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Effect of muscarinic ligands on the electrical activity recorded from the hippocampus: a quantitative approach

Paul Bevan

Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland

1 The electrical activity of the hippocampus was recorded from the CA 1 region in rats anaesthetized with halothane and the effects of compounds assessed following their intravenous injection. Quantification of the effects was achieved following on-line fast Fourier transformation of the signal.

2 The electrical activity recorded from the hippocampus of the halothane-anaesthetized rat demonstrated identical characteristics to that recorded from the freely-moving animal.

3 Three types of activity could be distinguished: rhythmical slow wave activity (RSA or theta); large amplitude slow wave activity (LIA); and small amplitude fast wave activity.

4 Muscarinic agonists induced RSA with a consequent reduction in power. The effects were dose-dependent and were reversibly antagonized by scopolamine, but not methyl-scopolamine, indicating that the effects are mediated centrally by muscarinic receptors.

5 The results show that, in halothane anaesthetized rats, a muscarinic RSA occurs which is unrelated to movement or behavioural arousal.

Introduction

The electrical activity recorded from the hippocampus of the freely moving rat consists of several distinguishable wave patterns (Vanderwolf *et al.*, 1978). These include (1) rhythmical slow wave activity (RSA or theta), which consists of trains of approximately sinusoidal waves; (2) large amplitude irregular activity (LIA), a pattern which lacks the rhythmical character of RSA, contains waves of lower frequency than RSA and includes irregularly occurring large amplitude spikes; (3) small amplitude activity and fast waves.

Hippocampal theta activity has generally been treated as a single entity reflecting the activity of a single neural system (Kramis *et al.*, 1975). However, it is becoming increasingly clear that the system is much more complex. The hippocampus receives two non-specific inputs from the brain stem (Swanson, 1982) which result in two pharmacologically distinct forms of RSA (Robinson, 1980). An atropine-resistant form appears if, and only if, an animal performs such motor patterns as head movements, walking or rearing, the so-called type 1 behaviours. This is the movement-related RSA (mRSA). An atropine-sensitive form of RSA may occur during complete immobility (iRSA). All species seem to have the mechanisms necessary for producing both mRSA and iRSA, although there are species differences in the occurrence and frequencies of each type (Robinson, 1980).

Atropine-sensitive iRSA is rare in the normal rat (Kolb & Whishaw, 1977) but is produced in the absence of any movement, and during ether or urethane anaesthesia (Vanderwolf, 1975). Atropine-resistant RSA, on the other hand, is sensitive to anaesthesia (Robinson, 1980). Indeed, mRSA cannot be induced by any known means during anaesthesia produced by ether or urethane (Kramis *et al.*, 1975). Thus, anaesthesia abolishes the higher frequency atropine-resistant type of RSA permitting the lower frequency atropine-sensitive type to be studied in isolation.

In this paper, it is shown that iRSA may also be recorded from the hippocampus in halothane-anaesthetized rats.

Moreover, a study of the effects of muscarinic agonists on this iRSA and a quantitative pharmacological study of the effects of muscarinic agonists on the power of the hippocampal electrical activity has been carried out.

Present address: Department of Pharmacology, DUPHAR B.V., P.O. Box No. 2, 1380 AA Weesp, The Netherlands.

Methods

Experiments were performed on male Sandoz OFA rats weighing between 200 and 300 g. Recordings were made from both normal animals and animals with lesions of the medial septal nucleus. The lesions were performed under barbiturate anaesthesia when the animals weighed 150 g. A stainless steel needle (25GI, insulated to within 2 mm of the tip) served as the electrode and was inserted into the medial septal nucleus (coordinates [König & Kippel, 1963]: A 0; L 0; V − 5; measured from bregma). The indifferent electrode was a stainless steel rectal probe. Lesions were produced by passing a current of 5 mA for 5 s. All wounds were closed and the animals allowed to recover for 5 days before being used for electrophysiological experiments.

For the electrophysiological experiments, anaesthesia was induced and maintained with halothane (Fluothane, ICI) delivered in oxygen through a face mask from a flow rate and temperature compensated vapourizer. The concentration of halothane during the experiment was 1% and all animals respired spontaneously. The rectal temperature was maintained at 38°C throughout the experiment by a heating pad controlled by a thermistor.

The animal's head was held in a stereotaxic instrument and the skull exposed. A small hole was drilled in the skull using a dental burr and the bipolar concentric recording electrode lowered into the CA 1 region of the hippocampus. The coordinates, (König & Klippel, 1963) measured from bregma, were A − 4; L − 2; V − 2.5. The recording pole of the electrode was 0.5 mm long (0.3 mm diameter) and was separated by 0.3 mm of insulation from the indifferent pole (0.3 mm long and 0.4 mm in diameter). The location in the CA 1 region was confirmed histologically post mortem.

The recording electrode was connected to a Grass 7P511 differential amplifier. The electrical activity was displayed on an oscilloscope and fed to a computer to permit real-time on-line analysis by Fourier transformation. The analysis was performed continuously with a sampling frequency of 50 Hz to give the power in the frequency bands B 1 (0.2–3.7 Hz) and B 2 (3.9–7.6 Hz). These bands were chosen so as to include iRSA and mRSA respectively (Robinson, 1980). The total energy and power for each band were stored on a floppy disc for post-processing and displayed colour-coded as a histogram on a monitor enabling drug-interaction studies to be performed on-line.

