Behaviour-related effects of physostigmine on the rat visual evoked potential

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Abstract. The present study compares behaviour-dependent and physostigmine-induced changes of the visual evoked potential (VEP) in unrestrained rats to provide further indications on the role of acetylcholine in the behavioural VEP modulation. On 30 rats the VEPs on the pial surface of the primary visual cortex were investigated during five spontaneous behavioural states. Physostigmine, carbachol and nicotine were intraperitoneally applied on 7 rats. Several VEP parameters were found changing in dependence of the arousal level: the VEP latency, the amplitude of the second negative component (N41), and the voltage of the positive component between 60 and 80 ms after the light flash. Physostigmine caused amplitude alterations like during high arousal states. The VEP latency, however, was altered in an opposite way. Nicotine specifically altered the N41 amplitude whereas carbachol delayed the latency of the VEP. The second negative component (N41) is assumed cholinergically modulated. Moreover, the negative component between 60 and 80 ms latency that emerged in high arousal states may be cholinergically generated and may represent a marker of the activation degree of the cholinergic system.

Key words: acetylcholine, physostigmine, visual evoked potential (VEP), unrestrained behaviour, rat

INTRODUCTION

The internal state of an animal such as the arousal level affects the waveform of evoked potentials (EP) (Brankačk and Klingberg 1982). Behavioural EP modulations influence the results in experiments with unrestrained animals. Evoked potentials can also be used as a tool in identifying and controlling the internal state of anaesthetized animals (Sisson and Siegel 1989). Previous studies indicate separate generation and distinct modulations of the different wave components that comprise the visual evoked potential (VEP). Depth recordings in awake monkeys revealed the origin of the VEP components in different cortical layers (Kraut et al. 1985). In unrestrained rats, however, the putative intracortical sinks that constitute the cortical VEP were found mainly within the fourth layer of the area 17 (Brankačk et al. 1990). The different VEP components may also dissociate by application of neuroactive drugs. The GABA blocker bicuculline, for example, diminished a late positive and increased an early negative potential of the cat VEP (Zemon et al. 1980). Conversely, physostigmine, a blocker of acetylcholineesterase, diminished a prominent negative wave of the VEP and increased the amplitude of the following positive wave in anaesthetized cats (Arakawa et al. 1993). This agrees with a model which suggested a primary excitation flow to the visual cortex that is modulated in the thalamic level by the GABAergic nucleus reticularis thalami (RET) (Sefton and Burke 1966). The recurrent inhibition by the RET nucleus is likely one of the main ways of the behaviour-dependent modulation of the visual information flow to the cerebral cortex. The synchronization degree of the RET activity is dependent on the momentary behavioural state. The RET activity itself is modulated by various subcortical nuclei that are involved in neural activation processes. The nucleus basalis magnocellularis (NBM) and cholinergic cells within the brainstem, for example, were shown to innervate the RET (Ahlsén and Lo 1982, Levey et al. 1987).

The VEP was suggested composed of distinct components that overlap temporally but can be sep-arately affected (Arakawa et al. 1993). The separate modulation of the distinct VEP components may be reflected in the behavioural VEP modulation that is partly mediated by cholinergic mechanisms. We assume a cholinergic modulation of the VEP components especially in high arousal states since the cholinergic neurones of the basal forebrain show a higher activity during desynchronization of the cortical EEG (Détári and Vanderwolf 1987, Buzsáki et al. 1988). Therefore, the present study compares behaviour-dependent and physostigmine- induced VEP changes in unrestrained rats. This study may provide further indications about the role of acetylcholine (ACh) in modulating VEP components in dependence of the momentary behavioural state.

METHODS

Experiments were carried out on two groups of female Long-Evans rats (6-10 month old). The first group of 30 rats served to examine the VEP changes during different behaviours. Fourteen epidural electrodes were implanted under hexobarbital anaesthesia (200 mg/kg i.p.) on the rostral-occipital extent of the brain surface. The electrodes were set on the bulbus olfactorius, on the frontal areas Fr2 and Fr1, on the anterior area 18, on the area 17, and on the cerebellum of the right hemisphere (electrode coordinates are given in Fig. 1B). An electrode within the frontal bone (12 mm anterior of the bregma in the midline) served to record slow respiration waves. Furthermore, the frontal bone was the reference area for the EP records since in this region no VEP was detectable (Fig. 1A). The electrodes and the plug for the cable to the EEG polygraph were embedded in dental acryl.

