3±6.4	11.9±4.3	5.1±1.1	36.9±8.7	45.3±5.9	12.3±5.2	6.4±1.9
GP	38.4±22.6	48.0±23.9	7.6±0.1	4.5±1.8	39.0±17.1	48.8±25.0	6.5±3.3	4.7±1.3

Relative EEG power (mean ± SD) expressed as the % between a single frequency band and the total power values in the striatum and globus pallidus (GP) before 7-NI *i.p.* treatment in animals belonging to C and D groups.

is able to strongly influence the bioelectric activity of neural structures belonging to the basal ganglia system (18, 24, 30, 31).

In this study, we aimed to verify whether systemic CCK pre-treatment influences the functional response of the striatum and of the globus pallidus to the decrease of NO levels obtained by means of systemic injection of 7-nitroindazole (7-NI). This selective neuronal NOS inhibitor was administered in order to decrease cerebral NO levels, while excluding possible side-effects due to the alterations of cerebral blood flow (28, 32-34). Power spectra analysis was used to quantitatively describe the effects of the nNOS inhibition on striatal and pallidal depth EEG activity in not pre-treated and CCK-8S pre-treated rats.

Materials and Methods

Thirty adult male Wistar rats, weighing 220-280 g, were anaesthetised with urethane (1.2-1.4 g kg^{-1} intraperitoneally – *i.p.*). The trachea was cannulated and the skull exposed. The animals were positioned in a stereotaxic apparatus (David Kopf Instruments, USA) and the body temperature was maintained at 37-38˚C using a heating pad. Heart rate and pupil diameter were monitored during all the experimental sessions. After the craniotomy, depth bioelectric activities from both the striatum and the globus pallidus were simultaneously recorded through stainless-steel coaxial bipolar electrodes (external diameter 0.5 mm; exposed tip 25-50 μm) inserted into the two structures according to the stereotaxic co-ordinates of the Atlas of Paxinos and Watson (35); (Striatum: 8.7-7.2 mm anterior to the interaural line; 6.0-5.0 mm dorsal to it and 3.5-4.5 mm lateral to the midline. Globus pallidus: 8.0-7.6 mm anterior to the interaural line; 4.0-5.0 mm dorsal to it and 3.0-3.5 mm lateral to the midline). The bioelectric activity of the structures examined was amplified, recorded and printed through an eight-channel polygraph Grass model 7B. The time constant was set at 0.04 sec and high frequency filters were set at < 30 Hz. Depth striatal and pallidal recordings were time aligned, such that each pharmacological treatment was preceded by a control epoch of 15 min and followed by 120 min of recovery period. EEG spectral quantification was accomplished using Fast Fourier Transforms (FFTs) algorithms on contiguous 15-min epochs of unprocessed data (sampling rate: 1024 Hz for each recording channel), through a software package provided by DataWave Technologies (Longmont, CO, USA). Spectral power

from 1 to 30 Hz was determined at 1-Hz increments. Power in each of the four conventional frequency bands (delta, 1-4 Hz; theta, 4-8 Hz; alpha, 8-13 Hz; beta, 13-30 Hz) was expressed as percentage (%) of the total power in the epoch. The study was performed on four groups of animals: A) control, not pre-treated (n=5); B) control, CCK-8S pre-treated (n=5); C) not pre-treated (n=10) and D) CCK-8S-pre-treated (n=10). In both the C and D groups, 7-Nitroindazole (7-NI), a selective inhibitor of nNOS, suspended in a 10% aqueous solution of Tween 80 (non-ionic tensioactive agent), was administered by *i.p.* injection in a volume of 1 ml/100 g body weight (vehicle) at the dose of 50 mg kg^{-1}, which causes the maximal inhibition of nNOS (33). Thirty minutes before 7-NI administration, the D group of animals received an *i.p.* injection of CCK-8S (100 nM kg^{-1}; injection volume 1-2 ml of saline). Control animals in group A, after 120 min of recording, received an *i.p.* injection of the vehicle. Animals in group B, after an observation period of 120 min, received *i.p.* CCK-8S at the same dose of the D group. Both CCK-8S and 7-NI were purchased from Sigma Chemical Co. (Sigma, St. Louis, MO, USA).

