Archives Italiennes de Biologie, 144: 33-43, 2006.

BEHAVIORAL AND ELECTROPHYSIOLOGICAL CHANGES INDUCED BY ACETYL-L-CARNITINE IN AGED FREELY-MOVING RATS

G. MARINI, M.CALVANI[1], P. CECCARELLI, AND M. MANCIA

Centro di Ricerca Sperimentale sul Sonno "Giuseppe Moruzzi", Università di Milano, Via Mangiagalli 32, 20133 Milano, Italy; [1] Sigma-Tau Laboratories, Pomezia, Italy

INTRODUCTION

Acetyl-L-carnitine (ALCAR) is the acetyl ester of carnitine (trimethylbetaine of γ-amino-β-hydroxybutyric acid) and is present in mitochondria in the central nervous system (see 3). The close structural similarity of this endogenous substance with acetylcholine (ACh) suggests cholinomimetic properties (28, 1, Brambilla et al., personal communication).

The brain is able to generate oscillations at a wide range of frequencies. Both normal and abnormal synchronized EEG activities depend on intrinsic properties and on synaptic interactions between neurons of the thalamocortical networks which are under the control of the brain stem cholinergic system (13, 27).

Cholinergic mechanisms have long been believed to be involved in electrocortical arousal (6, see for rev. 24). Acetyl-cholinesterase-rich afferents have been found to originate in the brain stem, particularly in the two neuronal groups at the mesopontine junction (pedunculo pontine tegmental nucleus, PPT, and laterodorsal tegmental nucleus, LDT). ACh has been identified as one of the main transmitters in such ascending pathways in different species (see 26). Activation of the ascending cholinergic system is responsible for EEG arousal and behavioral manifestations of attention in the rat too (29, 30).

Although attentive processes in the state of wakefulness are associated with fast (so called beta and gamma) rhythms, lower-frequency oscillations also develop when the animal expects targets that are not yet visible (2).

In some rat strains (e.g. Fischer, Wistar), in the awake but immobile animal generalized highly synchronized EEG patterns emerge. These neocortical rhythms, termed High Voltage Spindles (HVS), first described by Kingberg and Pickenhain (10), are characterized by large-amplitude rhythmic spike-and-wave discharges at 6-9.5 Hz. In view of these electrical characteristics associated with suppression of vigilance, attention, and exploratory activity, the HVSs have been considered an animal model of human "absence" (7).

Corresponding Author: Prof. Mauro Mancia, Via Mangiagalli 32, 20133 Milano, Italy - E-mail: mauro.mancia@unimi.it

Early experiments with intracerebroventricular (i.c.v.) injection of ALCAR showed that this substance disrupted the spindles of *cerveaux isolé* cats and woke them, as can be deduced from EEG desynchronization and dilatation of the pupils (16).

In Fischer rats, HVSs were observed to gradually and significantly increase in number and duration with age (5). Because of the high incidence of such intense EEG hypersynchrony, aged Fischer rats offer the best chance of studying the effect of i.c.v. and intraperitoneal (i.p.) injection of ALCAR on their EEG activity.

Here we report that, in chronically-implanted, drug-free, freely-moving aged Fischer rats, both i.c.v. and i.p. ALCAR injections dramatically boosted their vigilance, attention, and exploratory activity and abolished or substantially reduced the spontaneous immobility-related HVSs, in a dose-dependent manner.

METHODS

Eight male Fischer rats (F344, 23-26 months old, 350-450 g) were used. The studies were carried out in strict compliance with the EEC Council Directive 86/609 on the use and care of laboratory animals. All efforts were made to minimize suffering. Drug sessions were done over several days since long-term studies in behaving animals offer the best situation for assessing the time course of the effect.

The experimental set-up was essentially the same as used before (17, 18).

In brief, the animals, anesthetized with ketamine (10 mg/100 g, body weight, Ketavet®, Gellini, Italy), were implanted bilaterally with chronic standard electrodes along the anteroposterior axis (F1, F2, F7, F8, T3, T4, P3, P4) to record EEG activity. All recordings were monopolar and the reference was placed on the midline above the cerebellum. The EMG was recorded from the neck muscles.

Signals from the integrated circuit socket were fed through a rotating collector into a 24-channel analog-digital converter (sampling frequency 512 Hz sample/channel/s), interfaced with the digitalized STARII-Galileo system (EBNeuro, Firenze, Italia). In all rats, EEG activity was also studied in the frequency domain, using spectral analysis to study the rhythmic components. The power values were computed from the multiple monopolar leads in both hemispheres by a Fast Fourier Transform routine for 10 consecutive, artifact-free 2s epochs with a software specifically adapted for rodents (EBNeuro, Florence, Italy) over the frequency range 0.5-16/32 Hz and for the following frequency bands: 0.5-4.0 Hz; 4.5-8.0 Hz; 8.5-15.0 Hz; 15.5-32 Hz.