The EP records were carried out one week later by means of an EEG polygraph (0.1 s - 2,000 Hz). Light flashes were given from a photostimulator (intensity 6×10^4 cd). The VEPs were stored on computer disks (sampling frequency 3,000 Hz) by means of the "Global Lab" computer program (DATA TRÁNSLATION). Additionally, EEG epochs of 3 s length were recorded during the investigated behavioural states. Following behavioural
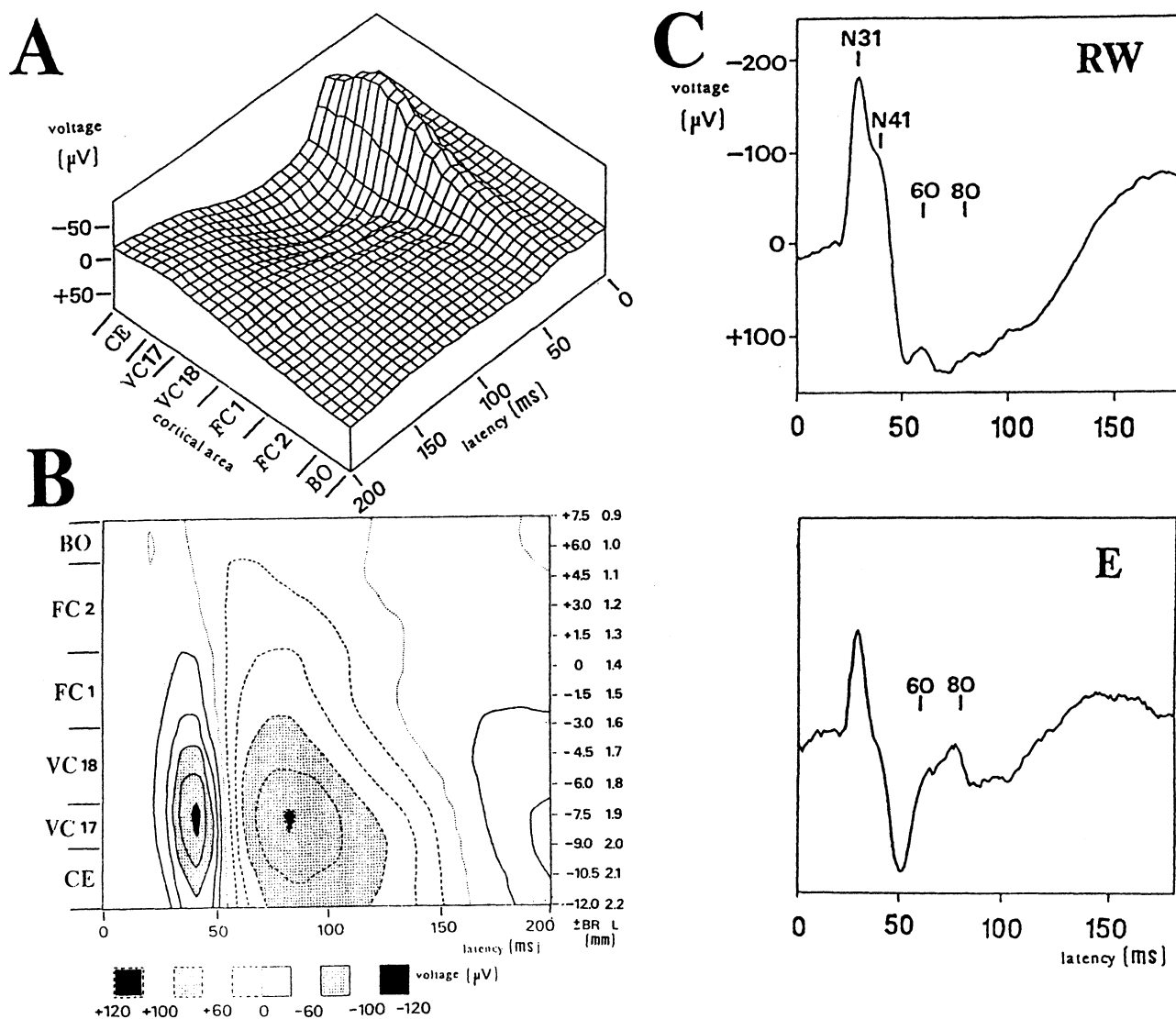
Fig.1. Visual EP of the unrestrained rat. A, mean topographic distribution of the VEP on different cortical areas during relaxed waking immobility as mean of 17 rats. B, mean VEP distribution during slow wave sleep. The electrode coordinates are given on the right side. C, averaged VEP of the same rat recorded on the area 17 during relaxed waking immobility (RW) and during exploratory sniffing (E). BO, bulbus olfactorius; FC2, frontal area Fr2; FC1, frontal area Fr1; VC18, visual cortex, area 18; VC17, area 17; CE, cerebellum; ±BR, anterior and posterior of the bregma, respectively; L, lateral.