Drugs were dissolved in 0.9% w/v NaCl solution (saline) and injected in a volume of 0.2 ml through an in-dwelling femoral or tail vein cannula. Drug effects were assessed as the percentage change in power for each band relative to its pre-drug level. The size of a response (units) to a drug was estimated as the total change in the power (for each band) produced in response to the drug application and is equivalent to the area underneath the response curve.

Antagonism was evaluated in the following way. When a response to an agonist had been established, the antagonist was injected and any intrinsic effects observed. Then the agonist was injected repeatedly at fixed intervals and the development of antagonism observed. The time-course of the recovery of the agonist response was also followed. On-line computation permitted quantitative drug interaction studies to be performed. Values quoted in the text are means ± s.e.mean from at least 5 measurements unless otherwise stated. Significant differences were determined at the 5% level using Student's t test.

Results

Electrical activity recorded from the hippocampus

The electrical activity recorded from the hippocampus often contained large amplitude rhythmical slow wave activity (RSA; typically 4 Hz, 0.6 mV) with superimposed higher frequency activity. This type of signal was peculiar to the hippocampus. Simultaneous recordings made from the cortex directly overlying the hippocampal recording site contained only low amplitude high frequency activity.

RSA alternated apparently at random with a less synchronized fast wave activity as well as with irregular large amplitude slow wave activity (Figure 1). The oscillation of the signal between these three types of activity was unpredictable. Fourier transformation of the signal, however, resulted in a stable image of the hippocampal electrical activity, a pre-requisite for quantitative drug interaction studies. Fourier analysis of the electrical signal revealed that 80 ± 4% of the total energy of the signal could be accounted for by the two spectral bands B 1 and B 2. An example of the recording signal and the resultant Fourier spectrum is shown in Figure 2.

Effect of lesioning cholinergic afferents

Recordings were made from the hippocampi of animals in which the major cholinergic afferent pathway (Krnjević & Ropert, 1981; Swanson, 1982) had been destroyed. This was achieved by electrolytic lesion of the medial septal nucleus 5 days before recording. Such lesions resulted in a reduction of 90% in the activity of the pre-synaptic cholinergic marker choline acetyltransferase in the hippocampus (data not shown). Recordings from animals with medial septal lesions never contained RSA. The signal consisted largely of unsynchronized fast wave activity

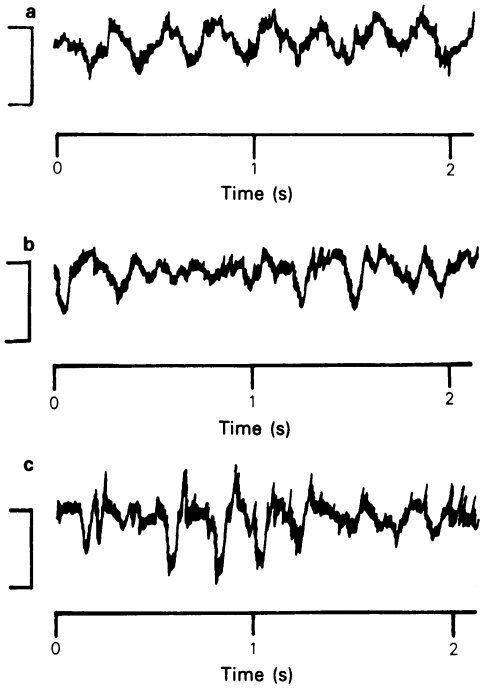

Figure 1 Hippocampal electrical activity: oscilloscope traces showing the different types of electrical activity found in the hippocampus. All recordings are from the same animal. (a) Rhythmical slow wave activity (RSA); (b) small wave and fast activity; (c) irregular large amplitude slow wave activity (LIA). Calibration bar: 1 mV.

with occasional bursts of large amplitude slow wave activity (Figure 3b). The lesions resulted in a reduction in the power of the recorded signal. In comparison with control animals, the total power was significantly reduced by $43 \pm 14\%$, which was reflected in a significant reduction in B 2 power by $67 \pm 20\%$. B 1 power was similarly reduced ($36 \pm 11\%$), but this reduction just failed to achieve statistical significance at the 5% level.

Medial septal lesions produced a shift of the hippocampal electrical activity to lower frequencies. Thus, whereas in control animals, the mean of the ratio B 1 : B 2 power was 4 ± 1, in lesioned animals this ratio was 25 ± 10.