For each EEG frequency band mean±SD % power values were compared across recording epochs, before and after drug treatment, by using one-way analysis of variance (ANOVA) for repeated measures and the Bonferroni *post hoc* test. Differences were considered to be significant at the level of $p<0.05$.

At the end of each experiment, the animals were killed with an overdose of pentobarbital and perfused with 10% buffered formalin. The brains were removed for histological examination: serial coronal sections were cut at 30-50 μm and stained by using Nissl thyonine and/or Nissl cresyl violet methods. All animal use procedures were in strict accordance with European Communities Council Directive (86/609/EEC) and the Italian Health Ministry guidelines (D.L. 116/1992) associated with the Animals Scientific Procedures Act 1986. All efforts were made to minimise the number of animals employed and to reduce their suffering.

Results

Depth EEG power spectra during the initial observation period (about 120 min) did not show any significant difference in the four groups examined. Moreover, the *i.p.* injection of the vehicle (group A) did not show alterations of striatal or pallidal EEG patterns during a further 120 min. Animals in the B group showed no modifications in the striatal and pallidal depth EEG within 120 min following CCK-8S *i.p.* injection. Table I shows the basal values of
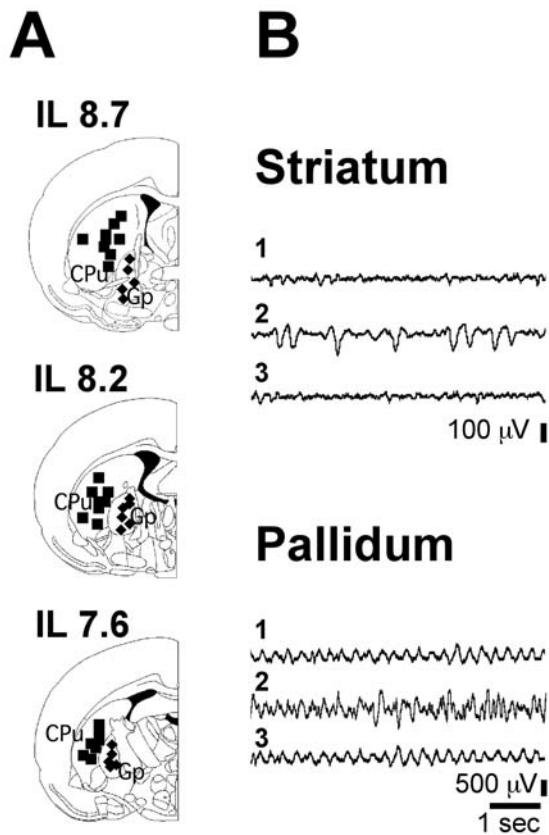
Recording sites and depth EEG

A

IL 8.7

IL 8.2

IL 7.6

B

Striatum

1

2

3

100 μV

Pallidum

1

2

3

500 μV

1 sec

Drug-induced effects on 1 - 4 Hz band

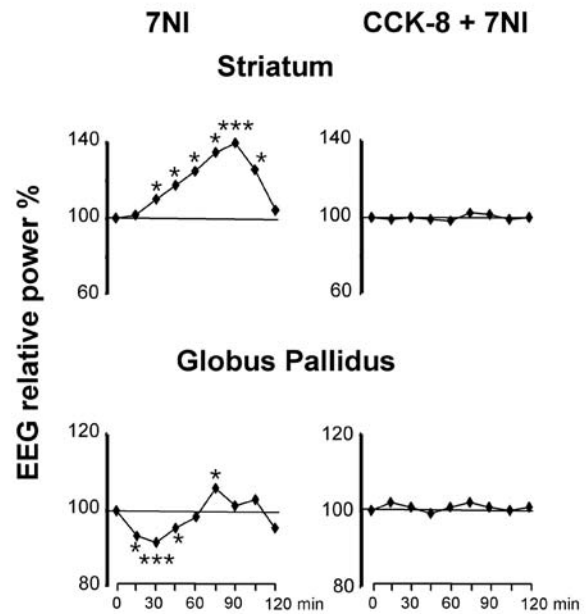7NI   CCK-8 + 7NI

Striatum

Globus Pallidus

Figure 2. *Drug-induced effects on 1-4 Hz band. Time course of % variations of EEG power in the 1-4 Hz band recorded from the striatum and the globus pallidus after 7-NI i.p. administration (50 mg kg^{-1}) in not pre-treated (n=10, left) and CCK-8S pre-treated rats (n = 10, right). Intervals of 15 min are reported in the abscissa axis. The horizontal line represents the control reference (100%). *p<0.05, ***p<0.001.*