Quantitative sequential analysis of power spectra enabled the computer program to provide the spatial distribution and to construct two-dimensional brain maps during different cortical rhythms. A colour scale in arbitrary units helped to visualize the distribution of the absolute power (μV^2) for each frequency band.

ALCAR was synthesized and provided by Sigma Tau Laboratories, Pomezia, Italy. The drug was dissolved in physiological saline. For intracerebroventricular injection (i.c.v., in the 3rd ventricle), polyethylene cannulae were stereotaxically placed at the following coordinates (22) from bregma: A-1; L-1; H2.5. ALCAR (or saline for control) was injected in four rats with a Hamilton microsyringe connected to a flexible tubing. ALCAR and saline i.c.v. injection in the awake unanesthetized immobilized animal was done at 11 a.m. and doses were given at least three days apart. In four rats, ALCAR was injected intraperitoneally (i.p.) at a dose of 150 mg/kg.

The effect of injection was assessed by comparing the number of HVSs measured in the 12 h before and after each dose of ALCAR. In each rat, comparison for significance was evaluated by means of a t-test and a one way ANOVA. The same statistical procedures were also applied by pooling together the data obtained in the two of the i.c.v. rats in which ALCAR doses were comparable. For each comparison, F and p values are given in the text.

The animals were freely-moving in Plexiglas cages, with water and food *ad libitum*. The animals' behavior was followed by simultaneous video-EEG recording, done continuously during the natural light/dark cycle for 12h before and after the injections.

To study the rats' head movements, three spherical infrared reflecting markers were placed on the head socket and three TV cameras (50 Hz) were placed in front of the cylinder and focused on the behaving animal. The reflected signals were captured by the camera and digitized. After careful calibration, the 3D trajectories were measured and calculated 2 h before and after the ALCAR injection by an opto-electronic system (SMART Capture, Emotion, Padua, Italy), which included the software for calculating kinematic data. This system and the software package for synchronizing the EEG with movements were specifically set up for the rat in our laboratory. The head movements and concomitant EEG activity were synchronized by means of an external trigger.

On completion of the experiments, the animals were given an overdose of chloral hydrate.

RESULTS

The effects of i.c.v. (4 rats) and i.p. (4 rats) ALCAR injections on behavioral and EEG activity were studied in chronic experiments using behaving, aged Fischer rats. The results were similar in all cases and included the following main features: 1) increase of movements, vigilance, attention, and exploratory behavior, documented by the increase in head movements toward stimuli of interest which in this species, due to the lateral position of the eyes, replace exploratory eye movements; 2) abolition or steep reduction of the spontaneous hypersynchronous EEG rhythmic patterns (HVS), characteristic of this rat strain.

Behavioral Effects of i.c.v. ALCAR

Healthy aged Fischer rats are usually rather quiet, still, slow-moving, with a drowsy attitude. ALCAR even at low doses (0.1 mg/kg, i.c.v.) dramatically boosted alertness, with an increase of general motor activity and particularly of head movements, suggesting increased attention and exploratory activity. Close inspection of the video-tapes taken before and after the injection indicated that in all cases ALCAR elicited an increase of spontaneous almost continuous exploratory behavior, which consisted in walking, circling, running, rearing, sniffing, whisker twitching and head movements. In particular, ALCAR increased head movements for about 2 h; this activity was quantified for 15 min before and after the injection by an opto-electronic system which measured the trajectories of the head movements as described in the method section. Figure 1 illustrates a representative case with the horizontal and vertical trajectories of the head movements before (Fig. 1a, b) and after (Fig. 1c, d) ALCAR. Very few head movements were present before injections, but the drug induced a marked increase in both axes. When full recovery of the EEG was attained, the rats returned drowsily to their calm position.

EEG effects of i.c.v. ALCAR

As noted in the introduction, the EEG of aged Fischer rats displays high incidence of generalized highly synchronized patterns (HVS) while immobile and wake. These