Effects of arecoline on electrical activity in the hippocampus

Intravenous injection of arecoline resulted in a synchronization of the electrical activity in the hippocampus (Figure 2). Thus, where there was little or no RSA present in the recording, arecoline could be said to generate RSA. An example is shown in Figure

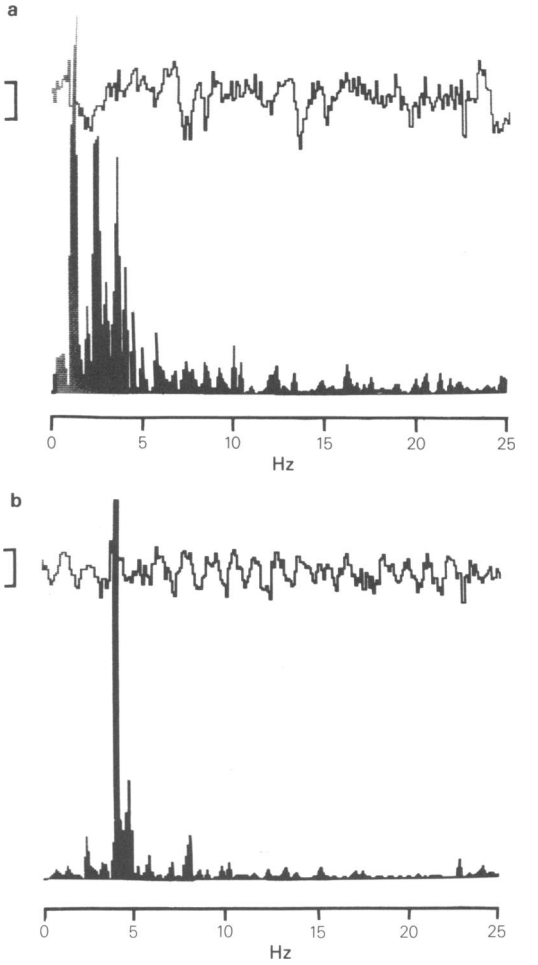

Figure 2 Fourier transformation: in (a) and (b) the upper trace shows a 5 s recording following analogue to digital conversion. Calibration bar: 1 mV. The lower trace shows the corresponding frequency spectrum. (Abscissa scale: Hz). (a) Control; (b) 2 min following injection of arecoline (0.5 mg kg^{-1} i.v.).

2. Following injection of arecoline (0.5 mg kg^{-1} i.v.) the signal was synchronized and RSA could clearly be seen (Figure 2b). This effect lasted some 10 min before the signal reverted to its pre-drug nature. As can be seen from the figure, a consequence of synchronizing the signal was a reduction in amplitude, and this is reflected in a reduction in power (see below).

The effects of intravenous arecoline injection in animals with medial septal lesions was more dramatic. An example is shown in Figure 3. The effect of the lesion itself is apparent from a comparison of Figure 3a, a recording from a normal animal, with Figure 3b, from a lesioned animal. Lesioning results in a poor

