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Noradrenaline neurotoxin DSP-4 effects on sleep and brain temperature in the rat

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Abstract

N-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) has a selective degenerative effect on noradrenergic fibers originating from locus coeruleus (LC) neurons. In the present study, we studied its effect on vigilance states and brain temperature by continuous recordings for periods of 1–5 days and 2–4 weeks following DSP-4 treatment. On the first day, paradoxical sleep duration was significantly decreased (–67%, $P < 0.05$), slow-wave sleep (SWS) duration increased (+16%, $P < 0.05$) up to 48 h after DSP-4 treatment (+8%, $P < 0.05$) and the wake period decreased (–8%, $P < 0.05$). The vigilance states returned to control values 4–5 days later. The brain temperature was decreased during the first night (–2°C) and then recovered the control values. Two and 4 weeks after DSP-4 treatment, paradoxical sleep was still decreased (–18% and –23%, respectively, $P < 0.05$), while SWS was significantly increased only at night during the fourth week (+23%, $P < 0.05$). These results therefore provide evidence for a differential involvement of the noradrenergic LC system in sleep mechanisms depending on the light-dark cycle. Different hypotheses are proposed.

Keywords: Locus coeruleus; Slow-wave sleep; Paradoxical sleep; *N*-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine; Neurotoxic lesion; Brain temperature; Rat

Since the initial description of paradoxical sleep (PS) in the cat, multidisciplinary studies have been carried out on various species to elucidate its mechanisms and functions, for review see [20]. The biogenic amines are one of the most important control systems for the induction and maintenance of the sleep process [10,11,20] and the locus coeruleus (LC) is the most important noradrenergic nucleus in the central nervous system. The spontaneous activity of LC neurons decreases during slow-wave sleep (SWS) and the neurons virtually cease firing during PS [1,20]. However, the relationship between the noradrenergic LC system (NA-LC) and PS mechanisms is still controversial. In rats, different authors have describe an increase in [17], a decrease in [16], or no effect on [19] PS duration following LC lesion.

It is well documented that [*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine] (DSP-4) has a preferentially neurotoxic effect on the NA-LC 2 weeks after administration. This drug produces a selective decrease in activity of noradrenergic neurons, together with degeneration of their axon terminals [7,9,14], and we therefore consider it to be a useful tool for evaluating the specific role of the NA-LC in PS mechanisms.

In the present study, we analyzed variations in the waking–sleep cycle in parallel with brain temperature by continuous recordings performed during days 1–5, 15 and 28 following DSP-4 injection.

Adult OFA Sprague–Dawley male rats (200 g; Iffa–Credo) were implanted under deep anesthesia (sodium pentobarbital 50 mg/kg, i.p.) for chronic sleep recordings (ECoG, EMG) and cerebellar temperature measurement (Tbr). Tbr was continuously recorded in parallel with the polygraphic activity. All the animals were treated according to the guidelines approved by French Ethical Committee

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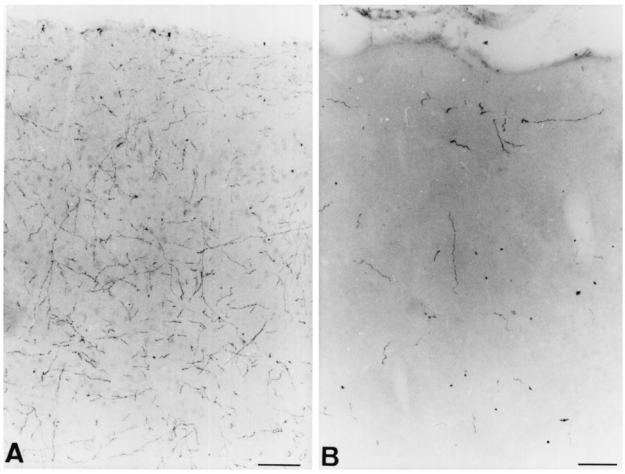

Fig. 1. Noradrenaline innervation (DBH immunocytochemistry) in the parietal cortex in control rats (A) and 30 days after DSP-4 treatment (B). Frontal plane; scale bar = 65 μm.

(decree 87–848). After surgery, rats were left for 10 days to adapt to the recording system in individual cages under a regulated light–dark cycle (light on: 0700 h, light off: 1900 h) at constant temperature (24 ± 1°C) and with food and water ad libitum. DSP-4 treatment consisted of a single i.p. injection (0.2 ml at 19.00 h) of a freshly-prepared 50 mg/kg saline solution of DSP-4 (Sigma); control animals received the same volume of vehicle (0.9% saline). Polygraphic recordings were scored visually over 30 s using classical criteria for the vigilance states of slow-wave sleep (SWS), paradoxical sleep (PS) and wakefulness (W) and fed into an in-house computer program. At the end of the experiment, we checked NA terminal degeneration at the cortex level by immunohistochemistry, using antibodies raised against dopamine-β-hydroxylase (DβH) (Eugene Tech) and previously-described techniques [8]. Only data from successfully-lesioned rats were used in the statistical analysis.