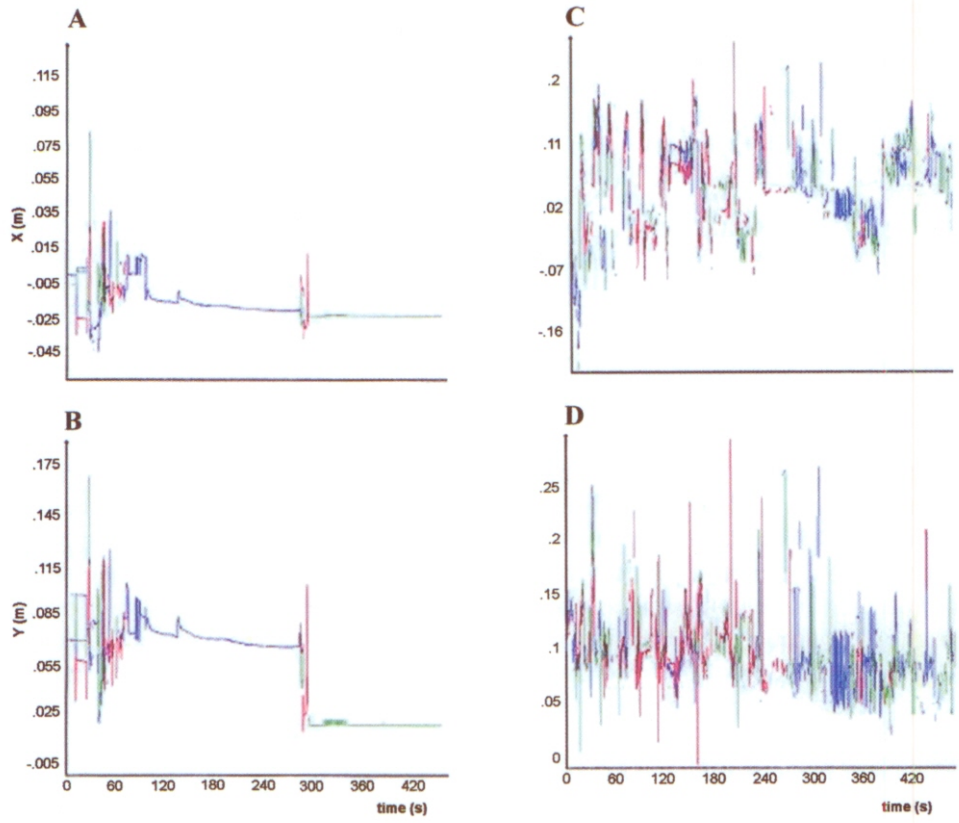

Fig. 1. - *Trajectories of head movements before (A and B) and after (C and D) ALCAR (0.25 mg/kg i.c.v.) in freely-moving aged Fischer rats.*
On the abscissa, the time (s), on the ordinate, the position (m) of the three markers on the rat's head. A, and C, trajectories of head movements on the x-axis. B, and D, trajectories of head movements on the y-axis. Note the dramatic increase in head movements after the ALCAR injection.

neocortical rhythms are characterized by large-amplitude (up to 1.5 mV) rhythmic spike-and-wave discharges at 6-9.5 Hz (17).

Figure 2A, B provide a typical example of the effect of a single dose of ALCAR (0.2 mg/kg body weight) on the duration of spontaneously occurring HVS recorded pre- and post-injection (2 h after) from frontal leads (F2 and F8) in the same animal. The wave-form and amplitude of the bursts, however, were not affected by the injection (Fig. 2C, D).

In Figure 3A, the effect of increasing doses of ALCAR on the number of HVSs is illustrated in one representative rat. The control is the mean obtained from the average of the number of HVSs measured each hour in the 12 h before each injection. When comparing each pre-drug control values with the corresponding post-drug values of each dose of ALCAR, the changes in HVS incidence were statistically highly significant (t-test: 0.1 mg/kg, $p < 0.001107$; 0.2 mg/kg, $p < 0.003048$; 0.3 mg/kg, $p < 0.000001$; 0.35 mg/kg, $p < 0.000002$). When comparing the pre-drug