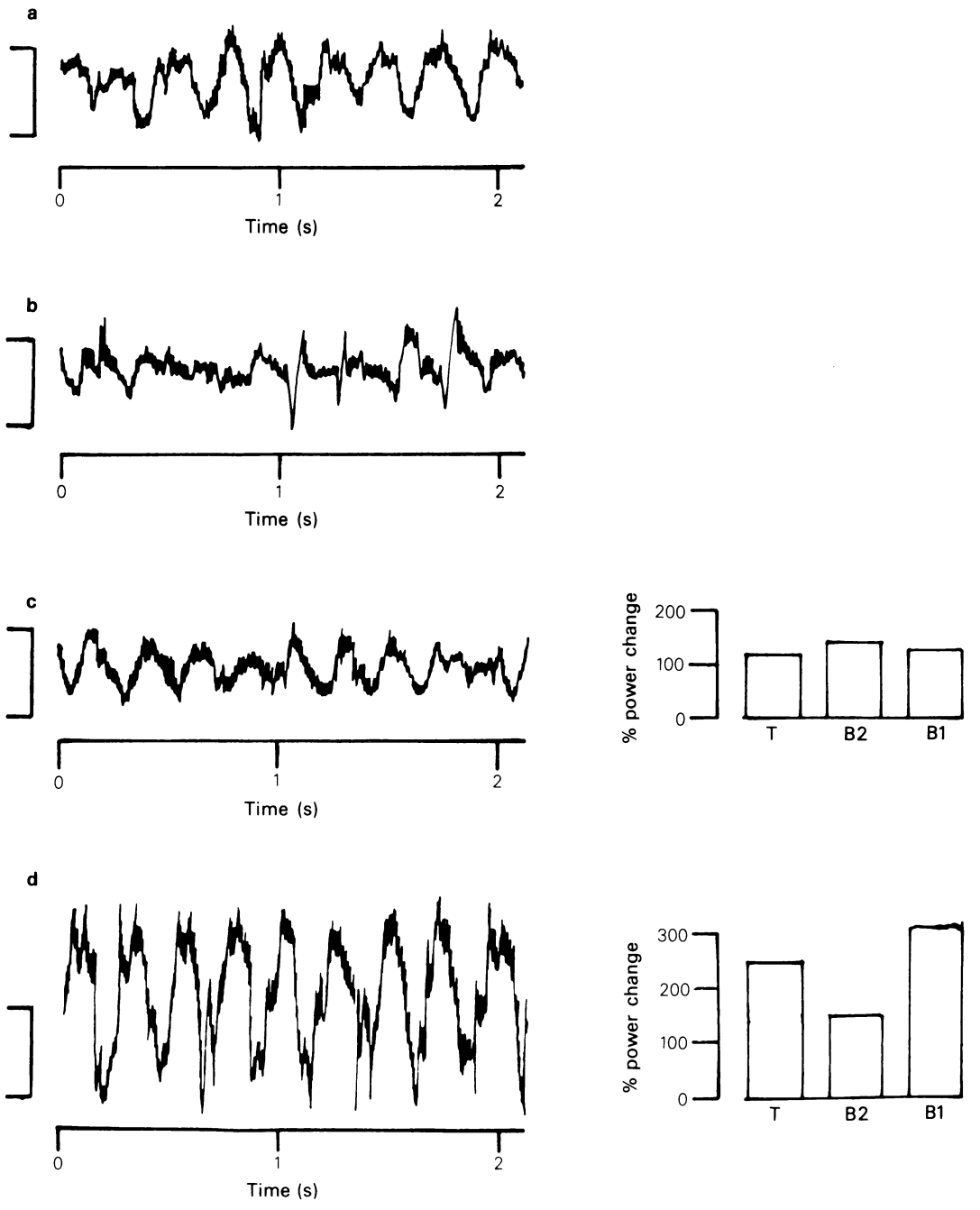

Figure 3 Hippocampal electrical activity in control animals and in animals with lesions of the medial septal nucleus. (a) Recording from a control rat showing a phase of RSA. (b) Recording from a lesioned animal showing LIA but no RSA. (c) Same animal as in (b), recorded 2 min after injection of arecoline (0.1 mg kg^{-1} i.v.). (d) Same animal as in (b) and (c), recorded 30 min after (c), and 2 min after injection of arecoline (0.5 mg kg^{-1} i.v.). Abscissae: Time (s). To the right of (c) and (d) is a representation of the Fourier transformation of the corresponding oscilloscope trace. Ordinates: % change in power compared to control (b). Abscissae: total power, Bands B 2 and B 1 respectively.