states were considered in the present study: exploratory sniffing (E), face washing as a part of grooming behaviour (FW), relaxed waking immobility (RW), slow wave sleep (SWS), and a state that is characterized by the appearance of rhythmic high-voltage spindles in the neocortical EEG (HVSs). HVSs are considered as epilepsy-like EEG paroxysms (Buzsáki et al. 1990, Kleinlogel 1990). Furthermore, the VEPs were recorded during scraping

on the head and ear (S) as well as during rhythmic jaw and vibrissae movements (JVM). On the basis of the hippocampal and cortical EEG morphology the states E, FW, S and JVM are considered as high arousal states (Gralewicz et al. 1994). Relaxed wakefulness (RW) was classified as a state of moderate arousal.

On the second group of 7 rats the VEPs were analyzed after intraperitoneal application of following substances: physostigmine salicylate 1 mg/kg (Arz-

neimittelwerk Dresden); carbachol (carbamylcholine chloride) 0.5 mg/kg (Jenapharm); nicotine tartrate 1 mg/kg (ICN); saline (0.9 % NaCl) as control. Substances were dissolved in 0.9 % NaCl and were administered in a fixed volume of 0.6 ml. VEPs were recorded 15, 30 and 45 min after substance application during waking immobility (RW-like states). The data of the three records were summarized since no significant differences of the drug effects were observed until 60 min after application. The protocols of the chronic experiments were accepted by the local Ethical Committee.

Fifteen single VEPs per animal were averaged off-line after selection according to the behaviour and the respiration rate. The spectral power of the EEG records were analyzed by fast Fourier transformation. For each behavioural state and animal the power of four EEG records (1.5 s) were averaged. Significant differences were evaluated by Mann Whitney U test.

RESULTS

Behavioural VEP changes

The visual EP of rat's area 17 during relaxed waking immobility begins with a primary negative wave complex (Fig. 1C). This complex consists of two components with peak latencies at 31 and 41 ms, respectively, after light flash. The primary negative complex is followed by a positive (P52 - P70) and a negative wave (N160) (Fig. 2). The VEP was recorded on all investigated cortical areas with the same latency (Fig. 1A). However, the greatest amplitude and the putative origin of the VEP were found in the area 17 (Fig. 1B).

While the VEP did not alter its origin in the cerebral cortex during different behavioural states, the amplitudes and latencies of different VEP components were clearly modulated (Fig. 3). The higher the level of arousal the shorter is the peak latency of the primary negative component (N31 in RW) (Fig. 3). The longest peak latencies were found during slow wave sleep (33 ms) and during hexobarbital anaesthesia (40 ms; both $P<0.001$ vs. RW). A
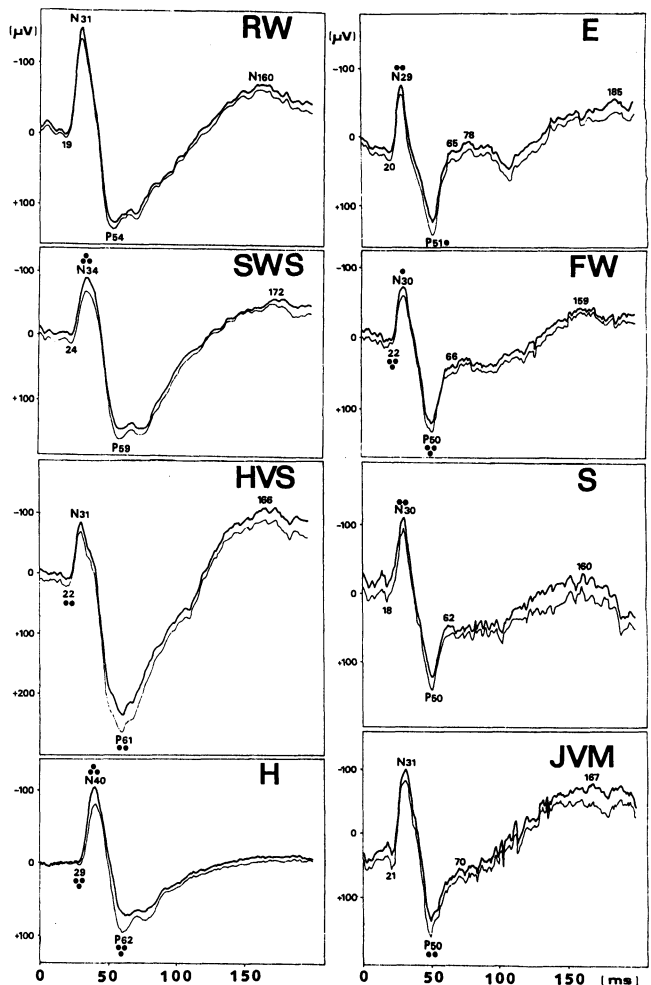

