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Nicotine-induced Perturbations on Heart Rate, Body Temperature and Locomotor Activity Daily Rhythms in Rats

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Abstract

The aim of this study was to evaluate the influence of nicotine on the daily rhythms of heart rate, body temperature and locomotor activity in unrestrained rats by use of implanted radiotelemetry transmitters.

The study was divided into three seven-day periods: a control period, a treatment period and a recovery period. The control period was used for baseline measurement of heart rate, body temperature and locomotor activity. During the treatment period three rats received nicotine (1 mg kg^{-1}, s.c.) at 0900 h. Three rats received saline under the same experimental conditions. Heart rate, body temperature and locomotor activity were continuously monitored and plotted every 10 min. During the three periods a power spectrum analysis was used to determine the dominant period of rhythmicity. If daily rhythms of heart rate, body temperature and locomotor activity were detected, the characteristics of these rhythms, i.e. the mesors, amplitudes and acrophases, were determined by cosinor analysis, expressed as means ± s.e.m. and compared by analysis of variance.

Nicotine did not suppress daily rhythmicity but induced decreases of amplitudes and phase-advances of acrophases for heart rate, body temperature and locomotor activity. These perturbations might result from the effects of nicotine on the suprachiasmatic nucleus, the hypothalamic clock that co-ordinates biological rhythms.

Nicotine is known to perturbate some physiological parameters such as heart rate, body temperature and locomotor activity in rats. Zarrindast et al (1995) demonstrated that nicotine injections (0·5, 1 and 2 mg kg^{-1}) induced dose-dependent hypothermia for 15 min after drug administration. Nicotine injections at different doses (6·25 μg kg^{-1} min^{-1}, 12·5 μg kg^{-1} min^{-1} and 25 μg kg^{-1} min^{-1}) in drug-naive and in chronic smoke-exposed rats induced tachycardia which increased during the first 15 min after drug administration (Barron et al 1988). Kita et al (1986) studied nicotine-induced variations of ambulatory activity in rats for 120 min after nicotine injection and demonstrated that during the light period a large dose (0·5 mg kg^{-1}) of nicotine induced a stimulant effect during the first 20 min after injection whereas after injection during the dark period the same dose of nicotine induced ataxia. A small dose (0·1 mg kg^{-1}) induced only ataxia during the first 20 min after injection during the dark period.

Nevertheless, most work has focused on the period immediately after nicotine injections and to the best of our knowledge the effects of nicotine on the daily rhythms of well-known markers such as body temperature, heart rate and locomotor activity have not been evaluated over a 24-h period. To clarify the longer-term effects of nicotine upon daily rhythms we have investigated the possible modifications induced by repeated injection of nicotine on the daily rhythms of temperature, heart rate and locomotor activity in rats and compared them with those of controls receiving saline. Such a protocol takes into account the possibility of stress-induced effects arising from handling or injection.

Materials and Methods

Animals and housing

For a minimum of three weeks before use six Wistar AF IOPS adult male rats from Iffa-Credo (St Germain-sur-l'Arbresle, France), mean weight 275 g, ten-weeks-old, were housed in individual

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transparent polypropylene cages (40 × 30 × 30 cm) under controlled environmental conditions—relative humidity (50–55%), temperature (24 ± 1°C) and synchronized by a 12-h light-dark cycle (light on from 0600 h to 1800 h). Lighting was provided by two 60-W fluorescent tubes and lighting intensity was approximately 300 lx at cage level. Food and water were freely available and changes occurred once a week on an irregular schedule. The experiments were conducted in accordance with internationally accepted principles concerning the care and use of laboratory animals (National Research Council 1985) after acceptance by our animal experimentation ethical committee (Commission Consultative d'Ethique Animale, Centre de Formation et de Recherches Expérimentales Médico-Chirurgicales, Faculté de Médecine de Marseille, France).

Experimental procedures

Heart rate, body temperature and locomotor activity were measured by radiotelemetry. Just before implantation calibration values for each transmitter were entered into the Dataquest III data-acquisition system. Surgical implantation of the transmitters (model TA11-CTA-F40, Data Sciences, St Paul, MN), as described by Kramer et al (1993), was performed under ketamine hydrochloride (100 mg kg^{-1} i.p.) general anaesthesia. Shortly after full anaesthesia was achieved a 2-cm incision was made in the peritoneum and the sensor was implanted into the abdominal cavity and sutured to the abdominal wall. ECG leads were extended subcutaneously to the right axilla and to the left lower rib area and sutured to muscle tissue. After closure animals were monitored for 2 h until they recovered from anaesthesia; they were then returned to their home cage and recording was started. Signals from the transmitter were received by an antenna mounted in a receiver board (model CTR86, Data Sciences) placed under the animal's cage. Data of heart rate (beats min^{-1}), temperature (°C) and locomotor activity (counts) were collected as described by Meerlo et al (1995) every 10 min over a 21-day period and processed by means of a PC with a specialized recording and analysis system (Dataquest III, Data Sciences).