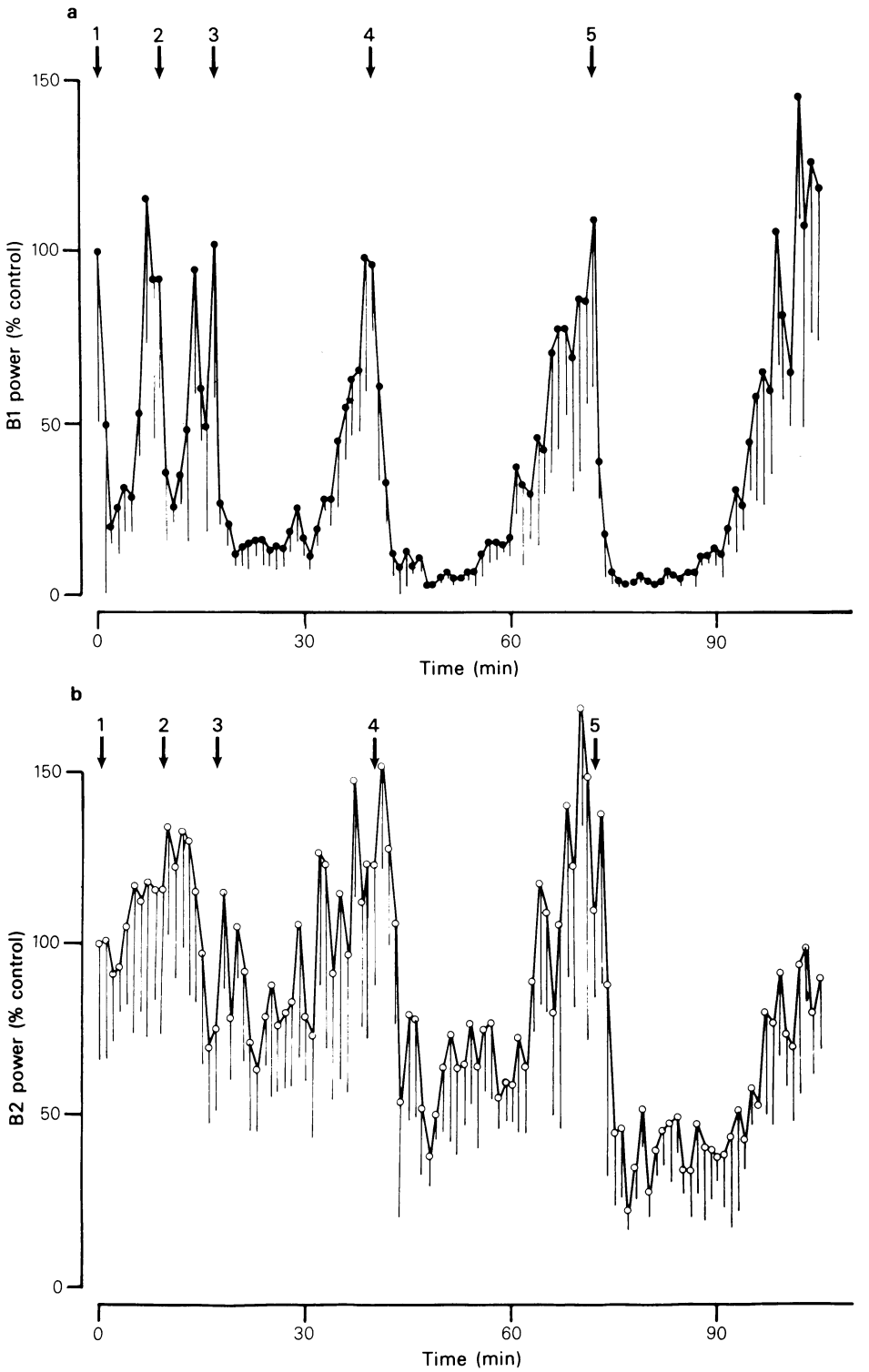

Figure 4 Effects of increasing doses of arecoline injected into one animal on B 1 (a) and B 2 (b) power. Each point is the mean (s.d. shown by vertical lines) of a 1 min spectral analysis expressed as a percentage of the respective control (pre-drug) values. Injections of arecoline i.v.: (1) 0.01 mg kg^{-1}; (2) 0.05 mg kg^{-1}; (3) 0.10 mg kg^{-1}; (4) 0.50 mg kg^{-1}; (5) 0.75 mg kg^{-1}.

signal amplitude and the total absence of RSA. Arecoline dramatically increased both the signal amplitude and generated RSA in a dose-dependent manner (Figure 3c and d). Again, the effects were quite short lasting (up to 15 min, depending on the dose).

Effects of antagonists on B 1 and B 2 power

Antagonists possess no intrinsic activity pharmacologically, by definition. Physiologically, however, antagonists may possess intrinsic activity *in vivo* manifested indirectly through antagonism of endogenous transmission.

The effects of scopolamine (in doses sufficient to produce muscarinic antagonism) were tested in 21 animals. Fourteen animals received injections of 0.1 mg kg^{-1} i.v. scopolamine. In one animal (7%) there was a decrease in B 1 power and B 2 power, whereas in 71% of the animals no effect was seen. The power in bands B 1 and B 2 was increased in 3 animals (22%). Similarly, following the injection of a higher dose of scopolamine (0.5 mg kg^{-1} i.v.), 3 animals showed an increase in B 1 power, whereas there was no effect in 4 animals.

The intrinsic effects of scopolamine were very weak compared to agonists (see below) and usually lasted not longer than 10 min. In contrast, the antagonistic effect of scopolamine lasted for up to 3 h (see below).

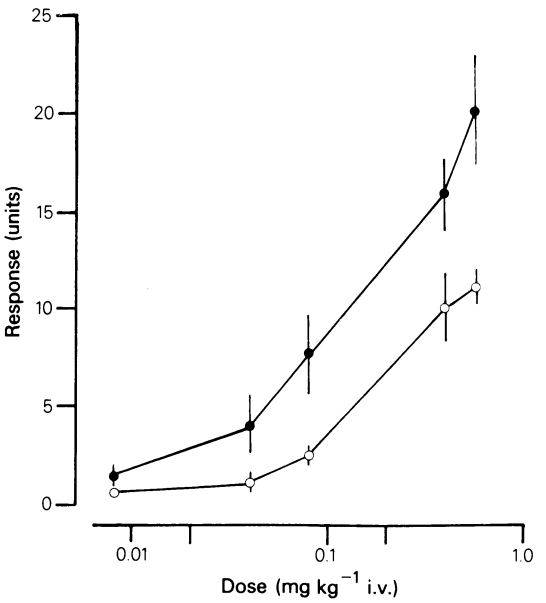

Figure 5 Effect of arecoline on B 1 and B 2 power plotted as a function of dose. Each point is the mean from 12 animals for B 1 (●) and B 2 (○); s.e.mean shown by vertical lines.

The effects of methyl-scopolamine were tested in 23 animals. No effect could be seen following injection of 0.1 mg kg^{-1} i.v. in 15 animals. Similarly, at a dose of 0.5 mg kg^{-1} i.v., the power in bands B 1 and B 2 was reduced in one animal whereas no effects were seen in a further 6 animals. The effect of methyl-scopolamine lasted 6 min.

Haloperidol (0.5 mg kg^{-1} i.v., dissolved in equimolar tartaric acid and diluted with water) was tested in 4 animals where no effects could be detected.

Effects of agonists on B 1 and B 2 power

Arecoline The effects of arecoline were tested in 12 animals. Arecoline produced a reversible dose-dependent decrease in both B 1 and B 2 power (Figure 4). The effect of arecoline lasted for 30 ± 6 min following intravenous injection of 0.5 mg kg^{-1}. The data from all the animals is shown in Figure 5 as a dose-response curve from which it can be seen that band B 1 was more sensitive than band B 2.

The effects of scopolamine (0.1 mg kg^{-1} i.v.) on responses to arecoline were tested in 11 animals. In all cases, the response to arecoline was reversibly antagonized by scopolamine. The maximum effect of the antagonist developed in 10 min and lasted for 3 h before recovery of the control response was obtained. An example of the effects of scopolamine on responses to arecoline is shown in Figure 6. Arecoline (0.5 mg kg^{-1} i.v.) resulted in a reversible decrease in power in both bands. Following injection of scopolamine (0.1 mg kg^{-1} i.v.), the response to arecoline was reduced to 55% (B 1) and 66% (B 2) of control values respectively. The antagonism developed further so that the next response to arecoline was 16% (B 1) and 26% (B 2) of controls.

In contrast to scopolamine, methyl-scopolamine (9 animals; 0.1 mg kg^{-1} i.v.) and haloperidol (4 animals; 0.5 mg kg^{-1} i.v.) had no significant effects on responses to arecoline.