Fig.2. Behaviour-dependent modulation of the visual EP recorded from the pial surface of the area 17 in unrestrained rats. The left side represents low arousal states, the right side high arousal states. Data are mean VEP (upper curves) - SEM (lower curves) of 30 animals. Light flash application at the beginning of the records. RW, relaxed waking immobility; SWS, slow wave sleep; HVS, high voltage spindling; H, hexobarbital anaesthesia; E, exploratory sniffing; FW, face washing; S, scrape; JVM, jaw and vibrissae movements. Dots indicate significant differences of the peak latencies vs. RW data. One dot $P<0.05$. Two $P<0.01$. Three $P<0.001$.

significant correlation was found between the N31 peak latency of the VEP and the delta wave power in the EEG of the area 17 ($r=0.949$; $P<0.05$). The first negative component (N31 in RW) had the greatest amplitude during RW and displayed slight amplitude reductions in states with higher as well as with lower levels of arousal (Fig. 3). The second ne-
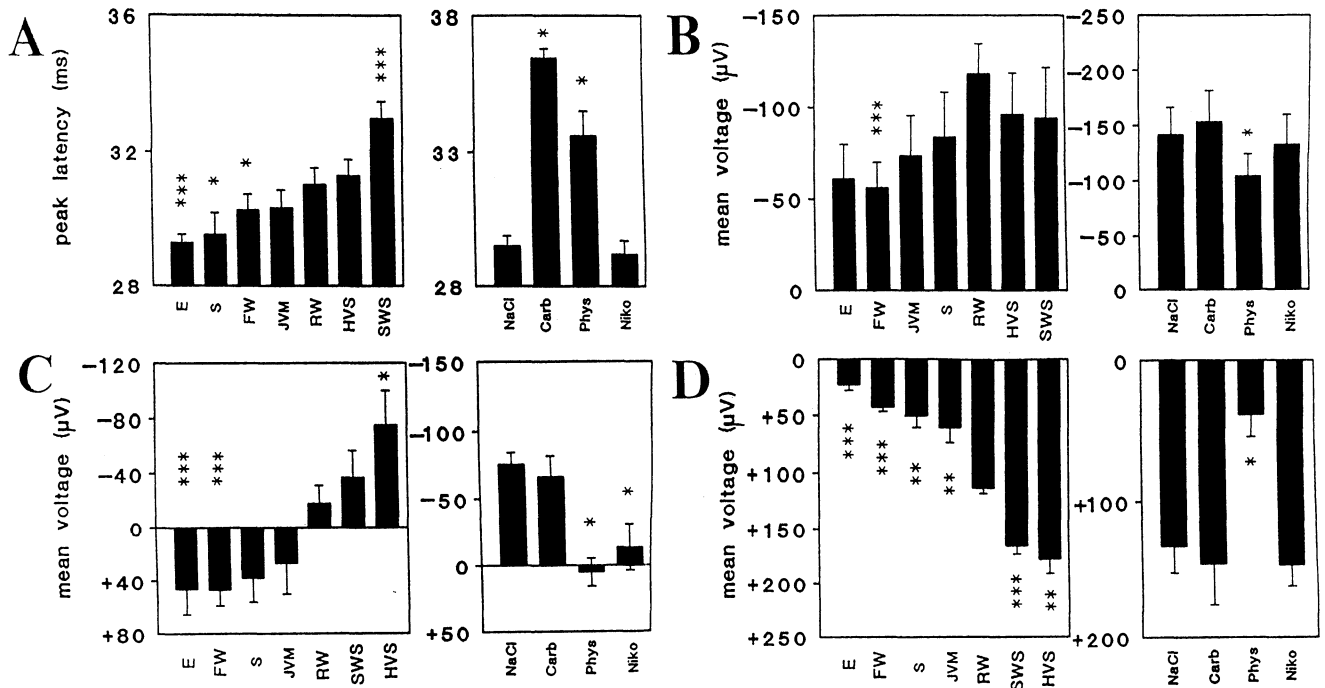
Fig.3. Modulation of different components of the visual EP of rat's area 17 during 7 behavioural states and 15-60 min after i.p. application of various neuroactive drugs. All drug effects were recorded during waking immobility. A, peak latency of the first negative component (N31 in RW). B, mean amplitude of the first negative component (N31 in RW). C, mean amplitude of the second negative component (N41 in RW). D, mean voltage between 60 and 80 ms latency. The behavioural states were arranged in dependence of the cortical arousal level (Gralewicz et al. 1994). Data are means ±SEM of 17 (behavioural modulation) and 7 animals, respectively (drug induced modulation). NaCl, saline control; Carb, carbachol; Phys, physostigmine; Niko, nicotine. For further abbreviations see Fig. 2. Asterisks indicate significant differences vs. RW data and vs. NaCl control, respectively. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