Drugs

Nicotine (Sigma, France) was dissolved in saline (0·9%) and injected subcutaneously at 1 mg kg^{-1}, a dose considered to be pharmacologically active and representing approximately 1/30th the LD50 (dose resulting in the death of half of the animals) in rats (Zarrindast et al 1995). The animals of the control group were injected with saline under the same experimental conditions.

Protocol

After a period of recovery from surgical implantation and anaesthesia (Prudian et al 1997), the study was divided into three seven-day periods. The first week was a control period for baseline measurement of daily rhythms in heart rate, body temperature and locomotor activity. This period was characterized by daily handling and weighing of the animals (0900 h) only. The second week was the treatment period—three animals (nicotine group) received daily nicotine at 0900 h and three animals (control group) received saline at the same time. The third week was a recovery period.

Data analysis

Heart rate, body temperature and locomotor activity were measured every 10 min and analysed by two methods by use of the Dataquest III data-acquisition system. Firstly, to determine the dominant period during the control, treatment and recovery periods a power spectrum analysis (Fourier transform) was applied to 30-min average data intervals. Then, to assess daily variations of heart rate, body temperature and locomotor activity, least-square cosine regression (cosinor analysis) was applied to individual data for the control, treatment and recovery periods and a rhythm was considered to be significantly detected when $P < 0{\cdot}05$ (Refinetti 1992). The daily rhythm characteristics of heart rate, body temperature and locomotor activity, i.e. mesor (midline estimating statistic of rhythm corresponding to the mean level, which is equal to the 24-h average), amplitude (half of the peak-to-trough difference of the fitted cosine function) and acrophase (the crest time of rhythm given in degrees (°), where 360° correspond to a 24-h cycle and the starting time of 0000 h was denoted by 0°) were estimated by the linear method of least squares (Morgan & Minors 1995) and expressed as means ± s.e.m.

Three-way analysis of variance was used for statistical analysis (Statview II program) using the three factors period (control period, treatment period or recovery period), the day of the period (day 1–7) and the treatment (nicotine or control). If no interaction related to the days within a period was detected, comparisons between control, treatment and recovery periods for nicotine and control groups and comparisons between nicotine and control groups for the control, treatment and recovery periods were performed by one-way analysis of variance; if a significant difference was found, Fisher's PLSD (protected last significant difference) test for multiple comparisons was applied.

Results

Fourier analysis

Fourier analysis showed that the daily rhythms of heart rate, body temperature and locomotor activity were significantly validated for each rat during each time-span of the protocol, i.e. each rhythm had a dominant period of 24 h.

Statistical analysis of cosinor parameters

Because Fourier analysis showed that all the rhythms were significantly detected for 24 h, cosinor analysis with a 24-h period was used to determine the characteristics of these rhythms, i.e. the mesors, amplitudes and acrophases. These parameters were expressed as means ± s.e.m. and compared by analysis of variance. As the three-way analysis of variance did not detect any interaction related to the day (day 1–7) and the period of the protocol (control, treatment and recovery periods), one-way analysis of variance was performed to compare: mesors, amplitudes and acrophases of the nicotine and control groups during the three periods of the protocol; and mesors, amplitudes and acrophases between the nicotine and control groups for each period of the protocol.

Tables 1–3 show the mean values of the mesors, amplitudes and acrophases for daily rhythms of heart rate, body temperature and locomotor activity, respectively.

Whatever the period of the protocol (control, treatment or recovery), mesor values of heart rate, body temperature and locomotor activity did not change for the control or nicotine-treated groups. Although the amplitudes of heart rate, body temperature and locomotor activity for both groups were significantly reduced during the treatment period in comparison with the control and recovery periods, the observed decreases were significantly ($P < 0.05$) more pronounced for the nicotine group than for the control group. Acrophases of heart rate, body temperature and locomotor activity were phase-advanced during the treatment period compared with the control and recovery periods, unlike the controls, for which acrophases were unmodified whatever the period.