The pooled data from all the animals showed that scopolamine antagonized responses to arecoline by 85 ± 4% (B 1) and 79 ± 7% (B 2) whereas the antagonism produced by methyl-scopolamine was 10 ± 5% (B1) and 5 ± 3% B 2 respectively.

Oxotremorine The effects of oxotremorine were tested in 6 animals. Oxotremorine produced a long lasting decrease in both B1 and B2 power. Even with a relatively low dose (0.05 mg kg^{-1} i.v.) the effects lasted for 79 ± 9 min, rendering impractical the construction of dose-response curves as with arecoline. The responses to oxotremorine could be reversed and antagonized by scopolamine (4 animals; 0.5 mg kg^{-1} i.v.) but not by methyl-scopolamine (4 animals; 0.5 mg kg^{-1} i.v.) (Figure 7).

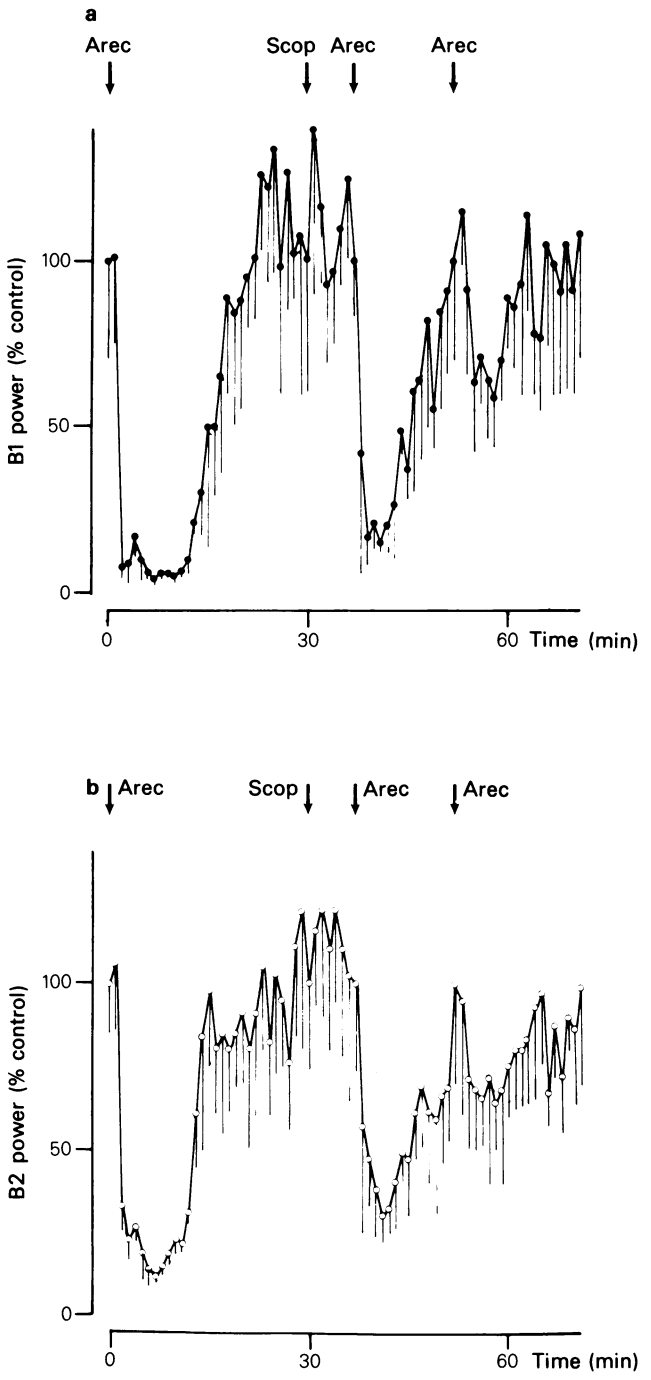

Figure 6 Effect of scopolamine on responses to arecoline in Bands B 1 (a) and B 2 (b). Each point is the mean of a 1 min spectral analysis expressed as a percentage of the respective control (pre-drug) values; s.d. shown by vertical lines. Arec: arecoline 0.5 mg kg^{-1} i.v.; Scop: scopolamine 0.1 mg kg^{-1} i.v. Responses to arecoline were antagonized by scopolamine with recovery occurring 3 h later.