gative component (N41 in RW) reduced its amplitude in states with high arousal and increased its amplitude in states of low arousal.

In high arousal states, e.g. exploratory sniffing and face washing, the positive wave was found superposed by a further negative complex that emerged between 60 and 80 ms after flash (Figs. 1C and 2). In low arousal states, e.g. SWS and HVS, this negative complex was not present and the visual EP displayed significant positivations between 60 and 80 ms (Fig. 3). High and low arousal states that also characterized by different respiration rates (Fig. 4) are well discernible by the presence of the negative complex between 60 and 80 ms after flash (Fig. 2).

Correlated alterations in dependence of the momentary behavioural state were found between the VEP latency and the N41 amplitude ($r=-0.757$;

$P<0.05$), between the VEP latency and the mean amplitude between 60 and 80 ms ($r=0.866$; $P<0.05$), and between the N41 amplitude and the mean amplitude between 60 and 80 ms ($r=-0.978$; $P<0.01$). The amplitude of the second negative component, the positivation degree between 60 and 80 ms, and particularly the peak latency of the first negative component are regarded as well markers of the degree and direction of the momentary arousal level.

Drug induced VEP changes

The three VEP parameters that were altered in a correlated way during different behavioural states were specifically modulated by the investigated cholinergic drugs. Physostigmine modulated all VEP amplitudes as in the case of behavioural states
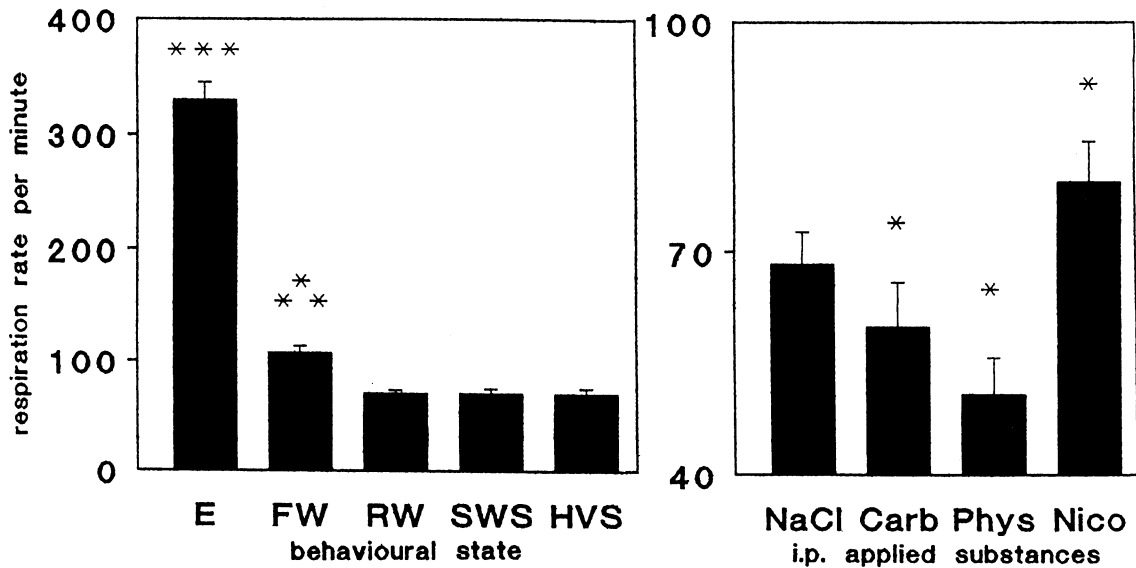
Fig.4. Behaviour-dependent and drug-induced changes of the respiration rate. Data are means ±SEM of 30 (behavioural modulation) and 7 animals (drug modulation), respectively. Asterisks indicate significant differences in relation to RW and NaCl data, respectively. *P<0.05; ***P<0.001. For abbreviations see Figs. 2 and 3.