Figures 1–3 show the mesors, amplitudes and acrophases of heart rate, body temperature and locomotor activity, respectively, for the nicotine and control groups over the three seven-day periods of the protocol. These data are expressed as means ± s.e.m.

Table 1. Mesor, amplitude and acrophase of heart rate daily rhythm for nicotine and control groups for each period of the protocol.

	Mesor (beats min^{-1})		Amplitude (beats min^{-1})		Acrophase (°)	
	Nicotine	Control	Nicotine	Control	Nicotine	Control
Control	341·29 ± 4·12	336·41 ± 3·09	44·48 ± 1·76	45·97 ± 2·10	356·56 ± 2·13	357·99 ± 1·65
Treatment	344·23 ± 3·89	338·06 ± 2·54	25·96 ± 1·74*‡	35·80 ± 2·08*	348·09 ± 2·39*‡	354·76 ± 2·41
Recovery	346·65 ± 5·80	338·06 ± 1·60	41·76 ± 1·89†	41·82 ± 2·35†	356·14 ± 1·66†	355·05 ± 1·92
Analysis of variance	$P = 0.6297$, $F = 1.056$		$P = 0.0001$, $F = 17.968$		$P = 0.0123$, $F = 3.094$	

Values are mean ± s.e.m. Results were compared by one-way analysis of variance. If a statistically significant difference was detected, post-hoc comparisons were performed by Fisher's PLSD test. *$P < 0.05$, significantly different from result for control period; †$P < 0.05$, significantly different from result for treatment period; ‡$P < 0.05$, significantly different from control result for the same period.

Table 2. Mesor, amplitude and acrophase of body temperature daily rhythm for nicotine and control groups for each period of the protocol.

	Mesor (°C)		Amplitude (°C)		Acrophase (°)	
	Nicotine	Control	Nicotine	Control	Nicotine	Control
Control	37·69 ± 0·03	37·67 ± 0·03	0·60 ± 0·03	0·60 ± 0·03	361·70 ± 2·38	361·51 ± 2·16
Treatment	37·77 ± 0·03	37·67 ± 0·03	0·36 ± 0·02*‡	0·45 ± 0·02*	347·68 ± 2·09*‡	361·53 ± 4·12
Recovery	37·68 ± 0·03	37·66 ± 0·04	0·62 ± 0·02†	0·59 ± 0·04†	354·36 ± 1·45†	357·66 ± 2·28
Analysis of variance	$P = 0.0654$, $F = 2.1388$		$P = 0.0001$, $F = 14.075$		$P = 0.0001$, $F = 17.342$	

Values are mean ± s.e.m. Results were compared by one-way analysis of variance. If a statistically significant difference was detected, post-hoc comparisons were performed by Fisher's PLSD test. *$P < 0.05$, significantly different from result for control period; †$P < 0.05$, significantly different from result for treatment period; ‡$P < 0.05$, significantly different from control result for the same period.

Table 3. Mesor, amplitude and acrophase of locomotor activity daily rhythm for nicotine and control groups for each period of the protocol.

	Mesor (Counts)		Amplitude (Counts)		Acrophase (°)	
	Nicotine	Control	Nicotine	Control	Nicotine	Control
Control	36·1 ± 1·9	38·4 ± 1·6	23·75 ± 1·09	28·61 ± 1·86	372·30 ± 4·31	378·39 ± 3·52
Treatment	36·8 ± 1·9	35·8 ± 1·8	12·48 ± 1·19*‡	19·64 ± 1·29*	347·83 ± 4·60*‡	389·53 ± 4·23
Recovery	34·1 ± 1·1	38·4 ± 2·5	21·55 ± 2·38†	27·63 ± 2·14†	374·96 ± 3·68†	387·26 ± 7·43
Analysis of variance	$P = 0·145$, $F = 1·6857$		$P = 0·0001$, $F = 11·832$		$P = 0·005$, $F = 3·59$	

Values are mean ± s.e.m. Results were compared by one-way analysis of variance. If a statistically significant difference was detected, post-hoc comparisons were performed by Fisher's PLSD test. * $P < 0·05$, significantly different from result for control period; † $P < 0·05$, significantly different from result for treatment period; ‡ $P < 0·05$, significantly different from control result for the same period.