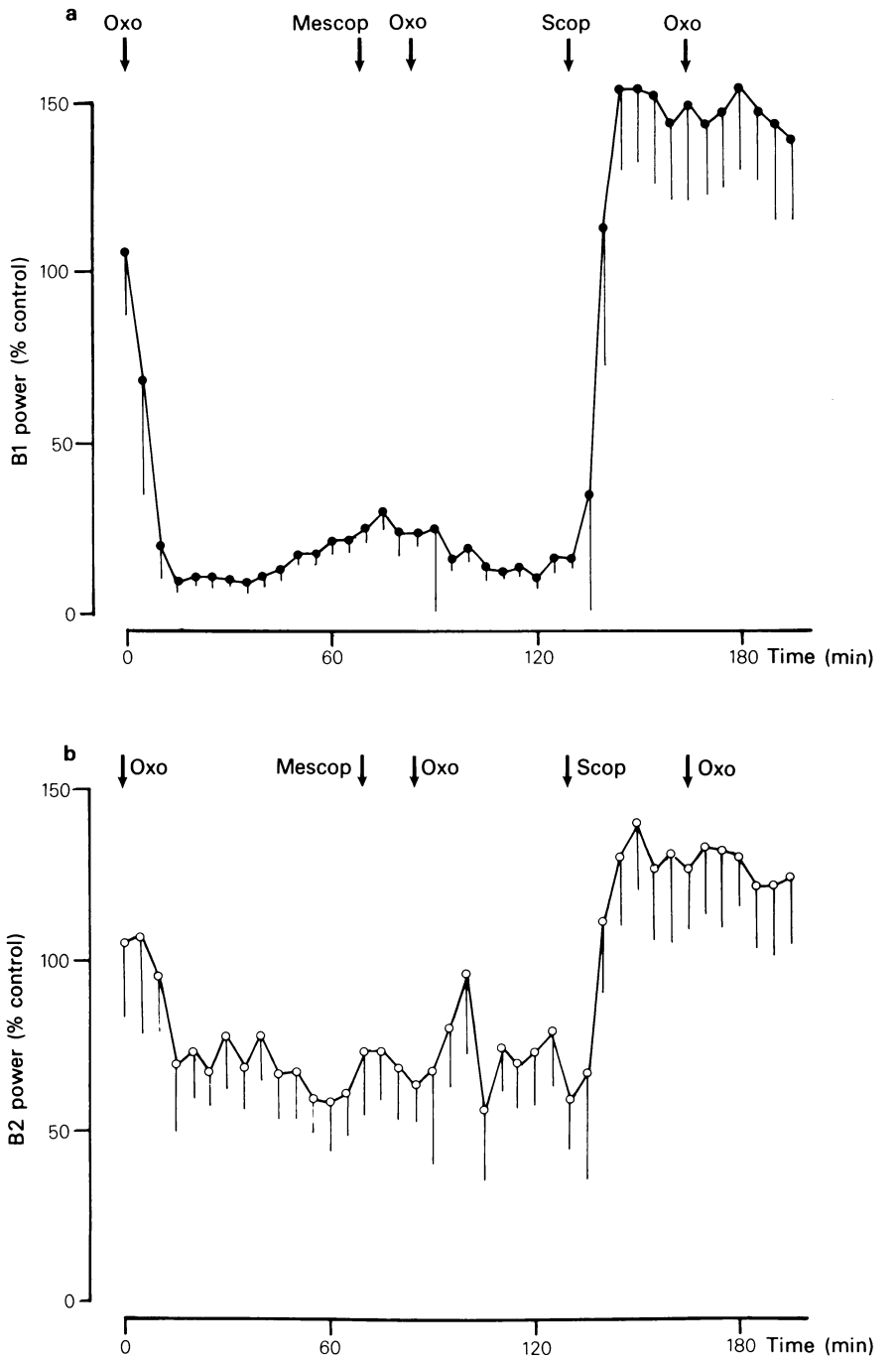

Figure 7 Effects of scopolamine and methyl-scopolamine on responses to oxotremorine in Bands B 1 (a) and B 2 (b). Each point is the mean of a 1 min spectral analysis expressed as a percentage of the respective control (pre-drug) values; s.d. shown by vertical lines Oxo: oxotremorine 0.05 mg kg^{-1} i.v.; Mescop: methyl-scopolamine 0.5 mg kg^{-1} i.v.; Scop: scopolamine 0.5 mg kg^{-1} i.v. The response to oxotremorine was methyl-scopolamine-resistant but was reversed and antagonized by scopolamine. Recovery of the response was not followed.

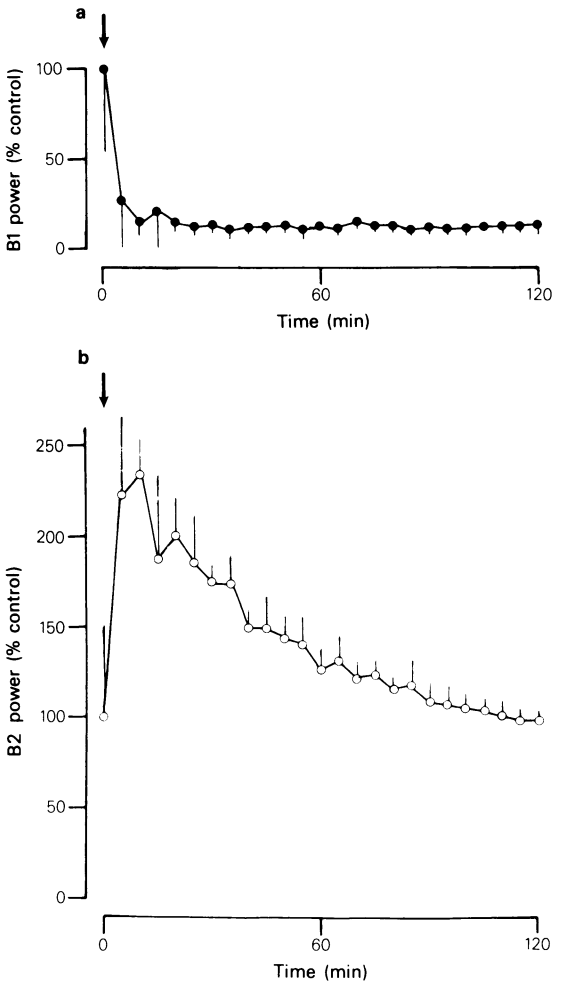

Figure 8 Effects of amphetamine on Band B 1 (a) and B 2 (b) power. Each point is the mean of a 1 min spectral analysis expressed as a percentage of the respective control (pre-drug) values; s.d. shown by vertical lines. Amphetamine (5 mg kg^{-1} i.v.) was injected at the time-point indicated by the arrows.