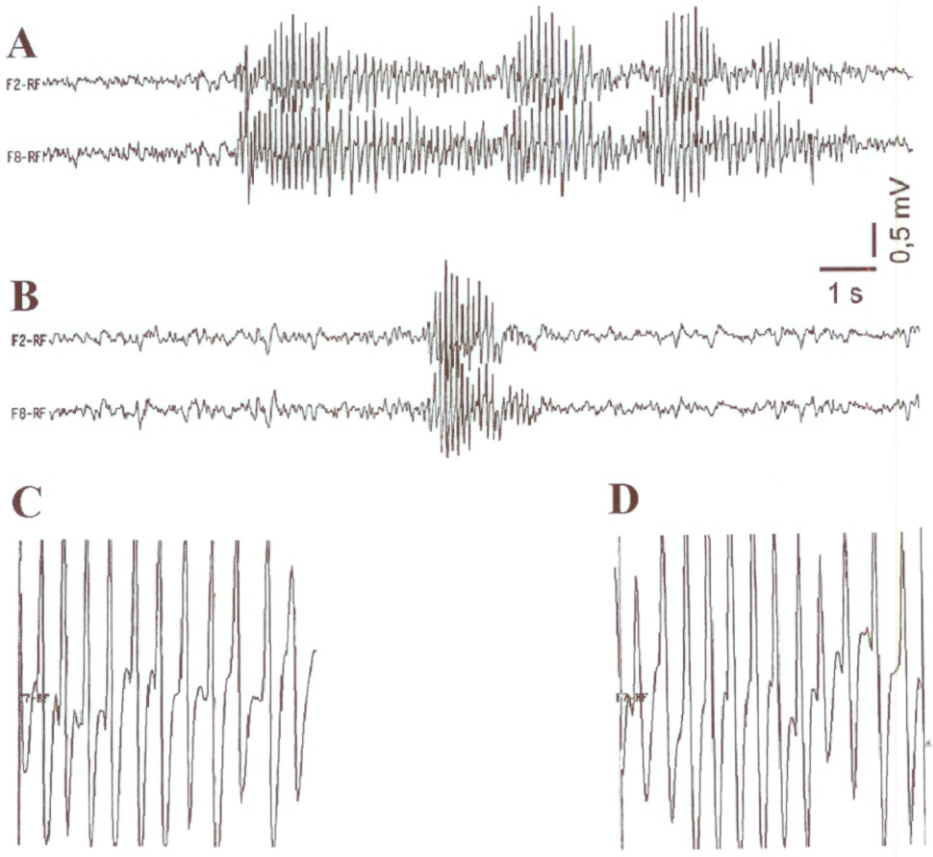

Fig. 2. - *Effect of i.c.v. Acetyl-L-Carnitina (ALCAR) on the duration of spontaneous High Voltage Spindle (HVS) in a representative, 23-months-old, freely-moving Fischer rat.*
A: monopolar EEG recordings obtained preoperatively from frontal leads, showing typical, spontaneously occurring sequences of HVS. HVSs were present in all leads, but were particularly prominent in frontal ones. B: spontaneous HVS recorded in the same rat 2 hours after i.c.v. injection of ALCAR (0.2 mg/kg). Note the dramatic decrease in the duration of the burst of HVS. C and D: enlarged view of the HVSs before and after injection respectively, showing that the wave-form and amplitude remained essentially unchanged.

control values with the post-drug values obtained after saline injection, no significant differences were found ($p < 0.962293$). A one way ANOVA demonstrated that the effect of the ALCAR on the HVS incidence was dose-dependent ($f(4,55) = 28,61$; $p < 0.000001$).

All the investigated rats showed similar results. When averaging data obtained in the two rats where administered doses were comparable (Fig. 3B), again the changes in HVS incidence after each dose were significant (t-test: 0.1 mg/kg, $p < 0.000001$; 0.2 mg/kg, $p < 0.000060$). One way ANOVA test demonstrated that the effect was dose-dependent ($f(2,69) = 40,73$; $p < 0.000001$).

Immediately after the i.c.v. ALCAR injection, the EEG was desynchronized and the HVSs were abolished for variable periods (from 1 to 12 h, depending on the

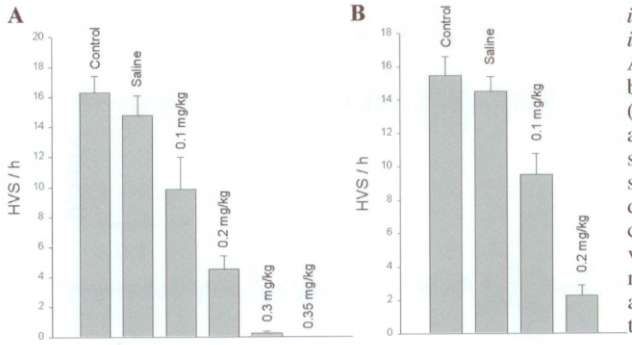

Fig. 3. - *Histograms showing statistical evaluation of the effect of i.c.v. ALCAR injections at increasing doses on the incidence of spontaneous HVSs.*
A: HVSs averaged in a period of 12 h before (control) and 12 h after injection (saline, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg and 0.35 mg/kg ALCAR) in one representative rat. Note the effect of increasing dose of ALCAR on the HVSs incidence; 0.35 mg/kg fully abolished HVSs complexes at least for 12 h. B: average values of HVSs evaluated in the 12 h recording before (control), after vehicle alone (saline) and after ALCAR injection (0.1 and 0.2 mg/kg) in two rats.