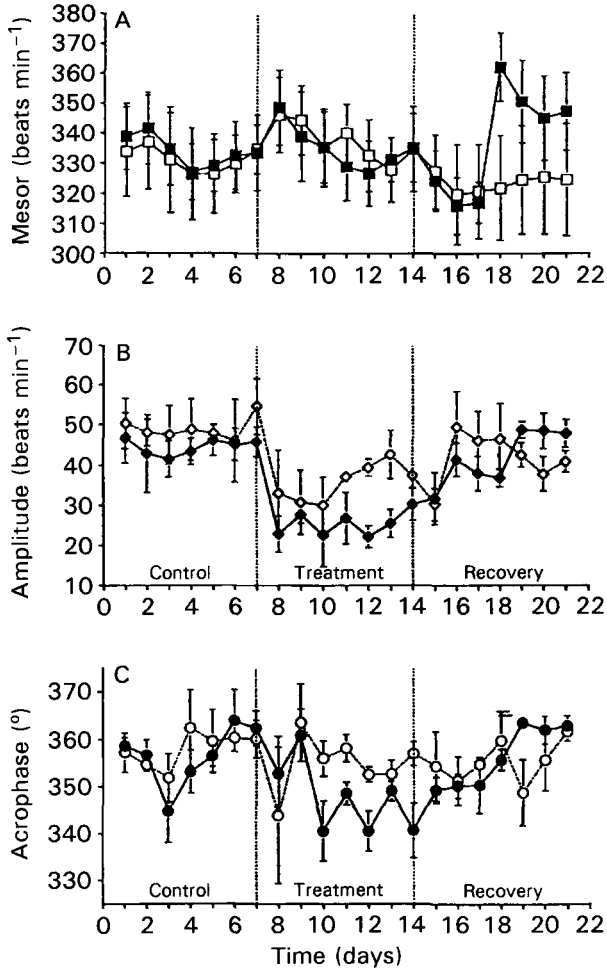

Figure 1. Mesor (A), amplitude (B) and acrophase (C) of heart rate for the nicotine (■, ◆, ●) and control (□, ◇, ○) groups over the 21-day control, treatment and recovery periods of the protocol. Each point represents the mean ± s.e.m. for the 24-h period for the three rats of each group.

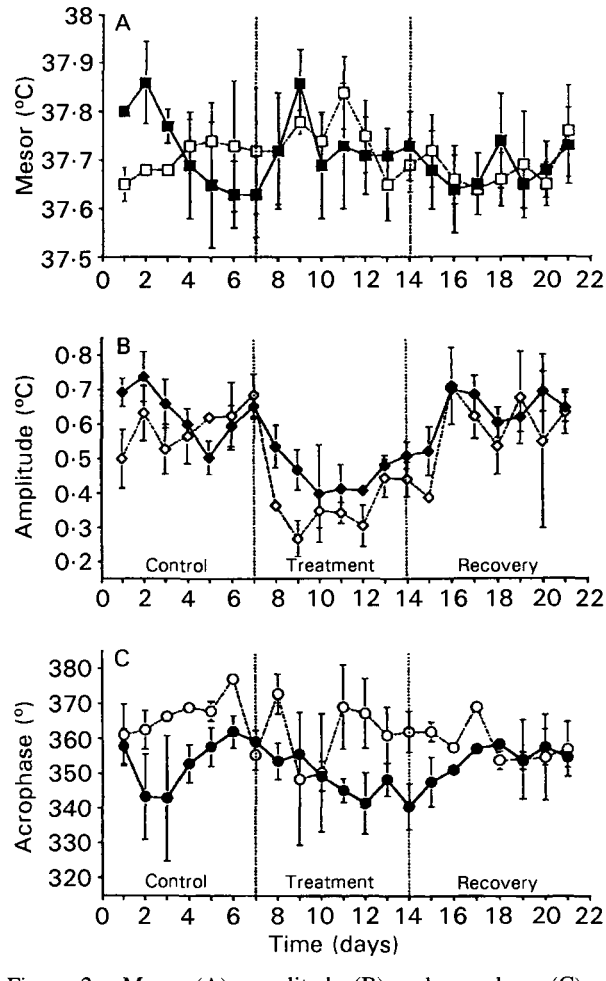

Figure 2. Mesor (A), amplitude (B) and acrophase (C) of body temperature for the nicotine (■, ◆, ●) and control (□, ◇, ○) groups over the 21-day control, treatment and recovery periods of the protocol. Each point represents the mean ± s.e.m. for the 24-h period for the three rats of each group.