Figure 1. *Recording sites and depth EEG. A: schematic serial reconstruction of depth recording sites within striatum and globus pallidus in three stereotaxic planes (IL: interaural line). B: representative example of striatal and pallidal depth bioelectric activity in basal conditions (trace 1) and after 7-NI i.p. administration (50 mg kg^{-1}) (trace 2) in not pre-treated rats; trace 3 shows the EEG activity after 7-NI treatment in CCK-8S pre-treated.*

striatal and pallidal % EEG total power for each frequency band in the C and D groups of animals. In particular, in both basal recordings a more marked % presence of the delta and theta frequency bands was observed. Figure 1 shows a schematic reconstruction of recording electrode positions and representative EEG traces before and after 7NI treatment.

Time course of 1-4 Hz (delta) band % power. Figure 2 reports the % mean variations of delta band power values recorded from the striatum and the globus pallidus after *i.p.* treatment with 7-NI. An increase of % spectral values in the striatum and a decrease in the globus pallidus was observed; the maximum effect was evidenced 90 min after drug administration in the

striatum (+ 39.09 %; $F_{1,8}$=1251.01, $p<0.001$) (Figure 2). On the contrary, the pallidal delta showed a significant decrease 30 min after 7-NI administration (- 8.13 %; $F_{1,8}$=8.22, $p<0.05$), followed by an increase which did not reach the level of significance (Figure 2).

CCK-8S pre-treated animals (Group D), when *i.p.* injected with 7-NI, did not show any sort of modification within the delta band recorded from the structures studied throughout the observation period (about 120 min) (Figure 2).

Time course of 4-8 Hz (theta) band % power. In this frequency band comparisons between pre- and post 7-NI treatment values showed a significant decrease of % spectral values in the striatum and an increase in the globus pallidus. The major efficacy of the cited modifications was highlighted 90 min after drug administration in the striatum (-19%; $F_{1,8}$=149.68, $p<0.001$) (Figure 3). In the globus pallidus, theta showed a significant increase (+8.07%; $F_{1,8}$=13.39, $p<0.01$) 30 min after 7-NI administration (Figure 3).

In the animals pre-treated with CCK-8S (Group D), the *i.p.* injection of 7-NI failed to induce significant modifications within the theta frequency band in the