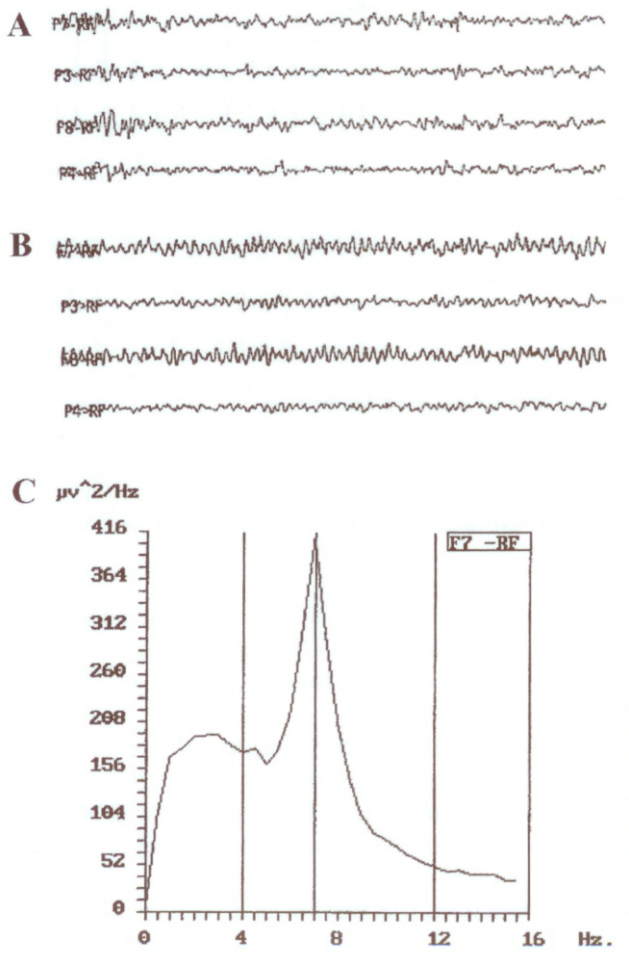

Fig. 4. - *EEG multiple recordings before (A) and after (B) i.c.v. ALCAR injection in freely-moving aged Fischer rats.*
The theta rhythm is particularly enhanced in frontal regions (F7, F8) after the injection. C: Quantitative spectral analysis of theta activity after i.c.v. ALCAR from a frontal (F7) lead. On the abscissa, the frequency (Hz), and on the ordinate the power spectrum values (μV^2/Hz). Note the theta peak at 7 Hz.

dose) and they gradually reappeared, being first disorganized and then displaying clear-cut amplitude and frequency characteristics. Unfortunately the 12 hours monitoring after injections was not enough to follow the recovery of HVS incidence after administration of higher does, so it is not possible to full discuss this issue here.

In the pre-drug condition, HVSs were nested within scanty neocortically monitored theta waves (Fig. 4A). After ALCAR, intense theta activity was observed (Fig. 4B) and documented by the quantitative spectral analysis of the EEG activity, calculated for 20s in the 30 min after the injection, during a period without HVSs (Fig. 4C). This effect mainly occurred in frontal regions (F7, F8). It is of interest that also humans EEG bands, in particular theta frequencies, exhibit frequency-specific regional changes (11).

An example of the effect of i.c.v. ALCAR (0.25 mg/kg) on the topographic distribution of the EEG spectral power is shown in Figure 5. Brain mapping was done during a sequence of HVSs lasting 10 seconds occurring 15 minutes before (Fig. 5A) and 2 h after the injection in the same animal (Fig. 5B). The color-coded maps before and after the injection showed very similar patterns in the power distribution, with frontal predominance. Comparing the different bands, however, decrease of power and spatial reduction were observed in all the frequency bands after ALCAR. Figure 5C and 5D show the power spectra of the F7 lead. After the injection, all the

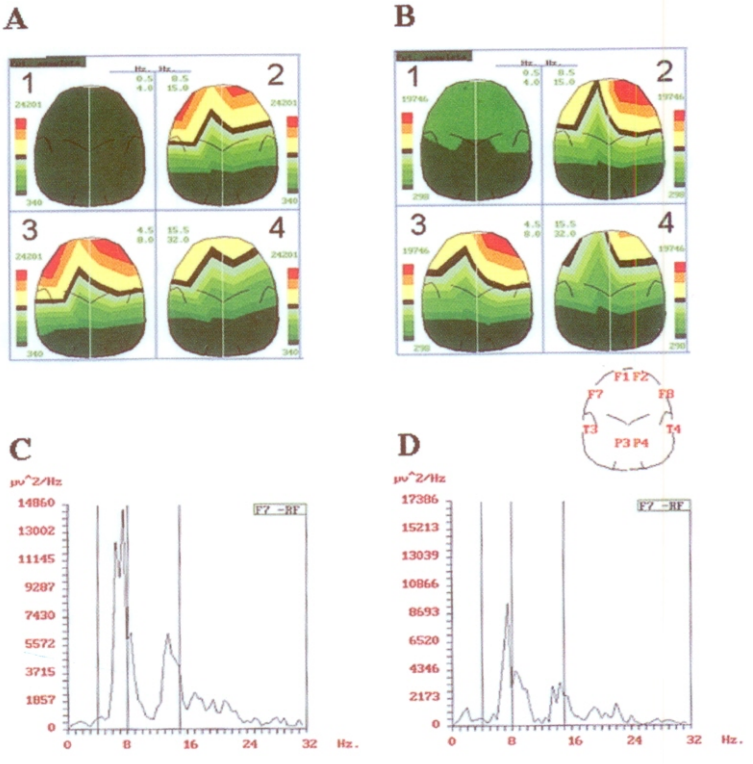

Fig. 5. - *Effect of i.c.v. ALCAR (0.25 mg/kg) on the topographic distribution of spectral power in freely-moving aged Fischer rats.*
Brain mapping of power spectra was done during a period with HVSs 15 min before (A) and 2 h after the ALCAR injection (B). Although the power distribution is similar, the power is slightly decreased after ALCAR (compare A1 and B1, A2 and B2, A3 and B3, A4 and B4). C and D: the power spectrum of the F7 lead (location shown in the insert) before and after the injection. The power spectra are expressed in μV^2. The density values are color-coded in arbitrary units, increasing from green to red.

components were reduced, but the power peak at 7 Hz gave the largest decrease (from 14860 to 8693 μV^2/Hz, see ordinate). This reflects the fact that the power peak component is specifically affected.