with high arousal level (Fig. 3). The N31 peak latency, however, was altered in an opposite way. Carbachol did not affect the VEP amplitudes whereas the N31 peak latency was significantly enhanced. Nicotine affected only the amplitude of the N41 component and had no effects on the other investigated VEP parameters. Carbachol and physostigmine significantly elevated the power of the cortical theta rhythm that was recorded on the area 17 at a peak around 4.7 Hz (P<0.05 vs. saline control). Both substances decreased the mean respiration rate during waking immobility (Fig. 4). Nicotine slightly increased the mean respiration rate.

DISCUSSION

The VEP of the rat was recorded over a wide range of rat's brain surface with the same latency. Besides the area 17 a VEP with smaller amplitude was recorded above the area 18, the frontal areas Fr2 and Fr1 as well as above the cerebellum. We assume that the VEPs in the latter regions may be a far field potential from the area 17. The visual activity within the area 18 may not be synchronized enough to form a regular large VEP. This assumption is supported by previous studies with depth profiles through the occipital cortex of the rat. A polarity reversal of the VEP was found within the area 17 but not within area 18b (Brankačk et al. 1990). The negative components N31, N41 and N162 were found mainly generated in the fourth layer of the area 17 (Brankačk et al. 1990). The excitatory synapses of the thalamocortical relay cells of the dorsal lateral geniculate body (DLG) on cells in this layer are most likely the generators of these negative components.

The present results indicate strong behaviour-dependent alterations of some VEP components that may be partly reflected in the drug-induced VEP changes. Physostigmine caused amplitude changes like during high arousal states. The VEP latency, however, was altered in an opposite way. Moreover, the results support the assumption that different VEP components may be modulated in an own way (Arakawa et al. 1993).

The VEP peak latency was found significantly shorter in behavioural states of high arousal level. The cholinergic drugs carbachol and physostigmine delayed the peak latency. This peak latency delay is

likely mediated by muscarinergic mechanisms since nicotine had no effect on this parameter. It is unclear where carbachol mediated the latency delay. Carbachol is assumed do not cross the blood--brain barrier. We assume a specific effect of carbachol on the VEP latency since the other behaviour-dependent VEP parameters were not changed in our experiments. Within the retina only a subpopulation of amacrine cells uses ACh as a transmitter (Hutchins 1987). Cholinergic receptors are located in different layers of the retina with the highest concentration of nicotinic receptors within the ganglion cell layer (Morgan and Mundy 1982) and of muscarinic receptors within the inner nuclear and plexiform layers (Zarbin et al. 1986). The delayed latency after carbachol and physostigmine remains a problem. High arousal states that are characterized by short latencies (Fig.3) are usually connected with a high cholinergic level within the brain (Phillis et al. 1968). This is supported by the correlation between the VEP latency and the delta wave power of the visual cortical EEG. A high delta wave power was found correlated with a reduced choline acetyltransferase activity in the rat cortex (Riekkinen et al. 1990). Within the retina or within the thalamus a putative muscarinergic mechanism may regulate the VEP latency.