Discussion

This study has demonstrated that: repeated administration of nicotine did not suppress the daily rhythmicity of heart rate, body temperature and locomotor activity; repeated administration of nicotine modified the amplitudes and acrophases of the three rhythms; and the modifications of the amplitudes were significantly more pronounced for the nicotine group than for the control group and the phase-shifts of acrophases, described during the

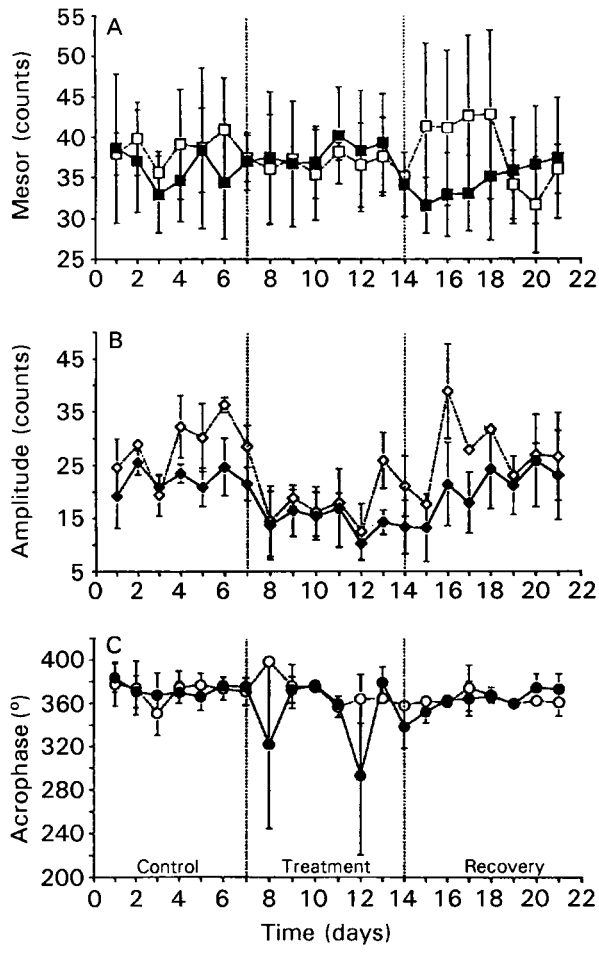

Figure 3. Mesor (A), amplitude (B) and acrophase (C) of locomotor activity for the nicotine (■, ◆, ●) and control (□, ◇, ○) groups over the 21-day control, treatment and recovery periods of the protocol. Each point represents the mean ± s.e.m. for the 24-h period for the three rats of each group.

treatment period, were only observed for the nicotine group.

These data confirmed that nicotine perturbated the daily rhythms of heart rate, body temperature and locomotor activity. The statistical differences between the two groups indicated that the effect of nicotine was genuine. Furthermore, it was of interest to show that the stress induced by saline injections significantly modified the amplitudes of the three rhythms, in agreement with previous reports of the modification of such rhythms induced by different kinds of stress (Harper et al 1996).

In rodents physiological and behavioural rhythms are co-ordinated by the suprachiasmatic nucleus (Mirmiran et al 1995; Sano et al 1995). In-vivo observations have revealed that the cholinergic system is involved in the regulation of these rhythms. Carbachol, a non-specific cholinergic agonist, phase-shifts circadian activity rhythms in rodents (Bina & Rusak 1996) and Trachsel et al (1995) demonstrated that nicotine phase-advanced the circadian neuronal activity rhythms in-vitro in rat suprachiasmatic nuclei explants. Our results were in total agreement with these observations and must be completed with further studies to determine the nicotinic receptors involved in the effects.

Another hypothesis should be explored. Dopamine is involved in the central effects of nicotine (Benwell & Balfour 1997) and Marshall et al (1997) reported that nicotine elicited a dose-dependent increase in dopamine release in the striatum and in the nucleus accumbens. As described by Zarrindast et al (1995), nicotine-induced modification of body temperature might be mediated by an indirect dopaminergic mechanism. Fung et al (1996) observed a significant decrease of locomotor activity and a reduction of the dopamine content of the nucleus accumbens in rats after the cessation of 14-day nicotine administration. According to Taylor (1996), the nicotine-induced increases of heart rate and blood pressure in mammals were a result of stimulation of the sympathetic ganglia together with the discharge of monoamines from sympathetic nerve endings. All these data, even if obtained under experimental conditions different from those used in our study, clearly indicated that dopamine participated in the effects of nicotine on the regulation of heart rate, body temperature and locomotor activity in rats. Smith et al (1992) reported that dopamine concentrations followed a daily rhythm and Pietilä et al (1995) showed that chronic oral administration of nicotine affected the circadian rhythm of dopamine in the striata of mice. These findings suggest that repeated administration of nicotine altered the circadian pattern of striatal dopamine and thus might affect the functions regulated by these transmitters.

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