Amphetamine The effects of amphetamine were tested in 5 animals. Amphetamine produced differential effects on B 1 and B 2 power. B 1 power was irreversibly reduced following the injection of amphetamine (5 mg kg^{-1} i.v.), whereas B 2 power was dramatically increased. This effect lasted for 2 h (Figure 8). An increase in B 2 power accompanied by a decrease in B 1 power is equivalent to an increase in the dominant frequency. Only one response to amphetamine could be evoked in each animal; the animal remained insensitive to further applications of amphetamine for at least 5 h although responses to muscarinic agonists, e.g. oxotremorine could still be evoked.

Bromocriptine The effects of bromocriptine (dissolved in lactic acid and diluted as appropriate with water) were tested in 11 animals. No effects of bromocriptine could be detected within the dose range 0.001 to 2 mg kg^{-1} i.v.

Discussion

The electrical activity recorded from the hippocampus in the halothane-anaesthetized rat demonstrates identical characteristics to that recorded from the freely moving animal. Thus, 3 types of recordings were obtained, namely RSA, LIA and small amplitude fast activity (Figure 1). Whereas in the awake, freely moving animal each type of electrical activity has been related to a particular behaviour (Kramis *et al.*, 1975), in the anaesthetized animal the probability of each type of electrical activity seems to be a function of time only.

Two pharmacologically distinct types of RSA have been reported, one of which is blocked by atropine (iRSA) and one which is resistant to atropine (mRSA) but is blocked by anaesthetics (Kramis *et al.*, 1975). Both types seem to require an intact medial septal nucleus, however, as no RSA has been reported following lesions of this nucleus (Kolb & Whishaw, 1977). Similarly, in the experiments described here, no RSA could be detected following lesions of the medial septal nucleus. In animals anaesthetized with ether, urethene (Vanderwolf, 1975) or halothane (this paper), then, RSA is solely cholinergic, is dependent on medial septal nucleus afferents and can be generated by the injection of cholinoceptor agonists such as arecoline (Figure 4). A consequence of such a synchronization of the signal (transforming LIA to RSA for example) is a reduction of the power of the signal revealed through Fourier transformation (Figure 4). In contrast, in animals with medial septal lesions, injection of arecoline generated iRSA with a concomitant increase in power (Figure 5). The explanation for this is not clear, as supersensitivity of muscarinic receptors following removal of presynaptic terminals is thought not to occur (Bevan, unpublished observations; Overstreet *et al.*, 1980; but see Westlind *et al.*, 1981).

Almost by definition, iRSA is a phasic phenomenon. Malisch & Ott (1982) have argued that the injection of muscarinic agonists should consequently inhibit RSA, as the continuous presence of the exogenously applied agonist at the synapse would mask the phasic rhythmical influence of the medial septal nucleus. As this is patently not the case, one may counter that, although a septal input to the hip-

pocampus is indeed required for iRSA, a phasic septal input is not pre-requisite. Moreover, exogenously applied muscarinic agonists (a 'tonic' stimulus) can evoke or potentiate phasic phenomena (Figures 2, 3). This is important in the context of the current interest in muscarinic agonists as a possible therapeutic intervention in Alzheimer's disease.

The results show that, in the anaesthetized animal, iRSA, LIA and small amplitude fast activity alternate with each other apparently at random. Muscarinic agonists synchronize the recordings, i.e. change for example LIA into iRSA (Figure 2) so that the proportion of iRSA present in a recording of fixed length increases (Lehmann *et al.*, 1971). Such an effect is, however, difficult to quantify and thus, for drug interaction studies, rather than study iRSA *per se*, a Fourier transformation was used to produce a steady quantitative image of the electrical signal. The results show that muscarinic agonists produce a dose-dependent decrease in the power of the hippocampal electrical activity, both in the total energy and in the energy in frequency bands B 1 and B 2. The responses, which could not be mimicked by amphetamine or bromocriptine, were scopolamine-sensitive but methyl-scopolamine- and haloperidol-insensitive, showing that the effects are indeed mediated by central muscarinic receptors.

Green & Arduini (1954) were perhaps the first to describe the hippocampal rhythmical activity as the hippocampal arousal rhythm. Since then, numerous studies have appeared attempting to establish a relationship between RSA and arousal and attention (for discussion, see Robinson, 1980). However, such a relationship is not universally accepted. RSA is unlikely to be a simple correlate of arousal as it can be absent in behavioural states where arousal is not apparent (this paper; Black & Young, 1972; Vanderwolf *et al.*, 1978). Thus, in agreement with Vanderwolf (1975), there would seem to be no compelling reason to relate atropine-sensitive RSA and arousal.

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