The amplitude of the second negative component (N41 in RW) is assumed to be modulated by nicotinergic mechanisms. Since nicotine affected only the N41 component and had no effects on the other behaviour-dependent parameters of the VEP we assume that nicotine centrally affected the N41 amplitude. A peripheral nicotine effect with a secondary activation of the general behaviour would cause also changes of the other behaviour-dependent VEP parameters. The origin of this second negative excitation wave remains unclear. The fast and slow X- and Y-channels of the visual tract may cause the N31- and N41-peaks of the VEP. A nicotinergic mechanism may be indicated by the high concentration of nicotinergic ACh receptors that was found within the fourth layer of the primary visual cortex in cats (Parkinson et al. 1988). These nicotinergic receptors were assumed to be located on the terminals of the DLG and may facilitate the input of visual information into the visual cortex (Parkinson et al. 1988). In rats the highest concentration of nicotinergic receptors within the area 17 was also found within the fourth layer (Kumar and Schliebs 1992). The second negative component (N41) may be a VEP reflection of a secondary excitation that could be evoked by the first excitation wave (N31) within the fourth layer of the area 17. This afterdischarge may be mediated by a nicotinergic mechanism that works in dependence of the momentary behavioural state.

The large positive wave P52-P70 is likely generated by recurrent inhibition of the DLG relay cells when the visual excitation transfers through the thalamus into the visual cortex (Sefton and Burke 1966). The recurrent inhibition that leads to a hyperpolarization of the thalamocortical relay cells may be caused by intrinsic GABAergic interneurones within the DLG and by GABAergic cells of the thalamic reticular nucleus (RET). This assumption is supported by the finding that the GABA blocker bicuculline diminished the positive component of the cat VEP (Zemon et al. 1980). During high arousal states the GABAergic inhibition is reduced. This is likely reflected in the amplitude reduction during E and FW in our experiments. Moreover, during high arousal states the positive wave was found superposed by a negative component that emerged between 60 and 80 ms latency. This negative component may reflect an excitation wave that inhibits the GABAergic activity, e.g. from RET. The negativation between 60 and 80 ms may reflect an additional excitation of the thalamocortical neurones. The negative component between 60 and 80 ms is likely cholinergically generated. Physostigmine caused an elevation of this component in our experiments. The failure of nicotine effects on this component indicates a muscarinic mechanism.

A candidate that may mediate the cholinergic inhibition of the RET is the nucleus basalis magnocellularis (NBM). This cholinergic nucleus has afferents to the RET (Levey et al. 1987). The NBM may be activated by efferent fibres from the visual cortex. During low arousal states, e.g. SWS and

HVS, the NBM is rather inactivated. This behaviour-dependent NBM activity is reflected in results of multi-unit records. Neurones within the cholinergic basal forebrain that project to the cortex showed a higher excitation degree during desynchronization of the cortical EEG in anaesthetized rats (Détári and Vanderwolf 1987). They increased their firing rates during movements and decreased their discharge frequencies during immobility (Buzsáki et al. 1988). Acetylcholine exerts a suppressive action on the GABAergic neurones of the RET (Ben-Ari et al. 1976). However, the component that emerged between 60 and 80 ms in high arousal states may also reflect a cholinergic excitation wave within the cortex, e.g. from the projections of the NBM. Whether the negative component between 60 and 80 ms really reflects the activation of the cholinergic NBM will be a question of further studies.

Our results of physostigmine effects agree with previous studies on cats. Several groups found a marked amplitude reduction of all VEP components by physostigmine (Harding et al. 1983, DeBruyn et al. 1986). However, VEP studies on cats were mainly carried out on anaesthetized animals. Arakawa et al. (1993) described a reduction of the negative VEP wave and an increased amplitude of the following positive wave after physostigmine in pentobarbital-anaesthetized cats. They concluded that the physostigmine effects on the cat VEP depends upon the anaesthetic used. During hexobarbital anaesthesia we found a rat VEP consisting of the primary negative complex and the following positive wave. These components are assumed to be generated within the thalamus. The components that are assumed depending on the cortical activity (N162 in RW, negative wave between 60 and 80 ms) were not present after hexobarbital. We assume that anaesthetized animals may be rather inappropriate models for studying the cholinergic modulation of the thalamic and cortical visual information processing since anaesthetics depress the cholinergic activity within the brain. This view is supported by the present results that revealed similar modulations of certain VEP amplitudes in high arousal states and after cholinergic drugs.

REFERENCES

Ahlsén G., Lo F.S. (1982) Projections of the brain stem neurons to the perigeniculate nucleus and the lateral geniculate nucleus in the cat. Brain Res. 238: 433-438.

Arakawa K., Peachey N.S., Celesia G.G., Rubboli G. (1993) Component-specific effects of physostigmine on the cat visual evoked potential. Exp. Brain Res. 95: 271-276.