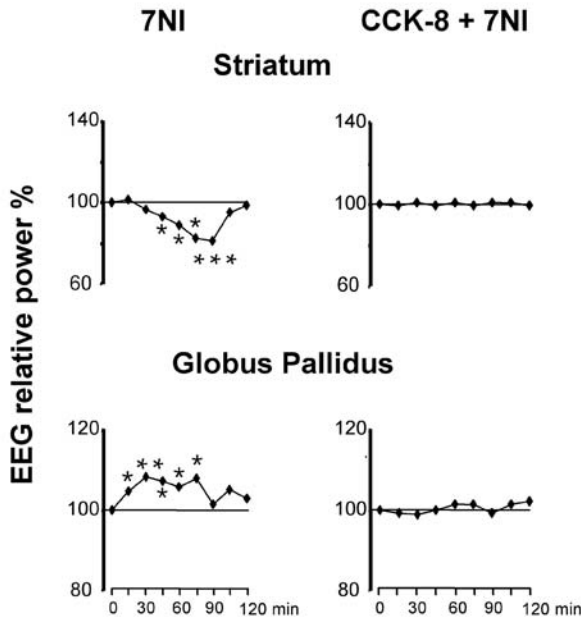## Drug-induced effects on 4 - 8 Hz band

Figure 3. *Drug-induced effects on 4-8 Hz band. Time course of % variations of EEG power in the 4-8 Hz band recorded from the striatum and the globus pallidus after 7-NI i.p. administration (50 mg kg^{-1}) in not pre-treated (n=10, left) and CCK-8S pre-treated rats (n=10, right). Intervals of 15 min are reported in the abscissa axis. The horizontal line represents the control reference (100%). *p<0.05, ** p<0.01, *** p<0.001.*
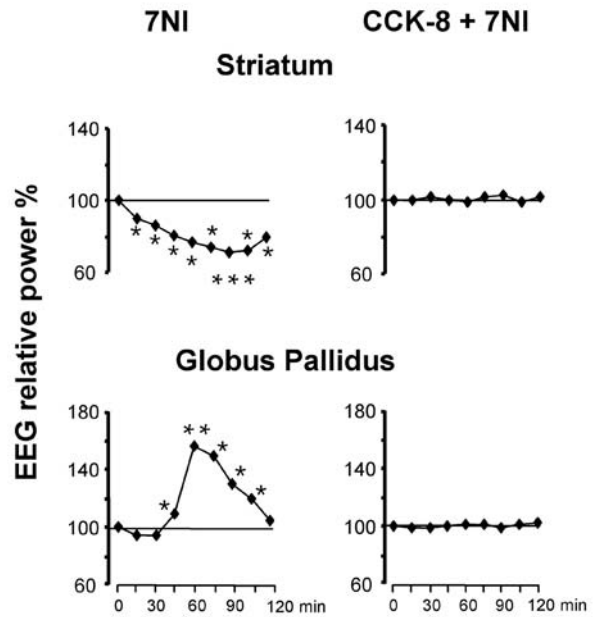
Drug-induced effects on 8 - 13 Hz band

Figure 4. *Drug-induced effects on 8-12 Hz band. Time course of % variations of EEG power in the 8-13 Hz band recorded from the striatum and the globus pallidus after 7-NI i.p. administration (50 mg kg^{-1}) in not pre-treated (n=10, left) and CCK-8S pre-treated rats (n=10, right). Intervals of 15 min are reported in the abscissa axis. The horizontal line represents the control reference (100%). *p<0.05; **p<0.01, ***p<0.001.*

striatum and in the globus pallidus during the total period of analysis (about 120 min) (Figure 3).

Time course of 8-13 Hz (alpha) band % power. A significant decrease of % spectral values with a peak at 90 min was observed after drug administration in the striatum (-29.3%; $F_{1,8}$=89.58, $p<0.001$) (Figure 4). In the globus pallidus an initial not significant decrease, followed by a significant increase (+55.58%; F_{1-8}=13.39, $p<0.01$) 60 min after 7-NI administration, was observed (Figure 4).

In the striatum and in the globus pallidus of CCK-8S pre-treated rats (Group D), no significant modifications within the alpha frequency band were noted during all the observation period (about 120 min) (Figure 4).

Time course of 13-30 Hz (beta) band % power. Comparisons between pre- and post 7-NI treatment showed a significant decrease of % spectral values in the striatum, with a peak 105 min after drug administration (-27.26%; $F_{1,8}$=51.47, $p<0.001$) (Figure 5). In the globus pallidus an initial decrease was followed by a significant increase, with a maximum 75 min after 7-NI administration (+27.82%; $F_{1,8}$=6.94, $p<0.05$) (Figure 5).

The pharmacological treatment with *i.p.* 7-NI in the group of animals pre-treated with CCK-8S (Group D) did not induce any sort of significant modifications within the beta frequency band in the striatum and globus pallidus throughout the experimental session (about 120 min) (Figure 5).

All 7-NI-induced modifications of % spectral frequency band were reversible within about 120 min.

Discussion

In different areas of the CNS, NO is able to influence the neuronal excitability by modulating two opposite neurotransmitter systems, such as inhibitory-GABA or excitatory-glutamate. In the striatum and in the globus pallidus the functional involvement of NO neurotransmission in the integrative processes of the motor function has been highlighted (15, 36). In particular, striatal NOS interneurons receive convergent information from cortical, pallidal and nigral afferents, significantly influencing the striatal GABAergic output (37).

Furthermore, the cortico-striatal glutamatergic projections and the striato-pallidal GABAergic transmission are also