In some rats, highest dose of ALCAR (> 0.5 mg/kg) could precipitate electrical manifestations characterized by hypersynchronuous spikes, often in sequences suggesting an epileptic-like attack. However, no depression was observed after the paroxysmal episodes and the rats' electrical and behavioral conditions were normal.

Behavioral and EEG effects of i.p. ALCAR

As already described, before the injection the aged animals were usually quiet, lying down and moving very little. ALCAR at the tested dose of 150 mg/kg i.p. dramatically changed the rats' attitude, with a latency of about 15 minutes. The changes involved body movements with circling, running, rearing, standing up on the hind limbs and, particularly, extremely active head movements in the different planes (Fig. 6). This behavior suggested intense attention and exploratory activity. The

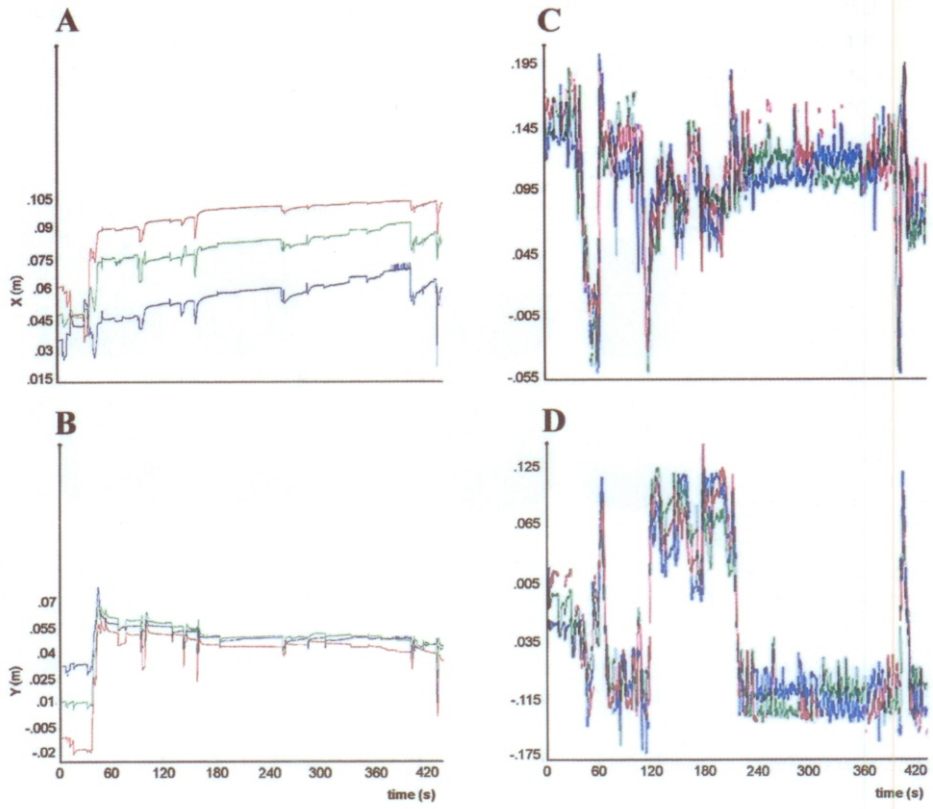

Fig. 6. - *Trajectories of head movements before (A and B) and after (C and D) ALCAR (150 mg/kg i.p.) in freely-moving aged Fischer rats.*
A and C, on the x-axis; B, and D, on the y-axis. Note the dramatic increase in head movements after the ALCAR injection.

behavioral effects lasted several hours, and were sometimes observed even 8 h after the injection.

The i.p. ALCAR injection induced the same EEG changes (i.e. EEG desynchronization with abolition of HVSs and enhanced theta activity) as the i.c.v. injection.

DISCUSSION

The main findings of this study concern the effects of i.c.v. and i.p. ALCAR injection on the behavior and EEG neocortical/hippocampal activity in freely-moving aged Fischer rats. In such animals, immobile behavior particularly of the head was associated with very high incidence of EEG hypersynchrony (HVS, 5). Since in the rat orienting head movements toward stimuli of interest replace exploratory eye movements, the immobility of the head suggests impairment of attention and exploration. I.c.v. ALCAR caused immediate EEG activation with abolition of the synchronous HVSs and enhancement of the neocortically monitored theta rhythm. Concomitantly, the aged rats quickly started general movements with sniffing, whisker twitching, standing up on the hind limbs and moving the head horizontally and vertically. This behavior persisted for several hours.