The durations of each vigilance state after DSP-4 were compared to baseline levels, since the vehicle injection has no significant effect (data not shown). Analysis of variance (ANOVA), followed by an LSD multiple range test, was used.

Thirty days after DSP-4 injection, the density of the cortical NA fibers was drastically decreased as compared to that seen in control animals (Fig. 1).

Starting at about 30 min after drug injection and lasting for a period of several hours, the animal adopted a 'frozen' attitude. As shown in Table 1, in the first 24 h following DSP-4 treatment, compared to controls, the total PS duration was decreased (–67%, $P < 0.05$), due to a decrease both during the dark (–71%, $P < 0.05$) and light (–66%, $P < 0.05$) periods. An increase in total SWS duration (+16%, $P < 0.05$) was seen, mainly due to an increase at night (+40%, $P < 0.05$). W was significantly decreased during the night (–15%, $P < 0.05$).

During the second and third nights, SWS duration was still increased compared to the baseline level (+39% and +18%, respectively; $P < 0.05$) while W was still significantly decreased (–18%, and –8%, respectively; $P < 0.05$); PS showed no significant variation. During the fourth and fifth days, the values for the vigilance states returned to the baseline level.

Two weeks after DSP-4 injection, PS duration was significantly decreased only during the light period (–18%, $P < 0.05$) while SWS and W did not differ significantly from those in control animals. Four weeks after DSP-4 administration, the PS/24 h duration was still decreased due to a significant decrease restricted to the light period (–23%, $P < 0.05$). A significant increase in SWS was restricted to the night period (+23%, $P < 0.05$) (Table 1).

Table 1

Nycthemeral variations in wakefulness (W), slow-wave sleep (SWS) and paradoxical sleep (PS) durations in control (baseline) and DSP-4-treated rats

		W (min ± SEM)	SWS (min ± SEM)	PS (min ± SEM)
Baseline (*n*)	Dark	491.88 ± 8.61	200.87 ± 7.44	27.25 ± 2.01
(17)	Light	192.33 ± 10.17	446.56 ± 9.24	81.12 ± 2.75
DSP-4 (D 1)	Dark	387.56 ± 18.18*	323.36 ± 19.27*	9.08 ± 3.26*
(17)	Light	211.09 ± 18.25	482.62 ± 18.70	26.29 ± 7.06
DSP-4 (D 2)	Dark	403.92 ± 33.09*	279.92 ± 32.20*	36.17 ± 2.73
(5)	Light	227.17 ± 25.76	421.54 ± 24.56	71.29 ± 9.90
DSP-4 (D 3)	Dark	450.40 ± 24.37*	237.00 ± 21.33*	32.60 ± 4.98
(5)	Light	210.00 ± 26.09	429.30 ± 25.41	80.70 ± 7.47
DSP-4 (D 4)	Dark	476.70 ± 17.48	217.80 ± 16.72	25.50 ± 2.77
(5)	Light	217.29 ± 23.87	429.47 ± 25.47	73.24 ± 12.11
DSP-4 (D 5)	Dark	513.41 ± 22.50	190.46 ± 45.67	16.13 ± 2.83
(5)	Light	203.22 ± 10.94	437.47 ± 16.88	79.31 ± 14.81
DSP-4 (D 15)	Dark	454.35 ± 17.56	239.85 ± 14.82	25.81 ± 3.82
(13)	Light	181.96 ± 10.09	471.57 ± 10.65	66.47 ± 4.28*
DSP-4 (D 30)	Dark	451.76 ± 16.11	247.35 ± 14.53*	20.89 ± 2.33
(13)	Light	190.63 ± 10.56	466.86 ± 11.04	62.51 ± 4.51*

D, day; *n*, number of animals. * $P < 0.05$, significantly different from baseline durations.

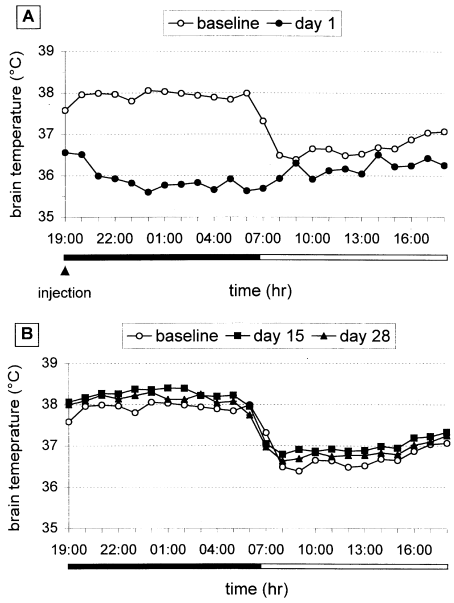

Fig. 2. Hourly variations in the brain temperature during the first day (A), 15 days and 28 days (B) after DSP-4 treatment.

Tbr recordings showed an marked decrease during the first 12 h following DSP-4 injection, then returned to control levels (Fig. 2, Table 2).