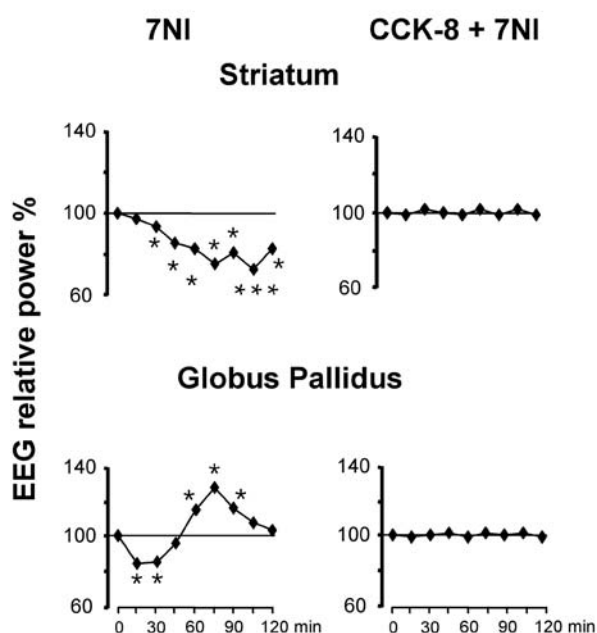## Drug-induced effects on 13 - 30 Hz band

Figure 5. *Drug-induced effects on 13-30 Hz band. Time course of % variations of EEG power in the 13-30 Hz band recorded from the striatum and the globus pallidus after 7-NI i.p. administration (50 mg kg⁻¹) in not pre-treated (n=10, left) and CCK-8S pre-treated rats (n=10, right). Intervals of 15 min are reported in the abscissa axis. The horizontal line represents the control reference (100%). *p<0.05, **p<0.01, *** p<0.001.*

under the functional modulation of the CCK, whose presence has been largely demonstrated by using immunohistochemical techniques (20, 29).

The functional mechanisms of the CCK modulation are not well understood yet: on one hand, central CCK is able to influence the neuronal excitability by acting on glutamatergic neurotransmitter systems (38, 39); on the other hand, systemically administered CCK is able to modulate different neurotransmitter systems (*e.g.* glutamate, GABA, dopamine).

Taking into consideration the contiguity and the shared features of CCK and NO neurotransmission, in this study we verified the possible influence of both neuromodulatory systems on the bioelectric activity of the corpus striatum and globus pallidus. In the striatum, 7-NI treatment caused an increase of the % power in the delta band, while the power in the other bands was significantly decreased. Previous results from our laboratory showed a strong excitatory action of 7-NI on the firing of striatal projection neurons, without a direct influence on the interneuronal population (10, 11). The transition from a basal condition, in which

striatal projection neurons are silent or show a sporadic random firing activity (40), to a new condition, in which they are recruited to fire, could explain the 7-NI-induced synchronising effect.

In the globus pallidus, after 7-NI treatment, the % power of the delta band was significantly decreased, while the % power of the theta, alpha and beta bands was significantly increased. In particular, the alpha and beta bands showed an early decrease followed by a wide increase centred at 60-75 min after drug injection. Our previous results have shown a direct effect of NOS inhibition on pallidal neurons (41); this influence could explain the present findings. Nevertheless, the evidence of a close temporal relationship between striatal EEG de-synchronisation and pallidal synchronisation suggests a possible parallel indirect influence exerted by the activation of the striatal output.

In the CCK-8S pre–treated rats, 7-NI administration failed to significantly modify the % power of EEG bands in both structures. Pre-treatment with CCK-8S increases central GABA and dopamine (19, 21, 42) and can decrease the responsiveness of striatal output neurons, counteracting the effect of NOS inhibition with a following influence of the globus pallidus activity. The results suggest a functional co-operation between CCK and NO, probably through the involvement of other neurotransmitters (*e.g.* GABA and dopamine) in the functional balance between the striatal NOS interneurons and the striato-pallidal projecting neurones (26, 29, 43).

NO and CCK could exert an analogous co-operative effect in terms of neuroprotection in several pathological events. In fact, the inhibition of neuronal NOS causes an increase of neuronal excitability up to the appearance of an epileptiform hyperactivity (3, 12, 44, 45). Furthermore, CCK exerts a protective action in the neurotoxicity phenomena associated with trauma (45) and its profile is down-regulated in different models of epilepsy (31, 46). Interestingly, peripheral CCK administration enhances endogenous NGF levels in the brain (47, 48). The increased availability of NGF causes a stimulatory effect of neuronal NOS synthesis, reducing, through a regulatory feedback loop, the effects of the inhibition of neuronal NOS activity (49-52).

In conclusion, our data suggest a functional interaction between the CCK and NO systems, which could influence the intrinsic and the reciprocal activity of the striatum and of the globus pallidus.

Acknowledgements

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