It has previously been shown that the i.c.v. ALCAR induced strong arousal in the cat (16) and it has been suggested that ALCAR stimulates the cholinergic component of the brain stem ascending activating system (21, 14, 24), which exert an inhibitory effect on the synchronizing thalamic system, particularly on the interneuronal circuits responsible for such processes (13, 15, 25). This is in line with EEG activation and vigilance induced by this drug also in *cerveaux isolé* cats (16).

This attractive hypothesis is based on results obtained in brainstem neurons *in vivo* (28) and more recently on the effects induced by ALCAR on identified cholinergic brainstem neurons *in vitro* (Brambilla et al., personal communication).

The increase of theta activity observed in the present experiments confirms the action of ALCAR on the brain stem reticular system, which not only excites the neocortex (20), but can also synchronize hippocampal structures, which are believed to play an essential role generating the rhythm of the theta patterns (8, 12).

The ALCAR effect on the cholinergic brainstem neurons may be direct or indirect. Direct action would be on the GABAergic thalamic reticular neurons, blocking or reducing their inhibitory post-synaptic activity (1) and therefore dis-inhibiting the thalamocortical neurons which would change from a bursting to a tonic mode of firing (see 19). Indirect action might inhibit the reticular thalamic neurons through activation of the cholinergic structures of the brainstem reticular formation at the mesopontine junction (PPT/LDT; 27) as well as of the cholinergic component of the hypothalamus (9). The two mechanisms are not mutually exclusive, but they may act in combination.

These observations take on particular significance in the light of the problems of normal neuronal aging in humans and decline of cortical function in patients with Alzheimer Disease (AD; 23) in whom the cholinergic system plays an essential role.

The present results are in agreement with evidence of the utility of clinical treatment of AD by pharmacological agents stimulating cholinergic neurotransmission.

It is particularly worth mentioning that similar behavioral and electrophysiolgical effects were seen after i.p. ALCAR. The similarity of the two routes of administration confirms the active transport of this substance through the blood-brain barrier (4).

SUMMARY

In chronically-implanted, drug-free, behaving aged Fischer rats, intracerebroventricularly (i.c.v.) and intraperitoneally (i.p.) acetyl-L-carnitine (ALCAR) injections powerfully enhanced motor behavior and head movements aimed at attention and exploratory activity. This effect was dose-dependent and associated with the abolition or substantial reduction of the incidence and duration of the spontaneous EEG generalized hypersynchronous patterns termed High Voltage Spindle (HVS), with an increase in EEG monitored theta activity.

The results suggest that ALCAR may stimulate the motivational system and disrupt the hypersynchronous processes by inhibiting the GABAergic thalamic reticular neurons and/or activating the brain stem cholinergic reticular system (pedunculo pontine tegmental, PPT and laterodorsal tegmental, LDT nuclei).

Acknowledgements. - This research was supported by Sigma-Tau, Pomezia, Italy. The authors thank Dr. Gabriella Cerri and Dr. Marcella Montagna who kindly helped for the statistical analysis and Mr. L. Trentin for photographic assistance.

REFERENCES

1. BAHRING, R., STANDHARDT, H., MARTELLI, E.A. AND GRANTYN, R. GABA-activated chloride currents of postnatal mouse retinal ganglion cells are blocked by acetylcholine and acetylcarnitine: how specific are ion channels in immature neurons? *Eur. J. Neurosci.*, **6:** 1089-1099, 1994.
2. BOUYER, J.J., TILQUIN, C. AND ROUGEUL, A. Thalamic rhythm in cat during quiet wakefulness and immobility. *Electroenceph. Clin. Neurophysiol.*, **55:** 180-187, 1983
3. BRESOLIN, N., FREDDO, L., VERGANI, L. AND ANGELINI, C. Carnitine, carnitine acyltransferases, and rat brain function. *Exp. Neurol.*, **78:** 285-292, 1982.
4. BURLINA, A.P., SERSHEN, H., DEBLER, E.A. AND LAJTHA, A. Uptake of acetyl-L-carnitine in the brain. *Neurochem. Res.*, **14:** 489-493, 1989.
5. BUZSÀKI, G., BICKFORD, R.G., ARMSTRONG, D.M., PONOMAREFF, G., CHEN, K.S., RUIZ, R., THAL, L.J. AND GAGE, F.H. Electric activity in the neocortex of freely moving young and aged rats. *Neuroscience,* **26:** 735-744, 1988.
6. CELESIA, G. AND JASPER, H. Acetylcholine released from cerebral cortex in relation to state of activation. *Neurology*, **16:** 1053-1064, 1966.
7. DANOBER, L., DERANSART, C., DEPAULIS, A., VERGNES, M. AND MARESCAUX, C. Pathophysiological mechanisms of genetic absence epilepsy in the rat. *Prog. Neurobiol.*, **55:** 27-57, 1998.