The present study shows that DSP-4 has drastic effects on vigilance states and brain temperature during the first day after injection, with W and PS decreasing, SWS increasing and a drop in Tbr. Once NA depletion was complete (2–4 weeks after DSP-4 treatment) [7,9], PS duration remained significantly decreased and SWS duration increased, whereas the values for W and Tbr returned to their normal values.

Only one study has been performed on the effects of DSP-4 on sleep [17]; this was restricted to its effects on vigilance states during the light period 5–6 days after DSP-4 treatment in the rat. These authors found similar changes in the duration of SWS and PS during the first days after injection to those in the present study; however, on days 5 and 6, they observed an increase in PS duration. This discrepancy may be due to methodology differences (rats exposed to dim light and DSP-4 injected during the light period) which might result in a different neurotoxic action of DSP-4. Indeed, it seems that more active NA neurons take up DSP-4 more efficiently [14].

In vivo anatomical and biochemical studies have shown long-term depletion of NA in those regions innervated by the LC within a few hours of DSP-4 administration [7]. This NA loss is similar to that seen after 6-hydroxydopamine (6-OHDA) treatment. Both drugs decrease the duration of W and increase that of SWS [present results, 16]. Slow waves, characteristics of SWS, have a thalamic and cortical origin and their appearance is inhibited by different systems activated during W, such as the noradrenergic, cholinergic, histaminergic and aspartate/glutamatergic systems. Thus, NA depletion would facilitate EEG slow-wave expression and decrease W a few hours after DSP-4 administration. Moreover, SWS might be overestimated, since slow waves have been described during behavioral arousal in 6-OHDA-treated rats [16]. Besides, the important depletion of NA by an LC lesion provokes an increase in blood volume and decreases the oxidative metabolism [13] which could be related to the Tbr drop and PS loss, respectively [12]. The NA depletion observed at the peripheral and hypothalamic levels the first days after DSP-4 treatment, could be also responsible for vasodilation [18] which in turn causes the Tbr drop [4]. However, during the first week, DSP-4 action is not selective, and other amine systems are affected [6,22]. Thus, NA-LC involvement in sleep and Tbr changes is difficult to interpret at this time.

After 2 weeks, peripheral and central aminergic systems other than the NA-LC have recovered [5,6,14,22], as has the Tbr. At this time, only the terminal portion of the NA axons shows degeneration, while the preterminal axons are spared [7]. The amounts of sleep seen in DSP-4-lesioned rats are comparable with those seen in normal BALB/c mice, (SWS increase during night and PS decrease during day) [24]. Interestingly, in this mouse strain, 38% of the NA-LC perikarya are genetically missing as compared with the standard C57BL6 strain [23] and this is associated with a decreased density of cortical NA terminals [2]. The SWS increase might be due to a decrease in NA inputs to the structures (thalamus and cortex) controlling EEG expression of this stage. Moreover, a metabolic hypothesis may be suggested. It is well known that monoamines promote glycolysis by stimulating cAMP formation. In particular, the NA modulates the availability of energy substrates (lactate and pyruvate) by activation of astrocyte β- and α_1-receptors [15]. DSP-4-induced NA depletion results in an increased density of these receptors [5,22]. Interestingly, we have noted an increased glial fibrillary acidic protein (GFAP) immunoreactivity (an index of astrocyte activity) in the cortex and

Table 2
Day–night variations in the brain temperature (°C ± SEM) 24h, 15 and 28 days following DSP-4 administration

Baseline		DSP-4 treatment					
		Day 1 (n = 8)		Day 15 (n = 10)		Day 28 (n = 9)	
Dark	Light	Dark	Light	Dark	Light	Dark	Light
37.92 ± 0.16	36.73 ± 0.10	35.92 ± 0.59	36.15 ± 0.50	38.24 ± 0.10	37.00 ± 0.08	38.11 ± 0.13	36.87 ± 0.05

n, number of animals.

hippocampus 4 weeks after DSP-4 administration (unpublished data). Taken together, these results suggest that DSP-4 treatment might have marked effects on astrocyte metabolism and, consequently, on neuronal activity in the NA-denervated areas. Recently, Cirelli and Tononi [3] have described decreased expression of the immediate early genes, in the cerebral cortex 10 days after DSP-4 treatment. Since there are direct cortical inputs to the basal forebrain , and from the hippocampus to the suprachiasmatic nucleus [21], we suggest that, 2–4 weeks following DSP-4 administration, changes in neuronal metabolic activity in NA-denervated areas (i.e. cortex and hippocampus) would, in turn, modify the activity of those target regions, such as the thalamus, basal forebrain, hypothalamus and suprachiasmatic nucleus directly involved in the sleep regulation, for review see [10].

In conclusion, the long-term effect of the NA-LC terminal degeneration induced by DSP-4 suggests that the LC is involved in the regulation of SWS and PS via different mechanisms. However, further studies on the consequences of NA depletion (i.e. glial and neuronal metabolism changes) are required for a better understanding of the involvement of LC in normal sleep mechanisms.

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