Ben-Ari Y., Dingledine R., Lanazawa I., Kelly J.S. (1976) Inhibitory effects of acetylcholine on neurons of the feline nucleus reticularis thalami. J. Physiol. 261: 647-671.

Brankačk J., Klingberg F. (1982) Behaviour-dependent changes of visually evoked potentials and their correlation to the respiration rate in freely moving rats. Acta Biol. Med. Germ. 41: 315-324.

Brankačk J., Schober W., Klingberg F. (1990) Different laminar distribution of flash evoked potentials in cortical areas 17 and 18b of freely moving rats. J. Hirnforsch. 31: 525-533.

Buzsáki G., Bickford R.G., Ponomareff G., Thal L.J., Mandel R., Gage F.H. (1988) Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. J. Neurosci. 8: 4007- 4026.

Buzsáki G., Smith A., Berger S., Fisher L.J., Gage F.H. (1990) Petit mal epilepsy and parkinsonian tremor: hypothesis of a common pacemaker. Neuroscience 36: 1-14.

DeBruyn E.J., Gajewski Y.A., Bonds A.B. (1986) Anticholinesterase agents affect contrast gain of the cat cortical visual evoked potential. Neurosci. Lett. 71: 311-316.

Détári L., Vanderwolf C.H. (1987) Activity of identified cortically projecting and other basal forebrain neurones during large slow waves and cortical activation in anaesthetized rats. Brain Res. 437: 1-8.

Gralewicz S., Łuczak C., Tomas T., Wiaderna D. (1994) Spontaneous seizure electrocortical activity in rats: relation to arousal level. Acta Neurobiol. Exp. 54: 19-31.

Harding T.H., Wiley R.W., Kirby A.W. (1983) A cholinergic-sensitive channel in the cat visual system tuned to low spatial frequencies. Science 221: 1076-1078.

Hutchins J.B. (1987) Review: acetylcholine as a neurotransmitter in the vertebrate retina. Exp. Eye Res. 45: 1-38.

Kleinlogel H. (1990) Analysis of the vigilance stages in the rat by fast Fourier transformation. Neuropsychobiology 23: 197-204.

Kraut M.A., Arezzo J.C., Vaughan Jr. H.G. (1985) Intracortical generators of the flash VEP in monkeys. Electroencephalogr. Clin. Neurophysiol. 62: 300-312.

Kumar A., Schliebs R. (1992) Postnatal laminar development of cholinergic receptors, protein kinase C and dihydropyridine-sensitive calcium antagonist binding in rat visual cortex. Effect of visual deprivation. Int. J. Devl. Neuroscience 10: 491-504.

Levey A.I., Hallanger A.E., Wainer B.H. (1987) Cholinergic nucleus basalis neurons may influence the cortex via the thalamus. Neurosci. Lett. 74: 7-13.

Morgan L.G., Mundy P.G. (1982) Ganglion cells of chicken retina possess nicotinic rather than muscarinic acetylcholine receptors. Neurochem. Res. 7: 267-274.

Parkinson D., Kratz K.E., Daw N.W. (1988) Evidence for a nicotinic component to the actions of acetylcholine in cat visual cortex. Exp. Brain Res. 73: 553-568.

Phillis J.W., Tebecis A.K., York D.H. (1968) Acetylcholine release from the feline thalamus. J. Pharm. Pharmacol. 20: 476-478.

Riekkinen Jr. P., Sirviö J., Riekkinen P. (1990) Relationship between the cortical choline acetyltransferase content and EEG delta-power. Neurosci. Res. 8: 12-20.

Sefton A.J., Burke W. (1966) Mechanism of recurrent inhibition in the lateral geniculate nucleus of the rat. Nature 211: 1276-1278.

Sisson D.F., Siegel J. (1989) Chloralhydrate anesthesia: EEG power spectrum analysis and effects on VEPs in the rat. Neurotoxicol. Teratol. 11: 51-56.

Zarbin M.A., Wamsley J.K., Palacios J.M., Kuhar M.J. (1986) Autoradiographic localization of high affinity GABA, benzodiaze pine, dopaminergic, adrenergic and muscarinic cholinergic receptors in the rat, monkey and human retina. Brain Res. 374: 75-92.

Zemon V., Kaplan E., Ratliff F. (1980) Bicuculline enhances a negative component and diminishes a positive component of the visual evoked cortical potential in the cat. Proc. Natl. Acad. Sci. USA 77: 7476-7478.