8. GREEN, J.D. AND ARDUINI, A. Hippocampal electric activity in arousal. *J. Neurophysiol.*, **17:** 533-557, 1954.
9. GRITTI, I., MARIOTTI, M. AND MANCIA, M. Gabaergic and cholinergic basal forebrain and preoptic-anterior hypothalamic projections to the mediodorsal nucleus of the thalamus in the cat. *Neuroscience,* **85:** 149-178, 1998.
10. KIINGBERG, F. AND PICKENHAIN, L. Das Aufreten von "Spindelenthadinigen" bei der Ratte in Beziehung zum Verhalten. *Acta. Biol. Med. Ger.*, **20:** 45-54, 1968.
11. LANDOLT H.-P. AND BORBÉLY, A.A. Age-dependent changes in sleep EEG topography. *Clin. Neurophysiol.*, **112:** 369-377, 2001.
12. LEE, M.G., CHROBAK, J.J., SIK, A., WILEY, R.G. AND BUZSÁKI, G. Hippocampal theta activity following selective lesion of the septal cholinergic system. *Neuroscience,* **62:** 10033-1047, 1994.
13. MANCIA, M. *Neurofisiologia e vita mentale*. Bologna: Zanichelli, 1980.
14. MANCIA, M. Some Physiological Properties of the Ascending Brain Stem Reticular System. *Arch. Ital. Biol.*, **120:** 72-94, 1982.
15. MANCIA, M. AND OTERO-COSTAS, J. Nature of the midbrain influences upon thalamic neurons. *Brain Res.,* **49:** 200-204, 1973.
16. MANCIA, M., IMERI, L. AND GIGLIO, R. Modifications of the cerebral electric activity induced by intracerebroventricular injection of acetyl-L-carnitine in cats. *Int. J. Clin. Pharmacol. Res.,* **10:** 115-121, 1990.
17. MARINI, G., CECCARELLI, P. AND MANCIA, M. Thalamocortical dysrhythmia and the thalamic reticular nucleus in behaving rats. *Clin. Neurophysiol.*, **113:** 1152-1164, 2002.
18. MARINI, G., CECCARELLI, P. AND MANCIA, M. Spontaneous K-complexes in behaving rats. *Arch. Ital. Biol.,* **142:** 59-67, 2004.
19. MCCORMICK, D.A. Cholinergic and noradrenergic modulation of thalamocortical processing. *Trends Neurosci.*, **12:** 215-229, 1989.
20. MORUZZI, G. AND MAGOUN, H.W. Brain stem reticular formation and activation of the EEG. *Electroenceph. Clin. Neurophysiol.*, **1:** 455-473, 1949.
21. MORUZZI, G. The sleep-waking cycle. *Ergebn. Physiol.*, **64:** 1-165, 1972.
22. PAXINOS, G. AND WATSON, C. *The rat brain in stereotaxic coordinates*, 2nd ed. Sidney, Academic Press, 1986.
23. PETTEGREW, J.W., LEVINE, J. AND MCCLURE, R.J. Acetyl-L-carnitine physical-chemical, metabolic, and therapeutic properties: relevance for its mode of action in Alzheimer's disease and geriatric depression. *Molecular Phychiatry*, **5:** 616-632, 2000.
24. RIEKKINEN, P., BUZSÀKI, G., RIEKKINEN, P. JR, SOININEN, H. AND PARTANEN, J. The cholinergic System and EEG slow waves. *Electroenceph. Clin. Neurophysiol.*, **78:** 89-96, 1991.
25. STERIADE, M. Mechanisms underlying cortical activation. In: POMPEIANO, O. AND AJMONE-MARSAN, C. (Eds). *Brain Mechanisms and perceptual awareness*. New York: Raven Press, 1981.
26. STERIADE, M., PARÉ, D., PARENT, A. AND SMITH, Y. Projections of cholinergic and non-cholinergic neurons of the brain stem core to relay and associational thalamic nuclei in the cat and macaque monkey. *Neuroscience*, **25:** 47-67, 1988.
27. STERIADE, M., JONES, E.G. AND MCCORMICK, D.A. *Thalamus*. Amsterdam, Elsevier, 1997.
28. TEMPESTA, E., JANIRI L. AND PIRRONGELLI, C. Stereospecific effects of acetylcarnitine on the spontaneous activity of brainstem neurons and their response to acetylcholine and serotonin. *Neuropharmacology,* **24:** 43-5, 1985.
29. VANDERWOLF, C.H. AND ROBINSON, T.H. Reticulocortical activity and behavior: a critique of the arousal theory and a new synthesis. *Behav. Brain Sci.*, **4:** 459-514, 1981.
30. WOOLF, N.J. AND BUTCHER, L.L. Cholinergic system in the rat brain. III Projections to the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia and basal forebrain. *Brain Res. Bull.,* **16:** 603-637, 1986.